

Dodecanedioic acid infusion induces a sparing effect on whole-body glucose uptake, mainly in non-insulin-dependent diabetes mellitus

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Even-numbered dicarboxylic acids (DA) have been proposed as an alternative fuel substrate in parenteral nutrition. In particular, dodecanedioic acid (C12) shows a rapid plasma clearance from tissues, a very low urinary excretion compared with other DA and a high oxidation rate. The aim of the present study was to investigate the effect of C12 infusion on insulin-stimulated glucose uptake in patients with non-insulin-dependent diabetes mellitus (NIDDM) compared with healthy volunteers. A primed-constant infusion of C12 (0.39 mmol/min) was administered over 240 min, and at 120 min a 2 h euglycaemic hyperinsulinaemic clamp was performed. Blood specimens were sampled every 30 min and fractionated urines were collected over 24 h. The levels of C12 were measured by HPLC. Indirect calorimetry was performed continuously during the entire session. Body composition was assessed in all subjects studied to obtain fat-free mass (FFM) values. Whole-body glucose uptake decreased significantly during C12 infusion in both groups, although this effect was much more evident ($P < 0.01$) in NIDDM patients (52.4 (SD 15.8) % decrease compared with saline) than in controls (25.9 (SD 12.1) % decrease). The M value ($\mu\text{mol}/\text{kg}_{\text{FFM}}$ per min) was reduced by C12 to lower levels in NIDDM patients than in normal controls (12.6 (SD 3.9) v. 25.9 (SD 4.5), $P < 0.01$). Urinary excretion of C12 over 24 h was significantly lower in NIDDM patients than in controls (4.26 (SD 0.30) mmol v. 5.43 (SD 0.48), $P < 0.01$), corresponding to less than 3 % of the administered dose. The infusion of C12 decreased non-protein RQ significantly in both groups of patients. In conclusion, this study shows, for the first time, that C12 significantly reduces glucose uptake in both normal controls and NIDDM patients, although this sparing effect on glucose uptake is much more pronounced in diabetic patients. These data suggest that C12 decreases glucose uptake and oxidation, mainly through a mechanism of substrate competition. Thus, it might be a useful alternative substrate in enteral or parenteral nutrition, sparing glucose utilization and increasing glycogen stores, in those clinical conditions, like NIDDM, where reduced insulin-induced glucose uptake and oxidation are observed.

Dicarboxylic acids: Dodecanedioic acid: Non-insulin-dependent diabetes mellitus

Alternative lipid substrates, such as medium-chain triacylglycerols (MCT) (Bach & Babayan, 1982; Johnson & Cotter, 1986; De Gaetano *et al.* 1994) and more recently structured triacylglycerols (Sandström *et al.* 1993) and inorganic salts of dicarboxylic acids (DA) (Raguso *et al.* 1994; Mingrone *et al.* 1994, 1996; Greco & Mingrone, 1995) have been proposed for use in parenteral nutrition because of their faster clearance than long-chain triacylglycerols (LCT) and due to their ability to provide energy effectively coupled with the prevention of fat accumulation in the liver.

Among DA the best characteristics, in view of their use for parenteral nutrition, seem to be displayed by dodecanedioic acid (C12). Low amounts of C12 are, in fact, lost in the urine compared with the shorter chain DA, azelaic (C9) and sebacic (C10) acids (Bertuzzi *et al.* 1991; Mingrone *et al.* 1994). The urinary loss of C12 in experimental animals (Mingrone *et al.* 1994) has been reported to be 3.90 (SD 1.62) % of the administered dose; while the urinary excretion of C12 in humans has been found to be about 7 % of the given dose (Mingrone *et al.* 1996). In addition, the kinetic profile of C12 (Mingrone *et al.* 1996) shows a short half-life (about 12 min) and a rapid plasma clearance.

C12, like other even-numbered DA, is completely oxidized in the cells to CO₂ and water, via succinyl-CoA formation, which, in turn, represents a gluconeogenic substrate being directly converted to glucose (Kou & Tserng Shioh-Jen, 1991). We have recently shown in man (Raguso *et al.* 1994) that, during a euglycaemic hyperinsulinaemic clamp (EHC), C10 acts as a glucose-sparing substrate. Also, in the rat, the simultaneous administration of both labelled C12 and glucose resulted in a preferential tissue uptake of C12 compared with glucose (Bertuzzi *et al.* 1997). Therefore, even-numbered DA might be particularly useful in those clinical conditions in which alternative gluconeogenic substrates, such as amino acids, are used preferentially with a consequent increase in protein catabolism. Among these conditions is non-insulin-dependent diabetes mellitus (NIDDM), in which the inhibition of the enzyme glycogen synthase (Beck-Nielsen *et al.* 1992), coupled with a reduced activity of the pyruvate dehydrogenase (*EC* 1.2.4.1; PDH) complex (Kelley & Mandarino, 1990; Beck-Nielsen *et al.* 1992) is well recognized. It has been hypothesized that in diabetes mellitus there is a shift in substrate utilization from carbohydrates to lipids (Kiens *et al.* 1987): a large proportion of the increase in lipid oxidation, characteristic of NIDDM, is accounted for by an increase in intramuscular triacylglycerol mobilization. An impaired activity of PDH (Beck-Nielsen, 1989) and an increased activity of β -oxidation enzymes (Randle *et al.* 1964) have been described in these patients. In decompensated NIDDM glucose metabolism is impaired, so that free fatty acids (FFA) are used as energy substrates instead, but due to a lack of intermediates of the Krebs' cycle, ketosis may occur. Even-numbered DA, through the formation of succinyl-CoA, can circumvent this problem and allow the oxidation of acetyl-CoA deriving from different substrates, like glucose, FFA and the DA themselves. Therefore, the use of DA in parenteral nutrition, in combination with LCT or MCT, might prove to be a useful practice in order to improve lipid metabolism through the increased oxidation of acetyl-CoA in Krebs' cycle.

