Original Paper

Ann Nutr Metab 1992;36:1–11

Metabolic Effects and Disposition of Sebacate, an Alternate Dicarboxylic Fuel Substrate

Aldo Virgilio Greco[a]
Geltrude Mingrone[a]
Comasia Raguso[a]
Antonio Tataranni[a]
Enrico Finotti[a]
Roberto M. Tacchino[b]
Esmeralda Capristo[a]
Andrea De Gaetano[b]
Marco Castagneto[b]

[a] Istituto di Clinica Medica e
[b] Istituto di Clinica Chirurgica, CNR Centro Studio Fisiopatologia Shock, Università Cattolica del Sacro Cuore, Roma, Italia

Abstract

Disodium sebacate is a 10-carbon-atom dicarboxylic acid, proposed as substrate for parenteral nutrition. We investigated its pharmacokinetic profile and thermogenic effect during a short-time infusion (5 h at 10 g/h) in 7 male volunteers. Sebacate in serum and urine was measured by high-performance liquid chromatography. A single-compartment model with two linear elimination routes was fitted. Metabolic measurements (VO_2, VCO_2, respiratory quotient, metabolic rate) were continuously performed for 8 h (5 h during and 3 h after the infusion) by a canopy indirect calorimeter. The apparent volume of distribution of sebacate was 8.39 ± 0.69 liters, and the plasma fractional removal rate constant was 0.0086 ± 0.00077 min^{-1}. The average half-life and plasma clearance were 80.6 min and 72 ml/min, respectively. The increase in metabolic rate, the decrease in respiratory quotient and the changes in ketone body, glucagon and insulin levels during the infusion were not significant. 24-hour catecholamine excretion was within normal limits. Calories administered by sebacate seem to be available for utilization without relevant metabolic side effects.

Key Words
Dicarboxylic acids
Sebacic acid
Thermogenesis
Pharmacokinetics
Parenteral nutrition

Introduction

Dicarboxylic salts (azelate and sebacate) in parenteral nutrition could be particularly useful in clinical conditions where the glucose or lipid utilization by tissues is impaired. Despite the fact that stoichiometric analysis [1] and indirect calorimetric data [2] suggest a good tissue utilization of azelate, the pharmacokinetic analysis of plasma azelaic acid con-

This study was supported by a grant from Sigma Tau, S.p.A., Pomezia (Rome), Italy.

Received: March 8, 1991
Accepted: June 1, 1991

Prof. A.V. Greco
Istituto di Clinica Medica
Largo Agostino Gemelli, 8
I-00168 Rome (Italy)

0250–6807/92/
0361–0001$2.75/0

centrations during constant intravenous infusion show a high level of urinary excretion (mean 76.9% of infused dose) and a mean renal clearance of 140.4 ml/min, demonstrating the presence of a tubular secretion mechanism. Recent studies [3, 4] have shown that sebacate, the superior homologue of azelate, which is a saturated dicarboxylic acid with 10 carbon atoms, is eliminated via the renal route to a much smaller extent than azelate (about 16% when infused at the rate of 3.3 g/h). Therefore, sebacate would seem to be a likely substrate for parenteral nutrition. This diacid does not exhibit either toxic or teratogenic effects in laboratory animals [5]. It is completely oxidized to H_2O and CO_2, and the intramitochondrial transport is carnitine-independent [6]. In addition, the oxidation of sebacate is also possible at the peroxisomal level [7]. Finally, the salts of sebacate, like those of azelate, can be directly administered through a peripheral vein and their preparation is inexpensive.

In this paper the pharmacokinetic parameters of sebacate in a single-compartment model as mean values of the whole subject population are presented. Moreover, the metabolic effects of sebacate infusion (50 g over 5 h) during the study period were also evaluated as were the plasma concentration of ketone bodies (β-hydroxybutyrate and acetoacetate), the plasma insulin and glucagon levels and the 24-hour urinary amounts of catecholamines. In order to analyze the thermic effect of sebacate, metabolic rate (MR) measurements by indirect calorimetry were made each minute for 30 min every hour during the 5-hour infusion and 3 h following the end of the sebacate infusion.

Materials and Methods

Experimental Design

Seven moderately obese (body mass index 29.8 ± 2.41 kg/m^2) male volunteers aged 33 ± 4 years were studied. All subjects were admitted to the Gastroenterology Department the evening preceding the experiments.

On the following morning a continuous infusion of 50 g disodium sebacate (10% in bidistilled water) was administered over a period of 5 h through a peripheral venous catheter with an Infusomat (Secura) infusion pump (Braun, Melsungen, FRG). A second intravenous catheter was inserted 1 h before the study was started to draw blood and to give the patient a slow drip of normal saline.

