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Dodecanedioic acid overcomes metabolic inflexibility in type 2 diabetic subjects

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Salinari, Serenella, Alessandro Bertuzzi, Alberto Gandolfi, Aldo V. Greco, Antonino Scarfone, Melania Manco, and Geltrude Mingrone. Dodecanedioic acid overcomes metabolic inflexibility in type 2 diabetic subjects. *Am J Physiol Endocrinol Metab* 291: E1051–E1058, 2006. First published June 20, 2006; doi:10.1152/ajpendo.00631.2005.—Metabolically healthy skeletal muscle possesses the ability to switch easily between glucose and fat oxidation in response to homeostatic signals. In type 2 diabetes mellitus and obesity, the skeletal muscle shows a great reduction in this metabolic flexibility. A substrate like dodecanedioic acid (C-12), able to increase skeletal muscle glycogen stores via succinyl-CoA formation, might both postpone the fatigue and increase fatty acid utilization, since it does not affect insulin secretion. In healthy volunteers and in type 2 diabetic subjects, the effect of an oral C-12 load was compared with a glucose or water load during prolonged, moderate-intensity, physical exercise. C-12 metabolism was analyzed by a mathematical model. After C-12, diabetics were able to complete the 2 h of exercise. Nonesterified fatty acids increased both during and after the exercise in the C-12 session. C-12 oxidation provided 14% of total energy expenditure, and the sum of C-12 plus lipids oxidized after the C-12 meal was significantly greater than lipids oxidized after the glucose meal ($P < 0.025$). The fraction of C-12 that entered the central compartment was 47% of that ingested. During the first phase of the exercise (~60 min), the mean C-12 clearance from the central compartment toward tissues was 2.57 and 1.30 l/min during the second phase of the exercise. In conclusion, C-12 seems to be a suitable energy substrate during exercise, since it reduces muscle fatigue, is rapidly oxidized, and does not stimulate insulin secretion, which implies that lipolysis is not inhibited as reported after glucose ingestion.

dodecanedioic acid; energy substrate; physical exercise; mathematical modeling

DODECANEDIOIC ACID (C-12), belonging to the family of straight-chain dicarboxylic acids, is completely oxidized to CO_2 and H_2O via formation of succinyl-CoA, a gluconeogenetic substrate that can be directly converted to glucose and activate the acetyl-CoA oxidation in the tricarboxylic acid cycle (30).

The intravenous infusion of C-12, under the form of sodium salt, determines a progressive and significant lowering of blood glucose levels in type 2 diabetic subjects compared with healthy volunteers matched by gender, age, and body mass index (17). Because plasma insulin levels were found to be unchanged, this finding might likely derive from a reduced hepatic glycogen breakdown. During C-12 infusion, lactate plasma concentration decreased, whereas pyruvate levels were increased only in diabetic patients (17). These results suggested that C-12 might represent a fuel substrate immediately available for tissue energy requirements, especially when glucose metabolism is impaired. Furthermore, C-12 infusion decreases glucose uptake and glucose oxidation during euglycemic hyperinsulinemic clamp, mainly through a mechanism of substrate competition. The subsequent conclusion (27) was that C-12 might be a useful alternative substrate in enteral or parenteral nutrition, sparing glucose utilization and increasing glycogen stores in those clinical conditions, like type 2 diabetes, where reduced insulin-induced glucose uptake and oxidation are observed.

Regular mild- to moderate-intensity exercise is regarded as one of the cornerstones in the management of type 2 diabetic patients (5). In part, this recommendation is aimed at improving insulin sensitivity (1, 6). Whole body uptake and oxidation of plasma nonesterified fatty acids (NEFA) is diminished by ~30% compared with controls during exercise in obese type 2 diabetic patients (5). Also, sedentary individuals show a reduced utilization of fat during exercise compared with trained subjects (1, 18, 25). Hyperglycemia is not usually rectified by exercise training in type 2 diabetics, although the contribution to energy expenditure from the net oxidation of muscle glycogen is reduced in diabetic compared with nondiabetic individuals (19).

Metabolically healthy skeletal muscle possesses the ability to switch easily between glucose and fat oxidation in response to homeostatic signals. In type 2 diabetes mellitus and obesity, skeletal muscle shows a great reduction in this metabolic flexibility (21). A substrate able to increase skeletal muscle glycogen stores might both postpone the development of fatigue and, should this substrate not affect insulin secretion, increase fatty acid utilization, resulting in weight loss over time.

In the hypothesis that C-12 might alter substrate utilization and improve exercise duration, we investigated the effect of an oral load of C-12 compared with a water and a glucose load during prolonged physical exercise in untrained type 2 diabetic subjects and in healthy volunteers. In this way, two different conditions with low glucose availability, low insulin circulating levels, and basal/high NEFA circulating levels, as obtained after both water and C-12 load, and a condition with high glucose availability with high insulin levels and low NEFA levels, were studied. Experimental data of C-12 disposition after an oral load have been analyzed according to a previously

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described mathematical model (9), suitably modified to take into account the oral C-12 administration and the occurrence of physical exercise.

MATERIALS AND METHODS

Subjects

Five healthy active male volunteers and five male type 2 diabetic subjects participated in this study. None of diabetic subjects was under hypoglycemic agents nor under insulin. The diabetes mellitus length was 5 ± 2 yr and glycated Hb was 8.5 ± 0.8%. Fat-free mass (FFM), body mass index, and age of the subjects were (means ± SD) 48.3 ± 7.2 kg, 26.1 ± 2.7 kg/m^2, and 46 ± 3.8 yr for controls and 52.9 ± 5.6 kg, 28.4 ± 4.3 kg/m^2, and 57.8 ± 7.0 yr for diabetic patients, respectively.

