0148-6071/94/1803-0225$03.00/0
JOURNAL OF PARENTERAL AND ENTERAL NUTRITION

Vol. 18, No. 3
Printed in U.S.A.

Pharmacokinetic Profile of Dodecanedioic Acid, a Proposed Alternative Fuel Substrate

G. MINGRONE,* A. V. GRECO,* A. DE GAETANO,† A. TATARANNI,* C. RAGUSO,* AND M. CASTAGNETO†

*From the *Istituto di Clinica Medica and †Istituto di Clinica Chirurgica, Centro per lo Studio della Fisiopatologia dello Shock, Università Cattolica S. Cuore, Roma*

ABSTRACT. Dodecanedioic acid (C12), a saturated, aliphatic dicarboxylic acid with 12 carbon atoms, was given as an intravenous bolus (800 μmol/kg of body weight [kg_{BW}]) in male Wistar rats to study its pharmacokinetic profile. Because total plasma C12, which results from the sum of both free and albumin binding fractions, was measured by high-performance liquid chromatography, an *in vitro* experimental session was carried out to determine the binding curve of C12 in rat plasma. These data were then used to calculate the plasma C12 free fraction in *in vivo* experiments. The best fit obtained for the experimental data of albumin binding was obtained with the equation of reversible, saturable binding to one, two, or three classes of noninteracting equivalent sites. Only a single binding site was clearly identified with a dissociation constant of 147 μmol/L and a maximal predicted binding of 1.57 mol/mol albumin. The urinary excretion of C12 was 3.90 ± 1.62% of the administered dose. The pharmacokinetic analysis was performed by one-compartment model with linear transfer to the tissues, taking into account simultaneously both plasma concentration and urine excretion data. The apparent volume of distribution of C12 was 0.248 ± 0.035 L/kg_{BW}, the apparent first order rate constant to the tissues was 0.0535 ± 0.0123 min^{-1} and that from plasma to urine was 0.00206 ± 0.00051 min^{-1}. The C12 plasma half-life was 12.47 minutes. Renal clearance was 0.00051 L/kg_{BW} per minute, whereas the systemic clearance was 0.0138 L/kg_{BW} per minute. Because the renal clearance was much less than the rat inulin clearance reported in literature, the presence of C12 passive back-diffusion was hypothesized. (*Journal of Parenteral and Enteral Nutrition* **18**:225–230, 1994)

The use of the salts of dicarboxylic acids (DAs) as a possible fuel substrate in parenteral nutrition[1–6] was recently proposed. The theoretical basis of this contention is that salts of DAs (1) are highly water soluble and, compared with both long-chain and medium-chain fatty acids, are bound less avidly by albumin[7–9]; (2) undergo β-oxidation at the level of both mitochondria and peroxisomes at a high rate[10–14]; (3) do not induce ketogenesis[5,15] as medium-chain triglycerides do,[16,17] but rather promote gluconeogenesis via succinate production during their β-oxidation[18]; (4) do not require hydrolysis before tissue uptake and therefore could represent an immediately available form of energy; and (5) can be easily and inexpensively prepared because they are water soluble.

In spite of these theoretical advantages over the usual lipid substrates (long-chain and medium-chain triglycerides), the DAs studied for nutritional purposes have been subject to high urinary excretion until now. Depending on the administered dose, 50% to 70% of azelate (C9) and 16% to 46% of sebacate (C10) are eliminated in the urine.[1–6,19]

Therefore, the aim of this study was to investigate the nutritional potential of the 12-carbon DA, C12, in the laboratory animal with special reference to its pharmacokinetic profile and urinary excretion characteristics, in view of its possible use as a fuel substrate in humans.

MATERIALS AND METHODS

Equilibrium Dialysis

Binding of C12 to rat serum albumin was determined by using equilibrium dialysis. Dialysis apparatus (Spectra/Por Equilibrium Dialyzer) and membranes were from Spectrum (Houston, TX). The chamber contains two 1-mL compartments separated by a dialysis membrane that is permeable to compounds with a molecular weight < 6000; albumin does not cross the dialysis membrane.[20] Preliminary experiments showed that C12 reaches equilibrium within 3 hours at 37°C and 25 rpm. Thus, we chose a 4-hour incubation period to ensure equilibrium. Binding was assessed by adding varying concentrations of C12 (100 to 6000 μmol/L) to samples of DA-free rat plasma. Plasma with added C12 was placed in one side (side A) of the chamber and a salt solution containing 0.116 mol/L sodium chloride, 0.0049 mol/L potassium chloride, and 0.016 mol/L sodium phosphate at pH 7.4 was added to the other side (side B). Dialysis chambers were put into a constant-temperature (37°C) water bath and were rotated at 25 rpm. At

