Original Paper

Ann Nutr Metab 1992;36:296–303

Tracer Study of Metabolism and Tissue Distribution of Sebacic Acid in Rats

P. Antonio Tataranni[a]
Geltrude Mingrone[a]
Andrea De Gaetano[b]
Comasia Raguso[a]
A. Virgilio Greco[a]

[a] Clinica Medica, and
[b] CNR, Centro Fisiopatologia Shock, and Clinica Chirurgica, Catholic University, Rome, Italy

Abstract

The present study investigates the metabolic disposition of sebacic acid in rats. Three groups of experimental animals received different doses of disodium sebacate with 25 μCi of ^{14}C-labeled molecule by intravenous injection. In the first group radioactivity plasma elimination curves were examined for two administered doses (80 and 160 mg). In the second group, expired $^{14}CO_2$, urine tracer and feces tracer were counted after intravenous administration of 160 mg of sebacate. The animals of the third group were sacrificed at different times after intravenous administration of 160 mg of sebacate, and tracer elimination curves were obtained for several organs. The plasma half-life of sebacate is 38.71 min; about 35% of the administered tracer was excreted in the urine as unchanged sebacate; about 25% was eliminated as $^{14}CO_2$ in expired air. Disposition of sebacate was complete within 4 h of administration. The sebacate half-life is longest in adipose tissue (135 min) and in liver (74 min), sites of likely transformation. In all other organs examined, the sebacate half-life is similar to that in plasma.

Key Words
Dicarboxylic acids
Sebacic acid
Tracer studies

Introduction

Recently, we proposed the use of the salts of dicarboxylic acids (DAs) in total parenteral nutrition as an alternate energy source [1–3]. The advantage of medium-chain DAs over conventional lipid substrates (both long and medium triglycerides) is related to the immediate availability of these compounds. The salts of DAs are highly water-soluble and thus can be directly administered through a peripheral venous route. Unlike long chain triglycerides or medium chain triglycerides (MCT), which are available under emulsion

Received:
March 9, 1992
Accepted:
July 25, 1992

Dr. Pietro A. Tataranni
Istituto di Clinica Medica
Università Cattolica del Sacro Cuore
L.go A. Gemelli, 8
I-00168 Roma (Italy)

0250–6807/92/
0366–0296$2.75/0

form for clinical use [4–6], they do not require complex and expensive production procedures.

As in the case of MCT, DAs are rapidly oxidized, but they do not require any hydrolysis prior to cellular utilization. Moreover, DAs have the advantage of being transported across the mitochondrial membrane via a carnitine-independent pathway [7]. This could prove particularly advantageous in certain clinical conditions where a carnitine deficiency state has been suggested such as in septic processes [8], liver cirrhosis [9], premature newborns [10], and hemodialyzed patients [11]. However, odd carbon atom DAs, e.g. azelaic acid (C9), are incompletely oxidized since the β-oxidation is terminated at the level of malonic acid. Malonic acid is then available for the synthesis of free fatty acids, thus creating a potential futile cycle.

Sebacic acid (Sb), the superior homologous with 10 carbon atoms appeared, in preliminary studies [3, 12], to be excreted in the urine in lesser amounts than azelate. Like azelate [13], Sb has been safely used in laboratory animals and has not shown any toxic effects during chronic administration. Similary no teratogenic effects have been observed [14].

Sebacic acid is oxidized to H_2O and CO_2 passing through acetyl-CoA and succinyl-CoA formation [3, 15]. Moreover succinyl-CoA can also be utilized as a starting point in the gluconeogenetic pathway [16, 17].

We calculated that equimolar amounts of Sb provide more calories than carbohydrates [3], with a comparably great increase of the carbon dioxide production. This could be a significant advantage in critically ill patients [18, 19] particularly when different degrees of pulmonary insufficiency are present [20–22].