The aim of the present investigation was, thus, to compare a sample of NIDDM patients and a sample of normal volunteers for the effect of C12 administration on whole body glucose uptake and oxidation, measured by EHC and indirect calorimetry.

EXPERIMENTAL

Dodecanedioate solution

C12 was purified by the company Real S.R.L., Como, Italy, and was free from pyrogens and contaminants with a degree of purification, ascertained using GLC and mass spectrometry, of 99.8 %. A 0.4 M solution of C12 salified with NaOH was used for the infusions. The infusions were sterilized by ultrafiltration through 0.25 μ m diameter Millipore filters (Molsheim, France) before administration.

Subjects

The study groups consisted of five male healthy volunteers (C) and five male NIDDM patients, matched groupwise for age and BMI. The anthropometric characteristics of the subjects studied are reported in Table 1.

Body composition was estimated on the basis of total body water (TBW) (Bonora *et al.* 1992) measured by isotopic dilution. On the day preceding the EHC evaluation each subject was given 2.96 MBq tritiated water (3700 MBq/ml) in 5 ml saline solution as an intravenous bolus injection. Blood samples were obtained every 30 min for 180 min and the plasma radioactivity counted with a β -scintillation counter (Canberra-Packard, Model 1600TR; Canberra, CT, USA). Each determination was obtained in duplicate on 0.5 ml plasma. The resulting disintegrations/min (dpm) values were plotted against time (min), obtaining the steady state concentration (dpm/ml) of the tracer, and consequently its apparent volume of distribution (TBW, litres). The fat-free mass (FFM; kg) was approximated by dividing the TBW by 0.73 (Bonora *et al.* 1992).

All subjects were clinically euthyroid, had no stigmata of renal, cardiac or hepatic dysfunction and were not being treated with drugs (other than those for diabetes) which could affect carbohydrate or insulin metabolism. None smoked.

Diabetic patients were treated with a therapeutic regimen consisting of three tablets daily of oral hypoglycaemic agents (glibenclamide 2.5 mg + metformin 400 mg) plus a bed-time dose (from 10 to 20 IU) of human intermediate action insulin (ProtaphaneHM, Novo Nordisk, Denmark).

All subjects consumed a weight-maintaining diet including at least 250 g carbohydrate/d for 1 week before the study.

The study protocol followed the guidelines of the hospital Ethical Committee and all subjects gave their written informed consent.

Experimental protocol

The subjects were studied in the postabsorptive state after a 12–14 h overnight fast. A primed-continuous infusion of either sodium dodecanedioate (14.78 mmol in bolus and 0.39 mmol/min as a constant infusion) or saline solution was administered over 240 min. The solutions were infused at a constant rate, by means of an electric syringe pump (Harvard Apparatus, Southnatick, MA, USA).

Table 1. *Anthropometric characteristics of male subjects with non-insulin-dependent diabetes mellitus (NIDDM) and their healthy male controls*
(Mean values and standard deviations)

	Controls (n 5)		NIDDM (n 5)		Statistical significance of difference, P =
	Mean	SD	Mean	SD	
Age (years)	49.8	6.98	52.2	6.22	NS
Height (m)	1.696	0.0826	1.692	0.0876	NS
Weight (kg)	72.2	4.92	76.8	13.55	NS
BMI (kg/m ²)	25.2	1.85	26.9	4.81	NS
Fat-free mass (kg)	56.6	3.30	53.7	6.62	NS
Fat mass (kg)	15.6	1.85	23.1	7.37	NS

The blood glucose concentration of diabetic patients was maintained below 6.1 mmol/l by small bolus doses of short-acting human insulin (Actrapid HM, Novo Nordisk) until the beginning of the study.

All subjects were admitted to the Department of Metabolic Diseases of the Catholic University, School of Medicine in Rome at 19.00 hours on the day before the study. At 07.00 hours on the following morning, indirect calorimetry monitoring was started; the infusion catheter was inserted into an antecubital vein; the sampling catheter was introduced in the contralateral dorsal hand vein and this hand was kept in a heated box (60°) in order to obtain arterialized blood. At 08.00 hours, after a 12–14 h overnight fast, the EHC was performed according to De Fronzo *et al.* (1979). A priming dose of short-acting human insulin was given during the initial 10 min in a logarithmically decreasing way, in order to raise acutely the plasma insulin to the desired concentration. Insulinaemia was then maintained constant with a continuous infusion of insulin at an infusion rate of 40 $\mu\text{U}/\text{m}^2$ per min for 110 min. During the clamp, the glucose level was monitored every 5 min and the infusion rate of a 200 g/l glucose solution was adjusted following the algorithm detailed by De Fronzo *et al.* (1979). Because serum K levels tend to fall during this procedure, KCl was given during each study at a rate of 15–20 mmol/h to maintain the serum K between 3.5 and 4.5 mmol/l. All the subjects underwent the study twice: sodium dodecanedioate was continuously infused for 4 h, starting 2 h before the EHC and continuing during the clamp. The subjects were restudied with the same routine on a different day when saline was infused instead of C12. The order of saline and C12 days was randomized.