Received for publication, August 18, 1992.
Accepted for publication, December 6, 1993.
Correspondence and reprint requests: G. Mingrone, MD, Istituto di Clinica Medica, Università Cattolica S. Cuore, Largo A. Gemelli, 8, 00168 Roma, Italy.

the end of the incubation period, 0.5 mL was removed from each side and the concentration of C12 was measured by high-performance liquid chromatography as previously described.[3] Azelaic acid (50 μg) was used as an internal standard. The concentration of bound C12 was determined by subtracting the concentration of C12 found in side B from the concentration of C12 in side A. This was then normalized for the measured albumin concentration and to the volume of the dialysis chamber.

In Vivo Experiments

Male Wistar rats weighing between 170 and 250 g were used in all the experiments. The rats were injected intravenously (intravenous tail vein injection) with 800 μmol of C12 per kilogram of body weight (kg_{BW}) in the form of disodium salt as a bolus. Blood samples were drawn by cardiac puncture, then the animals were killed by cervical dislocation. Heparinized blood samples were immediately centrifuged at 4000 × *g* and plasma was frozen at −20°C until analysis.

Five rats, treated as described above, were allocated to individual metabolic cages for the collection of 24-hour urine. DAs in both plasma and urine were measured by high-performance liquid chromatography as previously described.[3]

Albumin Binding Model

Dodecanedioic binding in plasma was assessed by a one-site model (Table I) according to the methods of Tonsgard and Meredith.[7] The model was fitted by unweighted nonlinear least squares, using the inverse Hessian method to derive the estimates of the parameter dispersions. A two-site model was not shown to be superior to the one-site model for explaining the binding curve. A stoichiometric approach was also followed: the two-step stoichiometric model was equivalent to the one-site model, and the one-site model was chosen for comparability with previously published results.[7,8] A three-step stoichiometric model was no better than the two-step model.

Pharmacokinetic Analysis

Two basic models were compared (Tables II and III); both were one-compartment models with linear transfer to the tissues, taking into account simultaneously both plasma concentration and urine excretion data. In model A, a simple linear renal excretion mechanism was presumed, represented by the apparent first-order rate constant k_{12}. In model B, a nonlinear renal excretion mechanism was allowed for, which resulted from the sum of a linear component ($k_{12}\ C_{1F}$) and a saturable transport [$T_{12}\ C_{1F}/(M_{12} + C_{1F})$]. Both models were fitted by nonlinear least squares with a quasi-Newton algorithm. Estimates of the parameter dispersions were obtained from the diagonal elements of the inverse Hessian at the minimum.

TABLE I
Dodecanedioic acid binding model

$r = S_B\ f/(K_B + f)$ $r = b/a$

where
f = free dodecanedioic acid (μmol/L);
b = bound dodecanedioic acid (μmol/L);
a = serum albumin concentration (μmol/L);
S_B = predicted saturation (mol C12/mol albumin); and
K_B = dissociation constant (μmol/L)

Fitted parameters
S_B = 1.57 ± 0.05 mol C12/mol albumin
K_B = 147 ± 23 μmol/L

RESULTS

The binding of monocarboxylic fatty acids to albumin modulates their transport to tissues and their cellular uptake.[21,22] Therefore, we have assumed albumin to be or to closely represent the principal species of DA carrier in plasma. Data are reported in the literature about C12 binding to defatted serum bovine albumin.[7,8] Such data, which are of great interest in elucidating the structure of the albumin binding sites for DAs, are of limited value in quantifying the real amount of DA bound in plasma, because many less-water-soluble compounds are normally present in plasma and compete for transport. Therefore, we chose to directly determine the binding curve of C12 in normal plasma by equilibrium dialysis.

Figure 1 shows the best fit obtained for the experimental data by using a one-site model. Only a single binding site for C12 can be clearly identified with a dissociation constant of 147 ± 23 μmol/L (coefficient of variation = 15.6%) and a maximal predicted binding of 1.57 ± 0.05 mol/mol albumin (coefficient of variation = 3.5%, R^2 = 79%). The mean ± SEM 24-hour urinary

TABLE II
One-compartment linear excretion bolus model with urinary data (model A)

