Effect of Oral Sebacic Acid on Postprandial Glycemia, Insulinemia, and Glucose Rate of Appearance in Type 2 Diabetes

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OBJECTIVE — Dicarboxylic acids are natural products with the potential of being an alternate dietary source of energy. We aimed to evaluate the effect of sebacic acid (a 10-carbon dicarboxylic acid; C10) ingestion on postprandial glycemia and glucose rate of appearance ($R_g$) in healthy and type 2 diabetic subjects. Furthermore, the effect of C10 on insulin-mediated glucose uptake and on GLUT4 expression was assessed in L6 muscle cells in vitro.

RESEARCH DESIGN AND METHODS — Subjects ingested a mixed meal (50% carbohydrates, 15% proteins, and 35% lipids) containing 0 g (control) or 10 g C10 in addition to the meal or 23 g C10 as a substitute of fats.

RESULTS — In type 2 diabetic subjects, the incremental glucose area under the curve (AUC) decreased by 42% ($P < 0.05$) and 70% ($P < 0.05$) in the 10 g C10 and 23 g C10 groups, respectively. At the largest amounts used, C10 reduced the glucose AUC in healthy volunteers also. When fats were substituted with 23 g C10, AUC of $R_g$ was significantly reduced on the order of 18% ($P < 0.05$) in both healthy and diabetic subjects. The insulin-dependent glucose uptake by L6 cells was increased in the presence of C10 (38.7 ± 10.3 vs. 11.4 ± 5.4%, $P = 0.026$). This increase was associated with a 1.7-fold raise of GLUT4.

CONCLUSIONS — Sebacic acid significantly reduced hyperglycemia after a meal in type 2 diabetic subjects. This beneficial effect was associated with a reduction in glucose $R_g$, probably due to lowered hepatic glucose output and increased peripheral glucose disposal.

The World Health Report launched in 2002 by the World Health Organization advised that more than 1 billion adults worldwide are overweight and at least 300 million are clinically obese. Type 2 diabetes can be considered a threatening obesity-related disease because hyperglycemia causes relevant complications such as micro- and macroangiopathy. Patients with type 2 diabetes exhibit increased hepatic glucose output, which is identified as the main cause of fasting hyperglycemia and is associated with impaired plasma glucose clearance (1) and reduced hepatic synthesis of glycogen of ~25–45% compared with that in nondiabetic subjects (2). Increased hepatic gluconeogenesis has been considered to be responsible for elevated hepatic glucose output in type 2 diabetes (3). When glycogen is available in adequate amounts, there is an autolimitation of hepatic glucose production. In diabetes, a breakdown of this autoregulation may occur if glycogenolysis is limited by glycogen depletion (4).

Jenkins et al. (5) have shown that spreading the nutrient load over a longer period of time by increased meal frequency, the so-called nibbling diet, is beneficial in terms of reduction of circulating levels of glucose, insulin, and free fatty acids in type 2 diabetes. Thus, the availability of snacks poor in fat and that do not induce hyperglycemia and/or overstimulate insulin secretion would be a good tool in the diet of insulin-resistant, type 2 diabetic subjects.

Dicarboxylic acids are naturally occurring substances produced by both higher plants and animals via ω-oxidation of fatty acids (6, 7). In plants, long-chain dicarboxylic acids are components of natural protective polymers, cutin and suberin, which support biopolymesters involved in waterproofing the leaves and fruits, regulating the flow of nutrients among various plant cells and organs, and minimizing the deleterious impact of pathogens (7). In animals and humans, medium chain dicarboxylic acids, which include prevalently sebacic (C10) and docosanedioic (C12) acids, derive from the β-oxidation of longer chain dicarboxylic acids (8). Long-chain dicarboxylic acids, in turn, are formed from the correspondent fatty acids by ω-oxidation in the microsomal membranes (9) or are taken in as a substitute but in addition to readily available carbohydrates, on glucose homostasis has never been studied.

We have shown previously that medium-chain dicarboxylic acids represent a suitable alternate energy substrate to glucose in clinical conditions with marked insulin resistance and/or impaired aerobic glycolysis (10). Interestingly, in type 2 diabetes, medium-chain dicarboxylic acids are rapidly oxidized, do not stimulate insulin secretion, and reduce muscle fatigue (11). Nevertheless, the effect of C10 or C12, not as a substitute but in addition to available carbohydrates, on glucose homostasis has never been studied.

On this basis, our aim was to investigate the effect of oral administration of C10 on postprandial glycemia, insulinema, and glucose rate of appearance ($R_g$) in type 2 diabetic subjects compared with that in healthy volunteers. To further elucidate the mechanism of action of sebacic acid in diabetes, insulin-mediated glucose uptake and GLUT4 protein expression were assessed in L6 cells in vitro.