The glucose uptake rates of the last 40 min were averaged to calculate the overall glucose disposal rate (*M* value). Arterialized blood samples were collected every 30 min during the study in order to measure insulin and C12 concentrations. The subjects voided before starting the study; urine was collected during the 5 h study to measure the urinary N loss for each subject, which was used for the calorimetry computations.

Respiratory gas exchange was measured by an open-circuit ventilated-hood system (monitor MBM-100, Deltatrac, Datex Instrumentarium Corp., Helsinki, Finland). Energy expenditure, RQ, and substrate oxidation rates were calculated from the O₂ consumption, the CO₂ production, and the N urinary excretion according to Ferrannini (1988). Respiratory gas exchange measurements were started 45 min before beginning the study to measure the resting energy expenditure (REE) and continued during the 120 min preceding the EHC and the 120 min of the clamp. The experimental design is summarized in Fig. 1.

Analytical methods

Serum glucose was measured by the glucose oxidase (*EC* 1.1.3.4) method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA). Plasma insulin was measured by microparticle enzyme immunoassay (Abbott Imx[®], Pasadena, CA, USA). DA were analysed in both plasma and urine using a previously described HPLC method (Mingrone *et al.* 1994).

Data Analysis

All results are expressed as means and standard deviations unless otherwise specified. Non-parametric tests were used due to the small sample size. Two different test procedures were employed: a Wilcoxon test was used to evaluate saline infusion *v.* C12 infusion differences in the metabolic variables; a Mann–Whitney U-test was used to compare the differences in

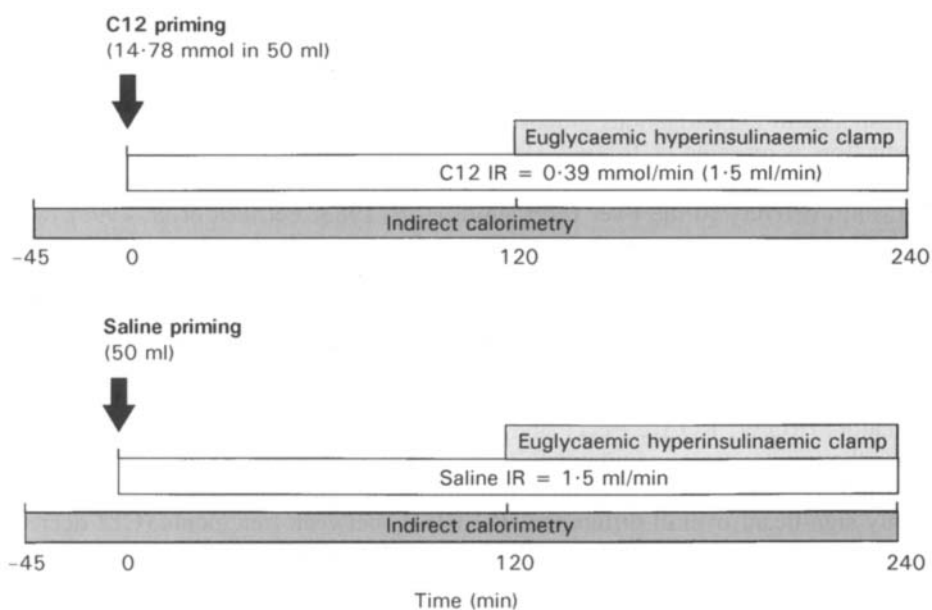


Fig. 1. Experimental design. A priming-constant infusion of dodecanedioic acid (C12) or saline was given for 240 min. After the first 120 min, a euglycaemic hyperinsulinaemic clamp was operated for a further 120 min. Energy expenditure was measured by indirect calorimetry throughout the experiment. IR, infusion rate.

metabolic variable values during C12 or saline infusion in normal controls *v.* NIDDM patients. A *P* value of less than 0.05 was considered statistically significant. It is not possible, by measuring only two variables (VO_2 and VCO_2), to derive simultaneously the amounts of C12, glucose and lipids oxidized because, in this case, the commonly used equations do not hold (there being more unknowns than equations). The effect of C12 administration on metabolism was explored in the following way: RQ Δ 1 and RQ Δ 2 variables were created, expressing respectively the increment in RQ from 0 min to 120 min (i.e. from basal to pre-clamp C12 infusion) and from 120 min to 240 min (i.e. from pre-clamp C12 infusion to end-clamp). At each period (basal, pre-clamp and clamp) results were averaged over 30 min. The EE Δ 1 and EE Δ 2 variables were created in the same way. A repeated-measures ANOVA was then performed on each of the four indicators (RQ Δ 1, RQ Δ 2, EE Δ 1 and EE Δ 2) using treatment (C12 *v.* saline) as within-subjects factor and disease (diabetic *v.* normal) as between-subjects factor.

RESULTS

The fasting insulin concentration in NIDDM patients was about threefold greater than in control subjects. No significant difference was found in the same groups between saline and C12 infusion. Plasma insulin concentration did not show any significant change during the 2 h of C12 infusion preceding the clamp, differences being respectively 1.24 and 5.55% of basal in control and diabetic patients.

During the EHC the average steady state plasma insulin concentrations were 491.6 (SD 14.76) and 503.0 (SD 12.48) pM (C12, control *v.* NIDDM, *P* = NS) and 486.6 (SD 16.68) and 507.6 (SD 14.88) pM (saline, control *v.* NIDDM, *P* = NS). During the insulin clamp

session the steady state plasma glucose concentration was maintained close to the initial values with CV ranging from 3.5 to 4.5 %.