$c_{1T}(0) = D/(V_1\ W)$

$dc_{1T}/dt = -(k_{12}\ c_{1F}) - (k_{10}\ c_{1F})$

$q_2(0) = 0$

$dq_2/dt = k_{12}\ c_{1F}\ V_1\ W$

$c_{1F} = 0.5\ [c_{1T} - C_{BS} - K_B + (C_{1T} - C_{BS} - K_B)^2 + 4\ K_B c_{1T}\ ] \div 2$

where
V_1 (L/kg_{BW}) is the apparent volume of distribution;
c_{1T} and c_{1F} (μmol/L) are total and free plasma C12 concentrations, respecitively
q_2 (μmol) is the amount of C12 loss with urine;
D (μmol) is the intravenously administered dose;
W (kg) is the body weight;
k_{10} (fraction/min) is the apparent first-order rate constant from plasma to tissues;
k_{12} (fraction/min) is the apparent first-order constant from plasma to urine;
C_{BS} (μmol/L) is the bound C12 at saturation = 1.57 × albumin (μmol/L); and
K_B (μmol/L) is the dissociation constant = 147

Fitted parameters
V_1 = 0.248 ± 0.035 L/kg_{BW}
k_{10} = 0.0535 ± 0.0123 min^{-1}
k_{12} = 0.00206 ± 0.00051 min^{-1}

kg_{BW}, kilogram of body weight.

excretion of C12 was 3.901 ± 1.62% of the administered dose.

Although the fit of model A to the data was good (R^2 = 69%) and parameter estimates were stable with narrow confidence limits, the fitting of the nonlinear model B was good but gave rise to very unstable estimates of parameters k_{12}, T_{12}, and M_{12}. Inasmuch as the urinary excretion of the compound is very small, the random scatter of the data points was greater than the quantifiable effect of a possible saturable transport mechanism. The T_{12} found ought to be regarded as uninformative, especially taking into account its very large standard error. For this reason, model A was chosen as being the most conceivable representation of DA disposition.

Figure 2 depicts the concentration profiles (predicted by the model by using the estimated values of the pharmacokinetic parameters) together with all the individual experimental data on plasma concentration. The apparent volume of distribution of C12 was 0.248 ± 0.035 L/kg_{BW}. The apparent first-order rate constant to the tissues (k_{10}) was 0.0535 ± 0.0123 min^{-1}, whereas that from plasma to urine (k_{12}) was 0.00206 ± 0.00051 min^{-1}. The C12 plasma half-life was 12.47 minutes. Renal clearance was 0.00051 L/kg_{BW} per minute, whereas systemic clearance was 0.0138 L/kg_{BW} per minute. Therefore, renal clearance is much less than the reported inulin clearance in the rat (0.0101 ± 0.0004 L/kg_{BW} per minute),[23] suggesting the presence of a C12 passive back-diffusion or an active reabsorption from the renal tubules to plasma.

DISCUSSION

Some years ago in a very interesting and original publication titled "Dicarboxylic Acids and the Lipid Metabolism," Mortensen stressed the concept of nonketotic dicarboxylic aciduria.[24] This term was used to indicate the presence of DA in the urine of subjects affected by congenital or acquired defects of fatty acid β-oxidation. Glutaric aciduria type II[25–29] is one congenital disease that causes dicarboxylic aciduria. Other similar congenital defects have been described in patients who died on the day of birth because of lactate acidosis[30–33] or survived the neonatal period but subsequently suffered a number of hypoglycemic attacks with simultaneous increases in DA excretion. All these patients showed a genetic defect of fat oxidation localized to the medium-chain acyl–CoA dehydrogenase. The authors[30–33] hypothesized that the DAs were formed from an ω-oxidation of medium-chain monocarboxylic acids that had accumulated because of the metabolic blockade.

Reye's syndrome was first described as a distinct clinical and pathologic entity in 1963.[34] This syndrome is characterized by encephalopathy and cellular fatty degeneration. The presence of a viral-type prodrome led investigators[35,36] to suspect viruses as causative agents. Histochemical analysis of mitochondrial enzymes in liver tissue from patients with Reye's syndrome showed that the activities of both succinic acid dehydrogenase and cytochrome oxidase were depressed in the presence of nicotinamide adenine dinucleotide–tetrazolium reductase and ubiquinone, suggesting an injury to either the tricarboxylic acid cycle or the electron transport system.[37] In these patients, the ingestion of toxins such as aflatoxins[38] and pesticides[39] or of some medications such as salicylates[40] may precipitate general conditions, acting as contributory causative agents. In a study on the causative role of DAs in Reye's syndrome, Tonsgard and Getz[41] showed that, when C10 was added to normal serum to obtain concentrations of 0.4 mmol/L and mitochondria were incubated in this medium, a reduction in adenosine triphosphate formation was observed (from 454 to 368 nmol) and the respiration was stimulated (77% compared with 33% in the presence of normal serum alone). However, the uncoupling effect of oleic acid at the same concentration was 173%.[41] Previously, Passi et al[42] hypothesized that there is a toxic effect of medium-chain-length DAs on mitochondrial respiration. This hypothesis was based on the observation that, in the presence of C8–C13 DAs, respiration was inhibited and the degree of inhibition correlated with the chain length of the diacids up to C12. However, this effect

