The Metabolic Effect of Dodecanedioic Acid Infusion in Non–Insulin-Dependent Diabetic Patients

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ABSTRACT

Dodecanedioic acid (C12) is an even-numbered dicarboxylic acid (DA). Dicarboxylic acids are water-soluble substances with a metabolic pathway intermediate to those of lipids and carbohydrates. Previous studies showed that contrary to other DAs, very low amounts of C12 are lost with urine. The effects of 46.6 mmol of C12 intravenous infusion for 195 min on blood glucose levels were investigated in five patients with non–insulin-dependent diabetes mellitus (NIDDM), with a good metabolic compensation, and in five healthy volunteers matched for gender, age, and body mass index. Blood samples were taken every 15 min for a period of 360 min to measure glucose, insulin, C-peptide, ketone bodies, and free fatty acid (FFA) levels, and 24-h urine samples were collected to measure C12 and urea excretion. Plasma and urinary C12 concentrations were determined by high-pressure liquid chromatography (HPLC). Indirect calorimetry was continuously performed both basally and during the study period. The average 24-h urinary excretion of C12 was 6.5% versus 6.7% of the administered dose, respectively, in NIDDM patients and in healthy controls. The area under the curve (AUC) values of plasma C12 were 279.9 $\pm$ 42.7 μmol in NIDDM patients and 219.7 $\pm$ 14.0 μmol in controls (P = ns). Plasma glucose levels significantly decreased in NIDDM patients during C12 infusion (from 7.8 $\pm$ 0.6 to 5.4$\pm$0.8 mM at the end of the study period, $P < 0.05$). Lactate plasma concentration decreased in NIDDM patients from 3.5 $\pm$ 0.2 to 1.5 $\pm$ 0.1 mM ($P < 0.001$), whereas blood pyruvate increased at the end of the experimental session from 26.0 $\pm$ 11.6 to 99.5 $\pm$ 14.9 μM ($P < 0.01$). Free fatty acids decreased in diabetic patients from the beginning until the end of C12 infusion, although this difference did not reach statistical significance. No significant increase was found between basal and final values in $\dot{V}O_2$ consumption and in the values of nonprotein respiratory quotient in both groups of subjects examined. The experimental data indicate that C12 infusion decreases plasma glucose levels in NIDDM patients to normal range without influencing plasma insulin levels. The balance between pyruvate and lactate was affected by C12 infusion only in diabetics patients. C12 might represent a fuel substrate immediately available for tissue energy requirements, especially in conditions such as diabetes mellitus in which glucose metabolism is impaired. *Nutrition* 1998; 14:351–357. ©Elsevier Science Inc. 1998

Key words: dicarboxylic acids, dodecanedioic acid, non–insulin-dependent diabetes mellitus, glucose metabolism

INTRODUCTION

Straight medium-chain, even-numbered dicarboxylic acids (DAs) are substances structurally similar to medium-chain free fatty acids (FFA) but are provided with two carboxylic terminal groups that confer to the molecule the characteristic of being water soluble. The metabolic pathway of these DAs is intermediate between those of lipids and carbohydrates because from their β oxidation, both acetyl-CoA and succinyl-CoA originate, the latter being an intermediate of the Krebs cycle.

The utilization of these DAs as an alternate fuel substrate was proposed in parenteral nutrition.[1–10] Recently, using the euglycemic-hyperinsulinemic clamp technique, we showed that sebacate, a DA with 10 carbon atoms, acts as a glucose-sparing substrate.[8]

Wada et al.[11] and Wada and Usami[12] found that the administration of dicarboxylic acids to starved or diabetic rats reduced the concentration of ketone bodies in blood, in contrast to the corresponding monocarboxylic acids. In addition, Mortensen[13,14] demonstrated that exogenously added adipic acid in fat-fed ketotic rats

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increased the subnormal excretion level of succinic acid and that there was a significant and rapid decrease in the concentrations of 3-hydroxy-butyric acid in both blood and urine. These investigations supported the hypothesis that adipic acid possesses antiketogenic properties.