It is well known that intravenous MCT-containing lipid emulsions, like oral MCT, increase serum ketones and insulin [23, 24]. An increase in ketone bodies is not generally desiderable because they induce metabolic acidosis, which may be less efficiently compensated for by the acutely ill patients receiving parenteral nutrition.

Berry et al. [25] suggested that the elevated thermogenesis found after MCT administration could be accounted for by an energy consumption process, uncoupled with ATP synthesis in the presence of hyperketonemia. We recently demonstrated that sebacic acid administration does not induce ketone body formation or a significant increase in energy expenditure [26].

In this study we evaluated the metabolism and tissue distribution of sebacate in rats measuring the time course of radiolabeled Sb in plasma and organs and of labeled CO_2 in expired air.

Materials and Methods

(1,10) ^{14}C-labeled sebacic acid tracer (specific activity 102 mCi/mmol) was purchased from Amersham (Buckinghamshire, UK). Sebacic and azelaic acids were obtained from Sigma (St. Louis, Mo., USA). All other chemicals were purity-available. Sebacic acid neutralized with NaOH was dissolved in doubly distilled water.

Male Wistar rats, 7–8 weeks old, weighing 220–250 g were used throughout the study. They had free access to water and a standard pellet diet containing 20% protein, 4% fat and 50% carbohydrate. Animals were divided into three treatment groups (A, B, C).

Experimental Procedure

Plasma Curves (Group A). Two different doses of sebacate (a low dose of 80 mg and a high dose of 160 mg), enriched with 25 μCi of tracer, were administered intravenously by tail vein. 14 rats were treated with each dose. Heparinized blood samples were taken prior to and 5, 10, 20, 40, 80, 160, and 320 min after injection.

$^{14}CO_2$ Collection (Group B). 4 rats were administered 160 mg Sb solution enriched with 25 μCi of tracer by intravenous injection. Since sebacic acid has been proposed as an alternate substrate in parenteral nutrition, we checked the oxidized amount at the highest dose, which is more interesting for energy delivery.

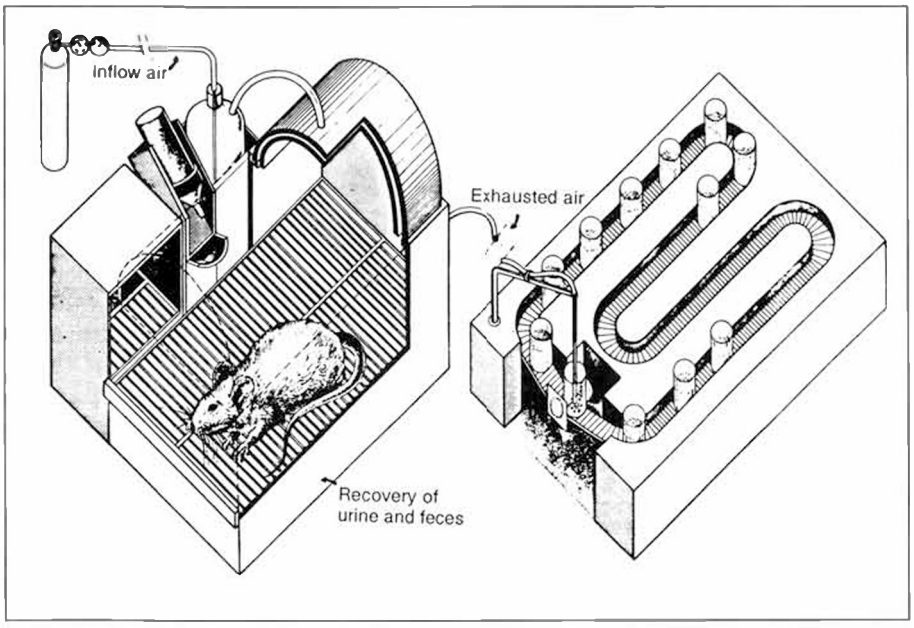


Fig. 1. Metabolic cage.