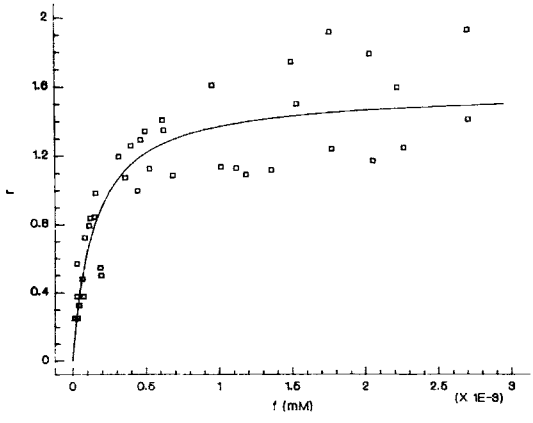

FIG. 1. Binding of disodic dodecanedioate to rat plasma proteins. Because albumin quantitatively represents the most important plasma protein fraction, the values of bound C12 are normalized for the concentration of plasma albumin. r is the molar ratio (mmol/mmol) between bound C12 and plasma albumin. The points (n = 49) are experimental data and the line is the best fit of the data to the theoretical equation.

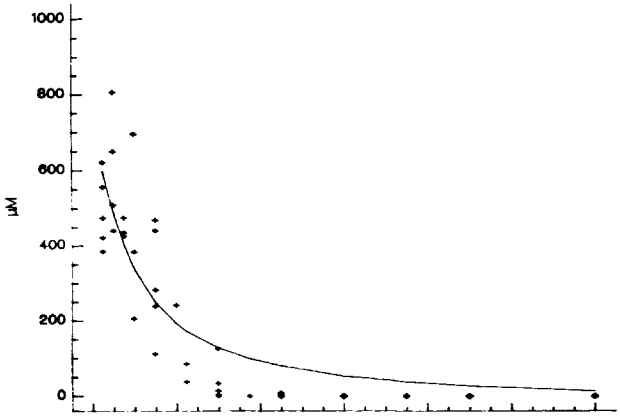

FIG. 2. Plasma concentrations of free C12 (μmol/L) *vs* time (minutes) after administration of 800 μmol/kg_{BW} of C12 as an intravenous bolus. Experimental data are fitted by nonlinear least squares with a quasi-Newton algorithm.

was obtained at DA concentrations in the medium on the order of 0.1 to 0.2 mol/L, which correspond, in the case of C12, to levels as high as 2.3 mg/mL. It is also necessary to point out that these elevated concentrations of C12 should be attained in the cell where the mitochondria are located. In addition, Passi et al[42] found that this inhibition was partially reversible after 1 hour of incubation. Moreover, the same group of researchers[43] stated that "C9 to C12 dicarboxylic acids do not have toxic effects on normal cells *in vivo*."

Among the acquired conditions of dicarboxylic aciduria are hypoglycine A intoxication and valproate treatment. Jamaican vomiting sickness[44] occurs after intake of unripe akee fruit. The fruit's content of hypoglycine A, acting through its metabolite, methylenecyclopropyl acetic acid, inhibits fatty acid oxidation and precipitates acute symptoms similar to those of Reye's syndrome. Mortensen et al[45] proposed that valproate, an unphysiologic monocarboxylic acid (2-propylpentanoic acid), also acts by inhibiting the oxidation of fatty acids. Therefore, in those pathologic conditions in which a partial or total block of fatty acid mitochondrial β-oxidation is present, dicarboxylic aciduria takes place. DA production represents a protective mechanism for the organism, inasmuch as it is well known that long-chain monocarboxylic acids, particularly if unsaturated, are highly toxic for mitochondria because of their uncoupling effect.[46,47] When free fatty acid β-oxidation is impaired, free fatty acids are ω-oxidized to long-chain DAs, which can then be β-oxidized at the level of peroxisomes and partially excreted with urine because they, unlike long-chain monocarboxylic acids, are relatively water soluble: their water solubility decreases with chain length. In addition, there is experimental evidence[48–51] that the ω-oxidation of even-chain monocarboxylic fatty acids makes it possible to convert them into metabolites with a gluconeogenic potential, whereby the oxidation of the synthesized DAs would result in a net production of the citric cycle intermediary, succinyl–CoA.

