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Approximate linear confidence and curvature of a kinetic model of dodecanedioic acid in humans

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Panunzi, Simona, Andrea De Gaetano, and Geltrude Mingrone. Approximate linear confidence and curvature of a kinetic model of dodecanedioic acid in humans. *Am J Physiol Endocrinol Metab* 289: E915–E922, 2005. First published June 21, 2005; doi:10.1152/ajpendo.00503.2003.—Dicarboxylic acids with an even number of carbon atoms have been proposed as an alternate energy substrate for enteral or parenteral nutrition in the acutely ill patient, due to their water solubility and their yielding TCA cycle intermediates upon β-oxidation. In the present work, a nonlinear compartmental model of the kinetics of dodecanedioic acid is developed, and its parameters are estimated from time concentration experimental observations obtained from six healthy volunteers undergoing a per os administration of 3 g of the substance. Although the model is linear in the transfer of the free substance from plasma to the tissues, the exchange between gut and plasma compartments is represented as a saturable function. Albumin binding is then incorporated to obtain the final model in terms of the measured total concentrations. Estimates of the model's structural parameters were computed for each experimental subject, and the usual single-subject approximate confidence regions for the parameters were derived by inversion of the Hessian at the optimum. To verify the applicability of this approximation, the nonlinearity of the expectation surface at the optimum was measured by computing the normal (intrinsic) component of curvature. Because the model curvature was excessive in all subjects, the usual approximation could not be trusted to provide acceptable approximations to the parameter confidence regions. A suitable Monte Carlo simulation yielded empirical joint parameter distributions from which the approximate parameter variances could finally be obtained.

mathematical models; metabolism; kinetics; nonlinear parameter estimation; confidence regions

DICARBOXYLIC ACIDS (DA) constitute a class of substances (1, 2, 11, 23) with two terminal carboxylic groups, which confer to the molecule an elevated water solubility. This peculiarity differentiates DA from monocarboxylic acids, which are lipophilic because of their aliphatic chain. DA, being water soluble, are bound to plasma albumin to a lesser extent than both medium- (11, 16, 23, 24) and long-chain monocarboxylic acids (10, 13, 21, 25). Their intravenous administration does not require emulsifying, because they can be injected as salts of inorganic cations.

Even-numbered DA are metabolized to acetyl-CoA and enter the TCA cycle. In addition, the metabolism of DA produces, as intermediate, succinyl-CoA, which is both a gluconeogenetic precursor and an intermediate of TCA cycle.

The possible use of DA as alternate fuel substrate for enteral or parenteral nutrition has been suggested (6, 7, 8, 14, 15, 17–20, 22, 27, 28), and their characteristics might make them useful in different pathological conditions. In dyslipidemia and late sepsis, where the tissue utilization of triglycerides administered as emulsion is impaired because of reduced clearance, DA can be more effectively administered and delivered to the tissues than conventional lipids. In decompensated diabetes mellitus or in those clinical conditions, like malnourishment or sepsis, where excessive gluconeogenesis from amino acids causes muscle mass wasting, they can help spare body protein.

However, DA with a chain length shorter than dodecanedioic acid (C12) are eliminated with urine to a high degree (7, 15, 20, 28) and are therefore not suitable in their pure form for the delivery of substantial amounts of energy in enteral or parenteral nutrition, despite their theoretical advantages.

Preliminary studies in rats (18) showed that only 3.9% of C12 administered as an intravenous bolus is lost with urine, that its half-life is short (12.47 min), and that its systemic clearance is good (0.0138 liter·kg body wt^{-1}·min^{-1}); in addition, the low C12 renal clearance indicates tubular reabsorption of dodecanedioic acid.

Within the general purpose of better understanding the absorption and distribution of this potentially useful artificial metabolic substrate, the goal of the present study is therefore that of quantifying the kinetics of per os-administered C12 in healthy volunteers.

In the course of the evaluation of C12 kinetics, a study of the degree of nonlinearity of the resulting mathematical model is conducted to verify whether the usual linearization procedure for the determination of parameter confidence regions is appropriate.

MATERIALS AND METHODS

Materials

C12 was purchased from Sigma (St. Louis, MO). C12 was then purified by Real S. R. L. (Como, Italy) and was free from pyrogens and contaminants with a degree of purification, ascertained using gas-liquid chromatography and mass spectrometry, of 99.8%. A 0.4 M solution of C12 salified with NaOH was used.

