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Comparison Between Dodecanedioic Acid and Long-Chain Triglycerides as an Energy Source in Liquid Formula Diets

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ABSTRACT. *Background:* Dicarboxylic acids (DA) are water-soluble substances with high-energy density proposed as an alternative lipid substrate for nutrition purposes. The aim of the present study was to investigate the interaction between glucose and DA or long-chain triglyceride (LCT) metabolism after oral administration. *Methods:* Two test meals containing either dodecanedioic acid (C12, the 12-atom DA) or LCT, together with glucose and amino acids, were each administered to five healthy volunteers. Tracer amounts of ^{14}C-dodecanedioic acid were added to the C12 meal to recover expired traced CO_2 and estimate the minimum rate of C12 oxidation. Glucose, insulin, and C12 plasma levels were measured for 360 minutes after the test meal. Indirect calorimetry was performed for the duration of the study. *Results:* LCTs proved ineffective in promoting their own oxidation after oral administration. On the contrary, C12 was promptly oxidized, a minimum of 21.9% ± 8.3% of the administered amount giving rise to the recovered expired CO_2. This difference in metabolic fate was reflected in a sparing effect on glucose: suprabasal respiratory quotient and suprabasal carbohydrate oxidation were significantly ($p < .05$) lower under C12 administration than under LCT administration, with a difference of 0.024 ± 0.015 in respiratory quotient (RQ) and a difference of 0.791 ± 0.197 kJ/min in carbohydrate oxidation. In particular, carbohydrate oxidation increased by 54% over basal with LCT but only by 28% with C12 administration. RQ increased over basal by 5.8% with LCT but only by 3.0% with C12 administration. *Conclusions:* These results show a fundamental metabolic difference between conventional lipids and DAs, which is the basis for a possible role of DAs in clinical nutrition. The fate of spared glucose is likely to be storage in glycogen form when dodecanedioic acid is made available as an energy source. (*Journal of Parenteral and Enteral Nutrition* **23**:80–84, 1999)

Dicarboxylic acids (DA) with 9 to 12 carbon atoms have been studied as possible alternate fuel substrates in parenteral nutrition.[1–15] This group of substances shares the characteristic of being water soluble because of their two carboxylic ends. The urinary loss of DA is variable, proportionally increasing with the decrease of their chain length: azelaic acid (C9) is actively secreted by the renal tubules;[7] sebacic acid (C10) and, especially, dodecanedioic acid (C12) are actively reabsorbed.[3,9,12] The β-oxidation of even-numbered DA leads to the formation of acetyl-coenzyme A (CoA) and succinyl-CoA, with complete oxidation in the Krebs cycle; malonyl-CoA, the end product of C9 oxidation, can only be diverted to fatty acid synthesis.

Using the euglycemic–hyperinsulinemic clamp technique, we recently showed that both sebacate and dodecanedioic acids act as glucose-sparing substrates.[8,14,15] Many investigations in experimental animals[16–19] also have shown that DAs possess antiketogenetic properties in the ketosis induced by starvation and diabetes mellitus. Both glucose-sparing and antiketogenetic effects probably derive from the prompt metabolic use of DA, which yield acetyl-CoA and Krebs' cycle intermediates. Therefore, even-numbered DA might be considered as useful energy substrates in some clinical conditions in which amino acids are used as gluconeogenetic substrate, with potentially wasting effects on lean body mass, or in which ketosis is likely to occur. Such conditions include decompensated diabetes mellitus, starving (in postoperative or elderly patients, and in anorexia nervosa), and sepsis.

No data from the literature are known to us regarding the comparison between dodecanedioic acid and long-chain triglycerides (LCTs) as energy substrates. The aim of the present investigation was to study the difference between C12 and LCTs in their effect on glucose metabolism. We therefore administered either a standard liquid meal containing LCTs or a similar meal containing C12 to healthy volunteers and compared the corresponding rates of substrate oxidation.

MATERIALS AND METHODS

Subjects

Five healthy male volunteers ages 50.4 ± 13.8 years were admitted to the study. Their mean weight was 69.7 ± 3.9 kg, and their height was 170.4 ± 3.6 cm (body mass index, 24.1 ± 2.1 [kg/m^2]). The subjects'

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weight had remained constant (±2 kg) for at least 1 year before starting the study. All subjects studied were on a free diet and were not trained for regular physical activity; none were smokers.