TABLE III
One-compartment linear + saturable excretion bolus model with urinary data (model B)

$c_{1T}(0) = D/(V_1 W)$

$dc_1/dt = -(T_{12} c_{1F})/(M_{12} + c_{1F}) - k_{12} c_{1F} - k_{10} c_{1F}$

$q_2(0) = 0$

$dq_2/dt = [(T_{12} c_{1F})/(M_{12} + c_{1F}) + k_{12} c_{1F}] V_1 W$

where quantities have the same meaning as in Table II and
T_{12} (μmol/min) = maximal transport (positive = secretion; negative = reabsorption)
M_{12} (μmol/L) = concentration of half-maximal transport

Fitted parameters
$V_1 = 0.221 \pm 0.065$ (L/kg$_{BW}$) (CV = 29%)
$k_{10} = 0.0438 \pm 0.0166$ (min^{-1}) (CV = 38%)
$T_{12} = 2.93 \pm 37.3$ (μmol/min) (CV = 1270%)
$M_{12} = 980 \pm 1176$ (μmol) (CV = 120%)
$k_{12} = 0.000117 \pm 0.0199$ (min^{-1}) (CV = 17,000%)

kg$_{BW}$, kilogram of body weight; CV, coefficient of variation.

In the light of previous studies on shorter-chain-length DAs, ie, azelaic and sebacic acids,[1,6] C12 appears to be a possible fuel substrate in parenteral nutrition, at least from a theoretical point of view. In the present paper, we have studied the pharmacokinetics of C12 in rats after intravenous bolus administration; an investigation of C12's *in vitro* binding kinetics was also performed to evaluate its affinity to rat albumin.

No data are reported in the literature concerning dodecanedioic renal elimination. In their papers, Ullrich et al[52,53] localized the transport systems for DAs with a chain length equal to or shorter than 10 carbon atoms at the level of the proximal renal tubule of rat kidney. Our data indicate that the renal elimination of C12 is mainly a linear phenomenon. It results from the glomerular filtration of the compound and from its possible passive back-diffusion along the concentration gradient created by tubular water reabsorption. A carrier-mediated, saturable transport mechanism by the tubules could also exist, but if this is the case, it is not likely to be of any major importance either in the excretion or active reabsorption of the compound.

Tonsgard et al[7] determined the parameters for binding of C12 (as well as other DAs) on defatted serum bovine albumin. Performing a new binding experiment of C12 on plasma was deemed necessary to quantify the actual amount of binding in actual physiologic conditions. However, the results we obtained are very close to those reported by Tonsgard et al.[7] In particular, the C12 binding affinity in rat plasma (147 μmol/L) is intermediate between that obtained by Tonsgard et al[7] without (75 μmol/L) and with (339 and 707 μmol/L) oleic acid at different concentrations. We therefore conclude that albumin binding of C12 is little affected by competitors normally present in plasma.

The loss of C12 in the urine is very small compared with that of other shorter-chain DAs (ie, azelaic and sebacic acids). Azelaic acid, in fact, showed a very high urinary excretion varying from 50%[1] to 76.9%[19] depending on the dose administered and the infusion rate. Sebacic acid was excreted in the urine to a lesser extent, varying from 16%[4] to 46%[5] of the given dose, again depending on the amount of sebacate infused, the infusion rate, and—as for azelaic acid—the interindividual variability, which appears to be considerable.

As already pointed out, DA β-oxidation can take place in both peroxisomes and mitochondria.[10,11] However, *in vitro* oxidation of medium-chain DAs of various lengths (including C12) by isolated hepatocytes seems to take place mainly in the peroxisomes.[11] On the other hand, Draye et al[54] in whole-animal studies showed that C12 is mainly a mitochondrial substrate. The high global oxidation of C12 observed by these authors in laboratory animals can probably be explained by the predominance of skeletal muscle tissue over other organs, such as the liver or kidneys, in this model. In fact, intact heart and skeletal muscle mitochondria from untreated and clofibrate-treated rats were capable of oxidizing C12.

Investigations of the chain-length dependency of DA β-oxidation showed, both *in vivo* and *in vitro*, medium oxidation for C12, with decreasing oxidizability for both

shorter and longer DAs.[55] The possible metabolic fates of DAs other than direct oxidation are gluconeogenesis and storage. This agrees with the data of Bergseth et al,[56] which showed that C12 is either oxidized or stored, inasmuch as only small amounts of it were found in the urine of rats given an intraperitoneal bolus, whereas approximately 28% to 39% of the radiocarbon administered as $(1\text{–}12)^{14}C\text{–}C_{12}$ was excreted as $^{14}CO_2$.