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RESEARCH DESIGN AND METHODS — From October 2006 to September 2007, 10 obese type 2 diabetic subjects and 10 healthy volunteers were enrolled. Women in fertile age were asked to avoid pregnancy during the study protocol. In addition, before starting each experimental session a pregnancy test was performed, and pregnant women were excluded from the investigation. All women were studied in the follicular phase of their menstrual cycle. None of the subjects previously or currently had major endocrinological, renal, cardiac, respiratory, liver, or gastrointestinal disorders.

All subjects underwent a 75-g oral glucose tolerance test to measure the Matsuda index, as an index of insulin resistance (12). None of the diabetic subjects were taking oral hypoglycemic agents or were diagnosed with type 2 diabetes 2–5 years before the study and their A1C was 5.5–7.5%.

The protocol was approved by the ethics committee of the Catholic University, School of Medicine, in Rome, Italy. All of the subjects signed a written informed consent form before starting the study.

Chemicals
Sebacic acid was purchased from Sigma-Aldrich (St. Louis, MO) and purified by Real s.r.l. (Como, Italy). It was pyrogen-free and contaminant-free with a degree of purification (gas-liquid chromatography/mass spectrometry) of 99.5%. 6,6-d-glucose was purchased from Mass Trace (Woburn, MA).

Experimental protocol
The subjects were studied on three separate occasions in a randomized way at a distance of 1 month. After an overnight fast, during which subjects were instructed not to drink, the study was initiated at 8:00 A.M. An indwelling catheter was placed into an antecubital vein for isotope infusion. A second catheter was inserted retrogradely into a wrist vein of the ipsilateral hand, and the hand was placed into a heated box (60°C) to achieve arterIALIZATION of venous blood. Basal rates of glucose turnover were assessed after 2 h of an adjusted primed (22 μmol/kg), continuous (0.22 μmol kg⁻¹ min⁻¹) infusion of 6,6-D₂-glucose (Cambridge Isotope Laboratory, Boston, MA) (13). Two hours after the beginning of the session, the subjects ingested a mixed meal (see Table 1 for meal composition).

Blood samples for the determination of 6,6-D-glucose enrichment, plasma C-peptide, and plasma insulin concentrations were obtained before the tracer infusion was started and every 10 min during the last 20 min of the 2 h preceding the meal ingestion (equilibration period). Starting from the meal ingestion time, plasma samples were obtained every 20 min for 5 h. Therefore, overall the study duration was 7 h or 420 min.

Each subject voided to empty his/her bladder before starting the experimental protocol. The subsequent 24-h urine samples were collected in a container with 0.1% sodium azide to prevent bacterial growth.

Body composition
After subjects voided and were weighed, body composition was assessed by dual-x-ray absorptiometry (Lunar Prodigy; GE Lunar, Madison, WI) to measure fat-free mass, fat mass, and percentage of fat mass.

Analytical methods
Plasma insulin and C-peptide were measured in duplicate by double-antibody radioimmunoassays (Linco Research, St. Charles, MO). Intra-assay variation was 4.9 and 2.4%, and inter-assay variation was 5.9 and 7.1%, respectively. Plasma glucose was monitored immediately after blood withdrawal with an Analox GM9 glucose-analyzer (Beckman Instruments, Fullerton, CA).

Plasma glucose enrichment due to 6,6-d-glucose was determined by gas chromatography-mass spectrometry on a gas chromatograph 5890/mass spectrometer 5972 (Hewlett Packard, Palo Alto, CA) equipped with a 30-m capillary column, as described by Wolfe (13). In brief, 100 μl of plasma was deproteinized using 2 ml of cold methanol, and the supernatant was dried and derivatized using acetic anhydride-pyridine (1:1) to form penta-acetate glucose. The samples were then dried again and dissolved with ethyl acetate for injection into the gas chromatograph-mass spectrometer. The fragments 200, 201, and 202 were monitored, and enrichment was calculated as the ratio of 202 to 200. C10 analysis was performed as described previously (14).

Calculations
A physiological and isotopic steady state was achieved during the last 20 min of the basal period and of the meal period; therefore, the glucose rate of appearance ($R_a$), which is equivalent to endogenous glucose production, and rate of disappearance ($R_d$) were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio. During the meal, glucose non–steady-state conditions prevail, and, thus, $R_a$ and $R_d$ were calculated using the trapezoidal method. AUC of meal glucose clearance was normalized to AUC of insulin to account for different insulin concentrations and of glucose $R_a$ and clearance were calculated using the trapezoidal method. AUC of meal glucose clearance was normalized to AUC of insulin to account for different insulin concentrations observed during the three tests. Insulin secretion rate was computed by C-peptide deconvolution (16).