Chemicals

C-12 was purchased from Isotec (Miamisburg, OH). C-12 and azelaic acid were from Sigma (St. Louis, MO). C-12 was purified by Real (Como, Italy) and was free from pyrogens and contaminants with a degree of purification, ascertained using gas-liquid chromatography and mass spectrometry, of 99.8%.

Experimental Protocol

On three separate occasions, the subjects arrived at 7:30 A.M. at the laboratory after 12-h fast. The subjects remained at rest for 1 h before starting the exercise. During this period, indirect calorimetry was performed to measure the resting energy expenditure. After the start of the session (30 min), the subjects ingested 200 ml of water or a 200-ml formula meal containing 281 kcal from 75 g glucose and 100 kcal from 8.2 g proteins, 0.2 g lipids, and 15 g of sucrose or 287 kcal from 40 g C-12 and 100 kcal from 8.2 g proteins, 0.2 g lipids, and 15 g of sucrose, added with a fixed amount of 200 mg [1, 12]^{13}C-12.

The water and the meal were ingested in no more than 10 min. After the beginning of the session (60 min), a moderate exercise was performed on an electrically controlled cycle ergometer (ESAOTE ETC9) at 45 ± 5% $\dot{V}O_{2\,max}$, asking the subjects to endure 2 h unless fatigue appeared. Immediately after the exercise, the subjects reclined in bed up to 360 min from the beginning of the experiment for recovery. The order of trials was randomized, and they were separated by a minimum of 48 h. Muscle fatigue was assessed using the Borg's (10) scale.

Heparinized blood samples (4 ml) were taken to measure glucose, insulin, lactic acid, NEFA, and C-12. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Plasma insulin was assayed by microparticle enzyme immunoassay (MEIA; Abbott, Pasadena, CA) with a sensitivity of 1 μU/ml and an intra-assay coefficient of variation (CV) of 6.6%. Whole blood lactate levels were determined spectrophotometrically on an ERIS analyzer 6170 (Eppendorf Garatebau, Hamburg, Germany). Plasma NEFA were measured spectrophotometrically (Wako, Neuss, Germany).

Plasma samples were taken at −15, −10, and −5 min and every 15 min after the beginning of the ingestion of the loads until 360 min from the beginning of the experiments, immediately centrifuged, and frozen at −20°C until analysis. Each subject voided his bladder before starting the experimental protocol, and the subsequent 24-h urines were collected in a container with 0.1% sodium azide to prevent the bacterial growth. The protocol was in conformity with the directives given by the Ethics Committee of the Institutional Health Review Board of the Catholic University, School of Medicine, in Rome (Italy). Informed consent was obtained.

Indirect Calorimetry and Expired Radiolabeled CO_2 Collection

Indirect calorimetry was continuously performed by a ventilated hood apparatus (Deltatrack; Datex Instrumentarium, Helsinki, Finland) that automatically gives values of $\dot{V}O_2$, $\dot{V}CO_2$, respiratory quotient (RQ), and energy expenditure each minute. The measured values of $\dot{V}O_2$ and $\dot{V}CO_2$ were corrected to obtain the nonprotein values. Expired air was collected over 2-min periods at intervals of 15–30 min for a period of 330 min after the labeled isotopic dodecanedioic meal was started by using a 20-liter Douglas bag. Samples of the expired air were taken by Vacutainer test tubes (BreathMat model 252, Thermo; Finningan, Bremen, Germany). Urea nitrogen was analyzed in the urine collected during the study by BUN Analyzer (Beckman Instruments).

Oxidation Rate of C-12

$\dot{V}O_{2\,max}$ (58.4 ± 14.4 ml·kg^{-1}·min^{-1} for controls and 45.1 ± 8.5 ml·kg^{-1}·min^{-1} for diabetics) was determined 1 wk before the experiment using an incremental cycle protocol lasting ~30 min. $\dot{V}CO_2$ resulting from C-12 oxidation was calculated on the basis of the ^{13}C-to-^{12}C ratio according to the theoretical approach given in Ref. 11.

Dicarboxylic Acid Analysis

Plasma samples. Azelaic acid (100 μg) was added to 1 ml of each plasma sample as an internal standard. Proteins were precipitated with 0.1 ml of NH_4Cl, and dicarboxylic acids were extracted two times with 8 vol of ethyl acetate, maintaining the solutions at 60°C for 15 min. The combined extracts were dried in a GyroVap apparatus (Howe, model GV1; Gio. De Vita, Rome, Italy), operating at 60°C, coupled with a vacuum pump and a gas trap FTS System (Stone Ridge, NY).

Urine samples. Samples (0.5 ml) from 24-h urine were added with 50 μg azelaic acid as an internal standard and then treated with cation-exchange resin (Dowex 50 W-XA, 100–200 μm mesh, H^+) to remove salts, concentrated under reduced pressure, and filtered through a Millipore HV (0.45 μm) filter. The samples were acidified to pH 1–2 with 4 N HCl, extracted two times with ethyl acetate, and evaporated in the GyroVap as previously described.

HPLC analysis. The extracted solutes were dissolved in 0.5 ml acetonitrile-methanol (1:1 vol/vol) and added to 10 mg of *p*-bromophenacylbromide and 30 μl of *N*,*N*-diisopropylethylamine as catalyst. The mixture is heated to 60°C for 15 min. The derivatives are dissolved in a final volume of 1 ml of acetonitrile-methanol (1:1 vol/vol), and an aliquot of 10 μl was automatically injected in a liquid chromatograph (Hewlett-Packard 1050) with an HP3396A integrator and a scanning spectrophotometer operating in 190–600 nm wavelength range [light source: deuterium lamp, noise $<2.5 \times 10$–5 absorbance units (AU) peak-to-peak at 254 nm with 4 nm bandwidth, flowing water at 1 ml/min].