Blood samples were taken at 0, 15, 30, 60, 120, 180, 240 and 300 min from the beginning of the sebacate infusion. Blood samples were also collected at 15, 30, 60, 90, 120 and 180 min after the sebacate infusion was stopped.

The study subjects voided before starting sebacate administration and their urine was collected over 24 h in a container with 0.1% sodium azide to prevent bacterial growth.

Measurement of Energy Balance Components

The basal MR (BMR) and the thermic effect of the infusion were measured during the entire experimental session using indirect calorimetry performed by a Deltatrac monitoring system (Datex Instrumentarium, Helsinki, Finland). The indirect calorimetry measurements were performed each minute for 30 min every hour, for a period of 8 h.

BMR was measured in the morning, after a 12-hour fast and 30 min before the start of the infusion. The thermic effect of the administered substrate was calculated using the difference between the MR measured during the 5th hour of sebacate infusion and the BMR.

All procedures were approved by the Ethical Committee of the Institutional Health Review Board of Science of the Catholic University School of Medicine in Rome. Written informed consent was obtained in all cases.

Analytical Procedures

Acetoacetic acid and β-hydroxybutyrate were measured enzymatically [8]. Plasma insulin [9] and glucagon [10] concentrations were measured by radioimmu-

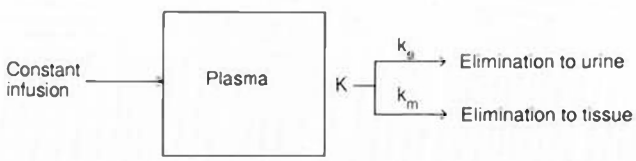


Fig. 1. Block diagram of the single-compartment model used for sebacate pharmacokinetic analysis. Symbols are defined in the text.

noassay. Catecholamine levels were measured enzymatically (Amersham kits, Amersham, UK) from urine collected over 24 h.

Dicarboxylic Acid Analysis

Serum Samples. 50 µg of azelaic acid (C9) were added to 1 ml of each serum sample as internal standard. Proteins were precipitated 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 ethylacetate. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under nitrogen stream.

Urine Samples. Urine samples (0.5 ml) were treated with cation-exchange resin (Dowex 50 W-X4, 100- to 200-µm mesh, H^+) to remove salts, concentrated under reduced pressure and filtered through a Millipore HV (0.45 µm) filter. The samples were extracted 3 times with 8 vol of ethylacetate and evaporated under N_2.

Analysis by High-Performance Liquid Chromatography. The extracted solutes from both sera and urine were dissolved in 2 ml acetonitrile/methanol (4:1, v/v) and added to 6 mg of *p*-bromophenacylbromide 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 chromatography on standard thin-layer plates (20 × 20 cm; Stratocrom SI-AP, Erba, Milan, 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/diethylether/acetic acid (92:7:0.5) and the spots corresponding to standard dicarboxylic *p*-bromophenacylesters were scraped off and extracted with 3 vol of acetonitrile. After evaporation of the solvent down to a final volume of 0.5–1 ml, aliquots from 20 to 40 µl were automatically injected into a liquid chromatograph (Hewlett-Packard 1050, Palo Alto, Calif., USA) with an integrator and a scanning spectrophotometer operating in the 190- to 600-nm wavelength range (light source: deuterium lamp).

The dicarboxylic acid derivatives were separated on a reverse-phase column (25 cm × 4 mm internal diameter) RP-18, 5 µm Hewlett-Packard.

After a 5-min isocratic elution with 60% CH_3CN in water adjusted to pH 3.10 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; range of absorbance from −0.3 to 1.000 absorbance units (AU; absorbance noise = 2.5×10^{-5} AU at 254 nm); chart speed = 0.25 cm/min; ultraviolet detector = 254 nm.

A mixture of derivatized dicarboxylic acids from adipic (C6) to dodecandioic (C12) acids was used as a reference standard.

Pharmacokinetic Analysis

A single-compartment model with two linear elimination routes was used (fig. 1).

