Kinetics of dodecanedioic acid triglyceride in rats

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De Gaetano, A., G. Mingrone, M. Castagneto, G. Benedetti, A. V. Greco, and G. Gasbarrini. Kinetics of dodecanedioic acid triglyceride in rats. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E497–E502, 1999.—The kinetics of the triglyceride of dodecanedioic acid (TGDA) has been investigated in 30 male Wistar rats after a rapid intravenous bolus injection. TGDA and its product of hydrolysis, nonesterified dodecanedioic acid (NEDA), were measured in plasma samples taken at different times using an improved high-performance liquid chromatographic method. The 24-h urinary excretion of TGDA was 1.54 ± 0.37 µmol, corresponding to ~0.67% of the administered amount. Several kinetics models were considered, including central and peripheral compartments for the triglyceride and the free forms and expressing transports between compartments with combinations of linear, carrier-limited, or time-varying mechanisms. The parameter estimates of the kinetics of TGDA and of NEDA were finally obtained using a three-compartment model in which the transfer of TGDA to NEDA was assumed to be linear, through a peripheral compartment, and the tissue uptake of NEDA was assumed to be carrier limited. TGDA had a large volume of distribution (~0.5 l/kg body wt) with a fast disappearance rate from plasma (0.42 min⁻¹), whereas NEDA had a very small volume of distribution (~0.04 l/kg body wt) and a tissue uptake with maximal transport rate of 0.636 mM/min. In conclusion, this first study on the triglyceride form of dodecanedioic acid indicates that it is rapidly hydrolyzed and that both triglyceride and nonesterified forms are excreted in the urine to a very low extent. The tissue uptake rate of NEDA is consistent with the possibility of achieving substantial energy delivery, should it be added to parenteral nutrition formulations. Furthermore, the amount of sodium administered with the triglyceride form is one-half of that necessary with the free diacid.

Dicarboxylic acids; triglyceride of dodecanedioic acid

In those clinical conditions in which plasma triglyceride lipolysis is impaired and/or long-chain fatty acid oxidation is altered, as in the advanced stages of sepsis, acute pancreatitis, and decompensated diabetes mellitus with acidosis, medium-chain triglycerides (MCT) and structured triglycerides (ST) have partially substituted long-chain triglycerides (LCT) as lipid fuel substrate (1, 2, 8, 17). Among the alternate lipid substrates, the use of the inorganic salts of dicarboxylic acids (DAs) has been recently proposed. DAs appear to have characteristics that might be suitable for parenteral nutrition (3, 4, 6, 9–14, 16, 18, 19). DAs do not have an aliphatic chain, like free fatty acids (FFA) do. They have, instead, two carboxylic groups, which confer water solubility to the molecule, at least up to a certain chain length. Therefore, although DAs can be given as water solution, FFAs cannot be administered directly but only as esters of glycerol, i.e., under triglyceride form (LCT or MCT). This could represent an advantage in those conditions where there is an impairment of lipoprotein lipase (LPL) function or of FFA disposition. In addition, the preparation of LCT or MCT emulsions is certainly more expensive than the preparation of water solutions of DAs. Urinary elimination of the administered DAs is inversely correlated with chain length, so that 50–70% of an intravenous dose of C-9 is lost with urine compared with 16–46% for C-10 and with 2–4% for C-12 (4, 10, 14, 19).

Even-numbered DAs are β-oxidized to acetyl-CoA and succinyl-CoA, thus producing an intermediate substrate for the tricarboxylic acid cycle, which should otherwise be derived from amino acids. Succinic acid, in addition, through pyruvic acid formation, is a gluconeogenic substrate that potentially increases the cellular glycogen pool.

Among the several DAs that could be considered for nutritional purposes, C-12, the even-numbered, straight chain length, so that 50–70% of an intravenous dose of C-9 is lost with urine compared with 16–46% for C-10 and with 2–4% for C-12 (4, 10, 14, 19).

The purpose of the present investigation was that of studying the kinetics of this newly synthesized derivative of C-12, TGDA, in rats.

EXPERIMENTAL METHODS

Materials

Suberic (C-8), azelaic (C-9), sebacic (C-10), and dodecanedioic (C-12) acids were purchased from Sigma (St. Louis, MO). C-12 triglyceride as sodium salt was purchased from Real (Como, Italy).

\[
\text{CH}_2\text{-O-CO-(CH}_2\text{)}_{10}\text{-COONa}
\]

\[
\text{CH-O-CO-(CH}_2\text{)}_{10}\text{-COONa}
\]

\[
\text{CH}_2\text{-O-CO-(CH}_2\text{)}_{10}\text{-COONa}
\]

Molecular weight of tridodecanedioyl glycerol was 728 as acid and 794 as sodium salt.