Body weight was measured to the nearest 0.1 kg by a beam scale. Bioelectric impedance measurements were performed with the subject lying quietly in the bed, using frequencies from 1 to 100 kHz between a set of four electrodes attached to the dorsum of the hand and the foot (Human-IM Scan; Dietosystem, Milan, Italy). The proximal hand electrode was placed in line with the ulnar tubercle, and the proximal foot electrode was in line with the medial malleolus. The distal electrodes were placed 4 cm distal to the respective proximal ones.[21] Three measurements were obtained, and their average value was used to obtain an estimate of total body water (TBW). Fat-free mass (FFM) was then obtained by dividing TBW by 0.73,[22] and fat mass (FM) was obtained by subtracting FFM from body weight. FFM and FM were 57.6 ± 2.6 and 12.3 ± 2.1 kg, respectively, in the studied subjects.

Experimental Protocol

Each subject received two test meals in randomized order: either a liquid formula diet containing LCTs (as soybean oil emulsion) or the same liquid formula diet in which the sodium salt of C12 was substituted for LCTs. Indirect calorimetry was performed and expired traced CO_2 from administered traced C12 and blood samples were collected throughout the experiments.

The subjects were admitted to the Metabolic Division of the Catholic University School of Medicine, Rome, Italy, 1 day before each test meal, at 7:00 AM. During the day spent in the metabolic ward, all subjects were assigned a diet with an energy content computed on the basis of their alimentary diary. Such a diet tried to reproduce the average food composition for each subject. The metabolic contents (carbohydrates, lipid, and protein) of all foodstuff items were derived from commercially available tables (Food Processor II; Hesha Research, Salem, OR) modified according to the food tables of the Istituto Nazionale di Nutrizione, Rome, Italy.

All subjects received the two test meals 1 week apart. All the experiments were performed after an overnight fast (10 to 12 hours) at 8:00 AM. After voiding, the subjects were placed in bed, and a venous catheter was inserted into an antecubital vein for blood sampling. The line was kept patent with physiologic saline. To measure the resting energy expenditure, respiratory gas exchange measurements were performed over 45 minutes using a ventilated hood metabolic monitor (Deltatrac; Datex Instrumentarium, Helsinki, Finland). Indirect calorimetry was continued until the 360th minute after the ingestion (in <3 minutes) of the test meals.

For each subject, the caloric amount administered was 12 kcal/kg body wt. The liquid formula test meals were prepared in the laboratory, and their composition in calories was as follows: 53.3% glucose; 30% soybean oil emulsion in soybean lecithine, or C12 as disodium salt; and 16.7% amino acids (L-aspartic acid 9.01 mmol/L, L-threonine 25.18 mmol/L, L-serine 14.27 mmol/L, L-glutamic acid 5.10 mmol/L, L-proline 13.03 mmol/L, glycine 169.84 mmol/L, L-alanine 16.84, L-valine 41.01 mmol/L, L-methionine 44.23 mmol/L, L-isoleucine 32.02 mmol/L, L-leucine 50.32, L-tyrosine 2.48 mmol/L, L-phenylalanine 39.95 mmol/L, L-tryptophan 7.35 mmol/L, L-lysine 32.83 mmol/L, L-histidine 43.50 mmol/L, and L-arginine 60.27 mmol/L).

Blood samples were drawn at 0, 10, 20, 30, 40, 60, 80, 90, 120, 150, 160, 180, 200, 240, 260, 270, 280, 300, 330, and 360 minutes from the beginning of the study. On samples at 0, 10, 20, 30, 40, 60, 80, 120, 160, 200, 240, 260, 280, and 360 minutes, C12 was determined. On samples at time 0 and every half hour afterwards, plasma glucose and insulin were determined. Plasma glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instrument, Fullerton, CA). Plasma insulin was measured by microparticle enzyme immunoassay (Abbott Imx, Pasadena, CA). C12 was quantitatively determined by high-performance liquid chromatography.[13]

Respiratory gas exchange was measured by an open-circuit ventilated-hood system (monitor MBM-100; Deltatrac, Datex Instrumentarium Corporation, Helsinki, Finland). Energy expenditure, respiratory quotient (RQ), and substrate oxidation rates were calculated from the oxygen consumption, the carbon dioxide production, and the nitrogen urinary excretion according to Ferrannini.[23] Respiratory gas exchange measurements were started 45 minutes before the beginning of the study to measure the resting energy expenditure and continued for 360 minutes after the test meal ingestion.