Experimental Protocol

A group of six healthy volunteers underwent per os administration of sodium dodecanedioate. They had no previous history of metabolic or endocrine diseases, no active illnesses, and were currently on no medications. The experimental subjects were admitted to the Day Hospital of the Division of Metabolic Diseases of the Catholic University School of Medicine Hospital (Rome, Italy) on the morning

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of the experiment, after an overnight fast. A bolus P.O. dose of 3 g of the substance was administered at *time 0* (around 8:30 AM). Heparinized blood samples (8 ml) were drawn from an arm vein at different times for about 4 h postadministration. Blood samples were immediately centrifuged. Plasma samples were frozen at −20°C until analysis. The protocol followed the directives of the Ethical Committee of the Institutional Health Review Board of the Catholic University School of Medicine and conformed to the principles of the Declaration of Helsinki. Informed consent was obtained in all cases.

DA Analysis

Plasma samples. One hundred micrograms of azelaic acid were added to each 1 ml of each plasma sample as an internal standard. Proteins were precipitated with 0.1 ml of 4 N HCl, and DA were extracted twice with eight volumes of ethyl acetate, maintaining the solutions at 60°C for 15 min. 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 from FTS Systems (Stone Ridge, NY).

High-performance liquid chromatography analysis. The extracted solutes were dissolved in 0.5 ml of acetonitrile-methanol (1:1, vol/vol) 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 min. The derivatives were dissolved in a final volume of 1 ml of acetonitrile-methanol (1:1, vol/vol), and an aliquot of 10 μl was automatically injected into a liquid chromatograph (Hewlett-Packard 1050) with an HP 3396A integrator and a scanning spectrophotometer operating in the 190-to-600-nm wavelength range (light source: deuterium lamp, noise $<2.5 \times 10-5$ AU peak-to-peak at 254 nm with 4 nm bandwidth, flowing water at 1 ml/min).

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

Modeling and Statistics

After per os administration, C12 is transferred from portions of the alimentary tract (henceforth briefly referred to as "gut") to a central compartment (plasma and quickly equilibrating interstitial fluid). Loss of the substance occurs from the central compartment toward the tissues. Urinary elimination is negligible, according to previously published results (8). Fecal elimination after per os administration is also negligible. Within tissues, C12 finally undergoes oxidation in the TCA cycle.

Two three-compartment models explicitly representing gastric emptying into the bowel were evaluated and discarded because of general nonidentifiability. Four two-compartment models of C12 kinetics, including a gut compartment and a central compartment, were thus compared. They differed according to the type of transport (linear or saturable) from gut to plasma and from plasma to peripheral tissues (Table 1 gives the detailed equations for each considered model). For each model, albumin binding was then taken into account (*Eqs. 3* and *4*), obtaining in every case a nonlinear model for the total drug concentration.

Each model was fitted on each experimental subject's observed C12 concentrations by ordinary least squares (OLS). Models were compared by visual inspection of the residuals plots and by consideration of the R^2 and the values of the Akaike and Schwartz criteria. The selected model [saturable/linear transfers (SL)] is sketched in Fig. 1 and is described below. Drug transfer from the gut to the central compartment is assumed to be saturable, whereas elimination from the central compartment is assumed to be linear in the free concentration. We assume that no protein-bound C12 is able to move out of the central compartment so that drug movement is limited to the free form. The model equations are

$$\frac{dG(t)}{dt} = -\frac{T_M G}{K_M + G} \quad G(0) = D \qquad (1)$$

and

$$\frac{dC_{TOT}(t)}{dt} = +\left(\frac{T_M G}{K_M + G}\right)\frac{1}{V} - k_{02}C_{FREE} \quad C_{TOT}(0) = 0 \qquad (2)$$

where G (mmol) is the amount of C12 in the gut compartment; t (min) is time; T_M (mmol/min) is the maximal rate of C12 transport from gut

Table 1. *Summary statistics of the chosen model (SL) and of three other models fitted to the experimental data (average values)*