After injection, the animals were placed in flow-through airtight metabolic cages. Air passed through the cage at 2 liters/min (total volume of the cage being 15 liters). The cage consisted of a Plexiglas box that is part of an open system in which both urine and feces can be separately collected (fig. 1). The air entering the cage was humidified (70%). Air exiting the cage was dried passing through a water trap (silica gel granular, self-indicating). At 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 540 and 1,440 min postdose $^{14}CO_2$ was trapped, bubbling the outflowing air into vials containing a solution of methylbenzethonium hydroxide (MH) or hyamine prepared as previously described [15]. Following the above procedure, solutions containing 3 mEq of MH were obtained: these solutions are capable of trapping exactly 3 mmol of CO_2 [27]. Thereafter, the MH solution trapping $^{14}CO_2$ was added with 10 ml of 0.4% 2,5-diphenyloxazole toluene in scintillation fluid and counted.

Urine was collected (separately for the periods 0–4 and 4–24 h) in containers with 1 ml 0.1% sodium azide to prevent bacterial growth and cooled in solid CO_2. 24-hour feces was also collected.

Tissue Distribution (Group C). 10 rats were administered 160 mg of Sb solution with 25 µCi of tracer by intravenous injection. The animals were sacrificed at 30, 60, 120, 240 and 360 min postdose. At each time and from each sacrificed animal the following tissue samples were collected for radioactivity measurement: liver, kidney, heart, lung, muscle, fat.

Analytical Procedures

Plasma Samples. Plasma samples were separated from the blood cells. 50 µg of azelaic acid were added to 1 ml of each serum sample as internal standard. Proteins were precipiated with ethanol kept at −20 °C overnight and removed by centrifugation at 5,000 rpm for 15 min. The pellet was washed 3 times with 0.6 ml of ethanol and the supernatants collected together. The supernatants were acidified to pH 1–2 with 1 *N* HCl and extracted 3 times with 8 vol of ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under a nitrogen stream. 1 ml of plasma was also counted for total radioactivity recovery.

Urine Samples. Urine samples (0.5 ml) mixed with 50 µg of azelaic acid were treated with cation exchange resin (Dowex 50 W-X4, 100–200 µm mesh, H^+) to remove salts, concentrations under reduced pressure and filtered through a Millipore HV (0.45 µm) filter. The samples were extracted 3 times with 8 vol of ethylacetate and evaporated under nitrogen stream. 1 ml of

urine was also counted for total radioactivity recovery.

Feces Samples. 24-hour feces was added with 100 µg of azelaic acid, acidified at pH 1–2 with 6 *N* HCl, extracted with 8 vol of ethylacetate, automatically shaken and warmed at 40 °C for 30 min. The ethyl acetate solutes were dried under a nitrogen stream. 1 mg of feces was also counted for total radioactivity recovery.

HPLC Analysis. The extracts from plasma, urine and feces were dissolved in 2 ml acetonitrile-methanol (4:1, v/v) and added to 6 mg of *p*-bromophenacyl bromide dissolved in CH_3CN and 14 µl of N,N-diisopropylethylamine as catalyst. The mixture was heated to 60 °C for 15 min. The derivatives were purified by thin-layer cromatography on standard thin-layer plates (20 × 20 cm) (Stratocrom SI-AP, Carlo Erba, Italy) coated with 0.25 mm of silica gel and activated by heating at 130 °C for 15 min. The plates were developed in pentane/ether/acetic acid (92:7:0.5, v/v/v) and dicarboxylic *p*-bromophenacyl esters were scraped off and extracted with 3 vol of acetonitrile.

After evaporation of the solvent up to a final volume of 0.5–1 ml, aliquots from 20 to 40 µl were injected into a 1050 liquid chromatograph (Hewlett Packard) equipped with a scanning spectrophotometer operating in the 190–600 nm wavelength range (light source: deuterium lamp). The HPLC includes an intergrator, so that areas and times are given for each peak in the chromatogram.