The value of the total body glucose disposal rate (M , in $\mu\text{mol}/\text{kg}_{\text{FFM}}$ per min), which corresponds to the exogenous glucose infusion rate plus possible residual hepatic glucose production, is reported in Table 2. Hepatic glucose output (HGO) was not estimated in the present study. However, attainment of HGO suppression does not require very high levels of insulin delivery to the liver (Ferrannini *et al.* 1983; Petrides *et al.* 1991), which were achieved, in any case, in the present study. Whole-body glucose uptake decreased significantly during C12 infusion in both groups, although this effect was much more evident in NIDDM patients than in controls ($P < 0.01$). In fact, in the face of a 25.9 (SD 12.1) % reduction of glucose uptake observed in healthy volunteers, NIDDM patients displayed a 52.4 (SD 15.8) % decrease ($P < 0.01$).

Table 3 reports the means and standard deviations of the four metabolic difference indicators (RQ Δ 1, RQ Δ 2, EE Δ 1 and EE Δ 2) in the groups studied (normal *v.* diabetics) under the two treatment regimens (C12 *v.* saline). Concerning the decrease in non-protein RQ (npRQ) after 2 h of C12 or saline infusion before the clamp session, ANOVA showed a highly significant overall difference ($P < 0.001$) between treatments (C12 decreasing RQ more than saline) and a significant ($P = 0.024$) interaction between treatment and diagnosis (C12 decreasing RQ more in diabetics than in normal controls). Between basal and pre-clamp periods, energy expenditure increased significantly more under C12 than under saline ($P = 0.002$), irrespective of disease; interaction was not significant. No effect of either disease, treatment or their interaction could be substantiated on the clamp-induced increases of both RQ and EE. The time courses of EE and npRQ values during both saline and C12 infusion, in controls and NIDDM patients, are depicted in Figs. 2 and 3.

The time course of plasma C12 concentration in the two groups under study is represented in Fig. 4: the levels of C12 peaked at about 90 min after starting the infusion and then remained constant until the end of the infusion. The area under the curve of plasma C12 concentration *v.* time was significantly ($P = 0.009$) lower in diabetic patients compared with controls (238.90 (SD 4.06) *v.* 252.94 (SD 5.77) μmol over 240 min). C12 urinary loss was 5.43 (SD 0.48) mmol in the control subjects and 4.26 (SD 0.30) mmol in NIDDM patients ($P = \text{NS}$).

At plasma C12 concentration steady state the amount of C12 infused equals the amount of C12 taken up by the tissues plus C12 urinary loss. Since very small amounts of C12 can be found in the urine (approximately 2–3 % of the administered dose), the steady state rate of C12 tissue uptake was about 0.38 mmol/min, corresponding to energy delivery of 2.65 kJ/min.

DISCUSSION

The present study provides evidence that C12 infusion significantly decreases whole body glucose uptake in normal subjects and in NIDDM patients, although this glucose-sparing effect is much more pronounced in the latter group.

C12 metabolism does not seem to be influenced by plasma insulin concentrations nor to stimulate insulin delivery from the pancreas, contrary to what was observed during MCT infusion (Pi-Sunyer *et al.* 1969). In fact, if on one hand C12 plasma levels were not changed during the hyperinsulinaemic clamp, on the other hand C12 infusion before EHC did not modify plasma insulin levels.

Contrary to C10 (Raguso *et al.* 1994) whose infusion during EHC inhibited to the same extent tissue glucose uptake in controls, insulin-dependent diabetic subjects and obese

Table 2. Basal values of plasma insulin, plasma dodecanedioic acid (C12) in the steady state (mean values taken from 120–240 min), and glucose uptake during euglycaemic hyperinsulinaemic clamp (EHC) in patients with non-insulin-dependent diabetes mellitus (NIDDM) and healthy controls receiving an infusion of C12 or saline†
(Mean values and standard deviations for five subjects per group)

	Controls + saline		Controls + C12		NIDDM patients + saline		NIDDM patients + C12		P ₁ †	P ₂ ‡	P ₃ ‡	P ₄ ‡
	Mean	SD	Mean	SD	Mean	SD	Mean	SD				
Basal plasma insulin (pmol/l)	24.7	4.80	25.6	8.16	62.8	14.70	66.2	15.12	**	**		
EHC plasma insulin (pmol/l)	486.6	16.68	491.6	14.76	507.6	14.88	503.0	12.48				
Whole body glucose uptake, M (µmol/kg _{BFM} per min)	35.3	5.44	25.9	4.55	28.7	10.83	12.6	3.89		**	*	*
% difference of saline v. C12 M			-25.9	12.1			-52.4	15.8		**		
Urinary C12 (mmol)			5.4	0.48			4.3	0.30		**		
Steady state plasma C12 (µmol/ml)			1.2	0.03			1.2	0.01				

* P < 0.05, ** P < 0.01.

† For details of subjects and procedures, see Table 1 and pp. 724–727.

‡ P₁, significance level for the difference between control and NIDDM subjects during the saline sessions; P₂, significance level for the difference between control and NIDDM subjects during C12 infusions; P₃, significance level for the difference between control subjects during the saline and C12 sessions; P₄, significance level for the difference between NIDDM patients during the saline and C12 sessions.