The elimination was assumed to be linear via the kidneys and the tissues; the plasma level curves during the infusion and postinfusion periods were then assumed to follow the relation:

infusion:

$$\frac{dC(t)}{dt} = \frac{r}{V} - kC(t),\ C(0) = 0 \rightarrow C(t) = \frac{r}{Vk}(1 - e^{-kt}),\ \text{and}$$

postinfusion:

$$\frac{dC(t-300)}{dt} = -kC(t-300),$$

$$C(300) = C_{300} \rightarrow C(t-300) = C_{300}\, e^{-k(t-300)},$$

where the concentration C (mg/l) is expressed as a function of time t (min), of infusion rate r (mg/min), of apparent volume of distribution V (l), of the elimina-

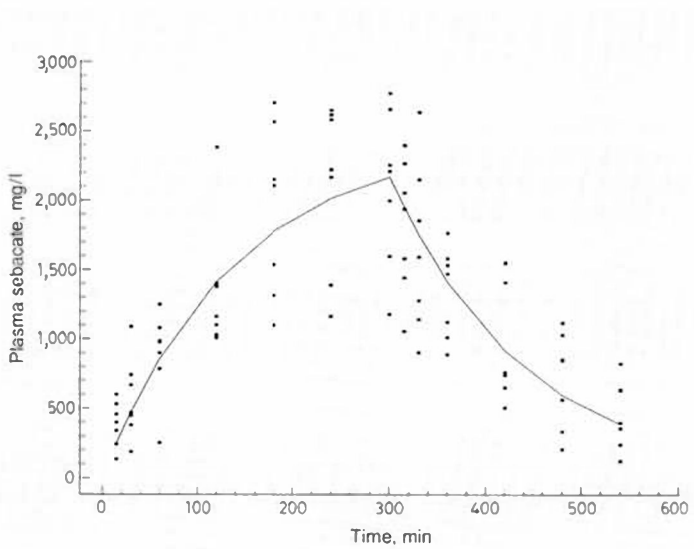


Fig. 2. Modal prediction of plasma sebacate concentration versus time (solid line) and experimental data of plasma sebacate concentration (closed squares).

tion rate constant k (min^{-1}) and of the 'true' concentration at the end of infusion C_{300}, forced to be:

$$C_{300} = \frac{r}{Vk}(1 - e^{-k \cdot 300}).$$

Plasma levels from all patients were fitted together.

Both plasma concentration curves (during and after infusion) were fitted simultaneously by minimizing the squared error (by a nonlinear Marquardt algorithm) with respect to the 2 parameters V and k in the composite model:

$$C(t) = q\left[\frac{r}{Vk}(1 - e^{-kt})\right] + (1 - q)[C_{300}e^{-k(t-300)}].$$

In the above formula, q is a dummy variable equal to 1 in the infusion period and to 0 in the postinfusion phase.

From the fitted parameters the following were computed:

total clearance: Cl[ml/min] = V·k, and

plasma half-life: $t_{1/2} = \frac{0.693}{k}$.

From the mean amount of the drug retrieved in the urine (X_u) and the total infused dose (X_o) the renal elimination rate constant (k_e) was evaluated as:

$$k_e = \frac{X_u}{X_o} \cdot k,$$

leaving a tissue elimination rate constant $k_m = k - k_e$. Renal and tissue clearances were estimated by $Cl_e = k_e \cdot V$, $Cl_m = k_m \cdot V$.

The indirect calorimetry results are expressed as means ± SD of the values measured each minute for 30 min every hour.

Results

The mean ± SD 24-hour urinary excretion of sebacate in the 7 subjects who received an infusion was 23.25 ± 9.8. This mean value corresponds to 46.5% of the administered dose.

Figure 2 shows the average plasma concentration curves obtained as described above. The data obtained from the examinated subjects (plasma sebacate concentration and 24-hour urinary excretion) were used to estimate the mean population values of the pharmacokinetic parameters. The model shows a reasonable fit to the experimental data with an $r^2 = 0.67$ over 91 points, $F = 487$, $p < 0.001$. The peak value of plasma sebacate was reached at

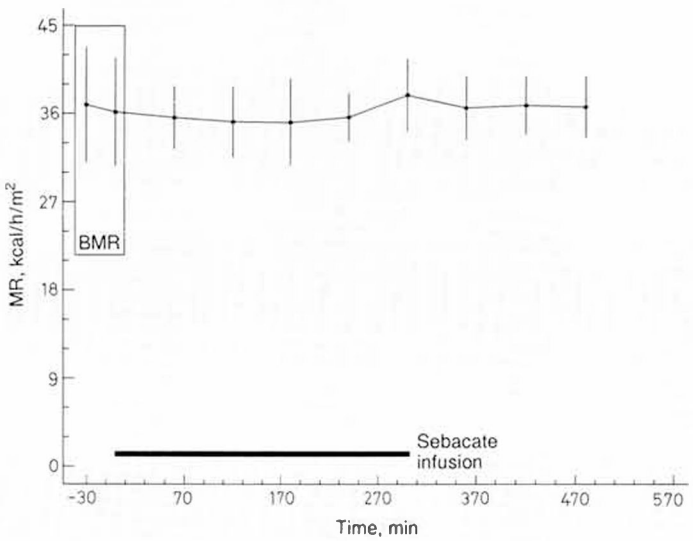


Fig. 3. MR values normalized for BSA (kcal/h/m^2) are shown before sebacate administration (BMR), during the 5-hour constant sebacate infusion (infusion rate = 5 g/h) and during the 3 h following the end of the infusion. Each point represents the mean ± SD of the values measured each minute for 30 min every hour.

the end of sebacate infusion and showed a high interindividual variability. The average peak value was 2,090.86 ± 562.5 mg/l.