DAs derivatives were separated on a reversed-phase LC-18 column (Supelco, Bellafonte, Pa., USA) 25 cm × 4.6 mm ID, particle size 5 µm according to Passi et al. [28]. Briefly, after 5 min isocratic elution with 60% CH_3CN in water adjusted to pH 3.1 with H_3PO_4, a gradient was performed to 100% CH_3CN in 60 min. The conditions were the following: flow rate 1 ml/min, absorbance noise 2.5 × 10^{-5} AU at 245–255 nm, chart speed 0.25 cm/min, UV detector 255 nm.

DAs as *p*-bromophenacyl diesters were fractionated by RP-HPLC, individually collected, placed in counting vials in the presence of standard scintillation solution and counted. A mixture of derived DAs from adipic (C6) to dodecanedioic (C12) acids was used as a reference standard.

Tissue Samples. Tissue samples were weighed and then homogenized by Polytron (Brinkman Instruments, Westbury, N.Y., USA), aliquots dissolved in tissue solubilizer, Soluene-350 (Packard Instrument, Downers Grove, Ill., USA), followed by the addition of scintillation fluid, and counted.

The radioactivity of plasma, urine, feces, tissues and expired CO_2 was determined by counting the β-emission of the samples with a β-scintillation counter (Packard Tri-Carb 460 C). Quenching was checked by the internal standard method.

Results

Data were first normalized by multiplying obtained µCi levels by the rat's weight, and referred to 100 g body weight. Plasma radioactivity elimination curves (group A) are shown in figure 2 for the two doses.

Data were fitted minimizing with the Marquardt algorithm, the unweighted squared error for a monoexponential decay, yielding the parameter estimates reported in table 1. The pooled estimates were obtained by fitting all points together.

The plasma half-life of labeled Sb was found to be 38.71 min and the apparent volume of distribution turned out to be 62.65 ml/100 g body weight.

Figure 3 shows the amount of labeled carbon dioxide elimination via expired air due to Sb tissue utilization (group B). Data were fitted for each animal as described above; the obtained values (table 2) were then averaged together and yielded the following parameter estimates: $k = 0.0074 \pm 0.001\ min^{-1}$; $R_0 = 0.0483 \pm 0.009$ µCi/min; $t_{1/2} = 93.64$ min. The area under curve (AUC) of the $^{14}CO_2$ elimination curve was computed by the trapezoidal rule for each of the 4 rats, yielding an average AUC of 6.209 ± 0.06 µCi/min equal to 25 ± 0.01% of the administered dose. After intravenous administration of 160 mg of Sb, 58.01 ± 3.57% of the radiocarbon dose was recovered in the urine over 24 h. The amount of sebacate retrieved from the 24-hour urine collection was 34.6 ± 1.97% of the administered dose, while suberic (C8) acid accounted for 5.08 ± 0.74%. Most of the compound was excreted in the first 4 h, while in samples col-

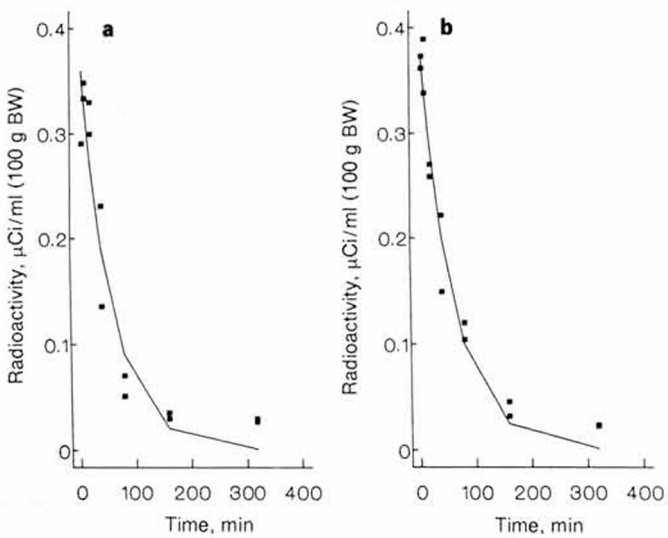


Fig. 2. Plasma radioactivity versus time after the administration of 80 mg Sb + 25 µCi tracer (**a**) and 160 mg Sb + 25 µCi tracer (**b**). Fitted curves are shown. BW = Body weight.