MODEL	Transfer between Compartments	Model equations	R^2	Akaike	Schwartz
SL	Carrier limited (gut to plasma) and linear (plasma to tissues)	1) $\frac{dG(t)}{dt} = -\frac{T_M G}{K_M + G}$	0.889	−65.376	−63.334
		2) $\frac{dC_{TOT}(t)}{dt} = +\left(\frac{T_M G}{K_M + G}\right)\frac{1}{V} - k_{02}C_{FREE}$			
LS	Linear (gut to plasma) and carrier-limited (plasma to tissues)	1) $\frac{dG(t)}{dt} = -k_{21}G$	0.821	−59.11	−57.068
		2) $\frac{dC_{TOT}(t)}{dt} = +(k_{21}G)\frac{1}{V} - \frac{T_M C_{FREE}}{K_M + C_{FREE}}$			
LL	Both linear	1) $\frac{dG(t)}{dt} = -k_{21}G$	0.750	−56.641	−55.109
		2) $\frac{dC_{TOT}(t)}{dt} = +(k_{21}G)\frac{1}{V} - k_{02}C_{FREE}$			
SS	Both carrier limited	1) $\frac{dG(t)}{dt} = -\frac{T_M G}{K_M + G}$	0.879	−63.058	−60.506
		2) $\frac{dC_{TOT}(t)}{dt} = +\left(\frac{T_M G}{K_M + G}\right)\frac{1}{V} - \left(\frac{T_{M_{02}} C_{FREE}}{K_{M_{02}} + C_{FREE}}\right)$			

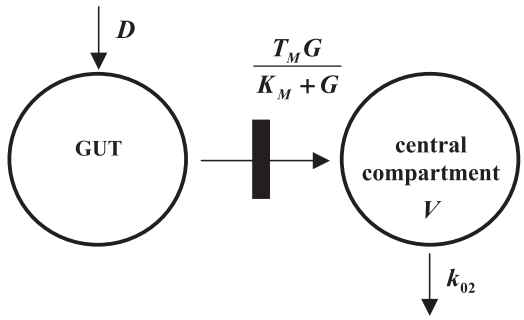

Fig. 1. Schematic representation of the two-compartment model used for analysis. V, central compartment distribution volume; k_{02}, transfer rate constant to tissues, T_M, maximal transport from gut to central compartment; K_M, relative half-maximal gut (C12) content; D, dose administered.

to plasma; K_M (mmol) is the quantity of half-maximal transport from gut to plasma; D (mmol) is the quantity of drug administered per os; V (l) is the apparent C12 distribution volume; k_{02} (min^{-1}) is the rate elimination constant of C12 from the central compartment; and C_{TOT} and C_{FREE} (mM) are the total and free concentrations, respectively, of C12 in the central compartment.

Previous results (8) indicate that a single-site model can describe C12 binding to human serum albumin

$$C_{TOT} = C_{FREE} + \frac{AnKC_{FREE}}{1 + KC_{FREE}} \quad (3)$$

where A (mM) is the concentration of albumin in plasma, *n* is the average number of albumin-binding sites for C12, and K (mM^{-1}) is the binding affinity.

Given the binding relationship (3), which expresses total drug concentration in terms of free drug concentration, the free concentration may be expressed in terms of total concentration as follows:

$$C_{FREE} = \frac{1}{2K}(-1 - AKn + KC_{TOT} + \sqrt{4KC_{TOT} + (1 + AKn - KC_{TOT})^2}) \quad (4)$$

For all subjects considered in the present study, the mean values for albumin-C12 binding parameters estimated in a group of healthy subjects (8) were utilized. The value of plasma albumin concentration was assumed to be equal to 0.61 mM. The number of binding sites per molecule *n* and the value of the association constant K, respectively, were equal to 3.1 and 6.4 mM^{-1}. The free parameters to be estimated in the final model, given by *Eqs. 1*, *2*, and *4*, were therefore V, k_{02}, T_M, and K_M.

For all parameters, the standard errors of the estimates were first determined from the inverse Hessian matrix computed at the optimum. The evaluation of model curvature at the optimum indicated potential problems in the above procedure, and parameter confidence regions were reassessed by Monte Carlo simulation.

In general, if nonlinearity of the model at the optimum is found to be excessive for the reliable determination of parameter confidence intervals, a Monte Carlo procedure may be performed to obtain empirical parameter confidence regions. This numerical procedure is based on the assumptions that *1*) the correct functional form of the model is known; *2*) the point parameter estimate is sufficiently near to the true value of the parameter itself; *3*) the observed variance, calculated as the mean squared residual, is close to the population variance, the expected squared error; and *4*) the errors are normally distributed with zero mean and variance-covariance matrix $\sigma^2 I$. Under these assumptions, the empirical distribution of a large number of parameter estimates obtained from randomly generated samples from the model will be close to the true distribution of the estimates. Confidence regions may thus be obtained from it.

To further explore the impact of variability in the binding parameters (*n* and K) onto the confidence regions of the structural model parameters (V, K_M, k_{02}, T_M), the entire Monte Carlo procedure was also repeated, letting *n* and K vary randomly.

Parameter estimates are expressed as sample mean ± SE unless otherwise specified.

To maintain as conceptually distinct the nonlinearity of the biochemical transport and the nonlinearity of the expectation surface at the optimum, the term "saturable" has been consistently used for the former.