During the administration of C12, the minimum rate of C12 oxidation was computed from the observed elimination of labeled CO_2; $\dot{V}O_2$ and $\dot{V}CO_2$ caused by C12 oxidation were subtracted from overall $\dot{V}O_2$ and $\dot{V}CO_2$, and the residual $\dot{V}O_2$ and $\dot{V}CO_2$ were used to compute carbohydrate and lipid consumption rates according to the usual metabolic formulas.[23]

Diet-induced thermogenesis was determined as the cumulative postmeal increase in energy expenditure above premeal basal values during the 360-minute measurement period: the area under the curve was computed using the trapezoidal rule.

Urine was collected for each subject at the end of the experimental session and from 360 minutes to 24 hours after the test meal. Nitrogen elimination was determined by a blood urea nitrogen Analyzer II (Beckman Instruments, Fullerton, CA). C12 urinary excretion also was measured.

Labeled C12 Administration and $^{14}CO_2$ Collection

Two hundred microcuries of [1–12-^{14}C]dodecanedioic acid under sodium salt form (specific activity 117 mCi/mmol; Amersham, Amersham Buckinghamshire, United Kingdom) were administered together with the liquid diet containing C12.

Expired air was collected during 2-minute periods at intervals of 10 to 30 minutes for a period of 360 minutes after starting the labeled dodecanedioic infusion by using a 20-L Douglas bag. Because ethanol solutions

of methyl-benzetonium hydroxide (MH) are not yet commercially available, methyl-benzetonium chloride (MC) from Sigma Chemical Co (St Louis, MO) was used. Equal parts of a 1 mol/L solution of MC in ethanol and of a mol/L NaOH ethanol solution were mixed at 45°C for 20 minutes. Then, the solution was filtered to eliminate the NaCl formed, and 0.1% phenophtaleine was added as a pH indicator to the moles per liter MH ethanolic solution obtained. Aliquots of 3 ml each of this solution were put into graduated tubes and titrated with 0.15 N HCl. Using this procedure, solutions containing 3 mEq of MH were obtained: these solutions are capable of trapping exactly 3 mmol of CO_2. The MH solution trapping $^{14}CO_2$ was added with 10 mL of scintillation solution.

Ethics

The study protocol followed the guidelines of the Catholic University Ethical Committee, and all subjects gave their informed consent before the enrollment.

Statistics

Results are given as means ± SD, unless specifically stated otherwise. Given the small number of subjects, a Wilcoxon signed-rank nonparametric test was used to compare metabolic indices across experimental maneuvers in the same subjects. p values <.05 level were considered significant. A repeated-measures analysis of variance (ANOVA), considering both time and substrate (C12 *vs* LCT) as within-subjects factors, was performed to check for differences in glucose and insulin curves.

RESULTS

The average time course of plasma C12 levels is depicted in Figure 1. C12 peaked at approximately 120 minutes after the test meal with mean values of 825.7 ± 109.5 mmol/L and then declined, reaching 372.6 ± 80.17 mmol/L at 240 minutes.

The average excretion rate of labeled $\dot{V}CO_2$ is shown in Figure 2. The maximum $\dot{V}^{14}CO_2$ value

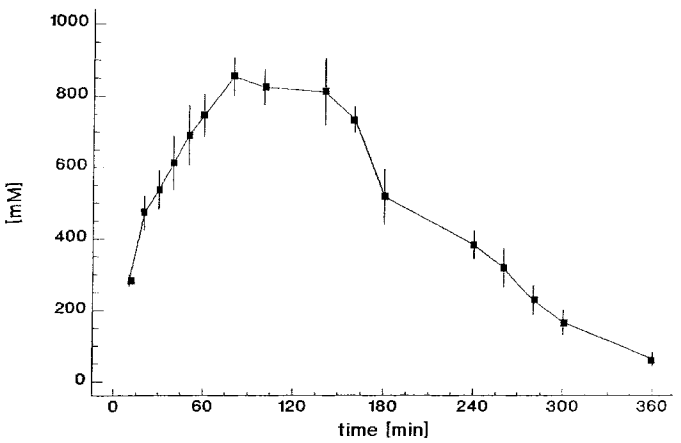

FIG. 1. Average time-course of plasma dodecanedioic acid. Values are given as means ± SD.