Even-numbered DA might be regarded as useful energy substrates in those clinical conditions, like decompensated diabetes mellitus, in which the organism uses alternate gluconeogenetic substrates, such as amino acids, with potentially damaging effects on reducing lean body mass.[10]

Although sebacic acid showed sparing effects on whole-body glucose utilization,[8] its effective clinical use is impaired by high urinary excretion, which reaches levels of up to 45% of the administered dose.[15] To the contrary, the urinary loss of dodecanedioic acid (C12) in experimental animals was low, corresponding to 3.90 ± 1.62% of the administered dose, and the analysis of its pharmacokinetic profile indicated the presence of tubular reabsorption.[9] These data have been confirmed in humans,[16] in whom after an intravenous bolus of 1 g of C12 (4.35 mmol), urinary excretion of C12 was 1.62% of the administered dose.

The aim of the present investigation was to study the relationship between C12 and glucose metabolism in healthy subjects and in patients affected by non–insulin-dependent diabetes mellitus (NIDDM). We performed a continuous intravenous infusion of C12 in NIDDM patients and in healthy controls and measured the time course of C12, the plasma levels of glucose, and the plasma concentration of insulin, ketone bodies, pyruvate, lactate, and FFAs.

MATERIALS AND METHODS

Monocarboxylic acids: myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4), erucic (C22:1); and dicarboxylic acids: suberic (C8:0), azelaic (C9:0), sebacic (C10:0), dodecanedioic (C12:0), were obtained from Sigma (St. Louis, MO, USA). Dodecanedioic acid was purified by Real S.r.l., (Como, Italy), and was free from pyrogens and contaminants with a degree of purification of 99.8% ascertained using gas-liquid chromatography and mass spectrometry. All other chemicals were purity available quality or of the highest purity available.

A 0.4 M solution of dodecanedioic acid salified with NaOH was used for the infusions. The infusions were sterilized by 0.25 μm ∅ Millipore filters (Molsheim, France) before administration.

Experimental Protocol

A continuous infusion of 0.24 mmol/min[17] sodium dodecanedioate or saline solution was performed, in a randomized way, for 195 min at a constant rate, by means of an electric syringe pump (Harvard Apparatus, South Natick, MA, USA) in 10 overnight-fasting subjects, 5 healthy volunteers, and 5 NIDDM patients. The NIDDM patients were treated with oral hypoglycemic agents (metformin 850 mg × 2 daily), showing good metabolic compensation (values of HbA_{1C} ranging from 5.0% to 6.2% in the last 4 mo). HbA_{1C} was determined using a ion-exchange microcolumn method (Biorad, Richmond, CA, USA) (normal range, 3.4–6.1%). Metformin administration was stopped 3 d before the experimental session, and short-acting human insulin (Actrapid HM, Novo Nordisk, Gentofte, Denmark) was administered before the three meals. Body composition was assessed by bioelectrical impedance measurements. Body weight was measured to the nearest 0.1 kg by a beam scale. Bioimpedance was performed using a radio frequency current of 800 μA at 50 KHz between a set of electrodes attached to the dorsum of the hand and the foot (Body Composition Analyzer, Medileader, Parma, Italy).[18]

The anthropometric characteristics of the subjects studied are described in Table I.

TABLE I.

ANTHROPOMETRIC CHARACTERISTICS OF SUBJECTS (MEAN ± SE)

	Controls (n = 5)	NIDDM (n = 5)	P
Age (y)	54 ± 5	62 ± 1	ns
Weight (kg)	74 ± 7	73 ± 1	ns
Height (cm)	175 ± 5	169 ± 1	ns
BMI (kg/m^2)	24.1 ± 0.2	25.5 ± 0.6	ns

ns, significant; BMI, body mass index.

Heparinized blood samples (8 mL) were taken every 15 min after the beginning of the infusion for a period of 360 min and immediately centrifuged. Plasma samples were frozen at −20°C until analysis. Aliquots of 5 mL of blood were used for pyruvate analysis.