Table 1. Parameter estimates for the model $R(t) = R_0\, e^{-kt}$ describing disappearance of the radioactive tracer from plasma

Parameter	Injected Sb		
	80 mg	160 mg	pooled
k, min^{-1}	0.0183	0.0174	0.0179
R_0, µCi/ml	0.393	0.403	0.399
$t_{1/2}$, min	37.86	39.82	38.71

Table 2. Estimated parameters for the model $R(t) = R_0\, e^{-kt}$ describing $^{14}CO_2$ decay from expired air

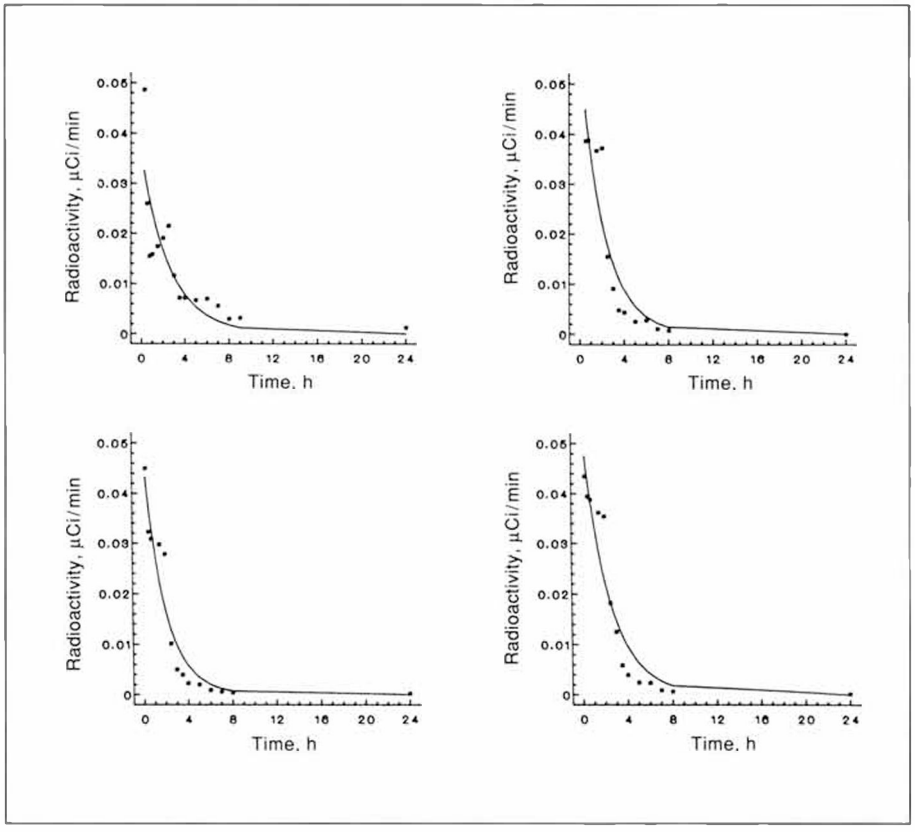
Group B	Parameters		
	k min^{-1}	R_0 µCi/ml	AUC µCi
1	0.0063	0.0357	7.04
2	0.0076	0.0564	5.69
3	0.0088	0.0493	5.83
4	0.0070	0.0527	6.25

Reported AUC is computed by trapezoidal approximation.

lected after this time only traces of the diacid were detected. Samples of 24-hour feces did not show any β-emission activity.