RESULTS

Model Selection

The anthropometric characteristics of the experimental subjects are reported in Table 2. Each model was fitted by OLS on each one of the six subjects, and in every case the R^2, Akaike criterion and Schwartz criterion were computed. Table 1 reports the average model selection criteria for all four considered models. All model selection criteria favored the SL model, which was therefore retained. This model is diagrammatically represented in Fig. 1.

Single-Subject Parameter Estimates

Table 3 reports the model parameter estimates obtained for each individual subject, together with the respective single-subject asymptotic coefficients of variation (CVs, computed via inversion of the Hessian at the optimum). The CVs for K_M and T_M were in some cases unacceptably high, leading to the conclusion that these constants could not be reliably identified on single subject data. Figure 2 shows the observed SL and LL model-predicted time courses of C12 plasma concentration for one subject.

The study of the degree of model nonlinearity at the optimum indicated nonadequacy of the usual approximation procedure for determining the parameter confidence regions in all six subjects. Table 4 reports, for each subject, the nonlinearity coefficients δ_1 and δ_2, defined in detail in the APPENDIX and each of the four components of the versor *h* (unit-length vector in the direction of maximum nonlinearity in parameter space). The obtained results evidence a high nonlinearity of the model for all subjects. Figure 3 shows, for one subject (*subject 6*), how the quantity δ_1 varies in all directions of the plane spanned by the couple of parameters V and K_M. Although over the V/K_M plane, nonlinearity seems to be limited (attaining a maximum of ~0.14), small departures from the plane, i.e., small increases of the k_{02} and T_M parameters, actually produce large increases in nonlinearity.

To obtain an empirical distribution of parameter estimates, a Monte Carlo procedure was conducted, generating 5,000 arti-

Table 2. *Anthropometric characteristics of the studied subjects*

Subject	Weight, kg	Height, cm
1	56	160
2	85	180
3	65	167
4	82	180
5	54	164
6	70	170
Means ± SE	68.67±5.28	170.17±3.39

Table 3. *Parameter estimates of the chosen kinetic model for all experimental subjects and their asymptotic CV together with the sample mean and SE*

	Parameter										
Subject	V	CV(V)	K_M	CV (K_M)	k_{02}	CV(k_{02})	T_M	CV(T_M)	R^2	Akaike	Schwartz
1	11.124	10.56	0.0005	*Undefined*	0.126	14.7	0.1	*Undefined*	0.89	−68.034	−65.774
2	13.551	12.18	0.0003	9.18	0.093	19.91	0.109	0	0.858	−62.528	−60.268
3	10.82	6.09	0.00007	56.52	0.137	9.25	0.109	0	0.965	−70.952	−69.361
4	11.461	10.68	0.0007	16.74	0.103	16.5	0.093	0.01	0.907	−65.2223	−62.963
5	12.282	21.01	2.178	166.81	0.08	33.5	0.181	46.42	0.819	−58.072	−56.132
6	12.102	15.03	1.824	104.65	0.11	20.99	0.18	24.6	0.901	−69.024	−67.085
Mean	11.890	12.608	0.667	70.780	0.108	19.142	0.129	14.206	0.890	−65.639	−63.597
SE	0.403		0.424		0.009		0.017				

V, central volume of distribution; k_{02}, elimination rate constant of C12 from the central compartment to peripheral tissues; T_M, maximal transport rate of C12 from gut to central compartment; K_M, gut C12 quantity of half-maximal transport; CV, coefficient of variation.

ficial data samples under the assumption that the model retained is correct by adding random Gaussian noise with variance s^2 to the values predicted using the optimum parameter. For each sample so generated, the estimation procedure was performed again and the parameter values were obtained. Table 5 reports, for each experimental subject, the results of the 5,000 Monte Carlo simulations. A direct comparison between Monte Carlo confidence regions and asymptotic confidence regions can be obtained by observing Tables 3 and 5. In general, the size of the confidence regions (reflected in the size of the CV) is markedly different for subjects with high model nonlinearity at the optimum and for those parameters for which nonlinearity is most marked (as evidenced by the components of the h versors). In particular, results for K_M indicate that this parameter is not usually identifiable in each single subject and that only sample estimates may actually be informative.

Table 6 reports the 50 and 90% absorption times computed for each studied subject from single-subject model parameter estimates, as well as their sample mean and standard deviations.

