Insulin effects on acetate metabolism

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PLASMA ACETATE IS DERIVED from an exogenous production by colonic fermentation of nondigestible carbohydrates and an endogenous source from amino acid, fatty acid, and glucose metabolism. In ruminant mammals, exogenous contribution is the major component, whereas in nonruminants such as rats or humans, the endogenous source is more important. Several studies (11, 14, 15) have investigated acetate kinetics in healthy human volunteers and in diabetic patients. Plasma acetate concentrations are increased in type 2 diabetic patients (3, 16, 18). It was suggested that blood glucose or insulin can control acetate metabolism (2, 7, 13). The relationship between acetate and glucose concentration in the blood remains unclear, but a key enzyme [acetyl-coenzyme A (CoA) synthetase (ACAS)] of acetate metabolism is regulated by insulin in in vitro models (17). The purpose of this study was to evaluate the effects of insulin on acetate metabolism in humans and its relationship to glucose metabolism. By using a hyperinsulinemic euglycemic clamp technique (hot clamp), we have measured acetate and glucose turnover in two groups of patients: control (normal insulin sensitivity; IS group) and insulin resistant (obese and diabetic patients; IR group).

METHODS

Subjects

Six healthy human volunteers of normal weight [body mass index (BMI): 25.5 ± 1.4 kg/m²] (Table 1), five abdominally fat obese patients (BMI 35.0 ± 0.7 kg/m² and height/hip ratio >1), and three type 2 diabetic patients (Hb A1c >7%; BMI 35.0 ± 3.0 kg/m²) participated in this protocol. These patients represented a wide range of insulin sensitivity. Their clinical and biochemical parameters are shown in Table 1. These 14 subjects (7 females and 7 males, 47.9 ± 8.3 yr old) were divided into two groups according to their insulin sensitivity. The IS group consisted of six volunteers (subjects 1–6) with normal insulin sensitivity. Eight insulin-resistant volunteers (subjects 7–14) formed the IR group. The subjects were considered to be insulin resistant when the glucose consumption required for maintaining baseline blood glucose during a euglycemic clamp was <7.5 mg·kg⁻¹·min⁻¹ (6). The subjects had no acute pathology and were not under any medication. The experimental protocol was approved by the Ethics Committee of the University Hospital of Nantes (France).

Experimental Design

To avoid exogenous acetate production, a low-fiber diet (<5 g/day) was consumed by the volunteers for 3 days before the protocol began. The study was conducted after a 12-h overnight fast. On the morning of the experiment, two short polyethylene catheters (20 gauge; Vigon, Paris, France) were inserted into the antecubital vein for tracer infusion and on the wrist of the second arm to sample venous blood.

A primed constant infusion technique with the use of [1,13C]acetate and [6,6-2H]glucose (99% enrichment; Cambridge Isotope Laboratories, Andover, MA) was performed. The primers were 19.25 and 18.3 μmol/kg, respectively. The rate of the acetate infusion was 0.5 μmol·kg⁻¹·min⁻¹ for 6 h and the rate of the glucose infusion was 9.75 μmol·kg⁻¹·h⁻¹ for 3 h. After 3 h of infusion, a hot clamp was started to obtain insulin concentration at ~100 μU/ml (6). During the clamp, the glucose tracer rate was adjusted to the glucose infusion rate (ml/h) to maintain the glycemia between 4.9 and 5.5 mmol/l. Arterialized blood samples were collected at −180, −60, −45, −30, −20, −10, 0, 60, 120, 150, 160, 170, and 180 min (Fig.

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Table 1. Clinical and biological features of subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Age, yr</td>
<td>43</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2</td>
</tr>
<tr>
<td>Height/hip ratio</td>
<td>0.9</td>
</tr>
<tr>
<td>FBG, mmol/l</td>
<td>5.2</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. IS, normal insulin sensitivity; IR, insulin resistance; BMI, body mass index; FBG, fasting blood glucose.

1). Respiratory gas exchanges were measured with the use of indirect calorimetry (Deltatrac, Helsinki, Finland) throughout the study. Expired breath gases were sampled before and after each blood sample.