Each patient voided before starting the C12 infusion, and the 24-h urine was collected in a container with 0.1% sodium azide to prevent bacterial growth.

The study protocol conformed with the guidelines of the Hospital Ethical Committee, and written informed consent was obtained in all cases.

Dicarboxylic Acid Analysis

Serum samples. One hundred micrograms of azelaic acid were added to 1 mL of each plasma sample as an internal standard. To obtain the free plasma concentrations of dicarboxylic acids, plasma proteins were removed by using syringe tip filters Dyna-Gard® 0.2 μm ME (Microgon Inc., Laguna Hills, CA, USA). Dicarboxylic acids were extracted twice from the ultrafiltrate with eight volumes of ethylacetate, maintaining the solutions at 60°C for 15 min. The combined extracts were dried in a GyroVap apparatus (Howe, model GV1, Gio. DeVita, Rome, Italy), operating at 60°C, coupled with a vacuum pump and a gas trap FTS System (Stone Ridge, NY, USA).

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-X4, 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 4N HCl and extracted twice with ethylacetate and evaporated in the GyroVap as just described.

Plasma Free Fatty Acid Analysis

After acidification at pH 2 with 1N HCl, plasma FFAs were extracted from 1 mL of plasma by two volumes of chloroform-methanol 2:1(v/v). As an internal standard, 100 μg of erucic acid (13-docosenoic acid) was added to plasma before the extractions. The extracted volumes were combined and dried in a GyroVap apparatus, operating at 70°C, coupled with a vacuum pump and a gas trap FTS System, as previously described.[19] Derivatives of FFA were obtained following the same procedure described for DA.[19]

High-Pressure Liquid Chromatography Analysis

The extracted solutes were dissolved in 0.5 mL acetonitrile and added to 10 mg of *p*-bromophenacylbromide and 30 μL of *N,N*-diisopropylethylamine as catalyst. The mixture was heated to 60°C for 15 min. The derivatives were dissolved in a final volume of 1 mL of acetonitrile and an aliquot of 10 μL was automatically

injected into a liquid chromatograph (Hewlett-Packard 1050; Hewlett Packard, Palo Alto, CA, USA) with a HP 3396A integrator and a scanning spectrophotometer operating in the 190- to 600-nm wavelength range (light source: deuterium lamp), noise < 2.5 × 10-5 AU peak-to-peak at 254 nm with 4-nm bandwidth, flowing water at 1 mL/min.

Monocarboxylic and dicarboxylic acid derivatives, extracted from plasma and mixed together in a final volume of 1 mL, were separated on an LC-18, 4.6 mm I.D., 25-cm length, 5 μm particle size, reversed phase column (Supelco Inc., Bellefonte, PA, USA). The HPLC conditions were as follows: solvent A bidistilled water/methanol (1:1, v/v), solvent B acetonitrile. After 10 min, isocratic elution with 10% acetonitrile A gradient elution was performed from 10% to 50% of B in 45 min, then a second gradient elution was performed from 50% to 68% B in 25 min and then the %B was adjusted to 100% within 30 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 absorbance units (AU).

This HPLC elution program allowed the separation of both monocarboxylic and dicarboxylic acids.[20] A mixture of derivatized monocarboxylic acids from myristic acid (C14) to arachidonic acid (C20:4) and a mixture of derivatized dicarboxylic acids from suberic acid (C8) to dodecanedioic acid (C12) were used as reference standards. At a sensitivity of 8.0 × 10-4 AU/cm, detection limits ranged from 20 to 40 ng for monocarboxylic acids and from 25 to 50 ng for dicarboxylic acids. Calibration curves were in the linear range from 150 to 2 000 ng for monocarboxylic acids and from 200 to 2 000 ng for dicarboxylic acids. Recovery of monocarboxylic acids and of DAs were obtained by adding 10 to 100 μg of both monocarboxylic and dicarboxylic acid standards to 1 mL of serum from 10 different untreated subjects. Sera were extracted, monocarboxylic and dicarboxylic acids derivatized as previously described and injected into the HPLC columns. The recovery of monocarboxylic acids ranged from 89% to 98% depending on the chain length; that of DAs ranged from 85% to 99%.

