Colonic fermentation from lactulose inhibits lipolysis in overweight subjects

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Ferchaud-Roucher, V. E. Pouteau, H. Piloquet, Y. Zaïr, and M. Krempf. Colonic fermentation from lactulose inhibits lipolysis in overweight subjects. Am J Physiol Endocrinol Metab 289: E716–E720, 2005; doi:10.1152/ajpendo.00430.2004.—One of the strategies to prevent insulin resistance is to reduce circulating free fatty acids (FFA). The aim of this study is to assess the effect of an oral lactulose load on fatty acid metabolism in overweight subjects. Eight overweight subjects received a primed constant intravenous infusion of [1-13C]acetate and of [1,1,2,3,3-2H5]glycerol for 9 h. After 3 h of tracer infusion, patients ingested 30 g lactulose, or saline solution. Arterialized blood samples were collected every 20 min. Basal plasma concentrations of acetate were similar before and between oral treatments as well as glycerol and FFA concentrations. Plasma acetate turnover was 11.4 ± 2.4 vs. 10.7 ± 1.4 μmol·kg−1·min−1 [not significant (NS)], and plasma glycerol turnover was 3.8 ± 0.4 vs. 4.8 ± 1.9 μmol·kg−1·min−1 (NS). After lactulose ingestion, acetate concentration increased twofold and then decreased to baseline. Acetate turnover rate increased to 15.5 ± 2.2 μmol·kg−1·min−1 after lactulose treatment, whereas it was unchanged after saline treatment (10.3 ± 2.2 μmol·kg−1·min−1, P ≤ 0.0001). In contrast, FFA concentrations decreased significantly after lactulose ingestion and then increased slowly. Glycerol turnover decreased after lactulose ingestion compared with saline, 2.8 ± 0.4 vs. 3.5 ± 0.3 μmol·kg−1·min−1 (P ≤ 0.05). A significant negative correlation was found between glycerol and acetate turnover after lactulose treatments (r = −0.78, P ≤ 0.02). These results showed in overweight subjects a short-term decrease in FFA level and glycerol turnover after lactulose ingestion related to a decrease of lipolysis in close relationship with an increase of acetate production.

Stable isotopes; acetate; turnover; obesity; lipolysis; nonabsorbable carbohydrates

Insulin resistance is a key factor in the pathogenesis of type 2 diabetes and cardiovascular diseases in obese patients (28). One of the strategies to prevent insulin resistance is to reduce plasma free fatty acids (FFA; see Refs. 9, 19, and 20). Obesity and insulin resistance are commonly associated with elevated plasma FFA concentrations, leading to lipotoxicity and metabolic complications. In the liver, FFA interfere with insulin suppression of hepatic glucose production (5). In peripheral glucose-dependent cells, lipotoxicity or high plasma FFA concentration inhibits insulin action on glucose transport through cell membranes and enhances peripheral insulin resistance. Lowering elevated FFA could reduce insulin resistance.

An improvement of insulin sensitivity has been reported in insulin-resistant middle-aged men with a moderate alcohol consumption (22) and in healthy men and women (8). Abramson and Arky (1) and Siler et al. (23) have shown that plasma acetate derived from ethanol inhibits lipolysis and decreases plasma FFA. Acetate production from bacterial fermentation of undigestible carbohydrates in the human colon could be another option to reduce plasma FFA and to prevent or reverse insulin resistance. To test this hypothesis, we have used lactulose, a nonabsorbable carbohydrate that is used to regulate intestinal transit and is entirely fermented in the colon.

The aim of this study was to assess the effect of lactulose intake on fatty acid metabolism in overweight subjects. We determined this using the stable isotope dilution principle, whole body acetate production rate, plasma glycerol appearance rate, and FFA concentrations that reflect lipolysis in adipose tissue (6) after an oral lactulose treatment in overweight volunteers.