Dicarboxylic acid derivatives were separated on an LC-18, 4.6 mm ID, 25 cm length, 5 μm particle size reversed-phase column (Supelco, Bellefonte, PA). The HPLC conditions were as follows: solvent A bidistilled water/methanol (1:1 vol/vol), solvent B acetonitrile; after 15 min of isocratic elution with 15% acetonitrile, a gradient elution was performed from 15 to 100% of B in 80 min. The flow rate was 1 ml/min, UV detector operating at 255 nm, chart speed 0.2 cm/min, range of absorbance from −0.300 to 1.000 AU.

Equilibrium dialysis. Binding of C-12 to normal human plasma proteins was determined by equilibrium dialysis according to Ashbrook et al. (3) as previously described for sebacic acid (7, 26).

Indirect calorimetry equations. Glucose and lipid oxidation rates were derived according to formulas reported in Ref. 15 for a nonprotein RQ.

Water and glucose meal

$$\dot{G} = 4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2$$
$$\dot{L} = 1.67 \times (\dot{V}O_2 - \dot{V}CO_2), \quad (1)$$

where $\dot{G}$ and $\dot{L}$ are the amount of glucose and lipids oxidized per minute (mg/min) and $\dot{V}O_2$ and $\dot{V}CO_2$ are the measured O_2 consumption rate and CO_2 production rate (ml/min), respectively.

C-12 meal (see APPENDIX)

$$\dot{G} = 4.55 \times \dot{V}_{CO_2} - 3.21 \times \dot{V}_{O_2} - 0.32 \times \dot{V}_{CO_{2C\text{-}12}}$$
$$\dot{L} = 1.67 \times (\dot{V}_{O_2} - \dot{V}_{CO_2}) - 0.49 \times \dot{V}_{CO_{2C\text{-}12}}, \quad (2)$$

where $\dot{V}_{CO_{2C\text{-}12}}$ is the CO_2 production rate resulting from C-12 oxidation.

Mathematical Model

The kinetics of C-12 were described by a one-compartment model with two routes of elimination: renal excretion and tissue uptake (8, 9). The input represents the intestinal absorption of C-12 decreased by the hepatic uptake. Because C-12 binds to albumin, the total C-12 concentration in the compartment (c_t) was represented as the sum of the free concentration (c_f) plus a bound concentration. On the basis of previous results, the latter term was described by assuming one class of independent and equivalent binding sites (9) giving

$$c_t = c_f + aKc_f/(1 + Kc_f), \quad (3)$$

where a is the concentration of protein binding sites and K is the association constant.

The renal excretion of C-12 was assumed to be linearly dependent on the concentration of free C-12, with an apparent renal clearance ρ (l/min). The rate of tissue uptake was assumed to be a linear function of free C-12 concentration with a tissue uptake coefficient γ (l/min).

Thus the kinetics of total C-12 concentration are described by the equation

$$V dc_t/dt = -\rho c_f - \gamma c_f + fI(t) \quad c_t(0) = 0 \quad (4)$$

where V is the distribution volume (liters), $fI(t)$ (mmol/min) is the C-12 entry in the compartment, and $t = 0$ is the time of the meal ingestion. Because C-12 was administered by an oral meal, the function $I(t)$ was assumed proportional to a third-order gamma function to take into account the dynamics of intestinal absorption:

$$I(t) = \frac{Dt^3}{6\tau_g^4} e^{-t/\tau_g} \quad (5)$$

where D is the administered dose (mmol) and τ_g is the time constant of the gamma function. Because a part of the administered dose could be stored in the liver, we considered that only a fraction f of $I(t)$ was the actual input to plasma. Assuming the equilibrium between free and bound C-12 to be instantaneous, we obtain:

$$V dc_f/dt = [-\rho c_f - \gamma c_f + fI(t)]/[1 + aK/(1 + Kc_f)^2] \quad c_f(0) = 0 \quad (6)$$

The outputs of the model were the total C-12 concentration measured in plasma

$$y_1(t) = c_t(t) \quad (7)$$

and the measured CO_2 expiration rate. We assumed that the production of CO_2 resulting from C-12 oxidation, as well as its transport and excretion in the expired air, can be represented by a fast pathway in which the C-12 taken up by tissues is instantaneously transformed into CO_2 and excreted in the expired air and, by a slow pathway, acting in parallel to the fast pathway, modeled by a first-order kinetics (9). Thus denoting by $y_2(t)$ the measured CO_2 expiration rate, in mmol/min, we have:

$$y_2(t) = \alpha' u^*(t) + \left[\alpha \int_0^t e^{-(t-s)/\tau} u^*(s) ds\right]/\tau \quad (8)$$

where $u^*(t) = 12\gamma c_f$ and α and α′ are the fractions of C-12 taken up by tissues conveyed by the slow and the fast pathway, respectively. Because the oxidation may be incomplete, causing a part of C-12 to be retained in the body as C-12 or other compounds oxidizable later than the horizon of the experiment, it will be $\alpha' + \alpha \leq 1$.

A diagram of C-12 kinetics and oxidation is shown in Fig. 1. To account for the effects of the exercise, the parameters γ, α, and α′ are assumed to be time dependent, as detailed in the following section.