Other Methods

Plasma glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instrument, Fullerton, CA, USA), and plasma insulin was measured by radioimmunoassay (Radim, Pomezia, Italy). Pyruvate was measured on total blood, and lactate was assessed in plasma by enzymatic methods (Boehringer Mannheim, Germany). Urinary urea excretion was determined by a BUN Analyzer 2 (Beckman Instrument, Fullerton, CA, USA). β-hydroxybutyrate and acetoacetate were assayed by a standard method:[21] reported data (ketone bodies) are the sum of the two concentrations.

Indirect calorimetry. Indirect calorimetry was continuously performed starting 45 min before and for 600 min after the experimental session by a Deltatrac apparatus (Datex Instrumentarium, Helsinki, Finland), which automatically gives values of $\dot{V}O_2$, $\dot{V}CO_2$, RQ, and energy expenditure (EE) each minute.

Statistical analysis. The results are given as mean ± SE. Independent-sample *t*-tests were used to assess the significance of differences between means of the examined variables. *P* values below the 0.05 level were considered significant.

RESULTS

The average (± SE) 24-h urinary excretion of C12 in the NIDDM patients was 3.0 ± 0.8 mmol (equal to 6.5% of the given dose) and 3.1 ± 0.1 mmol (corresponding to approximately 6.7% of the administered amount) in the control subjects. The urinary urea loss over 24 h was 4.8 ± 1.0 g in NIDDM patients and 5.1 ± 1.1 g in the controls. Urinary nitrogen loss was not significantly different in the two groups and was within a normal range.[22]

Plasma C12 concentration reached the peak after 135 min from the beginning of the infusion in both NIDDM and control subjects (respectively, 1.2 ± 0.2 versus 1.2 ± 0.2 μM; P = ns).

The area under the curve (AUC) values of C12 plasma curves were 279.9 ± 42.7 in NIDDM patients versus 219.7 ± 14.0 μM in controls; P = ns.

The curves of plasma glucose, insulin, lactate, and pyruvate levels versus time in the two groups of subjects examined are depicted in Figures 1 and 2. Fasting plasma FFA concentration was significantly higher in diabetic patients than in healthy controls (1 500 ± 250 versus 750 ± 128 μM, $P < 0.05$). Basal FFA plasma levels decreased in NIDDM patients at the end of the study, but this decrease failed to reach statistical significance (1 500 ± 250 versus 875 ± 341 μM, P = ns). No statistical differences were found in the values of plasma FFA in controls during the infusion.

No significant differences were observed between the basal values and the end values of insulin during the experimental section in both the groups examined. To the contrary, the plasma glucose levels significantly decreased in NIDDM patients during the C12 infusion session, reaching a normal range at the end of the experimental session (7.8 ± 0.6 versus 5.4 ± 0.8 mM, $P < 0.05$, in NIDDM patients, and 4.7 ± 0.1 versus 4.4 ± 0.04 mM, in control subjects; P=ns). No significant reduction of basal plasma glucose concentration was observed in NIDDM patients during saline infusion.

Lactate plasma concentration significantly dropped in the diabetic group (3.5 ± 0.2 to 1.5 ± 0.1 mM, $P < 0.001$), whereas its level remained unchanged in the control group. Blood pyruvate increased at the end of the experimental session to 99.5 ± 14.9 μM from the basal values of 26.0 ± 11.6 μM, $P < 0.01$ in NIDDM patients, whereas no significant difference was found in the control group. Plasma ketones did not change significantly after C12 infusion in the two groups of subject studied (235 ± 6 μmol basally to 241 ± 7 μmol at the end of the infusion in healthy controls and 240 ± 5 μmol to 246 ± 6 μmol in NIDDM patients).