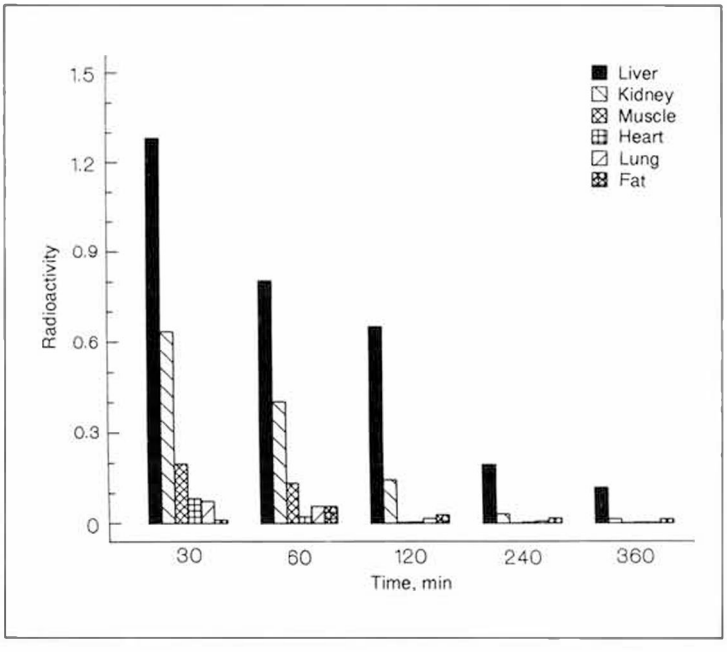
Figure 4 shows the radioactivity recovered from the dissected organs (group C). The radioactivity levels were fitted with a simple exponential decay model, obtaining the following estimates for tracer half-life in each organ: heart 16 min ($k = 0.043\ min^{-1}$), muscle 32 min ($k = 0.021\ min^{-1}$), kidney 42 min ($k = 0.016\ min^{-1}$), lung 48 min ($k = 0.014\ min^{-1}$), and liver 72 min ($k = 0.0096\ min^{-1}$).

The behavior of sebacate in fat seems to reflect an absorption phase still present 1 h



3

Fig. 3. Radioactivity of expired air versus time for each of the four group B rats. Tracer elimination is essentially zero at 24 h.



4

Fig. 4. Radioactivity of dissected organs (µCi; fat and muscle: µCi/g). Minimal residual radioactivity at 6 h.

after intravenous injection. However, the half-life obtained fitting the late portion of the curve turned out to be 135 min ($k = 0.0022\ min^{-1}$).

Discussion

Although little is known about in vivo DA utilization, for DAs from sebacic to dodecanedioic the mitochondrial transport system has been indicated to be identical to the one used by the corresponding monocarboxylic acid [29]. Peroxisomes seem to be able to readily β-oxidize dodecanedioic, sebacic and suberic acid [30].

Dicarboxylic acid β-oxidation was demonstrated with in vitro studies [29, 30]; however, no data are available about sebacic acid metabolism in rats.

In the present paper we demonstrated that sebacic acid can be oxidized by tissues and that this diacid is only partially eliminated in the urine. Since a linear model fitted well the plasma radioactivity decay for both administered doses and since the parameter estimates were very near each other, it seems reasonable to suppose that linear kinetics describes well the elimination of Sb from plasma in the tested dose range. Therefore we used all points to obtain a pooled estimate of the disposition parameters (table 1).

In our series we found a large apparent volume of distribution (62.65 ml/100 g body weight), which suggests wide diffusion or, more likely, tissue binding of Sb. The global plasma elimination rate (half-life 38.71 min) reflects the contribution of both sebacate uptake and metabolism by tissues and of sebacate elimination by the kidney.