Table 3. Differences between values measured at 120 min and 0 min ($\Delta 1$) and between values measured at 240 min and 120 min ($\Delta 2$) for energy expenditure (EE, kJ/min) and non-protein RQ in patients with non-insulin-dependent diabetes mellitus (NIDDM) and healthy controls infused with dodecanedioic acid (C12) or saline*

(Mean values and standard deviations for five subjects per group)

Infusion ...	Saline		C12	
	Mean	SD	Mean	SD
Controls				
RQ $\Delta 1$	-0.0013	0.0308	-0.0311	0.0384
EE $\Delta 1$	0.040	0.238	0.397	0.340
RQ $\Delta 2$	0.119	0.062	0.098	0.035
EE $\Delta 2$	0.226	0.162	0.163	0.146
NIDDM patients				
RQ $\Delta 1$	0.0131	0.0146	-0.0818	0.0319
EE $\Delta 1$	0.110	0.111	0.382	0.127
RQ $\Delta 2$	0.062	0.029	0.108	0.010
EE $\Delta 2$	0.324	0.262	0.190	0.148

* For details of subjects and procedures, see Table 1 and pp. 724–726. Significances of the differences are discussed on pp. 726–727.

patients, C12 reduced whole-body glucose uptake particularly in patients with NIDDM compared with normal controls. The different behaviour of these two DA might be ascribed to their different kinetics. In fact, C10 is taken up by the tissues to a lower extent than C12, while C10 urinary excretion largely exceeds that of C12 (Mingrone *et al.* 1996); in addition, in the face of the low oxidation rate found for C10, C12 is oxidized in amounts comparable to those of both MCT and LCT (Mingrone *et al.* 1996). C12 plasma concentration area under the curve values were significantly higher in NIDDM patients than in controls; this finding, together with the observation that C12 urinary excretion was lower in diabetic patients, indicates that NIDDM patients have a higher tissue uptake of C12 than controls, in other words that they utilize more C12 with respect to normal controls.

As stated earlier, when compared with normal subjects, NIDDM patients showed a much lower glucose uptake during C12 infusion. The hypothesis can be put forward that preliminary treatment with C12 increased glycogen synthesis via succinic acid formation from C12 β -oxidation and, as a consequence, increased the size of glycogen stores available during the hyperinsulinaemic clamp. As a function of the larger size of the glycogen stores, glycogen synthesis from glucose during the EHC would be diminished. Furthermore, this effect might be more evident in NIDDM patients than in controls because of their deficient glycogen stores. It is an accepted fact that in obese individuals, and particularly in NIDDM patients, there is an inhibition of the enzyme glycogen synthase (Beck-Nielsen *et al.* 1992) coupled with a decreased activity of the PDH complex (Kelley & Mandarino, 1990; Beck-Nielsen *et al.* 1992). It has been hypothesized that in diabetes mellitus there is a shift in substrate utilization from carbohydrates to lipids (Kiens *et al.* 1987): a large proportion of the increase in lipid oxidation, characteristic of obesity and obese NIDDM, is accounted for by an increase in intramuscular triacylglycerol mobilization. An impaired activity of PDH (Beck-Nielsen 1989) and an increased activity of β -oxidation enzymes (Randle *et al.* 1964) have been described in these patients. Furthermore, the activation of glycogen synthase by insulin is decreased in obese and