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All other chemicals used were of the highest purity available.

A 66.7 mM solution of TGDA sodium salt was used for the intravenous bolus injection. The solution was sterilized by ultrafiltration through 0.25-µm Millipore filters (Molsheim, France) before administration.

Experimental Protocol

The study followed the guidelines set forth by the Catholic University in-house animal experimentation ethical committee. Thirty male Wistar rats (bred at the Catholic University facilities in Rome) weighing on average 225 g were used. At least 1 wk before the experiment, the animals were housed in pairs in a light-controlled room at an ambient temperature of 22°C to allow monitoring of body weight gain and to ensure normal growth characteristics. During this time, the animals consumed standard laboratory food and tap water ad libitum. Twenty-four hours before the study, the animals were fasted. On the morning of the experiment, the rats were anesthetized with ethyl ether and then were rapidly injected intravenously through the vein of the tail with 230 µmol (53 mg) of C-12, administered as triglyceride diluted in 1 ml of bidistilled water. Blood samples (one from each animal) were obtained from intracardiac puncture at 1, 1.5, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 80, and 120 min after the end of the intravenous injection. Two or more animals underwent cardiac puncture at each stated time. The animals were then killed by cervical dislocation.

Heparinized blood samples were immediately centrifuged at 4,000 g, and plasma was frozen at −20°C until analysis.

Four rats, injected with TGDA as described above, were allocated to individual metabolic cages for 24-h urine collection.

DA Analysis

Plasma samples. From each sample, the concentrations of DA present as TGDA or as total NEDA, both free and albumin bound, were determined by high-performance liquid chromatography (HPLC). Total concentrations of NEDA were determined by direct extraction from plasma (both of free and albumin-bound fractions); the separation of the measured total concentration into free and bound fractions was made aromatically, according to the model outlined in Materials.

One hundred micrograms of azelaic acid were added to 1 ml of each plasma sample as an internal standard. Proteins were precipitated with 5 mg of trichloroacetic acid, and DAs were extracted twice with 8 ml of ethyl acetate, maintaining the solutions at 60°C for 15 min. The combined extracts were dried in a GyroVap apparatus (Howe, model GV1; Gio. DeVita, Rome, Italy), operating at 60°C and 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 added with 50 µg azelaic acid as internal standard and then were 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 ethyl acetate, and evaporated in the GyroVap as described previously.

HPLC Analysis

The extracted solutes were dissolved in 0.5 ml acetonitrile-methanol (1:1, vol/vol) and added to 20 mg of p-bromophenacetyl bromide and 70 µl of N,N-disopropylethylamine 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 in a liquid chromatograph (Hewlett-Packard 1050) with a HP 3396A integrator and a scanning spectrophotometer operating in the 190- to 600-nm wavelength range (light source: deuterium lamp, noise < 2.5 × 10⁻³ absorbance units 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 HPLC conditions were as follows: solvent A, bidistilled water-methanol (1:1, vol/vol); solvent B, acetonitrile; after 15 min isocratic elution with 15% acetonitrile, a gradient elution was performed from 15 to 100% of solvent B in 80 min. Conditions were as follows: flow rate, 1 ml/min; ultraviolet detector operating at 255 nm; chart speed, 0.2 cm/min; absorbance range, −0.300 to 1.000 absorbance units.

Recovery of Standard

Aliquots from 25 to 200 µg in 100 µl acetonitrile-methanol (1:1, vol/vol) of TGDA standard were added to 1 ml of plasma from five untreated rats to measure recovery. The triglyceride was extracted and analyzed as described under HPLC Analysis.

Modeling and Statistics

Previous published results were used to quantify the albumin binding of C-12. Binding of C-12 (molecular weight 230) to rat plasma albumin (molecular weight 60,000) was considered to follow a one-site, one-step kinetics with capacity 1.57 mol/mol and affinity 7.02 × 10³ M⁻¹. Briefly, the albumin binding experiment showed a good fit to the model with an R² of 0.78 and asymptotic coefficients of variation of 25.4 and 5.7% for affinity and capacity, respectively.