Parameter Estimation

The model of C-12 kinetics and disposition, after an oral load and at rest, presents the following unknown parameters: V, a, K, f, ρ, γ, τ_g, α, α′, and τ. For all subjects, we assumed the value of $K = 6.4 \times 10^3\ M^{-1}$ and $a = 0.86 \times 10^{-3}$ M, these values being the mean values previously estimated in a group of healthy subjects (8, 9). Moreover, we computed the value of the parameter ρ for each subject as the ratio between the measured value of the C-12 excreted in the 24-h urine and the area under the curve of the plasma concentration of free C-12. The area under the curve was calculated using *Eq. 3* to calculate the free C-12 concentration from the measured C-12 concentration and extrapolating the data, for $t > 360$ min (end of the experiment), by an exponential function. The identifiability of the model with respect to parameters (V, f, γ, τ_g, α, α′, and τ) was verified by the similarity transformation method (33).

To take into account the influence of the physical exercise on the parameter values, we subdivided the time interval after the meal into the following three phases: rest (0–30 min, assuming 0 as the start of the meal), exercise (30–150 min), and recovery (150–330 min). Because it seems reasonable that the exercise mainly affects the values of the parameters related to C-12 tissue uptake and oxidation, for γ, α, and α′ we considered different values in the three intervals. In particular, in the time period preceding the exercise, we set these parameters to "rest values" equal to the values estimated in Ref. 9. The rest value was also assigned to the parameters α and α′ during the recovery period, whereas the values during the exercise were denoted as α_e and α'_e, respectively. In addition, during the exercise, we considered two possibly different values for γ (γ_1 and γ_2) to take into account some oscillations observable in the experimental data in this phase. At the end of the exercise, we hypothesized that γ decays exponentially to its resting value with a time constant τ_γ. The intervals in which the exercise was subdivided were defined by inspection of

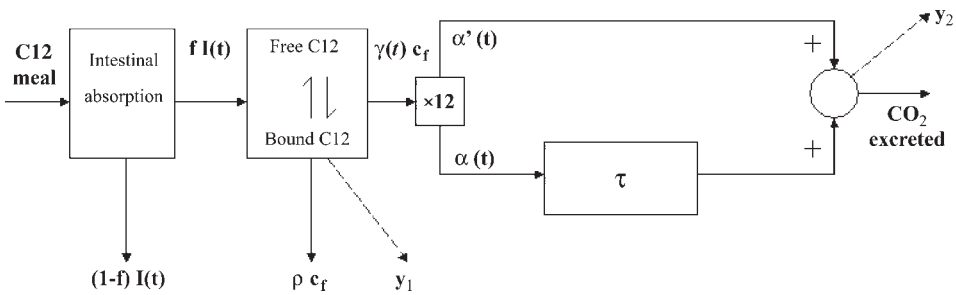

Fig. 1. Block diagram of the mathematical model used for the analysis of dodecanedioic acid (C-12) kinetics and oxidation. To account for the effects of exercise, the parameters γ, α, and α′ are time dependent, as explained in the text. See text for definitions of symbols.

the experimental data. Therefore, the vector of parameters to be estimated is given by V, f, τ_g, τ, τ_γ, γ_1, γ_2, α_e, and α'_e.

The model parameters were estimated for each subject by simultaneously fitting the individual data of plasma C-12 concentration and CO_2 expiration rate at the available time points. Under the assumptions that all the measurements had a constant CV, a weighted least-squares fit was performed, with weights given by the inverse of the CV times the experimental values (23). The least-squares index was minimized by means of a constrained quasi-Newton routine of the MATLAB library, and SD of the estimates were determined from the Jackknife method (31).

Statistical Analysis

All the data were expressed as means ± SD unless otherwise specified. Unpaired two-tailed *t*-test was used for intergroup comparisons. The ANOVA test for repeated measurements, followed by Tukey's test, was applied to calorimetric data.

RESULTS

Controls

All the controls were able to complete the exercise during the different experimental conditions. However, after the C-12-enriched meal, all subjects reported a lower perceived exertion of the fatigue (10.2 ± 0.8 vs. 11.6 ± 0.5 after glucose, $P < 0.05$). Figure 2, *left*, shows the average plasma glucose, insulin and NEFA time courses during the three trials. Plasma glucose concentrations increased up to 6.0 ± 0.5 mM after the glucose-enriched meal, whereas it remained around the basal levels both during and after the exercise when water or C-12 was ingested. The time course of plasma insulin showed a sharp first peak (232.2 ± 24.6 pM) followed by a second broadened peak (127.8 ± 43.8 pM) after the glucose load, whereas it remained constantly ~50 pM after C-12 and water load. Plasma NEFA concentration fell down (from 0.35 ± 0.04 to 0.15 ± 0.01 mM) after glucose ingestion then remained constant throughout the exercise. When the subjects ingested C-12, NEFA levels markedly increased both during and after the exercise. With the water load, NEFA concentrations remained around the basal level (0.33 ± 0.023 mM). Lactic acid plasma levels were regularly lower after C-12 and water than after glucose [1.50 ± 0.64 mM after C-12 meal ($P < 0.001$), 0.93 ± 0.16 after water ($P < 0.001$), and 2.60 ± 0.42 mM after glucose].

Figure 3*A* shows the best fit, obtained by the model, of C-12 plasma concentration and of $\dot{V}CO_2$ data, derived from C-12 oxidation during the experimental session, for a representative control subject.