Subjects and Methods

Subject Characteristics

Eight overweight volunteers (body mass index ≥ 28.5 kg/m2 and height-to-tip ratio 0.9 ± 0.1) without diabetes (HbA1c ≤ 6%) were involved in this study. Most of the subjects (6 females and 2 males, 36.6 ± 4.5 yr old) were insulin resistant, as defined by the homeostasis model assessment (HOMA) index > 2 (a mathematical estimate of insulin sensitivity based on fasted glucose and insulin concentrations: HOMA = glucose × insulin/22.5 (12); mean HOMA of 8 volunteers = 3.8 ± 1.1). Before the study (3 days), the subjects were asked to consume a low-fiber diet (<5 g/day) and to maintain a normal physical activity. Each volunteer was studied during both lactulose and placebo (saline solution) administration. The administration of placebo and lactulose was randomized. The possible risks were carefully explained to every subject before written consent was obtained. The experimental protocol was approved by the Ethical Committee of the University Hospital of Nantes (France).

Study Design

On the morning of the experiment, two short polyethylene catheters (20 gauge; Vigon, Paris, France) were inserted in the antecubital vein for tracer infusion and on the wrist of the second arm to sample venous blood. To collect arterialized venous blood, the hand was heated at 55°C in the isothermal box. After a 12-h overnight fast, a primed injection of [1-13C]acetate and [1,1,2,3,3-2H5]glycerol (19.25 and 1.60 μmol/kg, respectively, 99% enrichment; Cambridge Isotope Laboratories, Andover, MA) was followed by a constant infusion of 0.50 μmol·kg−1·min−1 acetate and 0.11 μmol·kg−1·min−1 glycerol for 9 h. Arterialized blood samples were collected at frequent intervals for 3 h (from −180 to 0 min) to measure basal acetate flux and to check the quasi plateau in acetate enrichment. Three hours after the beginning of tracer infusions (at time 0), the volunteers have received the same oral ingestion, either saline solution or 30 g of lactulose (Solvay Pharma, Suresnes, France) mixed with 100 ml water. Next, blood samples were collected every 20 min until time 360 min. No subject complained of adverse side effects (such as flatulence, abdominal pain, or diarrhea) during the clinical study and the day after.

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Sampling Procedures and Gas Chromatograph-mass Spectrometry Analysis

Total plasma cholesterol and triacylglycerol concentrations were measured using enzymatic kits (Biomérieux, Marcy l’Etoile, France). Plasma fatty acid concentrations were determined with a colorimetric method (13).

Acetate Analysis

Measurement of plasma acetate enrichment was previously described (16, 24). Briefly, the proteins of plasma samples containing an internal standard (D3-acetate) were precipitated by sulfosalicylic acid (10 mg). After centrifugation, the supernatants were acidified to pH 3 by 30 μl HCl (10 mol/l). Acetate and standards were extracted from plasma in 3 ml diethyl ether (Fluka Chemika, Buchs, Switzerland) by vortexing for 15 min. The tert-butylmethylsilyl (TBDM) derivatives were obtained by adding 8 μl TBDM imidazole (Fluka) in the separated organic phase and heating at 60°C for 30 min. Next, the samples were cooled and evaporated to 500 μl. Two microfilters were injected in a gas chromatograph (model 5890A; Hewlett Packard, Palo Alto, CA) using a 30 m × 0.25 mm capillary column (DB1; J&W Scientific, Folsom, CA) and connected with a quadrupole mass spectrometer (5971A; Hewlett Packard). The temperature program started at 50°C and ramped to 85°C at 5°C/min, then up to 250°C at 50°C/min followed by 1 min at 250°C. Selected ion monitoring mode with ions at mass-to-charge ratio (m/z) 117, 118, and 120 was used to measure acetate enrichment and concentration. The calibration curve for isotopic enrichments was prepared in the range of 0 to 20% for [1,1,2,3,3-2H5]glycerol. Plasma glycerol concentrations were determined with an enzymatic kit (Boehringer Mannheim, Germany). Plasma fatty acid concentrations were determined with a colorimetric method (13).