Figure 3 shows the average curves of $\dot{V}O_2$ (mL/min), and nonprotein (np) RQ during the period of study. No significant variations with respect to the basal values of $\dot{V}O_2$ were observed, indicating that the thermogenic effect of C12 was very small. The basal value of npRQ significantly ($P < 0.001$) decreased to 0.78 ± 0.02, which is a value close to the theoretical RQ value calculated for C12 oxidation:

$$C_{12}O_4H_{22} + 15.5\ O_2 \rightarrow 12\ CO_2 + 11\ H_2O$$

and

$$\frac{CO_2}{O_2} = \frac{12}{15.5} = 0.77$$

DISCUSSION

In the present study, we showed that dodecanedioic infusion in NIDDM patients caused a significant decrease in blood glucose levels, whereas no significant change of glycemia was observed in healthy controls. The reduced plasma concentration of glucose during C12 administration could be ascribed to the following mechanisms: an increased insulin secretion stimulated by C12 or an increased glucose uptake or a decreased hepatic glucose output (HGO) with concomitant rise in glycogen storage.

No significant changes from the basal values were observed in the levels of plasma insulin at the end of dodecanedioic acid infusion in both groups, indicating that C12 does not influence

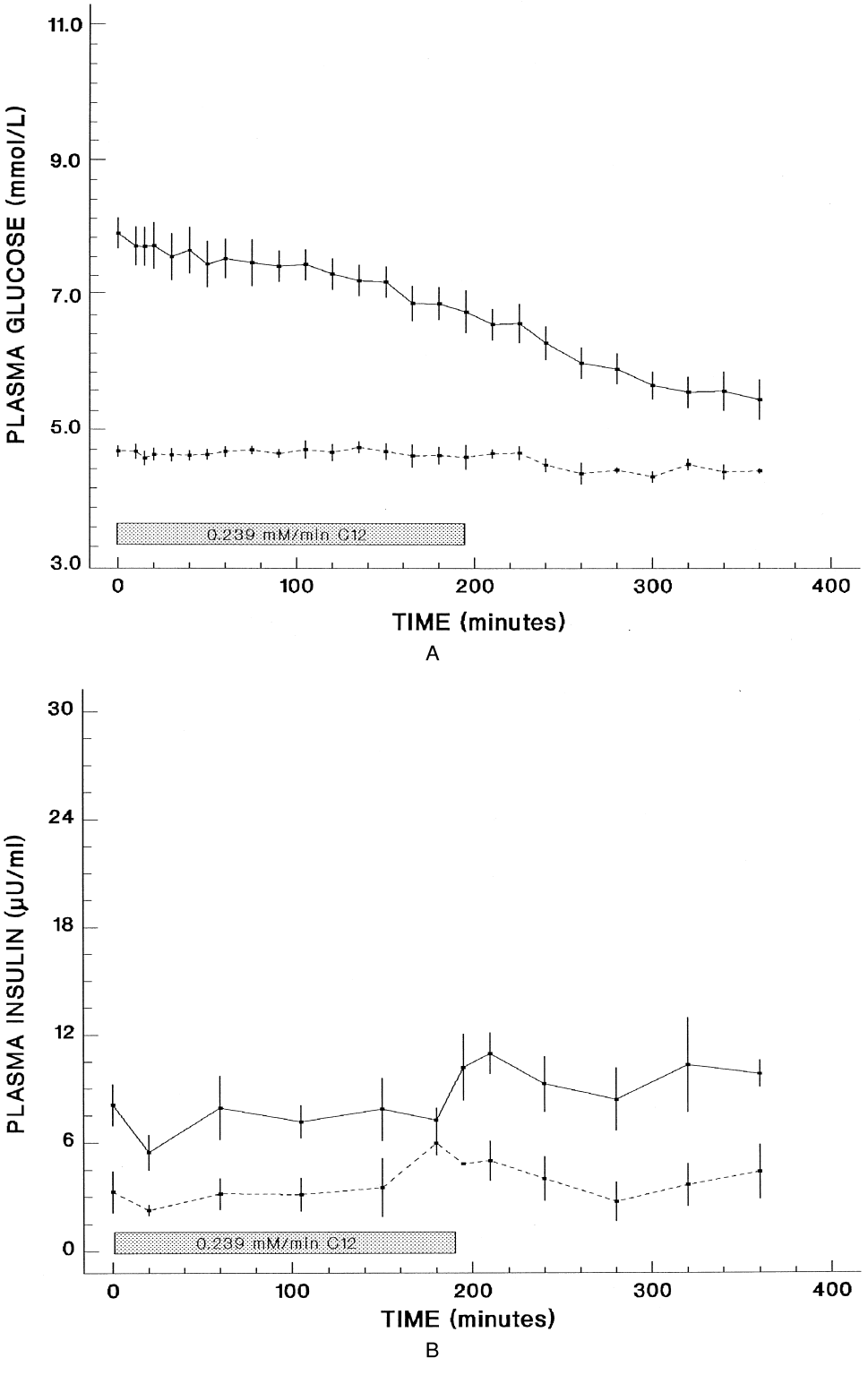

FIG. 1. Plasma glucose levels (A) and plasma insulin levels (B) during C12 infusion in controls (dashed line) and in NIDDM patients (solid line). The values are expressed as mean ± SD.