The average value of the apparent renal clearance ρ was 27.0 ± 10.0 ml/min, which corresponds to a total urinary excretion of 1.4% of the C-12 ingested. Table 1 reports the population mean ± SD of the parameter estimates. For each subject, the estimates of the individual parameters were determined with a CV always <20%. The estimated value of τ_g, the time constant of the gamma function, which represents the input to the central compartment, indicates that the intestinal absorption rate attained its maximum ($3\tau_g$) at 94.5 min after the meal. The fraction of C-12 that entered the central compartment, f, was ~83% of the C-12 content in the meal. The remaining 17% was the amount of C-12 that possibly entered the liver compartment, where it could be stored (likely as glycogen). During the first phase of the exercise (~60 min), the C-12 clearance from the central compartment toward tissues (the coefficient γ_1) rises from 0.08 l/min (basal level; see Ref. 9) to 3.41 l/min, corresponding to a mean value of the rate of tissue uptake in this time interval, R_{t1}, of 0.79 mmol/min. During the remaining phase (~60 min), the γ value halved, whereas the average value of tissue uptake remained close to the previous level. During the exercise, the mean value of α′ increased from the basal value 24 to 59%, whereas α decreased from 62 to 21%.

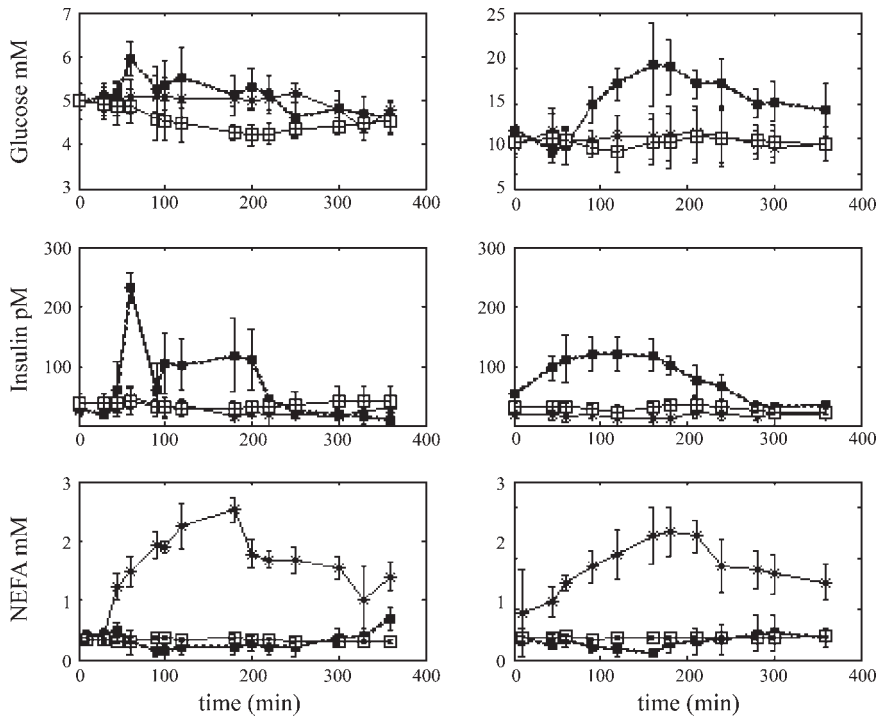

Fig. 2. Time courses of glucose, insulin, and nonesterified fatty acid (NEFA) plasma concentration in normal (*left*) and diabetic (*right*) subjects. ■, Glucose meal; *, C-12 meal; □, water load.