It was decided to disregard urinary elimination of TGDA and NEDA forms, given the minimal amounts retrieved in the 24-h urine collection (see RESULTS). Inclusion of the urinary output parameters would have been small in any situation where the physiological meaning of the added urinary output parameters would have been small in any case.

A number of different models were evaluated, all of which included at least two central compartments (compartment 1 for TGDA, compartment 2 for NEDA) with linear or carrier-limited transport terms between compartments. The respective volumes of distribution reflect both the actual central compartmental volume (i.e., plasma volume) and the binding characteristics to lipophylic structures, typically cellular plasma membranes.

These two compartments would correspond to two chemical species partially sharing the same physical space rather than to two distinct distribution spaces for the same compound. Transfer of substance occurs between compartments 1 and 2 (hydrolysis and possible reesterification of TGDA) and irreversible loss of substance occurs from compartment 2 (tissue uptake of NEDA). In other words, it was assumed that, while hydrolysis of triglycerides could happen in plasma as well as in selected peripheral tissues like the liver and skeletal muscle (their effects being in any case seen only in the change of plasma concentrations of the compounds), irreversible tissue uptake of triglycerides was not going to happen to any significant extent. Peripheral metabolizing
tissues were therefore assumed to use as fuel substrates only free diacids, in the same way as for LCT, MCT, and ST.

Several three- and four-compartment models with explicit representation of peripheral compartments for TGDA or NEDA (representing muscle or liver) were also tested, as well as some nonautonomous models with time-dependent transfer coefficients.

Each model was fitted to the time-concentration points by ordinary least squares using a quasi-Newton, variable metric algorithm. The numerical integration of all systems (linear, nonlinear, nonautonomous) of differential equations was performed using a fourth-order Runge-Kutta algorithm with 1.0-min fixed steps. Asymptotic standard errors of the parameters were obtained by inversion of the Hessian at the optimum. For all of the algorithms mentioned, a good general reference is Press et al. (15).

Model selection was made according to the following criteria. Any model showing a noninvertible Fisher’s information matrix was discarded as nonidentifiable (7). Of the identifiable models, preference was given to those with low Akaike information criterion and to those whose pattern of residuals better approximated a random scatter.

RESULTS

HPLC Analysis

The HPLC separation of a synthetic mixture of DAs (suberic acid, C-8; azelaic acid, C-9; sebacic acid, C-10; and dodecanedioic acid, C-12) and of the TGDA is reported in Fig. 1. An HPLC chromatogram of NEDA and TGDA extracted from rat plasma is shown in Fig. 2.

In the measured range, the HPLC calibration curve for TGDA was linear. The parameter estimates of the linear regression of peak area (pA·cm) on the injected amount (ng) and their standard errors were as follows: intercept = -4.55 ± 8.62 × 10^{-4}, slope = 3.43 ± 0.10 × 10^{-3}, with an $R^2$ of 96.71% ($P < 0.001$).

The intra-assay variation coefficient was 18.6 ± 3.2% for TGDA (on 35 couples of observations for the dose of 500 ng), whereas the interassay variation coefficient was 15.0 ± 6.7%, on 35 observations for the same amount injected. The detection limit for TGDA was 5 ng. The percent recoveries of the TGDA standard added to plasma ranged from 82.55 ± 0.77 (for 25 µg added) to 86.76 ± 0.65% (for 200 µg added).

Urinary Excretion of C-12

The 24-h urinary excretion of TGDA was 1.54 ± 0.37 µmol, corresponding to ~0.67% of the administered amount. In addition, small amounts of free dodecanedioic acid (2.18 ± 0.28 µmol or <1% of the administered dose) were excreted in the urine over 24 h.

Model Choice

A summary of the fitting characteristics of the most meaningful 11 models, out of the 37 models tested in all, is reported in Table 1. The best model (as judged from $R^2$ and Akaike criterion values) among those with approximately random scatter and no apparent systematic deviation from the recorded data was retained (model A in Table 1); it is presented as a block diagram.
The obtained letter; the number of compartments in the model; the type of transfers between compartments; the number and sites of exits from the system; linear and/or a carrier-mediated transport. TM, maximal transport

Elimination (tissue uptake) of NEDA is thought of as depending on an (enzyme) linearly dependent on the concentration of the precursor. (NEDA) is assumed to be produced by the action of an active carrier