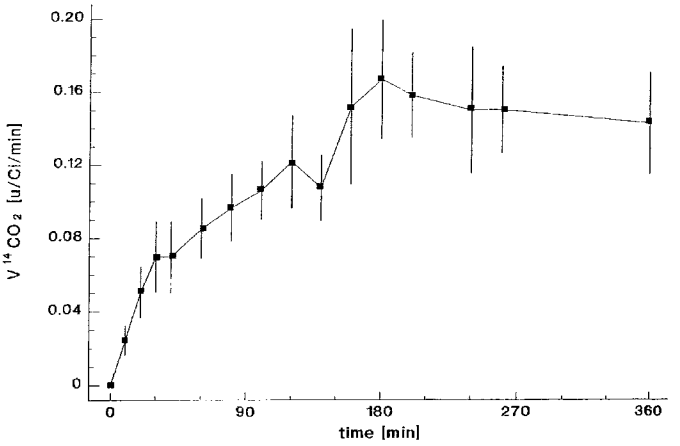

FIG. 2. Average time-course of labeled expired CO_2 after traced dodecanedioic acid administration. Values are given as means ± SD.

(0.167 ± 0.073 μCi/min) was reached 180 minutes after the test meal ingestion; after 360 minutes the $^{14}CO_2$ level was still high (0.136 ± 0.062 μCi/min). The average minimum oxidation of dodecanedioic acid within the first 360 minutes of observation was 33.2 ± 12.9 mmol, corresponding to 21.9% ± 8.3% of the administered C12.

Urinary nitrogen excretion over 24 hours was 12.2 ± 1.52 g/L and with C12 12.3 ± 2.06 g/L (p = NS). Twenty-four-hour urinary C12 loss was 6.3% ± 1.4% of the administered amount. Table I reports the metabolic variables measured in the two sessions.

The energy intake with the test meal, containing either LCT or C12, was 3496 ± 194 kJ. The differences observed between LCT and C12 studies in either total suprabasal energy expenditure or in suprabasal lipid oxidation were not significant (−0.17 ± 0.25 and −1.33 ± 0.53 kJ/min, respectively). On the other hand,

TABLE I
Metabolic variables measured under administration of the two liquid formulas

	LCT	C12
Basal CHO oxidation (kJ/min)	1.09 ± 0.54	0.88 ± 0.62
Average postmeal CHO oxidation (kJ/min)	2.31 ± 0.52	1.31 ± 0.76*
Suprabasal CHO oxidation (kJ/min)	1.22 ± 0.31	0.43 ± 0.32*
Basal lipid oxidation (kJ/min)	2.60 ± 0.76	2.79 ± 0.50
Average postmeal lipid oxidation (kJ/min)	2.16 ± 0.71	2.34 ± 0.64
Suprabasal lipid oxidation (kJ/min)	−0.44 ± 0.29	−0.45 ± 0.40
Average postmeal C12 oxidation (kJ/min)		0.64 ± 0.25
Basal energy expenditure (kJ/min)	4.74 ± 0.29	4.74 ± 0.40
Average postmeal energy expenditure (kJ/min)	5.49 ± 0.38	5.33 ± 0.42
Suprabasal energy expenditure (kJ/min)	0.75 ± 0.11	0.59 ± 0.17
Basal RQ	0.80 ± 0.03	0.78 ± 0.03
Average postmeal RQ	0.85 ± 0.03	0.81 ± 0.04

Values are means ± SD. LCT, long-chain triglycerides; C12, dodecanedioic acid; CHO, carbohydrates; RQ, respiratory quotient.
* p < .05 *vs* LCT administration.

both suprabasal RQ and suprabasal carbohydrate oxidation were significantly ($p < .05$) lower under C12 administration than under LCT administration, with a difference of 0.024 ± 0.015 in RQ and a difference of 0.791 ± 0.197 kJ/min in carbohydrate oxidation. In particular, carbohydrate oxidation increased by 54% over basal with LCT but only by 28% with C12 administration. RQ increased over basal by 5.8% with LCT but only by 3.0% with C12 administration.