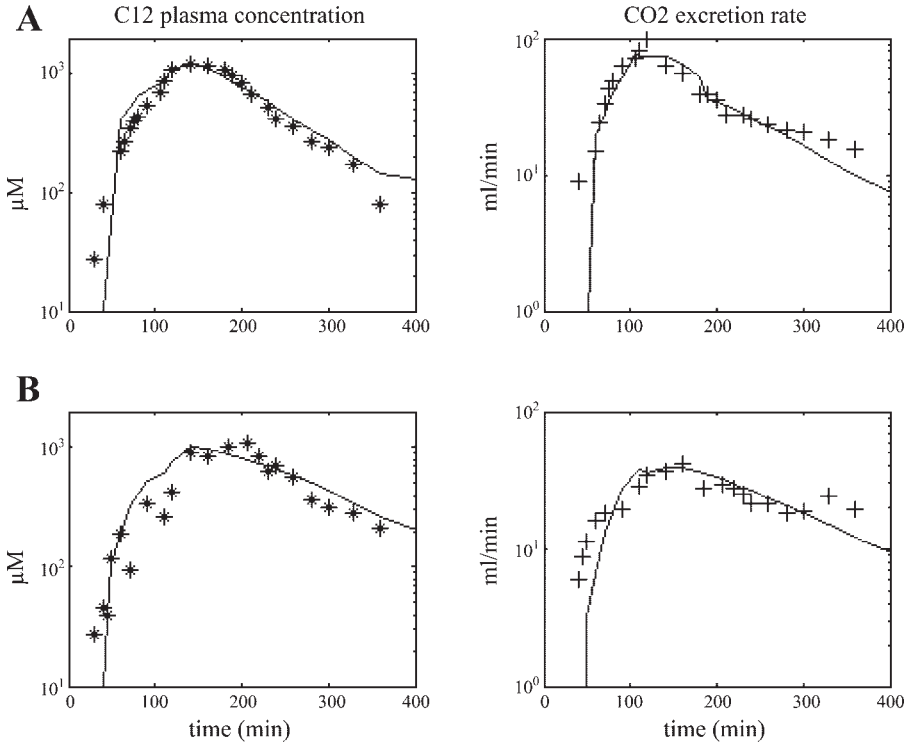

Fig. 3. Experimental data of total C-12 plasma concentration (*) and CO_2 excretion rate (+) vs. time after C-12 meal for a representative normal (*A*) and diabetic (*B*) subject. The continuous lines represent the optimal fitting curves predicted by the model with the following parameter values (mean ± SD of the estimate): *A*: γ_1 = 3.47 ± 0.67 l/min, γ_2 = 2.22 ± 0.15 l/min, V = 9.84 ± 1.5 l, τ = 155.32 ± 29.3 min, τ_γ = 200.19 ± 3.6 min, τ_g = 29.22 ± 1.4 min, α_e' = 0.52 ± 0.02, α_e = 0.10 ± 0.03, and f = 0.83 ± 0.014; *B*: γ_1 = 4.07 ± 0.37 l/min, γ_2 = 1.89 ± 0.09 l/min, V = 13.21 ± 0.42 l, τ = 150.00 ± 1.11 min, τ_γ = 100.00 ± 1.32 min, τ_g = 32.90 ± 0.97 min, α_e' = 0.35 ± 0.03, α_e = 0.34 ± 0.04, and f = 0.58 ± 0.02.

Table 2 reports the oxidation rate derived from glucose, lipid, and C-12 oxidation per unit FFM during the exercise session. C-12 oxidation (the energy yield for C-12 is 7.2 kcal/g) produced 36% of the total energy consumed during the exercise and was associated with a significant reduction of lipid oxidation. In contrast, the sum of C-12 plus lipid oxidation was not significantly different from the lipid oxidation after glucose load and water load.

Diabetic Subjects

The subjects stopped the exercise after 102 ± 4.5 min after the water load since they reported the appearance of fatigue, whereas all of them were able to complete the exercise session (120 min) after glucose and C-12 ingestion. The reported perceived exertion mean value was 18.8 ± 1.3 after glucose and 14.8 ± 0.8 after C-12 ($P < 0.05$).

Table 1. *Average values over control and diabetic subjects of the parameters of the C-12 disposition model*

Parameter Estimates	Controls	Diabetics	*t* Test
τ_g, min	31.46±3.26	33.16±4.45	NS
f	0.83±0.04	0.47±0.07	$P < 0.001$
V, liters	10.34±1.14	12.93±0.86	
γ_1, l/min	3.41±0.44	2.57±1.05	NS
R_{t1}, mmol/min	0.79±0.34	0.27±0.13	$P < 0.02$
γ_2, l/min	1.74±0.51	1.30±0.36	NS
R_{t2}, mmol/min	0.70±0.33	0.41±0.14	NS
τ_γ, min	214.89±21.18	131.83±35.69	$P < 0.005$
α_e'	0.59±0.12	0.37±0.02	$P < 0.005$
α_e	0.21±0.17	0.32±0.04	NS
τ, min	155.10±28.67	148.06±28.11	NS

Values are averages ± SD. τ_g, time constant of the input function I(*t*); f, fraction of I(*t*) reaching plasma; V, distribution volume; γ_1 and γ_2, clearances due to C12 tissue uptake; R_{t1} and R_{t2}, rate of C-12 tissue uptake on the two intervals in which the exercise was subdivided; τ_γ, decay time constant of γ; α_e and α_e', fraction of C-12 taken up by tissues conveyed by the slow and fast oxidation-excretion pathways, respectively; τ, time constant of the slow oxidation pathway. NS, not significant.

Plasma glucose concentrations, as shown in Fig. 2, *right*, increased up to 20.5 ± 5.9 mM after the glucose-enriched meal, whereas they remained around the fasting levels (9.67 ± 2.9 and 9.0 ± 1.5 mM) during and after the exercise after both water and C-12. After glucose ingestion, plasma insulin concentration showed a peak lower (~123 pM) than that observed in controls, whereas it remained constantly ~24 pM after the C-12 load and 33 pM after the water load. Lactic acid plasma levels were constantly lower after both C-12 or water than after glucose [1.2 ± 0.28 mM after C-12 ($P < 0.01$), 0.8 ± 0.25 mM after water ($P < 0.001$), and 1.9 ± 0.15 mM after glucose]. Plasma NEFA concentration fell down (from 0.45 ± 0.18 to 0.29 ± 0.05 mM) after glucose ingestion and then remained constant throughout the exercise. Plasma NEFA concentration was around the basal level (0.43 ± 0.04 mM) after water ingestion. After the C-12 load, NEFA levels increased both during and after the exercise.

Figure 3*B* shows the best fit of C-12 plasma concentration data and of $\dot{V}_{CO_2}$ derived from C-12 oxidation during the experimental session for a representative diabetic subject. The average value of the apparent renal clearance ρ was 42.9 ± 10.7 ml/min, corresponding to a total urinary excretion of 3.2% of the C-12 ingested.

Table 1 reports the population mean ± SD of the parameter estimates. The CV of the estimates for each subject was always <20%. The estimated τ_g was similar to that found in controls. The fraction of C-12 that entered the central compartment, f, was 47% of the ingested amount and was significantly lower than that of controls. During the first phase of the exercise, the mean coefficient γ_1 was 2.57 l/min, corresponding to an average uptake, on this time interval, of 0.27 mmol/min.