There is *in vitro* and *in vivo* evidence that C12[56] can be esterified and incorporated into tissue lipids. In addition, many authors[15,57–59] have suggested that even-numbered DAs are gluconeogenetic precursors *in vivo*. Osmundsen et al[59] showed that C12 is β-oxidized to succinate, and this represents the conclusive proof that net conversion of dicarboxylic fatty acids to glucose can take place in the animal. Kou et al[18] also found that C12 is either directly converted into succinate or the β-oxidation of dodecanedioate results in acetyl-CoA being channeled into the tricarboxylic acid cycle. Therefore, C12 does not give rise to acetoacetate production; in fact, after administration of even-numbered dicarboxylic salts, the concentration of ketone bodies in blood decreases.[58]

Our data confirm the above observations: not only is the urinary loss of C12 very small (3% to 5%), but also its tissue uptake is rapid (5% per minute, corresponding to a global half-life of about 13 minutes).

It is necessary to perform more detailed studies on DAs' long-term tolerance and their effects on protein metabolism. It will also be necessary to study administration forms different from disodium salts, which have a potential for sodium overloading and blood alkalinization. Possible forms include DA triglycerides and DA amino acid derivatives. However, C12 might be a promising fuel substrate for use in parenteral nutrition because of the combination of several distinct characteristics. It delivers a sizable amount of energy per gram administered (7.181 kcal/g with a theoretical respiratory quotient of 0.77 upon complete oxidation); it is quickly cleared from plasma and metabolized; it does not induce ketone body formation[5] but rather represents a good gluconeogenetic substrate[18]; and, by increasing the availability of Krebs cycle intermediates, it may favor the use of other conventional substrates as well.[58]

ACKNOWLEDGMENTS

We thank Mrs Anna Caprodossi and Miss Ilaria Cortopassi for their excellent technical assistance. This work was partially supported by Sigma Tau S.p.A., Pomezia, Italy.