The apparent volume of distribution of sebacate was 8.39 ± 0.69 liters. The plasma fractional removal rate constant was 0.0086 ± 0.00077 min^{-1} and the half-life was 80.6 min. The total plasma clearance was 0.072 l/min, and could be partitioned into a renal clearance (0.033 l/min, k_e = 0.0039) and a tissue clearance (0.039 l/min, k_m = 0.0047). Since we assumed that all the sebacate present in plasma samples was free and not vehicled by albumin or other proteins, an assumption which agrees with the high hydrosolubility of disodium sebacate, we evaluated the mean renal clearance as the ratio between excreted amount of sebacate and plasma area under the curve (AUC), which was equal to 0.0345 l/min. The AUC was computed from 0 to 480 min by trapezoidal approximation. To this value we added the AUC from 480 min to 24 h, computed extrapolating the concentration profile by an exponential decay with time constant determined from the data at 180 and 210 min. The renal clearance thus evaluated was very close to that calculated with the mathematical model, confirming the reliability of the model used.

The 8-hour average pattern of MR (kcal/h, mean ± SD) is plotted in figure 3, all data having been normalized for the body surface area (BSA) value of the examined subject. Since a plateau phase of plasma sebacate concentration curve was not reached during the experiments, the thermic effect was computed as the difference between MR at the time corresponding to the maximum value of plasma sebacate concentration (5th hour) and BMR. The average value of MR over the BMR, computed at the 5th hour (peak of plasma sebacate concentration), was 1.72 kcal/h/BSA equal to 4.77% of BMR (p = n.s.).

The respiratory quotient (fig. 4) gradually decreased from a basal value of 0.85 ± 0.043 to 0.76 ± 0.031; this value remained quite constant during the entire period of observation (p = n.s.).

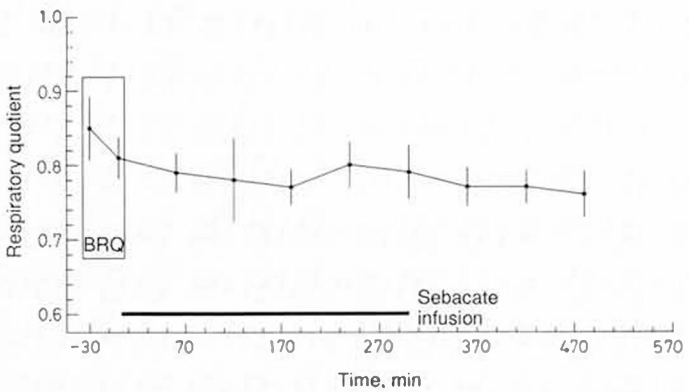


Fig. 4. Respiratory quotient (mean ± SD) before, during and after the sebacate infusion (infusion rate = 5 g/h). Like the MR, each point represents the mean value of the measurements taken each minute for 30 min every hour during the study period. BRQ is the basal value of the respiratory quotient before starting the infusion.

The average acetoacetate and 3-hydroxybutyrate concentrations during the period of the study are shown in figures 5a and b. No significant change in either acetoacetate or 3-hydroxybutyrate during or after sebacate infusion was observed. No increase in insulin or glucagon concentrations was noted (fig. 6a, b). Catecholamine excretion over 24 h was 10.8 ± 1.1 μg for epinephrine, 38.8 ± 6.5 μg for norepinephrine and 227.6 ± 21.4 μg for dopamine, all these being within a normal range.

Discussion

Experimental data have shown that sebacate is excreted in the urine to a very much lower extent than azelate, the inferior homologous dicarboxylic acid with 9 carbon atoms [1]. The latter appears to be actively excreted in the urine [11].

On the contrary, the value of renal sebacate clearance, which is very much smaller than that of inulin, suggests the presence of a tubular reabsorption mechanism. The simple mathematical model described in this paper is not capable of ascertaining whether the tubular reabsorption is due to a back diffusion or to active transport of sebacate. These data agree with those reported by Ullrich et al. [12, 13]. In fact, these authors demonstrated that in rat kidneys dicarboxylic acids have a different fate depending on their chain length. Dicarboxylic acids with a chain length longer than 1 nm are reabsorbed while those with a shorter chain length are excreted.