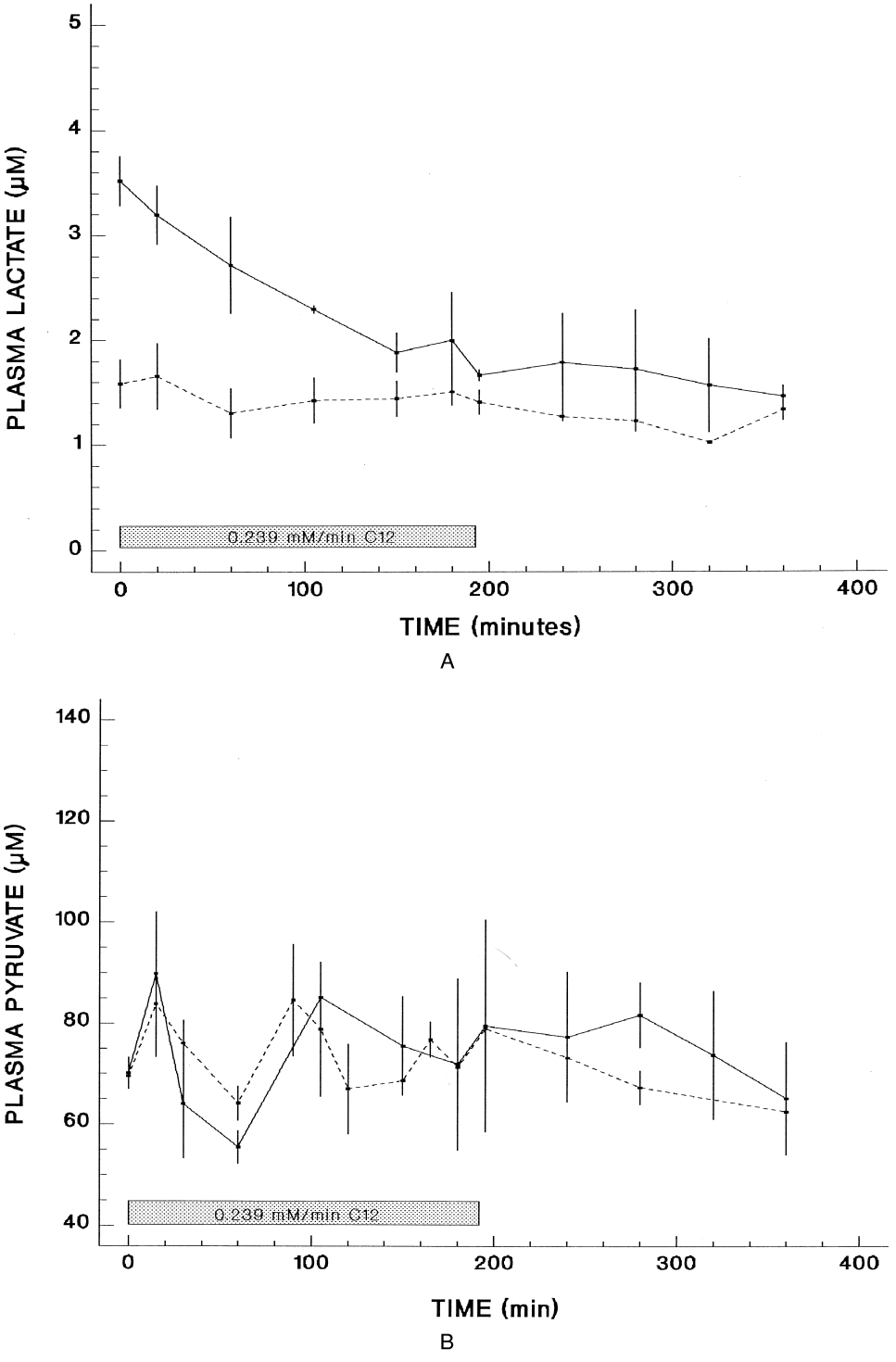

FIG. 2. Plasma lactate levels (A) and plasma pyruvate concentrations (B) during C12 infusion in controls (dashed line) and in NIDDM patients (solid line). The values are expressed as mean ± SD.

insulin secretion, contrary to FFAs, which promote insulin delivery.[23,24]

It is unlikely that glucose is taken up by the tissues at a higher rate during C12 administration, with respect to saline infusion, as the euglycemic-hyperinsulinemic clamp performed in NIDDM patients and in normal controls under C12 infusion showed a significant reduction of whole body glucose uptake.[25]

Another possibility that could be advanced is the decrease of HGO coupled with an increased glucose storage. Normally, blood glucose homeostasis is possible because other substrates, predominantly FFAs, acetoacetate, and β hydroxybutyrate are preferentially oxidized, sparing glucose oxidation. When C12 was administered to NIDDM patients, blood glucose levels were significantly reduced, probably as a consequence of an