To evaluate the impact of the uncertainty of albumin-binding parameter estimates onto the amplitude of the confidence regions of the model structural parameters, the Monte Carlo procedure was repeated on every subject (5,000 runs/subject), letting the generating values of K and *n* vary following a normal distribution with means of 6.4 and 3.1, and with standard deviations of 1.8 and 0.2, respectively (8). The results of this second round of Monte Carlo simulations are reported in Table 7; they can be compared with the corresponding results obtained when running the Monte Carlo in the absence of any variability in the binding parameters (Table 5). It can be appreciated that, in the presence of variability of the binding parameters, the structural parameter confidence regions are only a little wider. It can be concluded that, in the present case, the variability in the binding estimates does not practically affect the assessment of the model structural parameters.

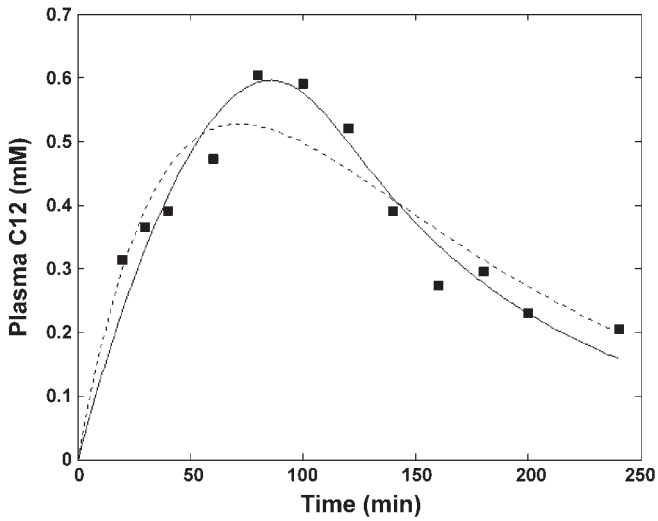

Fig. 2. Comparison between saturable/linear (SL) and linear/linear (LL) models. Experimental observations (■), SL model prediction (continuous line), and LL model prediction (dotted line) of C12 plasma concentrations vs. time.

Sample Parameter Estimates

The bottom two lines of Table 3 report the sample average estimates obtained together with their sample standard error and relative CV. The rate of elimination of C12 from the central compartment to the periphery, reflecting essentially tissue uptake and metabolism of the substance, was on average 0.108 ± 0.009 min^{-1}. The maximal rate T_M of gut-to-plasma transfer was 0.129 ± 0.017 mmol/min, whereas the mean values of K_M and the C12 distribution volume were 0.667 ± 0.424 mmol and 11.890 ± 0.403 l, respectively. All CVs except for K_M are below 20%, so we might be very confident of the obtained estimates.

DISCUSSION

As previously reported (8), the urinary elimination of C12 does not exceed 1% of the administered dose, even when the

Table 4. *Intrinsic model nonlinearity indicators*

Subject	δ_1	δ_2	h_v	h_{K_M}	$h_{k_{02}}$	h_{T_M}
1	0.40	0.64	0.419	−0.908	−0.007	−0.012
2	0.996	0.999	0.547	0.772	0.233	0.2225
3	1	1	0.471	0.535	0.2415	0.565
4	1	1	0.785	−0.607	0.12	0.015
5	0.84	0.97	−0.309	0.951	0.0001	0.012
6	0.98	0.99	−0.55	0.83	0.007	0.012

For each experimental subject (*1–6*) the nonlinearity coefficients δ_1 and δ_2 (defined in the text) and each of 4 components of the versor *h* (unit-length vector in the direction of maximum nonlinearity in parameter space) are reported.

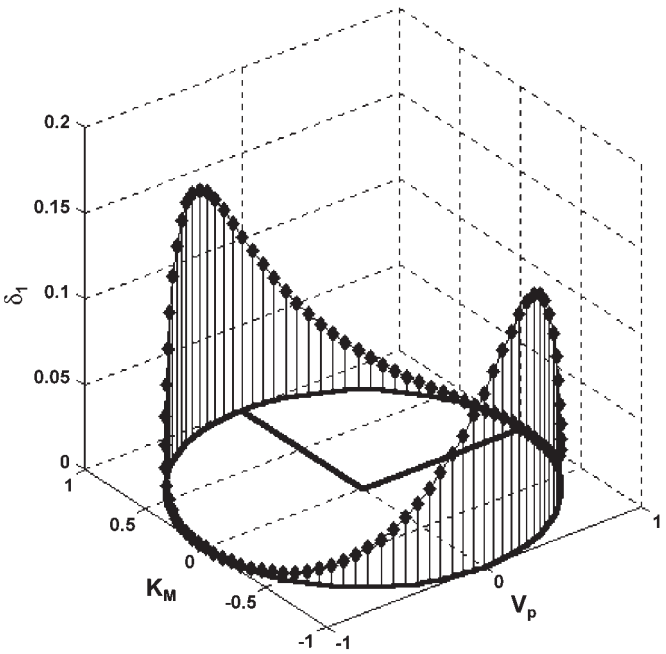

Fig. 3. Plot of the intrinsic nonlinearity indicator δ_2 relative to directions (unit vectors) in the (V, K_M) plane. Two unit vectors, one in the direction of the parameter *V* and the other in the direction of the parameter K_M, are drawn for reference.