Cell culture and glucose uptake
L6 myoblasts were grown and maintained in α-minimal essential medium containing 2% FBS and 1% antibiotic-antimycotic mixture in an atmosphere of 5% CO₂ at 37°C as described elsewhere (17,18). The cells were cultivated overnight in 12-well plates with or without 0.2 mmol/l albumin-bound C10 and serum starved for 4 h before being incubated for 20 min with 10 mmol/l insulin. Subsequently, myoblasts were washed twice, and glucose transport was assayed in HEPES-buffered saline solution (140 mmol/l NaCl, 20 mmol/l HEPES-sodium, 2.5 mmol/l MgSO₄, 1 mmol/l CaCl₂, and 5 mmol/l KCl, pH 7.4) containing 10 μM 2-deoxy-D-glucose (0.5 μCi ml⁻¹ 2-deoxy-D-[³H]glucose). The incubation medium was aspirated, the cells were washed with ice-cold saline, and 1 ml of NaOH...
(0.05 mol/l) was added to each well. Cell lysates were transferred to scintillation vials for radioactivity counting. Nonspecific uptake was determined in the presence of cytochalasin B (10 μM) and was subtracted from all values.

**Immunoprecipitation and Western blot**

To measure the total GLUT content, cell lysates were subjected to 10% SDS-PAGE and Western blotting (19). Filters were incubated with GLUT4 antibodies (Abnova, Prodotti Gianni, Milano, Italy) for 14 h at 4°C and then with peroxidated anti-antibodies (Amersham Biosciences, Cologno Monzese, Italy) for 1 h at room temperature. GLUT4 was finally revealed by detection of chemoluminescence with autoradiography.

**Statistical analysis**

Data are presented as means ± SD, unless otherwise specified. For the clinical study differences among the three sessions were analyzed by nonparametric (Wilcoxon's signed-rank test) tests for paired samples corrected for Bonferroni inference. Analyses were performed using SPSS for Windows (version 13.0; SPSS, Chicago, IL). Significance was accepted at *P* < 0.05.

For the in vitro study, Student's *t* test was used to evaluate the significance of the effect of sebacic acid and insulin on glucose transport. Results are expressed as means ± SD of five different experiments, each performed in triplicate; SE is reported for percent values.

**RESULTS**— Type 2 diabetic patients (five women and five men) and control subjects (four women and six men) were matched for sex distribution, age (52.1 ± 6.98 vs. 47.2 ± 6.03 years), and BMI (27.98 ± 4.08 vs. 26.63 ± 3.03 kg/m²). Diabetic subjects were insulin resistant with a Matsuda index of 5.65 ± 1.41 compared with 15.29 ± 2.63 in control subjects (*P* < 0.01).

All subjects ingested a mixed meal (see Research Design and Methods) containing either 0 g (control) and an additional 10 g C10 or 23 g C10 as a substitute of dietary fats. The time courses of plasma glucose and insulin in healthy control subjects are shown in Fig. 1. The ingestion of C10 together with the meal reduced to some extent the glycemic peak, but the glucose incremental AUC was significantly reduced only after 23 g C10 (Fig. 2). The insulin peak level was clearly reduced in both C10 groups, attaining a value of −39% in the 10 g C10 group and −71% in the 23 g C10 group (both *P* < 0.01) (Fig. 2).

The effect of C10 on plasma glucose and insulin time courses in type 2 diabetic subjects is depicted in Fig. 1. A reduction in the incremental glucose AUCs of 42 and 70% was observed in the 10 g C10 (*P* = 0.037) and 23 g C10 (*P* = 0.045) groups, respectively (Fig. 2). Similarly to what was observed in control subjects, the incremental AUCs of insulin were decreased by 39% after 10 g C10 intake and by 64% after 23 g C10 intake, respectively (*P* < 0.05) (Fig. 2C). Insulin secretion rate was similar across the three studies in both type 2 diabetic patients and healthy volunteers (Fig. 2).

Glucose Rₐ AUC was decreased similarly in control and diabetic subjects after C10 supplement (by ~18% after 23 g C10, *P* < 0.05) (Fig. 2D), whereas glucose clearance, which is an index of peripheral insulin sensitivity, tended to increase after C10 supplementation, but reached statistical significance only in control subjects after 23 g C10 (Fig. 2D).