Table 2. *Glucose, lipids, and C-12 oxidation rate per unit FFM during the exercise*

	Subjects					
	Controls			Diabetics		
Experimental Session:	Exercise glucose	Exercise water	Exercise C-12	Exercise glucose	Exercise water	Exercise C-12
Glucose oxidation rate	0.029±0.005 (34.9)	0.020±0.002 (24.4)	0.022±0.006 (25.6)	0.039±0.002† (54.9)	0.020±0.006 (30.8)	0.024±0.005 (30)
Lipid oxidation rate	0.054±0.010* (65.1)	0.062±0.016 (75.6)	0.033±0.002 (38.4)	0.032±0.002‡ (45.1)	0.045±0.005 (69.2)	0.045±0.008 (56)
C-12 Oxidation rate			0.031±0.002 (36)			0.011±0.0025 (14)
Total oxidation rate	0.083	0.082	0.086	0.071	0.065	0.08

Values are means ± SD. Units are kcal·kg fat-free mass $(FFM)^{-1}\cdot min^{-1}$. Values in parentheses are %reported oxidation rate over the total. *Significance between lipids oxidized after glucose meal and after C-12 meal ($P < 0.05$); †significance between glucose oxidized after glucose meal and after C-12 meal ($P < 0.05$); ‡significance between lipids oxidized after glucose meal and lipids + C-12 oxidized after C-12 meal ($P < 0.025$).

During the remaining phase, the γ value halved, whereas the average value of C-12 tissue uptake was greater than the previously observed level. In contrast with control subjects, the parameters α and α' during exercise attain similar values.

As shown in Table 2, C-12 oxidation provided 14% of the total energy consumed during the experiments. The glucose oxidized after the C-12 meal was significantly lower than that oxidized after the glucose meal. Lipid oxidation increased after the C-12 meal compared with the glucose meal. Moreover, the sum of C-12 plus lipids oxidized after the C-12 meal was significantly greater than lipids oxidized after the glucose meal. These findings suggest that C-12 can restore the metabolic flexibility in diabetic subjects by reverting the oxidative pathway to a quasi-physiological condition, as observed in controls.

DISCUSSION

The ingestion of 40 g of C-12 in type 2 diabetic subjects before a moderate exercise reduces muscle fatigue, thus allowing completion of the exercise cycle, and does not promote insulin secretion but rather is associated with an increase in triglyceride hydrolysis as shown by the significant rise of plasma NEFA. Forty seven percent (see f in Table 1) of C-12 is taken up by peripheral tissues and 69% of the rate of C-12 uptake, $(\alpha_e + \alpha_e') \times 100$, is enrouted in the oxidation pathway during the exercise, whereas the remaining fraction ($1 - f = 53\%$) is possibly taken up by the liver to synthesize glycogen. On the contrary, in healthy subjects, the C-12 tissue uptake is 33.4 g ($f = 83\%$), and 80% of the uptake is routed in the oxidation pathway. Urinary excretion of C-12 was increased in diabetic subjects still remaining at very low levels.

The production of CO_2 from C-12 and its excretion with the expired air was modeled by assuming the coexistence of two pathways, one fast and the other slow, to account for the observed delay in the $^{13}CO_2$ detection. A source of this delay might be the formation of glucose from succinic acid, an end product of C-12 oxidation together with acetyl-CoA, or the dilution of labeled CO_2 in the bicarbonate pool. During the exercise, the rapid pathway was enhanced, as shown by α_e' with respect to the resting values (9), although to a smaller degree in diabetic subjects. This finding appears to be in agreement with the accelerated kinetics of CO_2 excretion described by Barstow et al. (4) during light and moderate exercise.

In type 2 diabetic patients, irrespective of their treatment status, there is increased tendency for muscle to become fatigued (28). It is believed that efficient ATP production in response to continued high demand is a determinant of muscle performance at a high level on a continuous basis. Therefore, a decline in the muscle performance, often described as muscle fatigue, is observed if ATP production cannot be sustained during continuous activity. The current study demonstrates that, in subjects with type 2 diabetes, there is evidence of increased muscle fatigue, possibly as a consequence of an impaired ATP synthesis in response to a higher request, which might be secondary to a reduced mitochondrial function, largely described in type 2 diabetes (32). Magnetic resonance spectroscopy studies in humans suggest that a defect in insulin-stimulated glucose transport in skeletal muscle is the primary metabolic abnormality in insulin-resistant patients with type 2 diabetes (29). Excess of fatty acids appears to cause this defect in glucose transport by inhibiting insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-1-associated phosphatidylinositol 3-kinase activity (29). In type 2 diabetes, the incapacity to shift from glucose to lipids and vice versa as energy substrate, depending on the energy requirement, is defined as "metabolic inflexibility" (21). C-12 might overcome this impairment by providing intermediate substrates to mitochondrial oxidation and ATP synthesis. Other mechanisms might contribute to the reduction of muscle fatigue, such as the lower production of lactate during exercise in diabetic subjects. This might be a consequence of increased succinyl-CoA provided by C-12 oxidation with a subsequent improvement of tricarboxylic acid cycle efficiency: acetates deriving from decarboxylation of pyruvate in the anaerobic glycolysis can enter the tricarboxylic acid cycle instead of being diverted toward lactate. Furthermore, C-12 might exert a central effect in the brain, with a reduction of fatigue perception. In fact, the dicarboxylic acids are preferential metabolic substrates for the central nervous system. Alexander et al. (2) have shown that homogenates of rat brain catalyze the omega oxidation of monocarboxylic acids with a specific activity remarkably higher than that found in rat liver. Specific activity increases with increasing chain length of the substrate. Not only do cultured rat neurons, astrocytes, and oligodendrocytes all contain omega oxidation activity, but the product of omega oxidation in brain almost exclusively consists in dicarboxylic acids.