administration is intravenous, leading to higher peak plasma concentrations than per os. The physiological role of the substance as a potential fuel substrate is thus supported by its near-complete oxidation and storage.

To further investigate the pharmacological kinetics of C12 in humans undergoing per os administration of the substance, several different nonlinear two-compartment models were fitted to experimental data from six healthy volunteers. All of the compartmental configurations tested included alimentary tract (gut) and central (plasma) compartments and differed for transport mechanisms between compartments. Binding of C12 to serum albumin was always taken into account. According to a series of criteria (average Akaike information criterion, average Schwartz criterion and average R^2), the model including a saturable transport function of the Michaelis-Menten type from gut to plasma and a linear transfer from plasma to peripheral tissues was deemed to be the best. This does not imply in any way the absence of specific tissue receptors, particularly in the skeletal muscle. Rather, we might be in the domain of near-linearity of a possibly carrier-mediated transport.

Consistent estimates of the central volume of distribution were obtained, about 11–13.5 l for subjects weighing between 54 and 85 kg, reflecting plasma volume, a quota of quickly equilibrating interstitial space, and possibly a degree of binding to nonalbumin molecules. Maximal absorption from the alimentary tract to plasma was in the range of 0.1–0.2 mmol/min, corresponding approximately to 300–600 kcal/day (attainable, for instance, with continuous enteral nutrition). Linear transport from plasma to the periphery, at ~10%/min, seems on the other hand very brisk. The estimates of the volume of distribution that we obtain in the present work (11.89 ± 0.40 l) are substantially higher than those previously obtained (6.39 ± 0.44 l) (9). This is not surprising, given the longer time allowed for distribution in the case of oral administration compared with the case of intravenous administration, therefore recruiting a larger fraction of extracellular space. On the other hand, the fact of obtaining higher elimination rates than before (0.1 ± 0.01 vs. 0.013 ± 0.0023) can be explained by supposing an essential nonlinearity of tissue uptake. Bertuzzi et al. (9) had already assumed nonlinearity but could not meaningfully estimate the relative parameters and reverted to the simpler linear elimination on a patient-by-patient basis. We also could not substantiate nonlinear plasma to tissue transfer; moreover, we observed (due to po rather than iv administration) substantially lower plasma concentrations of the substance. It is therefore very likely that the linear rate we estimated on the present data, reflecting the initial steeper portion of the nonlinear transfer curve, is higher than that estimated by Bertuzzi et al. (9) on a flatter portion of the same curve.

The conclusion above is supported by a numerical argument: let us represent the (assumed real) nonlinear tissue uptake rate by means of a Michaelis-Menten kinetic [$T_{M02} \times C_{FREE}/(K_{M02} + C_{FREE})$] and let us suppose to set the T_{M02} and K_{M02} values to be 0.44 and 3.65 mM/min, respectively. Let us now hypothesize that, at the actual observed operating concentration C_{FREE}, we approximate the nonlinear transfer with a linear term of the form $K \times C_{FREE}$. We may find what the apparent value for the constant K is by solving the equation

$$(T_{M02} \times C_{FREE})/(K_{M_{02}} + C_{FREE}) = (K \times C_{FREE}) \qquad (5)$$

If we do this at the concentration $C_{FREE} = 0.4$ mM (typical of the concentration range observed in the present work), we find an apparent linear transfer rate $K = 0.108$; if, on the other hand, we repeat the computation, the concentration $C_{FREE} = 20$ mM (typical of the concentrations values observed in Bertuzzi' s work), we find a value of $K = 0.018$ for the apparent linear transfer rate constant.