As shown in Fig. 1, the concentration of plasma C10 in diabetic patients (Fig. 1F) tended to be higher than that in control subjects (Fig. 1E), without reaching statistical significance, however, and peaked at the same times. Furthermore, in diabetic subjects the two curves (10 vs. 23 g) overlapped up to 320 min after the meal, and then the concentration of C10 peaked at the same times. The effect of C10 on plasma glucose and insulin time courses in type 2 diabetic subjects is depicted in Fig. 1. A reduction in the incremental glucose AUCs of 42 and 70% was observed in the 10 g C10 (*P* = 0.037) and 23 g C10 (*P* = 0.045) groups, respectively (Fig. 2). Similarly to what was observed in control subjects, the incremental AUCs of insulin were decreased by 39% after 10 g C10 intake and by 64% after 23 g C10 intake, respectively (*P* < 0.05) (Fig. 2C). Insulin secretion rate was similar across the three studies in both type 2 diabetic patients and healthy volunteers (Fig. 2).

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declined slower in the patients fed 23 g C10 compared with those fed 10 g C10. The in vitro experiments were conducted by using a concentration of 0.2 mmol/l, on the basis of the average plasma concentration of C10 reached in humans after C10 ingestion. In fact, a level of 40 μg/ml plasma C10, whose molecular weight is 202, corresponds to a concentration of 0.198 mmol/l.

The insulin-dependent glucose uptake from L6 cells increased more in the presence of C10 (1.83 ± 0.40 vs. 1.37 ± 0.28 pmol/mg • 10 min [P = 0.01] vs. 1.73 ± 0.19 vs. 1.57 ± 0.14 pmol/mg • 10 min [P = 0.019]), corresponding to a percent increase of 38.7 ± 10.3% (mean ± SE) in presence of C10 vs. 11.4 ± 5.4% in its absence (P = 0.026). This increase was associated with a 1.74 ± 0.27-fold increase of glucose transporter GLUT4 (Fig. 3), in agreement with the slight increase in glucose clearance observed after C10 (Fig. 2D).

**CONCLUSIONS** — The major finding of the present study is that sebacic acid significantly reduces postprandial hyperglycemia in type 2 diabetic subjects. This reduction occurs when C10 is either added to a mixed meal or substituted for lipids in an equivalent caloric manner. The above effect is less pronounced in healthy control subjects. In L6 cells, C10 significantly increases insulin-mediated glucose uptake and GLUT4 expression.

There is general agreement that postprandial hyperglycemia in type 2 diabetic subjects mainly depends on a defect in the endogenous glucose output (EGO) suppression. The defect in suppressing EGO after a meal has been estimated to account for 35–50% of the excess circulating glu-
expression. Therefore, with all the precautions of incomplete information, we can remark that sebacic acid induces a significant reduction in the glucose $R_n$, which can be ascribed to a reduced hepatic glucose output and/or to reduced glucose absorption.

A possible mechanism through which sebacic acid might reduce EGO after a meal could be related to its mitochondrial $\beta$-oxidation with production of succinic acid (10), which enters the mitochondrial tricarboxylic acid cycle. Reduced in vivo mitochondrial function in skeletal muscle under resting conditions as well as during postexercise recovery has been observed in type 2 diabetic subjects (23). This impaired mitochondrial oxidative capacity seems to be attributable to a reduced mitochondrial tricarboxylic acid cycle flux (24). C10 might overcome this defective mitochondrial tricarboxylic acid function in diabetic skeletal muscles by providing succinic acid and thus increasing whole-body glucose clearance, skeletal muscle tissue being the major contributor to glucose uptake.

In this regard, we have observed that in L6 cells, 0.2 mmol/l C10 significantly increased glucose uptake by $\sim$30% compared with insulin alone and increased GLUT4 protein expression 1.7-fold, probably improving insulin signaling and the docking of GLUT4 to the cell membrane. This in vitro effect of C10 might partially explain the marked decrease in plasma glucose concentrations because it is well known that the skeletal muscle mass represents the most important consumer of glucose.

We note that a true dose-response effect of C10 on glucose disposal was not present in our investigation. In this regard, it is necessary to point out that, as previously demonstrated (25), the whole-body rate of C10 tissue uptake in male healthy volunteers was $180.9 \pm 4.5 \mu$mol/min, which equals $\sim 0.5$ mg/kg body wt/min or 2.1 g/h in a subject weighing 70 kg. Therefore, in the 5 h after C10 ingestion with the mixed meal, 10.5 g would be metabolized, thus explaining why the effects obtained with 10 or 23 g were similar.

In summary, although more detailed studies are required to elucidate the mechanism of action of sebacic acid in type 2 diabetes, this study provides relevant clues about its effect in reducing glucose circulating levels when added to standard meals.

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A.I., D.G., and A.F. participated in the study coordination, participant enrollment, medical oversight of participants, and data collection. A.G. participated in the analysis of stable isotopes and reviewed/editing the manuscript. C.C. participated in the in vitro studies. C.B. reviewed/editing the manuscript. K.M. participated in the conception and design of the study and reviewed/editing the manuscript. G.M. participated in the conception and design of the study and in the data analysis and interpretation and wrote the article.

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