Glycerol Analysis

Plasma samples (500 μl) were deproteinized with 80 μl sulfosalicylic acid solution (0.5 M), vortexed, and centrifuged at 2,000 g for 15 min at 4°C. The supernatants were neutralized with KOH solution (0.1 M) and deposited directly on a sequential anion and cation exchange chromatography. The ion-exchange columns were prepared by adding 1 ml Dowex AGW ×4 cation exchange (1:1, vol/vol, resin-water; Supelco, Sigma, St. Quentin Fallavier, France), then 1 ml of Dowex AG 1×8 anion exchange (1:1, vol/vol), and washing with 3 ml deionized water. The neutral compounds were eluted by 3 × 1 ml water and dried under a nitrogen vacuum at room temperature. The samples were derivatized with 100 μl acetic anhydride-pyridine (Aldrich, Sigma) mixture (1:1, vol/vol) at 80°C for 1 h (27), evaporated and diluted in 500 μl ethyl acetate. Triacetate derivative (1 μl) was injected in the GC-MS system described above. The temperature program was started at 80°C and ramped to 250°C at 30°C/min followed by 3 min at 250°C. Selected-ion-monitoring modes were used on ions of m/z 145 and 148 (4). The calibration curve was obtained from known isotopic enrichment solutions prepared in ethyl acetate ranging from 0 to 10% enrichment for [1,1,2,3,3-2H5]glycerol (D5-glycerol). Plasma glycerol concentrations were determined with an enzymatic method (26).

Calculations

Acetate and glycerol. The rates of total appearance of acetate and glycerol were calculated according to the equations described in steady-state conditions (17).

Statistics

Data were reported as means ± SE, unless otherwise stated. Statistical analysis was performed using Instat Statistical software (GraphPad, San Diego, CA). Differences in plasma concentrations and turnover with saline and lactulose were evaluated by ANOVA (repeated measures). Student’s t-test was used to compare clinical and kinetic data of subjects. A two-tailed probability level of 0.05 was considered statistically significant.

RESULTS

Baseline State

As expected, plasma acetate, glycerol, and FFA concentrations remained relatively stable and not different in the basal state (Table 1 and Fig. 1). Plasma acetate and glycerol turnover were similar for both treatments (Table 1). Mean basal glucose and insulin concentrations were 5.1 ± 0.3 and 74 ± 15 pmol/l (see Fig. 4). Plasma total cholesterol and triacylglycerol concentrations were 210 ± 20 and 93 ± 20 mg/dl, respectively.

Lactulose Ingestion

After oral ingestion of lactulose, plasma acetate concentrations increased and reached a plateau at 396 ± 70 μmol/l (compared with baseline, P = 0.004) from 160 to 280 min, and then decreased until the end of the acetate infusion (360 min) to 278 ± 27 μmol/l (Fig. 1A). Glycerol concentrations decreased with lactulose, but not significantly, and remained stable until the end of glycerol infusion (Table 1). Plasma FFA concentrations decreased significantly with lactulose until 120 min (P ≤ 0.01 compared with saline) and then increased slowly to reach the concentration observed with saline (Fig. 1B).

Acetate turnover rate increased compared with saline (P = 0.01; Fig. 2A). Glycerol turnover decreased with lactulose ingestion (P ≤ 0.05; Table 1 and Fig. 2B). An inverse correlation was found between glycerol and acetate turnover in the postingestion state (r = -0.78, P ≤ 0.02; Fig. 3).

With lactulose, an early and small increase in plasma insulin appeared (P ≤ 0.05) and followed the similar pattern of plasma glucose (no significant), with a maximal peak of 89.8 ± 15.4 pmol/l at 100 min for insulin and of 5.3 ± 0.2 mmol/l at 40 min for glucose (Fig. 4). No correlation was found between insulin concentration, acetate, and glycerol turnover at a maximal peak

| Table 1. Plasma concentrations and turnover of acetate and of glycerol at basal state (from −45 to 0 min) and after oral ingestion (from 120 to 360 min) of saline or lactulose |
|------------------|------------------|------------------|
|                  | Acetate          | Glycerol         |
|                  | Concentration, μmol/l | Turnover, μmol/kg−1·min−1 | Concentration, μmol/l | Turnover, μmol/kg−1·min−1 |
| Basal Saline     | 201 ± 32         | 11.4 ± 2.4       | 61.3 ± 10.9        | 3.8 ± 0.4       |
| Basal Lactulose  | 222 ± 34         | 10.7 ± 1.4       | 61.0 ± 8.8        | 4.8 ± 1.9       |
| Postingestion Saline | 197 ± 27     | 10.3 ± 2.2       | 81.1 ± 15.9      | 3.5 ± 0.3       |
| Postingestion Lactulose | 333 ± 54a  | 15.5 ± 2.2*      | 51.2 ± 12.5      | 2.8 ± 0.4*      |

Values are means ± SE. *P ≤ 0.05 vs. saline state postingestion.
of insulin after lactulose intake. No significant change of total cholesterol and triacylglycerol concentrations was observed after lactulose ingestion (cholesterol 190 ± 20 mg/dl; triacylglycerols 95 ± 20 mg/dl).