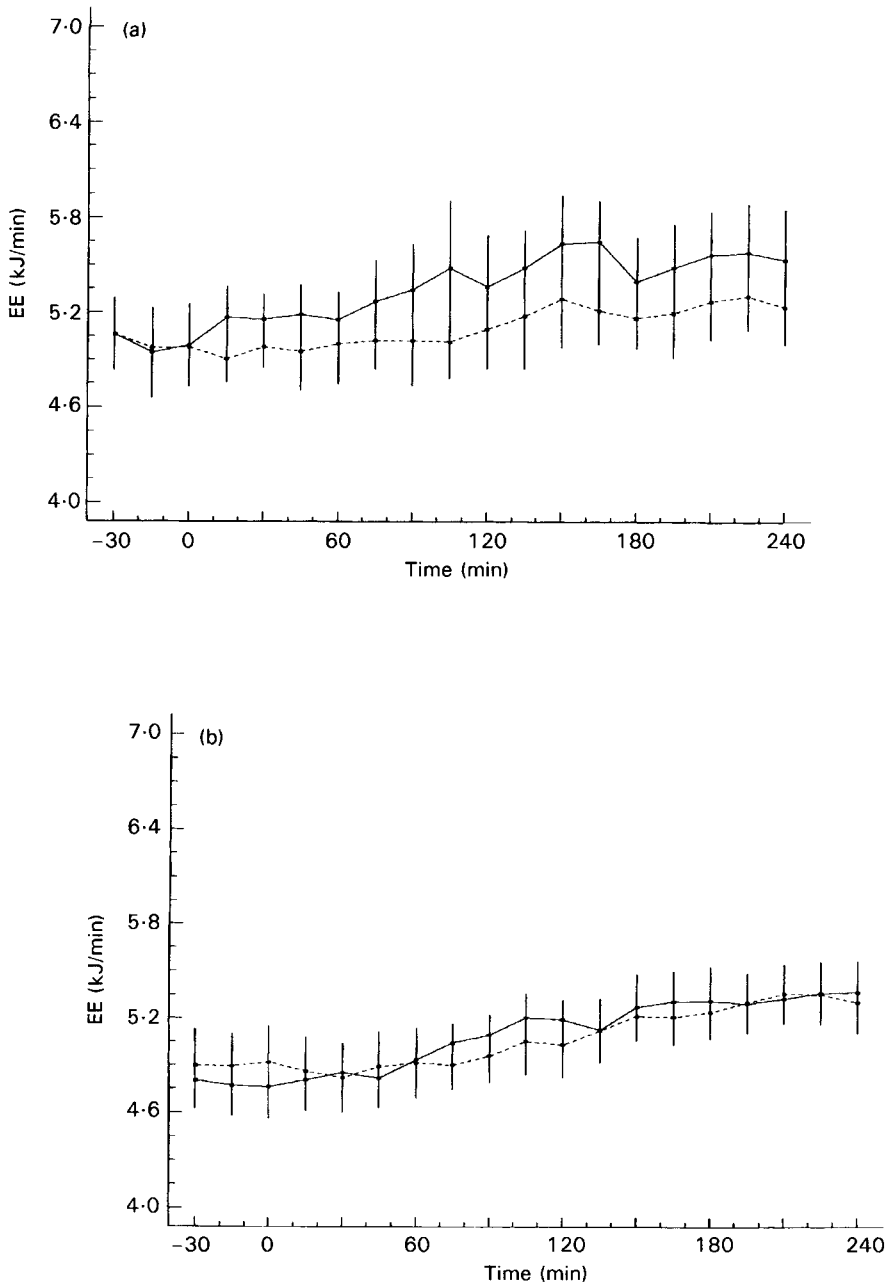


Fig. 2. Time course of energy expenditure (EE, kJ/min) during 240 min infusion of saline (---) or dodecanedioic acid (—), in (a) control subjects and (b) subjects with non-insulin-dependent diabetes mellitus. Values are means for five subjects, with standard deviations represented by vertical bars.

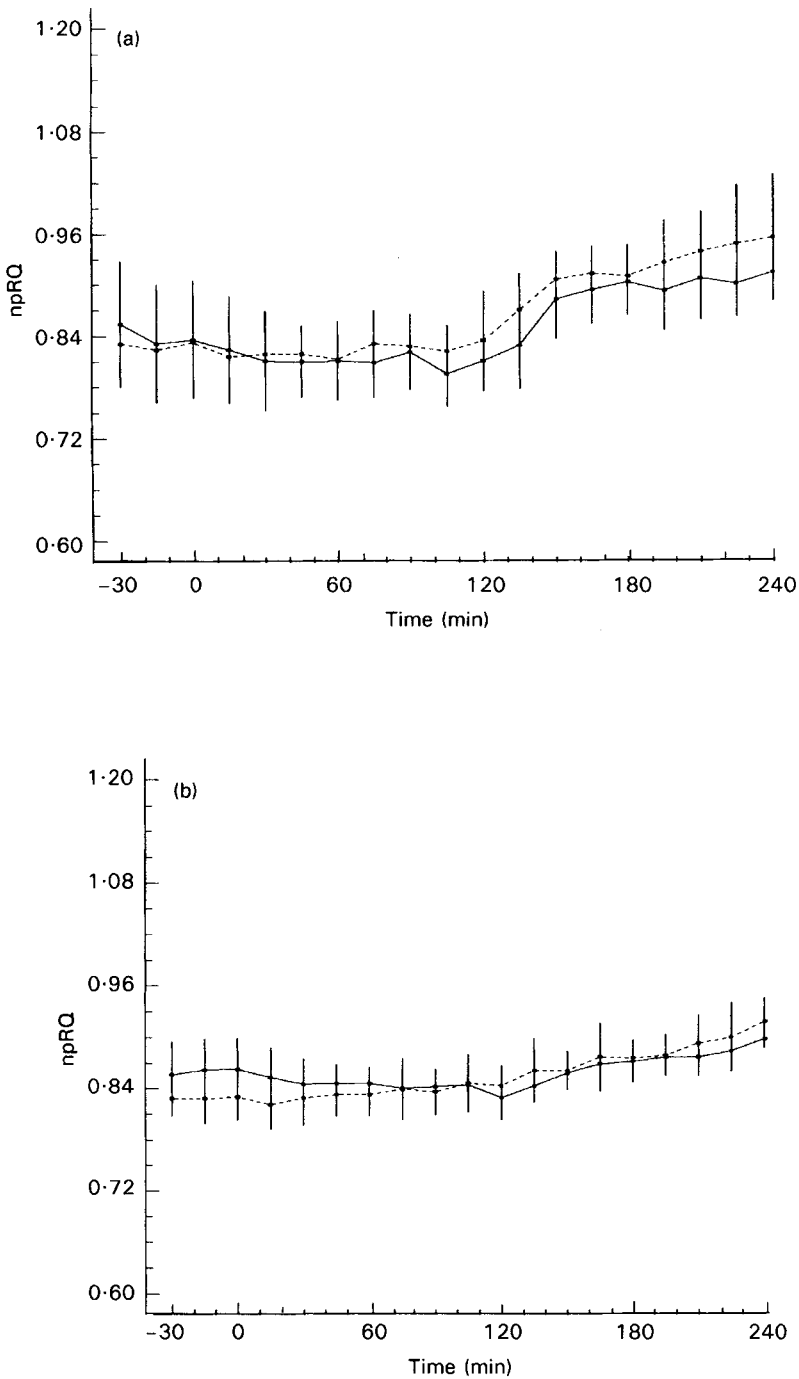


Fig. 3. Time course of non-protein RQ (npRQ) values during 240 min infusion of saline (---) or dodecanedioic acid (—) in (a) control subjects and (b) subjects with non-insulin-dependent diabetes mellitus. Values are means for five subjects, with standard deviations represented by vertical bars.