The estimated volume of sebacate distribution is near to the 'inulin space' (10.7 liters for a 70-kg man, 12.78 liters for the average 82.28 kg of our male patients) [14], suggesting that sebacate quickly enters the tissues where it does not remain unchanged (in this case the apparent volume of distribution would be larger) but rather it is metabolized.

Concerning the indirect calorimetry data, our results showed a small and nonsignificant increase in MR at the end of the intravenous sebacate administration. These data agree with the theoretical estimates of obligatory thermic effect of food which are around 3% of the calories given by sebacate (see Appendix).

On the contrary, in a group of 9 healthy male volunteers, Weissman et al. [15] reported a significant increase in VO_2 after 5 h

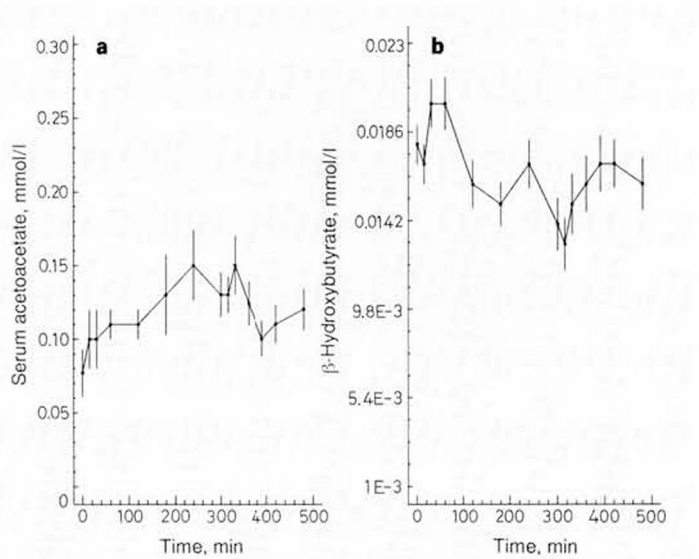


Fig. 5. Serum levels (mean ± SD) of acetoacetate (**a**) and β-hydroxybutyrate (**b**) during the study period.

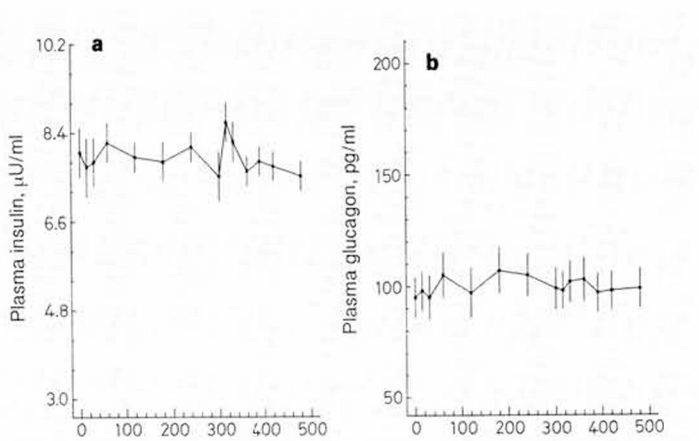


Fig. 6. Plasma levels (mean ± SD) of insulin (**a**) and glucagon (**b**) before, during and after sebacate infusion.

infusion of an emulsion containing 56% medium-chain triglycerides (MCT) and 44% long-chain triglycerides (LCT) given at two different infusion rates of 1.0 g/kg in 15 h or 1.5 g/kg in 15 h. Similar observations were made by these authors after infusion of LCT alone at the same infusion rates. The results obtained by Weissman et al. [15] by a continuous intravenous infusion of an MCT-enriched mixture were similar to those obtained by other authors with an oral bolus of MCT [16–19].

In relation to the above observation, we think that sebacate could be a useful fuel substrate, in spite of the high amount of loss with urine. In fact, at an infusion rate of 5 g/h it is expected that, on the basis of the previous studies [3] and of the present one, only about