**DISCUSSION**

In this study, a 50% increase of total acetate production rate and acetate concentration in plasma were observed after 30 g lactulose intake. The FFA concentrations and glycerol turnover in plasma decreased significantly after lactulose intake compared with saline solution. The maximum decreases of glycerol turnover and FFA concentrations were 30 and 35% after lactulose intake compared with baseline and were sustained for 160 and 120 min before returning slowly to fasting levels at the end of kinetics. The decrease in FFA concentrations and
glycerol turnover was inversely correlated to the whole body acetate production rate.

As previously reported, a significant increase in whole body turnover of acetate was observed after acute lactulose ingestion (11, 15, 17). In the present work, acetate turnover at baseline was higher compared with previous studies, probably related to the different studied subjects (11, 15). In this study, plasma FFA concentrations increased with the saline solution (baseline and postingestion) because of a progressive lipolysis increase during fasting in overweight subjects, as reported in healthy subjects (2, 7, 10). After lactulose intake, FFA concentrations and glycerol turnover decreased simultaneously to an increase of plasma acetate. These results are supported by a previous report from Abramson and Arky (1) and Siler et al. (23) in healthy volunteers in which intravenous acetate injection sharply decreased plasma FFA concentrations (1, 23). In the present study, the same effect was observed with a dietary fermented disaccharide producing a large amount of acetate.

The measurement of glycerol appearance rate that is considered to reflect lipolysis (4) gives further information on the decrease of plasma FFA. Glycerol turnover followed the same plasma profile as FFA, e.g., an increase with saline and a decrease with lactulose (≥30%). This change of glycerol turnover after the lactulose oral intake was directly related to the production of acetate. The fall in plasma FFA linked to the acetate increase is the result of an inhibition of lipolysis. By comparison with ketone bodies, which also inhibit lipolysis by a mass action on intramitochondrial acetyl-CoA concentration (21, 3), an increased production of acetyl-CoA from acetate could induce the same effect.

A poor insulinemic response appeared shortly after the lactulose intake and lasted 1 h. We assume three explanations for the modest change in insulin concentration. 1) It could be explained by the trace amounts of free fructose and galactose, since these two sugars are present in a very small amount in the lactulose syrup. 2) The increase of plasma insulin could be related to intestinal incretins that stimulate insulin secretion as plasma glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide concentrations, which increase within a few minutes after ingestion of meals (14). Unfortunately, we couldn’t measure these hormones after lactulose ingestion. 3) Increase of short-chain fatty acids can be involved, but human data have shown that there are no changes in hepatic glucose production, insulin or glucagon concentrations after sodium acetate ingestion or infusion (10). Regardless of the mechanisms, this small increase of plasma insulin cannot explain our data on glycerol kinetics. Two studies have explored the relationship between insulin and glycerol production in volunteers (18, 25). Using a standard hyperinsulinemic-euglycemic clamp (1 mU·kg⁻¹·min⁻¹) in combination with an infusion of [³H₃]glycerol, Stumvoll et al. (25) showed a suppression of glycerol turnover rate (≤60% from baseline, P < 0.001) related to the high plasma insulin concentrations after 120 min (386 ± 22 vs. 37 ± 5 pmol/l at baseline) in healthy subjects. Robinson et al. (18) reported a decrease of 50% of glycerol turnover correlated with a significant increase in plasma insulin concentration (variation from baseline = 78 ± 18 pmol/l) in obese adolescents. In the present study, the increase of insulin concentration was far lower compared with the two previous studies (variation from baseline =15 pmol/l), but it could not be excluded that it could be in part responsible for the decrease in plasma glycerol.

The decrease in FFA and glycerol observed in this study is therefore mainly originated from and sustained by the high increase in colonic acetate production rate. Next, acetate produced from colonic fermentation appears to be an anti-lipolytic agent that can reduce lipotoxicity and enhance insulin sensitivity. This could explain the beneficial effect of dietary fibers on insulin resistance. However, how this decrease in lipolysis can modify body weight or negatively interact with restrictive diets remains to be clarified with long-term dietary studies.

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