Peripheral TGDA compartment) to nonesterified dodecanedioic acid

TGDA is supposed to diffuse from the central compartment (including TGDA and NEDA, respectively; D (mmol) is the dose of TGDA administered as intravenous bolus; Q3 (mmol) is the quantity of TGDA available for hydrolysis (in the peripheral compartment); k0 (min⁻¹) is the apparent first-order rate constant from compartment j to compartment i; TM (mM/min) is the maximal transport rate of NEDA out of plasma; K M (mM) is the concentration of half-transport of NEDA out of plasma; A (mM) is the plasma concentration of NEDA; B (mol/mol) is the capacity of albumin for NEDA, i.e., the maximal binding of NEDA to albumin; n B (mol/mol) is the capacity of albumin for NEDA, i.e., the maximal binding of NEDA to albumin; and f(x) (mM) is the free concentration of NEDA corresponding to a total concentration x.

Fig. 3. Diagram of the model chosen to represent the kinetics of C-12; TGDA is supposed to diffuse from the central compartment (including plasma) to a peripheral metabolically active compartment (including liver and/or endothelium). Transfer (hydrolysis) of TGDA (from the peripheral TGDA compartment) to nonesterified dodecanedioic acid (NEDA) is assumed to be produced by the action of an active carrier (enzyme) linearly dependent on the concentration of the precursor. Elimination (tissue uptake) of NEDA is thought of as depending on a linear and/or a carrier-mediated transport. TM, maximal transport rate of NEDA out of plasma; KM, concentration of half-transport of NEDA out of plasma; k0, apparent first-order rate constant from compartment j to compartment i.

Table 1. Summary statistics of the chosen model (A) and of ten additional models fitted to the experimental data.

<table>
<thead>
<tr>
<th>Model</th>
<th>No. of Compartments</th>
<th>Transfers Between Compartments</th>
<th>Exits</th>
<th>R²</th>
<th>Akaike</th>
<th>Maximum CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>Linear transfers, linear plus carrier-limited exit</td>
<td>1 (NEDA)</td>
<td>0.948</td>
<td>546.3</td>
<td>71.7</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>Linear exit; nonautonomous transfer</td>
<td>1 (NEDA)</td>
<td>0.938</td>
<td>551.9</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>Linear; all linear transfers</td>
<td>1 (NEDA)</td>
<td>0.904</td>
<td>575.0</td>
<td>30.0</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>Carrier-limited transfer, linear exits</td>
<td>1 (NEDA)</td>
<td>0.942</td>
<td>550.8</td>
<td>168.4</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>Linear transfer, linear TGDA exit, carrier-limited NEDA exit</td>
<td>1 (NEDA)</td>
<td>0.929</td>
<td>563.0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>Same as model A, plus direct linear transfer from plasma TGDA to plasma NEDA</td>
<td>1 (NEDA)</td>
<td>0.935</td>
<td>558.6</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>Same as model A, plus direct linear transfer from plasma TGDA to plasma NEDA</td>
<td>1 (NEDA)</td>
<td>0.946</td>
<td>550.6</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>H</td>
<td>3</td>
<td>Linear exit; nonautonomous transfer</td>
<td>1 (NEDA)</td>
<td>0.942</td>
<td>550.3</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>Same as model A, plus direct linear transfer from plasma TGDA to plasma NEDA</td>
<td>1 (NEDA)</td>
<td>0.949</td>
<td>550.6</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>Same as model A, plus direct linear transfer from plasma TGDA to plasma NEDA</td>
<td>1 (NEDA)</td>
<td>0.942</td>
<td>550.3</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>Same as model C, with added peripheral compartments, one for TGDA and one for NEDA</td>
<td>1 (NEDA)</td>
<td>0.917</td>
<td>575.5</td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>

NEDA, nonesterified dodecanedioic acid; TGDA, triglyceride of dodecanedioic acid. For each model the following are reported: an identifier letter; the number of compartments in the model; the type of transfers between compartments; the number and sites of exits from the system; the obtained R² and Akaike criterion values; the maximum parameter estimate coefficient of variation obtained.