The appearance of a $^{14}CO_2$ peak value in expired air few minutes after intravenous injection of labeled Sb indicates that this compound is readily used as an energy substrate.

Bergseth et al. [31] performed a study on the metabolism of suberic (C8) and dodecanedioic (C12) acids administered to rats by intraperitoneal injection. These authors found that 9–22% of C8 and 28–39% of C12 were oxidized and that the ability of the organism to retain and oxidize DAs improved with the chain's length.

Our data agree very well with those of these authors in that we retrieved 25% of administered tracer in expired CO_2 using an intermediate chain length DA (C10). However, the total recovered tracer was only about 85% of that administered, and it seems likely that the oxidation is somewhat underestimated at 25%. Moreover, part of the metabolites produced with the breakdown of sebacate for energy purposes can be excreted with urine, like $^{14}CO_2$-containing bicarbonates or succinate.

While 60% of the total administered radioactivity was recovered from urine, only 35% of administered sebacate was recovered unchanged from urine. From all the above considerations, it seems likely that about 30–50% of administered sebacate may be used by tissues for energy purposes.

No appreciable accumulation of radioactivity is present in the body: after 24 h practically all of the administered tracer has disappeared from the sampled organs, fat included, although fat has the longest elimination time. A rough comparison between tissue elimination times seems to indicate that a delay in tracer elimination occurs also at the liver level. This suggests that fat and liver are possible preferential sites of Sb metabolism. On the other hand, heart, kidney, lung and skeletal muscle seem to have a faster sebacate elimination, consistent with the hypothesis of a simple dilution of the molecule in these areas, or of its use there eminently for energy production.

In conclusion, sebacic acid appears to be a promising molecule for nutritional purposes.

References

1 Mingrone G, Tacchino RM, Greco AV, Arcieri Mastromattei E, Finotti E, Castagneto M: Preliminary studies of dicarboxylic acid as an energy substrate in man. JPEN 1989;13: 299–305.

2 Tacchino RM, Mingrone G, Marino F, Arcieri Mastromattei E, Greco AV, Castagneto M: Short term infusion of azelaic acid versus intralipid in healthy subjects evaluated by indirect calorimetry. JPEN 1990;14: 169–175.

3 Mingrone G, Tacchino RM, Castagneto M, Finotti E, Greco AV: Use of even numbered carbon atoms dicarboxylic salts in parenteral nutrition as fuel substrate. JPEN 1992;16:32–38.

4 Schubert O, Wretlind A: Intravenous infusion of fat emulsified phosphatides and emulsifying agents: Clinical and experimental studies. Acta Clin Scand 1961;278(suppl):1–12.

5 Johnson RC, Cotter R: Metabolism of MCT lipid emulsion. Nutr Intern 1986;2:150–158.

6 Sailer D, Muller M: MCT in parenteral nutrition. JPEN 1981;5:115–119.

7 McGarry JD, Foster DW: The regulation of ketogenesis from oleic acid. The role of tricarboxylic acid cycle and fatty acid synthesis. J Biol Chem 1971;246:1149–1159.

8 Nanni G, Pittiruti M, Giovannini I: Plasma carnitine levels and urinary carnitine excretion during sepsis. JPEN 1985;9:483–488.

9 Rudman D, Sewell CW, Ansley JD: Deficiency of carnitine cachetic cirrhotic patients. J Clin Invest 1977; 60:716–726.

10 Schmidt-Sommerfield E, Penn D, Wolf H: Carnitine blood concentration and aft utilization in parenterally alimented premature newborn infants. J Pediatr 1982;100:260–264.

11 Bohmer T, Bergrem H, Eiklid K: Carnitine deficiency induced during intermittent hemodialysis for renal failure. Lancet 1978;i:126.

12 Bertuzzi A, Gandolfi A, Salinari S, Mingrone G, Arcieri Mastromattei E, Finotti E, Greco AV: Pharmacokinetic analysis of azelaic disodium salt, a proposed substrate for total parenteral nutrition. Clin Pharmacokinet 1991;20:411–419.