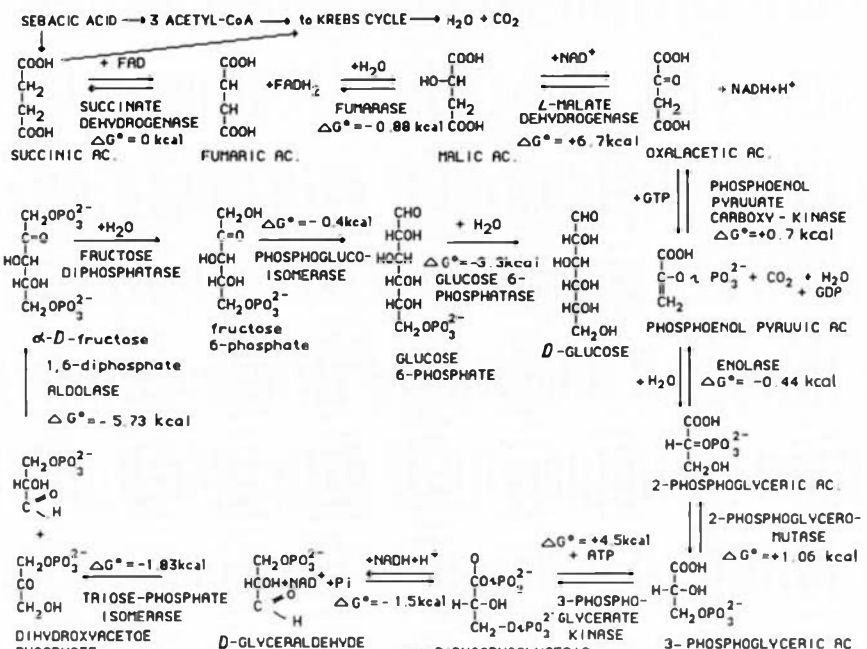


Fig. 7. Metabolic pattern of sebacic acid. The figure shows that sebacic acid can be completely oxidized to H_2O and CO_2 in the Krebs cycle through acetyl-CoA and succinyl-CoA formation as intermediate metabolites; the latter can also be used for gluconeogenesis.

20% will be excreted in the urine, thus 4 g/h of sebacate would be available for energy requirements. In this way, the caloric equivalent for sebacate being equal to 6.643 kcal/g, this gives 26.57 kcal/h, corresponding to 637.73 kcal/24 h. This could be a good energy supply for the organism if it is given together with glucose solutions.

Sebacate infusion does not appear to influence the serum levels of either insulin or glucagon. This suggests that sebacate metabolism is independent of these hormones which, having mainly opposite actions, control the balance between glucose and lipid metabolism. On the contrary, an increase in serum insulin concentration following the ingestion of both LCT and MCT has been extensively reported [16–19]. MCT stimulate a higher insulin release than LCT, and this effect has been deemed responsible for the suppression of lipolysis after MCT intake.

These differences could be due partly to the fact that sebacate was infused intravenously while generally, in the literature concerning both LCT and MCT, these lipids were ingested with a meal. In addition, sebacate is metabolized in a different way to LCT or MCT and could produce lower obligatory thermogenesis than has been theoretically estimated (fig. 7).

The independence of sebacate metabolism from glucagon and insulin hormones could be very useful in some particular clinical conditions such as ketosis in diabetes mellitus and starvation, and in the last stages of sepsis, in which the metabolism of both glucose and lipids is impaired. In these clinical conditions ketosis is a frequent phenomenon. Ketotic episodes are typically characterized by a relative lack of intracellular glucose, coupled with an accelerated gluconeogenesis which generally starts from gluconeogenetic amino acids and from lactate and pyruvate in Cori's cycle. Since sebacate is a potentially gluconeogenetic substrate (via the formation of succinate), its use in the above diseases could be most useful. In fact, metabolites which take part in the citric acid cycle (oxaloacetate, succinate, malate) have a reductive effect on ketoses induced by butyric acid [20]. Other authors also have shown that dicarboxylic acids reduce diabetic ketosis [21, 22].

No significant change in either acetoacetate or β-hydroxybutyrate concentrations during or after sebacate infusion was observed, in contrast to reports made about MCT. This indicates that sebacate, contrary to MCT or LCT which give high amounts of acetyl-CoA which cannot be further oxidized, thus originating ketone bodies, is completely metabolized in the cells either through the oxidative pathway or through gluconeogenesis and successive oxidation.

Although sebacate represents a fuel source which is immediately available as MCT, due to the different metabolic fate sebacate could be more useful for long-term therapy. In fact, it has been demonstrated [17, 19] that replacing LCT with MCT over long periods of time produces weight loss without any restriction of energy intake, due to the significantly greater thermic response to meals containing MCT.

Appendix

Using Flatt's procedure [23] to compute the specific dynamic action (SDA) of foods and assuming an energy expenditure of 20 kcal per ATP required for the absorption, transport and storage of glucose, we estimated the value of SDA of sebacate (expressed as a percentage) to be 2.91 % in the case of complete oxidation.