In Fig. 3 and is described using the following equations

\[
\frac{dc_1}{dt} = \frac{k_{13}Q_3}{V_1} - k_{31}c_1, c_1(0) = D/V_1
\]

\[
\frac{dc_2}{dt} = \frac{k_{23}Q_3}{V_2} - (k_{02} + k_{32})f(C_2) - \frac{T_n f(C_2)}{K_m + f(C_2)}, c_2(0) = 0
\]

\[
\frac{dQ_3}{dt} = k_{31}V_1 c_1 + k_{32}V_2 f(C_2) - (k_{13} + k_{23})Q_3, Q_3(0) = 0
\]

\[
f(x) = \frac{-1 - Ak_b n_B + k_b x + 4k_b x + (1 + Ak_b n_B - k_b x)^2}{2k_b}
\]

where C1 and C2 (mM) are the plasma concentrations of TGDA and NEDA, respectively; V1 and V2 (l/kg body wt) are the central volumes of distribution of TGDA and NEDA, respectively; D (mmol) is the dose of TGDA administered as intravenous bolus; Q3 (mmol) is the quantity of TGDA available for hydrolysis (in the peripheral compartment); k0 (min⁻¹) is the apparent first-order rate constant from compartment j to compartment i; TM (mM/min) is the maximal transport rate of NEDA out of plasma; KM (mM) is the concentration of half-transport of NEDA out of plasma; A (mM) is the plasma albumin concentration; KB (mol/mol) is the affinity of albumin for NEDA, i.e., the inverse of the concentration of free C-12 producing half-maximal binding; nB (mol/mol) is the capacity of albumin for NEDA, i.e., the maximal binding of NEDA to albumin; and f(x) (mM) is the free concentration of NEDA corresponding to a total concentration x.

Specifically, none of the linear models that we tested could reproduce the quickly rising and quickly decaying time course of NEDA.

All parameters of the chosen model were initially fitted. Because the estimated values of k32 and k0 were in the 10⁻⁸ through 10⁻⁶ range, with corresponding asymptotic percent coefficients of variation in the tens of thousands and above, these two constants were set to zero, effectively admitting that no significant reesterifi-
To quantify the kinetics of TGDA, in the present work a mathematical model of its conversion to NEDA and of its eventual peripheral tissue uptake was built.

We tried to fit the available experimental data with several different models, including a variety of compartmental configurations and transport mechanisms. In particular, we assessed the advantages of explicitly representing peripheral pools for either TGDA, NEDA, or both. One such model, identifying a peripheral compartment for the triglyceride, was in fact the best-fitting representation to the data. Together with a peripheral compartment for TGDA, it was also necessary to postulate some nonlinearity in the tissue uptake of NEDA, which we represented as consisting of a carrier-limited transport out of plasma. On the basis of the chosen representation, it would appear that the TGDA has a large volume of distribution and a very fast elimination rate from plasma, with bulk transfer of substance to the peripheral tissues and relatively fast appearance of NEDA in plasma. On the other hand, NEDA seems to have a rather small central compartment volume. Because the hypothesized peripheral compartment could contain either TGDA, NEDA, or both, the small central volume of distribution found for NEDA could underestimate the total distribution space available to these compounds. The estimate that we found for this volume is however of the same order of magnitude as that previously reported in humans (6).

The tissue uptake of NEDA has been estimated in the present work to be transport limited. The relatively low Michaelis constant with respect to the experimentally observed NEDA concentrations (≈0.027 mM, with plasma NEDA concentrations ranging up to 0.9 mM) implies that a substantial fraction of the available transport capacity is used. On the other hand, transport is very fast; the maximal transport rate of 0.636 mM/min and the central volume of distribution of NEDA of 40 ml/kg body wt, if applied to an average human being of 70 kg body wt, would translate to a maximal transport of 590 g/day, corresponding to a delivery of ≈17,500 kJ/day. This is approximately the same result previously obtained in rats using a different model to account for direct administration of NEDA (5); in that case, the maximal rate of NEDA tissue uptake in the rat was estimated to be 17.8 µmol·min⁻¹·kg body wt⁻¹, corresponding, for a 70-kg human, to a maximal load of 412 g/day or to a caloric delivery of ≈12,500 kJ/day. The correspondence of the present results with those previously obtained is therefore very good, even if the underlying model used is different. These results indicate that, in rats, C-12 administration achieves even higher rates of caloric delivery (≈174 J·min⁻¹·kg body wt⁻¹) than glucose administration (89.0 J·min⁻¹·kg body wt⁻¹; see Ref. 5).

We may draw a comparison between the present results in rats and those previously obtained in healthy volunteers (6). It must be considered that, in that work, no saturable transport mechanism could be demonstrated to exist at the achieved plasma concentrations. Therefore, a linear kinetics representation was employed, with an estimated rate constant of 0.0248 min⁻¹ for elimination from plasma toward peripheral tissues. In the present work, the steepness of the transport vs. concentration curve (equivalent to the apparent linear fractional elimination constant required to produce that transport at that concentration) equals 0.062.