No significant differences in plasma insulin concentrations between LCT and C12 studies were detected, either basally or during the study period. A borderline significant difference ($p = .042$) in the average glycemia was detected, with LCT studies lower than C12 studies (5.57 ± 10.31 *vs* 5.63 ± 10.29 mmol/L).

DISCUSSION

The present study shows a high oxidation rate of dodecanedioic acid after an oral load, similar to what was observed previously using the IV route.[13] In spite of the long observation period (360 minutes), the values of labeled $\dot{V}CO_2$ remained high until the end of the experiment. It was then impossible to establish the type of decay in the elimination of labeled CO_2. The value of the area under the curve reported in the Results section is that obtained during the study period but it is likely that in the following hours an additional considerable amount of C12 was oxidized.

It is well-known that with the simultaneous administration of lipids and carbohydrates within a balanced meal, the utilization rate of glucose substantially increases, whereas that of lipids does not change. The main result of the present work is that in the same circumstances, the administration of C12 instead of traditional LCT induces a much lower increase in the glucose oxidation rate, paralleled by a maintained overall energy expenditure increase, which is sustained by oxidation of C12 itself. The increase of glucose oxidation under C12 administration was, in fact, less than half that observed under LCT administration; further, the oxidation of C12 was approximately equal in caloric amount to the difference in increase of glucose oxidation between the two meals. We may, therefore, argue that, contrary to traditional lipids, C12 is promptly available for energy production, allowing a considerable sparing of glucose, which would otherwise be uniquely used as immediately available substrate.

Flatt et al[24] showed in normal subjects that the fat content of a mixed meal does not influence either the glycemic or the insulinemic postprandial responses. Furthermore, the time course of the changes in the respiratory quotient after a meal is not affected by its fat content, suggesting that the mixture of nutrients oxidized after the meal is independent of the amount of fat intake. In another study,[25] in which the fat oxidation rate was measured over 24 hours in a respiratory chamber, the addition of 106 ± 6 g of fat to a mixed maintenance diet failed to promote the use of fat as a metabolic fuel. In this study, the authors concluded that the whole fat supplement was stored because there was no evidence of fat malabsorption.

The same lack of response in the oxidation of lipids to an LCT challenge was observed in the present study: in contrast, C12 was promptly and substantially oxidized after oral administration. This result underscores a fundamental metabolic difference between conventional lipids and DA, which forms the basis for a possible role of DA in clinical nutrition. It is to this difference that the glucose-sparing effect should probably be ascribed. The sparing effect of C12 on glucose can have an important entail especially in pathologic states such as diabetes mellitus and obesity. In fact, it is generally agreed that the resistance to glucose storage and, to a lesser extent, to glucose oxidation is the cause of increased plasma glucose and insulin concentrations that compensate for the impairment of glucose storage and oxidation.[26] The contention of a limited glycogen storage capacity, with its effect on glycogen synthase and phosphorylase activities, explains the resistance to glucose storage observed in obesity and its consequences on the impairment of glucose tolerance and insulin resistance.[27]

The glucose and insulin plasma concentration curves over time did not show substantial differences in the two sessions: maximum pointwise average differences were 0.176 mmol/L for glucose and 12.6 pmol/L for insulin. Although the application of a repeated-measures ANOVA yielded a .042 p level for the average difference in glucose (the difference for insulin being nonsignificant), the magnitude of such difference is small. It is, therefore, reasonable to suppose that glucose absorption and tissue uptake were essentially similar in the two experimental conditions. As a consequence and given the different degree of glucose oxidation, glucose ought to be stored in glycogen form when dodecanedioic acid is made available as an energy source. The fate of spared glucose should, in any case, be the object of further detailed investigations.

In conclusion, dodecanedioic acid also seems to be a promising lipid-alternate energy substrate for enteral feeding. It might be particularly useful in those physiologic and pathologic conditions in which glycogen stores are depleted, because it can be observed during sustained physical exercise, in decompensated diabetes mellitus, or in patients requiring prolonged enteral nutrition for chronic debilitating diseases.

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