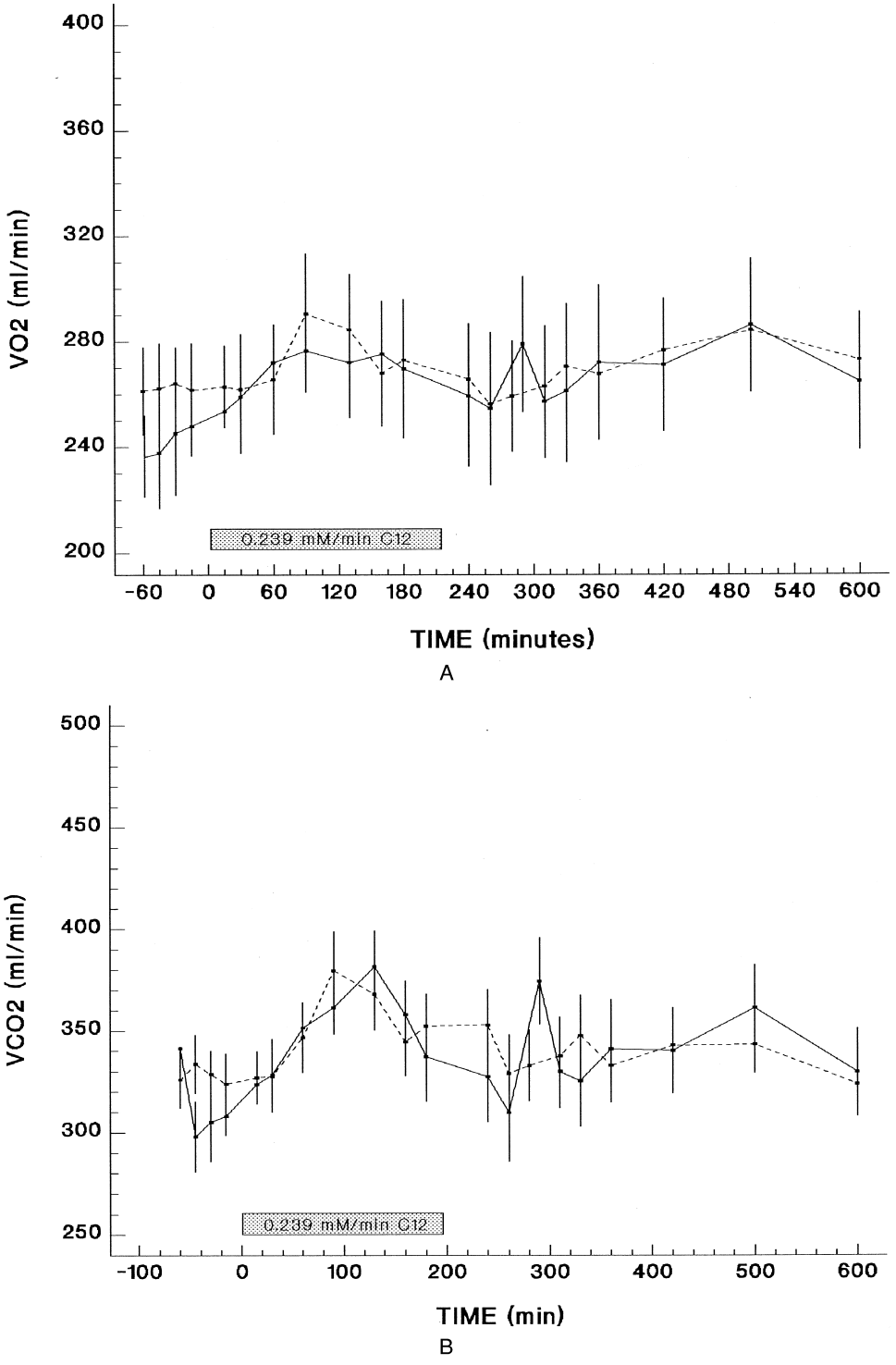

FIG. 3. $\dot{V}O_2$ (A) and $\dot{V}CO_2$ (B) values before and during C12 administration in controls (dashed line) and in NIDDM patients (solid line). Each points represents the mean ± SD of the values measured each minute for 30 min every hour.

increased gluconeogenesis from pyruvate and glycogen formation, as suggested by the decreased amounts of lactate found in the circulatory stream. In its turn, pyruvate might be increased in the liver and kidney, as well as in the blood, owing to the availability of acetyl-CoA and succinyl-CoA coming from dodecanedioic oxidation,[26] which could be utilized as fuel substrate instead of glucose. In this way, the inflow of C12 metabolites in the Krebs cycle could compete with the acetyl-CoA

derived from pyruvate and, therefore, from glucose. Cellular glucose in excess might determine increased glycogen storage. Glycogen should accumulate in the liver and in the kidney cortex so that glucose homeostasis can be maintained.

The body tries to maintain caloric homeostasis. Therefore, the quantity of glucose burned must be considered in relationship to the quantity of alternate fuel oxidized to CO_2 and water. In other terms, the reduction in lactate in relation to pyruvate could be due to a diminished hepatic output of glucose coupled with an increased glycogen storage.

In conclusion, in type II diabetes mellitus,[27,28] in which glucose oxidation is impaired and there exists a resistance to glucose storage, the use of C12 as an alternate fuel substrate, intermediate between lipids and carbohydrates, could be useful because it is promptly oxidized in high amounts[17] and gives succinic acid for gluconeogenesis.

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