Table 5. *Results of Monte Carlo procedure: average parameter estimates and relative empirical CVs over 5,000 generated samples*

	Parameter											
Subject	V	SD	CV(V)	K_M	SD	CV (K_M)	k_{02}	SD	CV(k_{02})	T_M	SD	CV(T_M)
1	11.37	1.40	12.35	0.004	0.16	4303.30	0.12	0.02	16.28	0.10	0.009	8.51
2	13.44	1.64	12.18	0.0005	0.01	1609.15	0.094	0.02	18.64	0.11	0.01	8.84
3	10.70	0.75	7.05	0.00007	0.000005	7.66	0.139	0.01	10.67	0.108	0.004	3.41
4	11.35	1.81	15.96	0.01	0.03	2622.37	0.108	0.03	24.04	0.093	0.008	9.01
5	11.23	2.96	26.38	3.21	10.19	317.02	0.10	0.05	48.59	0.188	0.21	109.76
6	11.755	1.91	16.21	1.868	1.77	94.62	0.119	0.03	24.66	0.176	0.04	20.17

Table 6. *Computed 50 and 90% gut absorption times of orally administered C12 in studied subjects*

Subject	Time at 50% of absorption, min	Time at 90% of absorption, min
1	66.5	118.5
2	61	109
3	61	109
4	71	127
5	90	186.5
6	87.5	178
mean (SD)	72.83 (12.91)	138.00 (35.03)

We can see, therefore, that the explanation that the difference in apparent linear transfer rate constants is produced by observing an inherently nonlinear transfer at two different operating concentrations is possible. If we further take into account the obvious uncertainty in the *K* value estimate, the conclusions arrived at in the present work seem consistent with those reported by Bertuzzi et al. (9).

The maximal caloric intake that can be provided with enteral C12 administration is comparable with the entire lipid quota of an isocaloric diet of 2,000 kcal/day. It is not likely that a physician would consider administering C12 as the only supplemental lipid substrate. However, an association of dicarboxylic and traditional monocarboxylic acids would be feasible and would exploit the metabolic benefits of both fuels. For instance, administering 420 kcal/day as C12 would substitute about 70% of the lipid component of commonly employed nutrition formulas (with 30% lipid).

In case larger amounts of dicarboxylic acids were thought to be useful, parenteral administration of the substance is likely to allow very substantial energy delivery. This form of administration is safe (8, 18), inexpensive, and practical because of C12 water solubility and good tolerability of peripheral vein infusion.

Coupled with the lack of energy and synthetic requirements for hepatic complexing and the production of precious TCA cycle intermediates from its β-oxidation, C12 therefore appears to be a viable and desirable substrate for artificial nutrition.

As far as the parameter K_M is concerned, it would appear that the six patients reflect two essential modes of behavior: K_M near zero (*subjects 1–4*), implying an essentially all-or-none response to increasing C12 plasma concentrations, where maximal transport is already immediately present as soon as minimal C12 circulates in plasma; and substantially nonzero K_M (*subjects 5* and *6*), where maximal transport is attained gradually with increasing C12 plasma concentrations. These differences might be due to a genetic variability of the transport system, a different expression of the transport system secondary to physiological determinants such as the diet, or the possible coexistence of more than one population of gut transporters with different affinities expressed to a different degree in the studied subjects. Further investigation in this direction is warranted, possibly obtaining a richer data set by the use of stable isotope tracers.

The speed of gastric emptying (essentially a zero-order process) could also well account for the observed divergence in K_M. To investigate this possibility, we also built and fitted two models, including explicitly a "stomach" compartment, into which the bolus dose was injected and from which C12 entered the gut with zero-order or first-order transport, respectively. Although neither of the two models (presenting 5 free parameters each) could be reliably identified, still, in the second one, high stomach-to-gut transfer rates tended to be associated with high K_M values, suggesting that when gastric emptying is fast, plasma uptake depends on gut concentration, whereas when gastric emptying is slow, plasma uptake of C12 from the gut proceeds at an essentially constant rate. These models, however, provide no identification advantage over the simpler four-parameter, two-compartment model. Lacking the ability to identify more than two compartments, we would retain the original model (*eqs. 1* and *2*) with the proviso that low (unidentifiable) K_M may indeed indicate slow gastric emptying and, hence, apparent zero-order kinetics. A further observation supporting this interpretation is that forecast time-to-peak values were larger in the first four and smaller in the last two subjects (120, 120, 120, 140, 100, 80 min), again indicating that subjects with faster stomach emptying tended to have first-order gut-to-plasma transfer.

From the methodological point of view, it is instructive to note how in the present work the suitability of the standard computational procedures for the determination of parameter confidence regions was seriously challenged. It is clear that the usual linear procedure, based on the inversion of the Hessian at the optimum (26), fails when the assumption that the expectation surface can be approximated in the neighborhood of the estimate by the tangent linear subspace is not realistic.