The caloric expenditure per mole of ATP formed is based on the heat of combustion of 1 mol of substrate divided by the total number of moles of ATP generated in its oxidation; thus, the energy released from 1 mol of sebacate per ATP made is:

$$\text{sebacate} \rightarrow 10\,CO_2: \frac{1{,}374\ \text{kcal}}{72} = 18.82\ \text{kcal/mol}.$$

In fact, the complete oxidation of 1 mol of sebacate to CO_2 and H_2O gives a gain of 70 ATP, 2 energy-high phosphate bonds being necessary to activate sebacic acid before starting its oxidation.

The energy released per ATP gained is therefore:

$$\frac{1{,}374\ \text{kcal}}{70\ \text{ATP}} = 19.63\ \text{kcal/mol}.$$

These ratios are quite similar to the average ones of glucose and free fatty acids as calculated by Flatt [23] and reported as follows:

energy released per ATP made:

$$\text{glucose} \rightarrow 6\,CO_2: \frac{637\ \text{kcal}}{38\ \text{ATP}} = 17.7\ \text{kcal/mol};$$

$$\text{palmitate} \rightarrow 16\,CO_2: \frac{2{,}398\ \text{kcal}}{131} = 18.3\ \text{kcal/mol},$$

and

$$\text{protein} \rightarrow CO_2 + (NH_3): \frac{475\ \text{kcal}}{28.8} = 16.5\ \text{kcal/mol}.$$

In the hypothesis that sebacate is being administered in such clinical conditions where a lack of intracellular glucose is present, and is therefore utilized for glycogen synthesis, we have calculated the stoichiometry of sebacate oxidation through glycogen formation as an intermediate step.

It should be emphasized that the above computations are only valid when sebacate β-oxidation takes place within the mitochondrion. During peroxisomal sebacate oxidation the ATP yield is only 61 (the first

step in β-oxidation being uncoupled with ATP formation and the second step yielding 2 ATP if the glycerophosphate shuttle is involved in the transport of H into mitochondria). However, the contribution of peroxisomal oxidation to total sebacate disposal ought to be low since the thermic effect, computed by indirect calorimetry, was negligible.

$$2 \text{ sebacyl-CoA} + 18\ O_2 \rightarrow 2 \text{ succinyl-CoA} + 12\ CO_2 + 12\ H_2O$$

$$2 \text{ succinate} + FAD + GTP + H_2O \rightarrow \text{glucose} + FADH_2 + GDP + Pi + 2\ CO_2$$

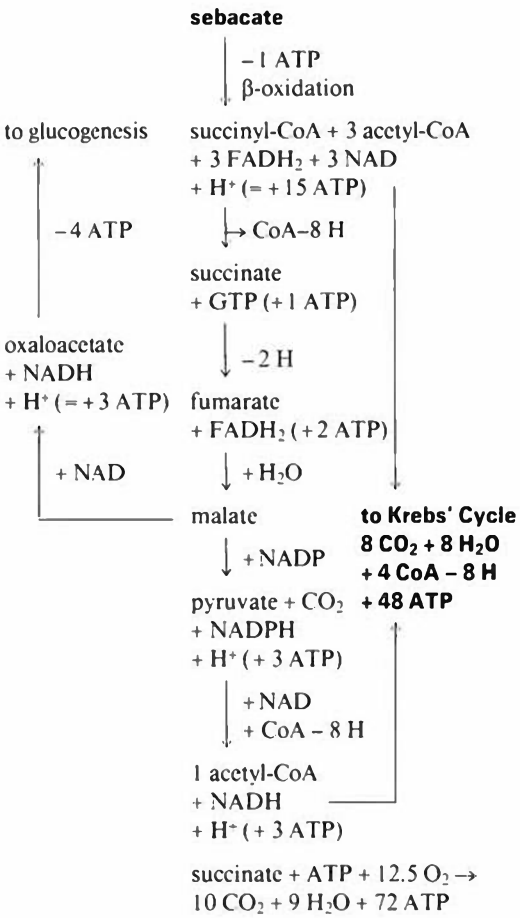
$$2\ HOOC\text{-}(CH_2)_8\text{-}COOH + 18\ O_2 \rightarrow C_6H_{12}O_6 + 14\ CO_2 + 10\ H_2O + H_2 + H_2$$

$$RQ = \frac{14}{18} = 0.77$$

2 molecules of succinate are necessary to synthesize 1 molecule of glucose 6-phosphate. The number of ATP gained during glucose 6-phosphate formation from 2 molecules of sebacate via the gluconeogenesis pathway and successive complete oxidation via the Cori and Krebs cycles equals 79, if we consider that 2 ATPs are consumed in both the first step of β-oxidation and during gluconeogenesis. Since glucose 6-phosphate is synthesized from oxaloacetate, no further energy expenditure is made, contrary to the case of glycolysis from glucose, which must be activated to glucose 6-phosphate to start oxidation.