REFERENCES

1. Mingrone G, Tacchino RM, Greco AV, et al: Preliminary studies of a dicarboxylic acid as an energy substrate in man. JPEN 13:299–305, 1989
2. Tacchino RM, Mingrone G, Marino F, et al: Short term infusion of azelaic acid versus Intralipid in healthy subjects evaluated by indirect calorimetry. JPEN 14:169–175, 1990
3. Mingrone G, Greco AV, Bertuzzi A, et al: Tissue uptake and oxidation of disodium sebacate in man. JPEN 15:454–459, 1991
4. Mingrone G, Tacchino RM, Castagneto M, et al: Use of even-numbered carbon atom dicarboxylic salts in parenteral nutrition as fuel substrate. JPEN 16:32–38, 1992
5. Greco AV, Mingrone G, Raguso C, et al: Metabolic effects and disposition of sebacate, an alternate dicarboxylic fuel substrate. Ann Nutr Metab 36:1–11, 1992
6. Tataranni A, Mingrone G, Greco AV, et al: Tracer study of metabolism and tissue distribution of sebacic acid in rats. Ann Nutr Metab 36:296–303, 1992
7. Tonsgard JH, Mendelson SA, Meredith SC: Binding of straight-chain saturated dicarboxylic acids to albumin. J Clin Invest 82:1567–1573, 1988
8. Tonsgard JH, Meredith SC: Characterization of the binding sites for dicarboxylic acids on bovine serum albumin. Biochem J 276:569–575, 1991
9. Bertuzzi A, Finotti E, Mingrone G, et al: Sebacic binding to human plasma albumin. Biochem Pharmacol 45:697–702, 1993
10. Petterson JE: Metabolism of hexadecanedioic acid and its mono-L-carnitine ester. Biochim Biophys Acta 306:1–19, 1973
11. Mortensen PG, Gregersen N: The biological origin of ketotic dicarboxylic aciduria. In vivo and in vitro investigations of the ω-oxidation of C6-C10-monocarboxylic acids in unstarved, starved and diabetic rats. Biochim Biophys Acta 666:394–404, 1981
12. Mortensen PB, Kolvraa S, Gregersen N, et al. Cyanide insensitive and clofibrate enhanced β-oxidation of dodecanedioic acid: Evidence of peroxisomal β-oxidation of dicarboxylic acids. Biochim Biophys Acta 713:393–397, 1982
13. Vamecq J, Draye JP: Peroxisomal and mitochondrial beta-oxidation of monocarboxylyl-CoA, omega-hydroxymonocarboxylyl-CoA and dicarboxylyl-CoA esters in tissues from untreated and clofibrate-treated rats. J Biochem 106:216–222, 1989
14. Vamecq J, Draye JP: Comparison between the formation and the oxidation of dicarboxylylcarnitine esters in rat liver and skeletal muscle: Possible implications for human inborn disorders of mitochondrial β-oxidation. J Inherit Metab Dis 12:59–63, 1989
15. Wada F, Usami M: Studies on fatty acid omega-oxidation. Antiketogenic effect and gluconeogenecity of dicarboxylic acids. Biochim Biophys Acta 487:361–368, 1977
16. Weissman C, Chiolero R, Askanazi J, et al: Intravenous infusion of medium-chain triglyceride-enriched lipid emulsion. Crit Care Med 16:1183–1190, 1988
17. Hill JO, Peters JC, Yang D, et al: Thermogenesis in humans during overfeeding with medium-chain triglycerides. Metabolism 38:641–648, 1989
18. Kou Y, Tserng, Shiow-Jen J: Metabolic conversion of dicarboxylic acids to succinate in rat liver homogenates. J Biol Chem 266:2924–2929, 1991
19. Bertuzzi A, Gandolfi A, Salinari S, et al: Pharmacokinetic analysis of azelaic acid disodium salt: A proposed substrate for total parenteral nutrition. Clin Pharmacokinet 20:411–419, 1991
20. Ashbrook JD, Spector AA, Fletcher JE: Medium chain fatty acid binding to human plasma albumin. J Biol Chem 247:7038–7042, 1972
21. Brown JR, Shockley P: Serum albumin: Structure and characterization of its ligand binding sites. IN Lipid-Protein Interactions, Vol I, Jost PC, Griffith OH (eds). John Wiley and Sons, New York, 1982, pp 25–68
22. Lowe JB, Sacchettini JC, Laposta M, et al: Expression of rat intestinal fatty acid-binding protein in *Escherichia coli*. J Biol Chem 262:5931–5937, 1987
23. Shirley DG, Zewde T, Walter SJ: Renal function in normal and potassium depleted rats. Pflugers Arch 79:416–474, 1990
24. Mortensen PB: Dicarboxylic acids and the lipid metabolism. Thesis, Aarhus University, 1983
25. Przyrembel H, Wendel U, Becker K, et al: Glutaric aciduria type II: Report on a previously undescribed metabolic disorder. Clin Chim Acta 66:227–239, 1976
26. Dusheiko G, Kew MC, Joffe BI, et al: Recurrent hypoglycemia associated with glutaric aciduria type II in an adult. N Engl J Med 301:1405–1409, 1979
27. Gregersen N, Kolvraa S, Rasmussen K, et al: Biochemical studies in a patient with defects in the metabolism of acyl-CoA and sarcosine: Another possible cause of glutaric aciduria type II. J Inherit Metab Dis 3:67–72, 1980
28. Sweetman L, Nyhan WL, Trauner DA, et al: Glutaric aciduria type II. Pediatrics 96:1020–1026, 1980
29. Goodman SI, McCabe ERB, Fennessey PV, et al: Multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II) with transient hypersarcosinemia and sarcosinuria: Possible inherited deficiency of an electron transfer flavoprotein. Pediatr Res 14:12–17, 1980