A study of the degree of nonlinearity of the model in a neighborhood of the optimum was conducted, exploring all lifted directions from parameter space and computing the relative normal curvature.

For the present model and design points, it appears that the directions in parameter space, along which intrinsic nonlinear-

Table 7. *Results of the Monte Carlo procedure letting the values of K and n vary randomly: average parameter estimates and relative empirical CVs over 5,000 generated samples*

	Parameter																	
Subject	V	SD	CV(V)	K_M	SD	CV (K_M)	k_{02}	SD	CV (k_{02})	T_M	SD	CV(T_M)	K	SD	CV(K)	*n*	SD	CV(*n*)
1	11.32	1.48	14.00	0.0015	0.05	2953.26	0.12	0.02	16.93	0.10	0.008	8.01	6.36	1.65	26.00	3.10	0.20	6.33
2	13.38	1.77	13.25	0.0017	0.05	2944.79	0.09	0.02	20.76	0.11	0.01	9.70	6.38	1.67	26.21	3.10	0.20	6.46
3	10.67	0.79	7.41	0.00007	0.000004	6.08	0.14	0.01	10.87	0.11	0.004	3.39	6.35	1.66	26.19	3.11	0.20	6.50
4	11.39	1.90	16.70	0.002	0.04	2338.83	0.10	0.03	24.23	0.09	0.009	9.23	6.35	1.64	25.87	3.10	0.20	6.53
5	11.40	3.05	26.80	3.00	9.72	325.28	0.10	0.05	48.81	0.19	0.21	113.49	6.33	1.63	25.68	3.10	0.20	6.42
6	11.73	2.00	17.03	1.84	1.76	95.60	0.12	0.03	25.13	0.18	0.04	20.25	6.33	1.66	26.30	3.10	0.20	6.45

ity is highest, are represented by simultaneous changes in the parameters V and K_M. For all studied subjects we had to conclude that the maximum curvature index was so much above an acceptable threshold, and that therefore the model was so highly nonlinear that the linear inferential procedure could not be accepted. Therefore, the confidence regions obtained with the linear procedure in this case either could or could not have reflected the actual confidence regions. To obtain more reliable parameter confidence regions we employed a Monte Carlo procedure, generating the empirical distribution of the parameter estimates, assuming that the model was correct and that the point estimates of the parameters and the error variance were not too far from the true values. The results of the Monte Carlo procedure show that the model employed fits well, but that the uptake constant K_M is not reliably identified from a single subject's data set. One important methodological conclusion from the present work is that the study of model curvature at the optimal parameter value should be part of all physiological model evaluation procedures.

APPENDIX

Treatment of Highly Nonlinear Models

Study of the degree of the nonlinearity at the optimum. In nonlinear problems the approximate confidence region for the parameter vector, as commonly obtained by inverting the Hessian at the optimum, depends on the linear approximation of the expectation surface in a neighborhood of the optimum. A generally neglected problem is that of verifying whether this linear approximation is warranted. The study of the nonlinearity of the model at the optimum, as conducted in the present work, follows the treatment by Bates and colleagues (3–5) and Seber and Wild (26), to which the reader is referred for more details.

Briefly, two measures of curvature of the expectation surface at the estimation point, with respect to a generic direction h in parameter space, are obtained.

These two measures, named K_{Th} and K_{Nh}, are the tangential or "parameter effects" curvature (which depends on the parameterization used), and the normal or "intrinsic" curvature, respectively (which is invariant with respect to the parametric system used).

If γ_{Th} and γ_{Nh} are the corresponding normalized curvatures and F_α is the upper α-quantile of the $F_{p,n-p}$ distribution, one possible measure of intrinsic, structural departure from linearity (in the direction h) would be the quantity

$$\delta_1 = (1 - \{-\gamma_{Nh}^2 F_\alpha\}^{\frac{1}{2}}). \qquad (A1)$$

If

$$\gamma_{Nh\max} = \max_h \gamma_{Nh} < \frac{1}{2\sqrt{F_\alpha}} \qquad (A2)$$

then $\delta_1 = 0.134$ and the maximum departure from linearity would be $<14\%$. In this case, the planar assumption would appear acceptable. Another possible measure of departure from linearity would be the quantity

$$\delta_2 = \gamma_{Nh}^2 F_\alpha,$$

which should be small and in any case less than unity. The advantage of using δ_2 over δ_1 is that the former is always computable, whereas the latter fails to be meaningful for strongly nonlinear models, when $\delta_2 > 1$. Although less directly informative, δ_2 therefore appears to be a more robust indicator than δ_1, and for this reason it has been used in the present work.

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