13 Mingrone G, Greco AV, Nazzaro Porro M, Passi S: Toxicity of azelaic acid. Drugs Exp Clin Res 1983;9: 447.

14 Greco AV, Mingrone G, Arcieri Mastromattei E, Finotti E: Toxicity of sebacic acid. Drugs Exp Clin Res 1990;16:531–536.

15 Mingrone G, Greco AV, Bertuzzi A, Arcieri Mastromattei E, Tacchino RM, Marino F, Finotti E, Castagneto M: Tissue uptake and oxidation of disodium sebacate in man. JPEN 1991;15:454–459.

16 Wada F, Usami M: Studies on fatty acid omega-oxidation: Antiketogenetic effect and gluconeogenicity of dicarboxylic acids. Biochim Biophys Acta 1977;487:261–268.

17 Tserng KY, Jin SJ: Metabolic conversion of dicarboxylic acids to succinate in rat liver homogenates, a stable isotope tracer study. J Biol Chem 1991;226:2924–2929.

18 Siegel JH, Cerra FB, Colemann B, Giovannini I, Shetye M, Border JR, McMenamy RH: Physiological and metabolic correlations in human sepsis. Surgery 1979;86:163–193.

19 Nanni G, Siegel JH, Colemann B, Fader P, Castiglione R: Increased lipid fuel dependance in the critically ill septic patients. J Trauma 1984;24:24–30.

20 Sundeston G, Zannder CW, Arborelins M: Decrease in pulmonary diffusing capacity during lipid infusion in healthy man. J Appl Physiol 1973;34:816–822.

21 Silberman H, Silberman AW: Parenteral nutrition biochemistry and respiratory gas exchange. JPEN 1986;10:151–154.

22 Nordenstrom J: Utilization of Exogenous and Endogenous Lipids for Energy Production during Parenteral Nutrition. Stockholm, Gatab, 1982.

23 Yeh Y, Zee P: Relation of ketosis to metabolic changes induced by acute medium-chain triglycerides feeding in rats. J Nutr 1976;106:58–67.

24 Weissman C, Chiolero R, Askanazi J, Gil K, Elwyn D, Kinney J: Intravenous infusion of a medium-chain triglyceride-enriched lipid emulsion. Crit Care 1988;16:1183–1190.

25 Berry MN, Cleark DG, Grivell AR: The calorigenic nature of hepatic ketogenesis. An explanation for stimulation of respiration induced by fatty acid substrates. Eur J Biochem 1983;131:205–214.

26 Greco AV, Mingrone G, Raguso C, Tataranni PA, Finotti E, Tacchino RM, Capristo E, De Gaetano A, Castagneto M: Metabolic effects and disposition of sebacate, an alternate dicarboxylic fuel substrate. Ann Nutr Metab 1992;36:1–11.

27 Wolfe RR: Tracer in Metabolic Research. New York, Liss, 1984.

28 Passi S, Nazzaro Porro M, Picardo M, Mingrone G, Fasella P: Metabolism of straight, saturated medium-chain length (C9–C12) dicarboxylic acids. J Lipid Res 1983;24:1140–1147.

29 Osumi T, Hashimoto T: Purification and properties of mitochondrial and peroxisomal 3-hydroxyacyl-CoA dehydrogenase from rat liver. Arch Biochem Biophys 1980; 203:372–383.

30 Mortensen PB, Kolvraa S, Gregersen N, Rasmussen K: Cyanide insensitive and clofibrate enhanced β-oxidation of dodecanedioic acid: Evidence of peroxisomal β-oxidation of dicarboxylic acids. Biochim Biophys Acta 1982;713:393–397.

31 Bergseth S, Hokland BM, Bremer J: Metabolism of dicarboxylic acids in vivo and in the perfused kidney of rat. Biochim Biophys Acta 1988; 961:103–109.