Table 2. Parameter estimates for the chosen kinetic model, accompanied by the respective coefficients of variation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (± SE)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>V₁, l/kg body wt</td>
<td>8.0 ± 0.514</td>
<td>94.9</td>
</tr>
<tr>
<td>V₂, l/kg body wt</td>
<td>71.7 ± 0.040</td>
<td></td>
</tr>
<tr>
<td>k₁₁, min⁻¹</td>
<td>13.0 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>k₁₂, min⁻¹</td>
<td>47.1 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>k₂₁, min⁻¹</td>
<td>34.7 ± 0.043</td>
<td></td>
</tr>
<tr>
<td>k₂₂, min⁻¹</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>k₁₂, min⁻¹</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>k₂₁, min⁻¹</td>
<td>49.2 ± 0.636</td>
<td></td>
</tr>
<tr>
<td>K_M, mM</td>
<td>47.6 ± 0.0272</td>
<td></td>
</tr>
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</table>

Values are means ± SE. Units are %. V₁ and V₂, central volumes of distribution of TGDA and NEDA, respectively; k_ij, apparent first-order rate constant from compartment j to compartment i; T_M, maximal transport rate of NEDA out of plasma; K_M, concentration of half-transport of NEDA out of plasma.

DISCUSSION

The appearance of NEDA in plasma. On the other hand, transport is very fast; the maximal transport rate of 0.636 mM/min and the central volume of distribution of NEDA of 40 ml/kg body wt, if applied to an average human being of 70 kg body wt, would translate to a maximal transport of 590 g/day, corresponding to a delivery of ≈17,500 kJ/day. This is approximately the same result previously obtained in rats using a different model to account for direct administration of NEDA (5); in that case, the maximal rate of NEDA tissue uptake in the rat was estimated to be 17.8 µmol·min⁻¹·kg body wt⁻¹, corresponding, for a 70-kg human, to a maximal load of 412 g/day or to a caloric delivery of ≈12,500 kJ/day. The correspondence of the present results with those previously obtained is therefore very good, even if the underlying model used is different. These results indicate that, in rats, C-12 administration achieves even higher rates of caloric delivery (≈174 J·min⁻¹·kg body wt⁻¹) than glucose administration (89.0 J·min⁻¹·kg body wt⁻¹; see Ref. 5).

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Fig. 4. Observed and model-predicted time courses of plasma concentration of TGDA and NEDA. (open diamonds: observed; continuous line: predicted) and NEDA (solid circles: observed; dashed line: predicted).

Table 2. Parameter estimates for the chosen kinetic model, accompanied by the respective coefficients of variation

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<tr>
<td>k₂₁, min⁻¹</td>
<td>34.7 ± 0.043</td>
<td></td>
</tr>
<tr>
<td>k₂₂, min⁻¹</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>k₁₂, min⁻¹</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>T_M, mM/min</td>
<td>49.2 ± 0.636</td>
<td></td>
</tr>
<tr>
<td>K_M, mM</td>
<td>47.6 ± 0.0272</td>
<td></td>
</tr>
</tbody>
</table>
min\(^{-1}\) at a C-12 plasma concentration of 0.5 mM and equals 0.020 min\(^{-1}\) at a concentration of 0.9 mM. The plasma elimination rates observed in rats in the present work thus agree very well with that observed in humans.

It is also relevant that TGDA is rapidly converted into the corresponding free DA (NEDA peaks in plasma after \(\sim 15\) min) and is then quickly cleared.

The urinary excretion of TGDA and of NEDA is confirmed by the present study to be very small, \(<2\%\) of the administered dose.

In conclusion, this first study on TGDA indicates that this substance is quickly hydrolyzed to free diacid and that C-12 is lost with urine to a small extent and, conversely, is quickly taken up by peripheral tissues at rates compatible with substantial caloric delivery. In addition, the amount of sodium given with TGDA is one-half of that administered using the corresponding DA alone; a further reduction of sodium intake and a detailed study of the possible side effects of the administration of sodium in these amounts are desirable. Although dodecanedioic acid by itself already appears to be a promising energy substrate (6, 12), its triglyceride possibly represents a more interesting form of energy delivery.

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REFERENCES