In addition, the administration of succinic acid dimethyl ester before 60 min of exercise in overnight-starved Goto-Kakizaki diabetic rats is able to compensate for the increased consumption of endogenous nutrients during exercise (22). Furthermore, succinate carbon incorporation into protein is markedly reduced in diabetic or insulin-treated diabetic rat hepatocytes, whereas most of these carbons have been found to

be diverted into the gluconeogenesis pathway (24). Therefore, C-12, which provides succinyl-CoA, might supply succinic acid to diabetic subjects, thus enhancing glycogen production. To support this hypothesis, the mathematical model showed that hepatic extraction of C-12 (the fraction $1 - f$) is higher in diabetics than in controls (53 vs. 17%). Although gluconeogenesis has not been measured in the current study, increased glucose production and increased gluconeogenesis are reported to occur in type 2 diabetic patients when they are not treated (16). C-12 might contribute to restore the depleted hepatic glycogen stores in our diabetic population, as suggested by the increased C-12 liver uptake with respect to controls. The defect in glucose metabolism and glycogen synthesis is not only present at the level of the liver (5, 13) but also in skeletal muscle tissue. In fact, a reduction in intramyocellular glycogen stores associated with increased triglyceride content has been shown in subjects affected by type 2 diabetes mellitus (12). Furthermore, muscle glycogen oxidation is slower in diabetic patients than in control subjects (11). Moreover, in diabetic subjects, lipid oxidation is increased by C-12 compared with glucose load (Table 2), meaning that the higher mobilization of NEFA from adipose tissue translates into an increased fat oxidation, possibly contributing in the long term to weight loss. C-12 does not stimulate pancreatic insulin secretion and is associated with a rise of NEFA circulating levels. It has been reported that lipolysis was suppressed to >60% at rest after an elevation in plasma insulin concentration to only ~180 pM (14, 20). This suppression, together with the high rate of triglyceride reesterification at rest (14, 20, 34), appears to account for the observation of Campbell et al. (12) that similar small elevations in plasma insulin concentration in resting subjects reduced the rate of NEFA appearance in plasma, plasma NEFA concentration, and the rate of NEFA disappearance from plasma ($R_{d\ NEFA}$) to the point that $R_{d\ NEFA}$ equaled fat oxidation. Therefore, at rest, a reduction in NEFA mobilization from adipose tissue after a relatively small increase in plasma insulin concentration may limit fat oxidation, whereas whole body lipolysis remains in excess of fat oxidation.

Fat oxidation rates increase from low to moderate exercise intensities and then decrease when the intensity becomes high. Maximal rates of fat oxidation have been shown to be reached at intensities between 59 and 64% of maximum oxygen consumption in trained individuals and between 47 and 52% of maximum oxygen consumption in a large sample of the general population. However, carbohydrate ingestion before physical exercise limits fat oxidation by inducing plasma insulin elevation (20). Because, contrary to glucose, C-12 ingestion does not affect insulin secretion, a simultaneous lipid mobilization is promoted as shown in our series by the increase of plasma NEFA levels associated with a trend toward an increased lipid oxidation in diabetic subjects. Long-term physical exercise promotes lipid oxidation in healthy subjects. As a consequence of the metabolic inflexibility, diabetic subjects are unable to increase lipid oxidation under similar conditions. As reported in RESULTS, C-12 ingestion allows an increase of lipid oxidation, together with a significant reduction of glucose oxidation, compared with the glucose meal. We hypothesize that C-12 might overcome the metabolic inflexibility of diabetic patients by providing succinyl-CoA as an intermediate substrate of the tricarboxylic acid cycle, which results in an improvement of mitochondrial oxidative efficiency. This is a relevant feature of C-12 as a fuel substrate and indicates that it might be used in overweight, diabetic subjects to reduce fat mass during exercise. The effect of C-12 in stimulating lipid oxidation might be masked in controls because of the higher availability of C-12 in peripheral tissues and possibly because of the competition with NEFA for skeletal muscle uptake.

We acknowledge that the study has been performed on a small sample of type 2 diabetic patients with relatively poor metabolic control; therefore, the results might be extended to all type 2 diabetic patients with some caution. In conclusion, C-12 seems to be a suitable energy substrate during exercise, since, in diabetic subjects, it reduces muscle fatigue, is rapidly oxidized, and does not stimulate insulin secretion, which implies that lipolysis is not inhibited as reported after glucose ingestion.

APPENDIX

According to Ferrannini (15) we have

$$1\ \text{g glucose (G)} + 0.746\ \text{l}\ O_2 \rightarrow 0.746\ \text{l}\ CO_2 + 0.6\ \text{g}\ H_2O \quad (1A)$$

$$1\ \text{g lipid (L)} + 2.029\ \text{l}O_2 \rightarrow 1.43\ \text{l}\ CO_2 + 1.09\ \text{g}\ H_2O \quad (2A)$$

For C-12 we have:

$$1\ \text{g C-12} + 1.509\ \text{l}\ O_2 \rightarrow 1.169\ \text{l}\ CO_2 + 0.86\ \text{g}\ H_2O \quad (3A)$$

From *Eqs. 1A* and *2A* and taking into account the oxidation of C-12, it follows:

$$\dot{V}O_2 = 0.746\ \dot{G} + 2.029\ \dot{L} + \dot{V}O_{2C\text{-}12} \quad (4A)$$

$$\dot{V}CO_2 = 0.746\ \dot{G} + 1.43\ \dot{L} + \dot{V}CO_{2C\text{-}12} \quad (5A)$$

From *Eq. 3A* we have:

$$\dot{V}O_{2C\text{-}12} = 1.509/1.169\ \dot{V}CO_{2C\text{-}12} \quad (6A)$$

Solving the system of *Eqs. 4A–6A*:

$$\begin{aligned} \dot{G} &= 4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2 - 0.32 \times \dot{V}CO_{2C\text{-}12} \\ \dot{L} &= 1.67 \times (\dot{V}O_2 - \dot{V}CO_2) - 0.49 \times \dot{V}CO_{2C\text{-}12} \end{aligned} \quad (7A)$$

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