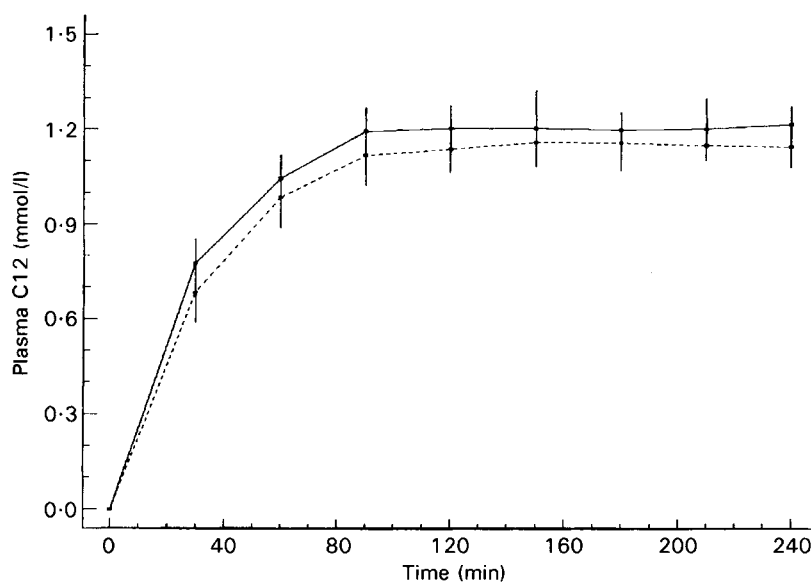


Fig. 4. Time course of plasma dodecanedioic acid (C12) concentration in control subjects (—) and subjects with non-insulin-dependent diabetes mellitus (- - -) during 240 min infusion of C12. Values are means for five subjects, with standard deviations represented by vertical bars.

NIDDM patients during the EHC compared with control subjects (Damsbo *et al.* 1991) with reduced storage of glycogen. The decreased activation of glycogen synthase by insulin in these patients is related to a decreased activation of glycogen synthase phosphatase (Freymond *et al.* 1988). Insulin-dependent PDH activity also appears to be reduced in NIDDM patients (Beck-Nielsen *et al.* 1987) and this reduced enzymic activity is considered to be the cause of the reduced insulin-stimulated glucose oxidation observed in these patients (Beck-Nielsen *et al.* 1987).

Unfortunately, the glucose and lipid oxidation rates could not be computed in the present study from the energy expenditure and the nprQ since the infusion of a third fuel substrate, C12, took place during the experimental session. However, the lower nprQ observed during C12 infusion compared with saline infusion, nearer the theoretical value of RQ for C12 (0.774), suggests that C12 is readily oxidized by the cells.

The inhibitory role of increased FFA levels on glucose disposal, mediated by an increased substrate-driven lipid oxidation, was first hypothesized by Randle *et al.* (1964). Subsequently, it has been shown that the infusion of triacylglycerol emulsions in man determines a decrease in both oxidative and non-oxidative (Bonadonna *et al.* 1989) glucose metabolism. Bonadonna *et al.* (1989) showed that the metabolic competition between lipid and glucose is time-dependent in normal subjects, and that mitochondrial oxidative processes are affected earlier than the cytosolic metabolic pathway leading to glucose storage. These data were confirmed by Boden *et al.* (1991) in a study where an infusion of Intralipid (a parenteral feed containing LCT and phospholipids) was associated with EHC. These authors demonstrated that changes in glucose metabolism due to lipids do not result from an immediate competition between glucose and fatty acids, while glucose oxidation is directly affected by changes in lipid oxidation. Experimental data in the present paper indicate that C12 shows metabolic behaviour similar to conventional lipid substrates.

In conclusion, this study demonstrates for the first time in the literature that C12 significantly reduces glucose uptake in both normal controls and NIDDM patients, although this sparing effect on glucose uptake is much more pronounced in diabetic patients. These data suggest that C12 might be a useful alternative substrate in parenteral nutrition, which increases glycogen stores in those clinical conditions, like decompensated NIDDM, in which reduced insulin-induced glucose uptake and oxidation are observed.