Since 3 acetyl-CoA are formed from 1 sebacyl-CoA during β-oxidation, the further complete oxidation of these acetyl-CoA in the tricarboxylic acid cycle produces 48 ATP.

Thus, in total, from 2 sebacyl-CoA, 6 acetyl-CoA and 1 glucose 6-phosphate are formed, giving a net gain of 173 ATP which corresponds to 86.5 ATP for each molecule of sebacate.



Using the information summarized above it is possible to compare the amounts of 'ATP gained' (i.e. 'ATP made' – 'ATP used') when sebacate is directly oxidized or stored first and utilized later. The ratio 'ATP gained' to 'ATP made' is 0.986% when sebacate is first converted to glucose 6-phosphate or glycogen.

References

1 Mingrone G, Tacchino R, Greco AV, Arcieri Mastromattei E, Marino F, Castagneto M: Preliminary studies of a 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, Arcieri Mastromattei E, Marino F, Finotti BS, Greco AV: Use of even numbered carbon atoms dicarboxylic salts in parenteral nutrition as fuel substrate. JPEN, in press.

4 Mingrone G, Greco AV, Bertuzzi A, Finotti E, Castagneto M: Tissue uptake and oxidation of disodium sebacate in man. JPEN, in press.

5 Greco AV, Mingrone G, Arcieri Mastromattei E, Finotti E, Castagneto M: Toxicity of sebacic acid. Drugs Exp Clin Res, in press.

6 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.

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

8 Williamson DH, Mellanby J, Krebs HA: Enzymatic determination of *D*(−)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J 1962;82:90–96.

9 Herbert V, Lav KS, Gottlieb GW, Bleicher SJ: Coated-charcoal immunoassay of insulin. J Clin Endocrinol Metab 1965;25:1375–1384.

10 Faloona G, Unger RH: Glucagon; in Jaffe B, Behrman H (eds): Methods of Hormone Radioimmunoassay. New York, Academic Press, 1974, pp 317–330.

11 Bertuzzi A, Gandolfi A, Salinari S, Mingrone G, Arcieri Mastromattei E, Finotti E, Greco AV: Pharmacokinetic analysis of azelaic acid disodium salt, a proposed substrate for total parenteral nutrition. Clin Pharmacokinet, in press.

12 Ullrich KJ, Fasold H, Rumrich G, Kloss S: Secretion and contraluminal uptake of dicarboxylic acids in the proximal convolution of rat kidney. Pflügers Arch 1984;400:241–249.

13 Ullrich KJ, Rumrich G, Fritzsch G, Kloss S: Contraluminal para-aminohippurate (PAH) transport in the proximal tubule of the rat kidney. II. Specificity: Aliphatic dicarboxylic acids. Pflügers Arch 1987;408:38–45.

14 Bishoff KB: Some fundamental considerations of the applications of pharmacokinetics to cancer chemotherapy. Cancer Chemother Rep 1975;59:777–793.

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

16 Flatt JP, Ravussin E, Acheson KJ, Jequier E: Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J Clin Invest 1989;76:1019–1024.

17 Contaldo F, Scalfi L, Coltorti A: Thermogenic effect of dietary medium chain triglycerides in man; in Crepaldi G, et al (eds): Diabetes, Obesity and Hyperlipidemias. New York, Elsevier, 1985, pp 209–216.

18 Seaton TB, Welle SL, Warenko MK, Campbell RG: Thermic effect of medium-chain and long-chain triglycerides in man. Am J Clin Nutr 1986;44:630–634.

19 Hill JO, Peters JC, Yang D, Sharp T, Kaler M, Abmurad NN, Greene HL: Thermogenesis in humans during overfeeding with medium-chain triglycerides. Metabolism 1989;38: 641–648.

20 Beatty CH, West ES: Effect of succinic and malic acids and fructose on ketosis in alloxan-diabetic rats. J Biol Chem 1955;25:661–668.

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

22 Mortensen PB: Urinary excretion of C4-C10 dicarboxylic acids and antiketogenic properties of adipic acid in ketogenic-stimulated rats due to diabetes, long-chain and short-chain monocarboxylic acids. Biochim Biophys Acta 1981;664:335–348.

23 Flatt JP: The biochemistry of energy expenditure; in Bray GA (ed): Recent Advances in Obesity Research II. Proc 2nd Int Congr on Obesity, Washington, Newan Publ, 1977, pp 211–228.