30. Borg L, Lindstedt S, Steen G: Aliphatic C6-C14 dicarboxylic acids in urine from an infant with fatal congenital lactic acidosis. Clin Chim Acta 41:363–366, 1972
31. Lindstedt S, Norberg K, Steen G, et al: Structure of some aliphatic dicarboxylic acids found in the urine of an infant with congenital lactic acidosis. Clin Chem 22:1330–1338, 1976
32. Gregersen N, Rosleff F, Kolvraa S, et al: Non-ketotic C6-C10-dicarboxylic aciduria: Biochemical investigations of two cases. Clin Chim Acta 102:179–189, 1980
33. Naylor EW, Mosovich LL, Guthrie R, et al: Intermittent non-ketotic dicarboxylic aciduria in two siblings with hypoglycemia: An apparent defect in β-oxidation of fatty acids. J Inherit Metab Dis 3:19–24, 1980
34. Reye RDK, Morgan G, Baral J: Encephalopathy and fatty degeneration of the viscera: A disease entity in childhood. Lancet 2:749–752, 1963
35. Corey L, Rubin RJ, Hattwick MAW, et al: A nationwide outbreak of Reye's syndrome: Its epidemiologic relationship to influenza B. Am J Med 61:615–625, 1976
36. Mortimer EA Jr, Lepow ML: Varicella with hypoglycemia possibly due to salicylates. Am J Dis Child 103:91–98, 1962
37. Bove KE: The character and specificity of the hepatic lesion in Reye's syndrome. IN Reye's Syndrome: Proceedings of the Reye's Syndrome Conference, Pollack JD (ed). Grune & Stratton, New York, 1975, pp 93–116
38. Ryan NJ, Hogan GR, Hayes AW, et al: Aflatoxin B1: Its role in the etiology of Reye's syndrome. Pediatrics 64:71–75, 1979
39. Crocker JFS, Rozee KR, Ozere RL, et al: Insecticide and viral interaction as a cause of fatty visceral changes and encephalopathy in the mouse. Lancet 2:22–24, 1974
40. Giles HC: Encephalopathy and fatty degeneration of the viscera. [Letter] Lancet 1:1075, 1965
41. Tonsgard JH, Getz GS: Effect of Reye's syndrome serum on isolated chincilla liver mitochondria. J Clin Invest 76:816–825, 1985
42. Passi S, Picardo M, Nazzaro-Porro M, et al: Antimitochondrial effect of saturated medium chain-length (C8-C13) dicarboxylic acids. Biochem Pharmacol 33:103–108, 1984
43. Nazzaro-Porro M, Passi S, Balus L, et al: Effects of aging on fatty acids in skin surface lipids. J Invest Dermatol 73:112–117, 1979
44. Entman M, Bressler R: The mechanism of action of hypoglycine on long-chain fatty acid oxidation. Mol Pharmacol 3:333–340, 1967
45. Mortensen PB, Gregersen N, Kolvraa S, et al: The occurrence of C6-C10-dicarboxylic acids in urine from patients and rats treated with dipropylacetate. Biochem Med 24:141–158, 1980
46. Borst P, Loos JA, Crist EJ, et al: Uncoupling activity of long-chain fatty acids. Biochim Biophys Acta 62:509–518, 1962
47. Mingrone G, Greco AV, Arcieri-Mastromattei E: Free fatty acids stimulate mucin hypersecretion by rabbit gallbladder epithelium in vitro. Clin Sci 78:175–180, 1990
48. Wada F, Usami M, Goto M, et al: Studies on the physiological significance of fatty acid omega-oxidation. J Biochem 70:1065–1067, 1971
49. Wada F, Usami M: Studies on fatty acid omega-oxidation. Antiketogenic effect and gluconeogenicity of dicarboxylic acids. Biochim Biophys Acta 478:261–268, 1977
50. Pettersen JE: Urinary excretion of n-hexanedioic and n-octanedioc acid in juvenile diabetics with ketonuria. Diabetes 23: 16–20,1974
51. Mortensen PB: The possible antiketogenic and gluconeogenic effect of the omega-oxidation of fatty acids in rats. Biochim Biophys Acta 620:177–185, 1980
52. Ullrich KJ, Rumrich G, Fritzsch G, et al: Secretion and contraluminal uptake of dicarboxylic acids in the proximal convolution of rat kidney. Pflugers Arch 400:241–249, 1984
53. Ullrich KJ, Rumrich G, Fritzsch G, et al: Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. II. Specificity: Aliphatic dicarboxylic acids. Pflugers Arch 408:38–45, 1987
54. Draye JP, Veitch K, Vamecq J, et al: Comparison of the metabolism of dodecanedioic acid in vivo in control riboflavin-deficient and clofibrate-treated rats. Eur J Biochem 178:183–189, 1988
55. Mortensen PB, Gregersen N: The biologic origin of ketotic dicarboxylic aciduria II: In vivo and in vitro investigations of β-oxidation of C8-C16-dicarboxylic acids in unstarved, starved and diabetic rats. Biochim Biophys Acta 710:477–484, 1992
56. Bergseth S, Hokland BM, Bremer J: Metabolism of dicarboxylic acids in vivo and in the perfused kidney of the rat. Biochim Biophys Acta 961:103–109, 1988
57. Wada F, Usami F, Goto M, et al: Studies on the physiological significance on fatty acid oxidation. J Biochem 70:1065–1067, 1971
58. Mortensen PB: The possible antiketogenic and gluconeogenic effect of the omega-oxidation of fatty acids in rats. Biochim Biophys Acta 620:177–185, 1980
59. Osmundsen H, Bremer J, Pedersen J: Metabolic aspects of peroxisomal β-oxidation. Biochim Biophys Acta 1085:141–158, 1991