REFERENCES

- Bach, A. C. & Babayan, V. K. (1982). Medium-chain triglycerides: an update. *American Journal of Clinical Nutrition* **36**, 950–962.
- Beck-Nielsen, H. (1989). Insulin resistance in skeletal muscles of patients with diabetes mellitus. *Diabetes Metabolism Review* **5**, 487–493.
- Beck-Nielsen, H., Vaag, A., Damsbo, P., Handberg, A., Nielsen, O. H., Henriksen, J. E. & Thye-Rønn, P. (1992). Insulin resistance in skeletal muscles in patients with NIDDM. *Diabetes Care* **15**, 418–429.
- Beck-Nielsen, H., Wright, K., Verity, L., Bell, J. M., Kolterman, P. & Mandarino, L. J. (1987). Reduced glucose oxidation and pyruvate-dehydrogenase activity (PDH) in Type I diabetics (insulin-dependent) in poor control. *Diabetes* **36**, Suppl. 1, 30A.
- Bertuzzi, A., Gandolfi, A., Salinari, S., Mingrone, G., Arcieri-Mastromattei, E., Finotti, E. & Greco, A. V. (1991). Pharmacokinetic analysis of azelaic acid disodium salt: a proposed substrate for total parenteral nutrition. *Clinical Pharmacokinetics* **20**, 411–419.
- Bertuzzi, A., Mingrone, G., De Gaetano, A., Gandolfi, A., Greco, A. V. & Salinari, S. (1997). Kinetics of dodecanedioic acid and effect of its administration on glucose kinetics in rats. *British Journal of Nutrition* **78**, 143–153.
- Boden, G., Jadali, F., White, J., Liang, Y., Mozzoli, M., Chen, X., Coleman, E. & Smith, C. (1991). Effects of fat on insulin-stimulated carbohydrate metabolism in normal man. *Journal of Clinical Investigation* **88**, 960–966.
- Bonadonna, R. C., Zych, K., Boni, C., Ferrannini, E. & De Fronzo, R. A. (1989). Time dependence of the interaction between lipid and glucose in man. *American Journal of Physiology* **257**, E49–E56.
- Bonora, E., Del Prato, S., Bonadonna, R. C., Gulli, G., Solini, A., Shank, M. L., Ghiatas, A. A., Lancaster, J. L., Kilcoyne, R. F., Alyassin, A. M. & De Fronzo, R. A. (1992). Total body fat content and fat topography are associated differently with in vivo glucose metabolism in nonobese and obese nondiabetic women. *Diabetes* **41**, 1151–1159.
- Damsbo, P., Vaag, A., Hother-Nielsen, O. & Beck-Nielsen, H. (1991). Reduced glycogen synthase activity in skeletal muscle from obese patients with and without Type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **34**, 239–245.
- De Fronzo, R. A., Tobin, J. D. & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *American Journal of Physiology* **273**, E214–E223.
- De Gaetano, A., Castagneto, M., Mingrone, G., Gangeri, G., Sganga, G., Tataranni, P. A., Raguso, C. & Greco, A. V. (1994). Kinetics of medium chain triglycerides and free fatty acids in healthy volunteers and surgically stressed patients. *Journal of Parenteral and Enteral Nutrition* **18**, 134–140.
- Ferrannini, E. (1988). The theoretical basis of indirect calorimetry: a review. *Metabolism* **3**, 287–301.
- Ferrannini, E., Barrett, E. & Bevilacqua, S. (1983). Effect of fatty acids on glucose production and utilization in man. *Journal of Clinical Investigation* **72**, 1737–1747.
- Freymond, D., Bogardus, C., Okubo, M., Stone, K. & Mott, D. (1988). Impaired insulin-stimulated muscle glycogen synthase activation in vivo in man is related to low fasting glycogen synthase phosphatase activity. *Journal of Clinical Investigation* **82**, 1503–1509.
- Greco, A. V. & Mingrone, G. (1995). Dicarboxylic acids, an alternative fuel substrate in parenteral nutrition: an update. *Clinical Nutrition* **14**, 143–148.
- Johnson, R. C. & Cotter, R. (1986). Metabolism of medium-chain triacylglycerol lipid emulsion. *Nutrition International* **2**, 150–158.
- Kelley, D. E. & Mandarino, L. J. (1990). Hyperglycemia normalizes insulin-stimulated skeletal muscle glucose oxidation and storage in noninsulin-dependent diabetes mellitus. *Journal of Clinical Investigation* **86**, 1999–2007.
- Kiens, B., Essen-Gustavson, B., Gad, P. & Lithell, H. (1987). Lipoprotein lipase activity and intramuscular triglyceride stores after long-term high-fat and high-carbohydrate diets in physically trained men. *Clinical Physiology* **7**, 1–9.
- Kou, Y. & Tserng Shiow-Jen, J. (1991). Metabolic conversion of dicarboxylic acids to succinate in rat liver homogenates. *Journal of Biological Chemistry* **266**, 2924–2929.
- Mingrone, G., De Gaetano, A., Greco, A. V., Benedetti, G., Capristo, E. & Castagneto, M. (1996). Plasma clearance and oxidation of dodecanedioic acid in man. *Journal of Parenteral and Enteral Nutrition* **20**, 38–42.

- Mingrone, G., Greco, A. V., De Gaetano, A., Tataranni, P. A., Raguso, C. & Castagneto, M. (1994). Pharmacokinetic profile of dodecanedioic acid, a proposed alternative fuel substrate. *Journal of Parenteral and Enteral Nutrition* **18**, 225–230.
- Petrides, A. S., Groop, L. C., Riely, C. A. & De Fronzo, R. A. (1991). Effect of physiologic hyperinsulinemia on glucose and lipid metabolism in cirrhosis. *Journal of Clinical Investigation* **88**, 561–570.
- Pi-Sunyer, F. X., Hashim, S. A. & Van Itallie, T. B. (1969). Insulin and ketone responses to ingestion of medium and long-chain triglycerides in man. *Diabetes* **18**, 96–100.
- Raguso, C., Mingrone, G., Greco, A. V., Tataranni, A., De Gaetano, A. & Castagneto, M. (1994). Dicarboxylic acids and glucose utilization in humans: effect of sebacate. *Journal of Parenteral and Enteral Nutrition* **18**, 9–13.
- Randle, P. J., Garland, P. B., Hales, C. N. & Newsholme, E. A. (1964). The glucose fatty acid cycle and diabetes mellitus. In *Ciba Foundation, Colloquia on Endocrinology 15, Aetiology of Diabetes Mellitus and its Complications*, pp. 192–216. London: J. & A. Churchill, Ltd.
- Sandström, R., Hyltander, A., Korner, U. & Lundholm, K. (1993). Structured triglycerides to postoperative patients: a safety and tolerance study. *Journal of Parenteral and Enteral Nutrition* **17**, 153–157.