**Sampling Procedures and Gas Chromatograph-Mass Spectrometer Analysis**

**Acetate analysis.** Measurement of plasma acetate enrichment was performed as previously described (14). Briefly, an internal standard (24 μl of [2H3]acetate, 2.35 mmol/l) was added to 500 μl of plasma sample to measure plasma acetate concentration. Plasma samples were deproteinized with 10 mg of sulfosalicylic acid (Sigma, St. Quentin Fallavier, France) and centrifuged at 2,200 g for 30 min. The supernatants were transferred into a glass tube and acidified by the addition of 30 μl of HCl (10 mol/l) (Fluka, Buchs, Switzerland). Acetate and standards were extracted from plasma into 3 ml of diethyl ether (Fluka) by being vortexed for 15 min. The samples were centrifuged for 15 min at 1,200 × g, and the supernatants were transferred into a glass tube and acidified with a mixture of 50 μl of acetic anhydride and 50 μl of pyridine (Sigma), at 80°C for 1 h, evaporated, and diluted in 1 ml of ethyl acetate (19).

Two microliters of acetate were injected into a split/splitless injector (splitless mode) into a gas chromatograph (GC; model 5890A, Hewlett-Packard, Palo Alto, CA) with the use of a 30 m × 0.25 mm capillary column (model DB1, J&W Scientific, Folsom, CA) and connected with a quadrupole mass spectrometer (MS; model 5971A, Hewlett-Packard). The injector temperature and the transfer line between GC and MS were 250°C and 290°C, respectively. The temperature program started at 80°C and ramped to 250°C at 30°C/min, followed by 3 min at 250°C. Electron impact ionization and selected ion monitoring mode were used on ions with m/z 98 and 100. Calibration curve was obtained from known isotopic enrichment solutions prepared in ethyl acetate ranging from 0% to 10% enrichment for [6,6-2H2]glucose.

**CO2 analysis.** CO2 production (VCO2) was determined by indirect calorimetry and isotopic enrichment of expired CO2 was measured by GC-IRMS (Breathmat Plus, ThermoFinnigan, Bremen, Germany) with the use of a packed steel column (3 m Hayes Sep-D). A laboratory CO2 standard calibration against the international P Belemnite (PDB) carbonate was used as a reference. The data were obtained in δ13C (in ‰).

**Calculation Methods**

**Acetate.** The rate of total appearance of acetate (Ra) was calculated according to the equations for steady state as follows

\[
R_a = \left( \frac{E_t}{E_p} - 1 \right)
\]

where \(i\) was the infusion rate (in μmol·kg⁻¹·min⁻¹), \(E_t\), and \(E_p\) were, respectively, the isotopic enrichment of the tracer ([l-13C]acetate), and the plasma at plateau given as mole percent excess (MPE).

Extracellular pool (μmol/kg) was C·V_d, where \(V_d\) is the distribution volume of acetate (0.2 l/kg) (12).

**Glucose.** Before the hot clamp treatment was started, the [6,6-2H2]glucose infusion was constant for 3 h. Therefore, the glucose \(R_a\) was calculated according to the equation used for acetate.

During the hot clamp treatment, the glucose \(R_a\) was calculated with the Steele equation for nonsteady state, as follows

\[
R_a = \left( F - \left[ pV \cdot (G_1 + G_p)/2 \cdot (E_p - E_p,1) \cdot (t_2 - t_1) \right] \right)/\left( E_p,2 + E_p,1 \right)
\]

where \(F\) was the infusion flow of the tracer (in mg·kg⁻¹·min⁻¹), \(p\) was the pool fraction (0.65), \(V\) was the distribution volume of glucose (0.2 l/kg), \(G_p\) was the plasma concentration of glucose at time \(t\) (in g/l), and \(E_p\) was the plasma isotopic enrichment of glucose at time \(t\).
For the constant isotopic enrichment in time, the equation used was

$$R_a = F/E_p$$

**Oxidation of acetate.** Enrichment of CO₂ was expressed in δ¹³C (in ‰). The values were transposed in atom percent (AP)

$$AP = 100R(0.0015¹³C + 1)/[1 + R(0.0015¹³C + 1)]$$

R was the ¹³C/¹²C ratio of international PDB standard (R = 0.0112372), and δ¹³C was the ¹³C enrichment of the samples. The isotopic enrichment of expired CO₂ was calculated in AP excess (APE) as

$$APE = AP_0 - AP_s$$

AP₀ and AP₀ were the AP of the sample and at time 0, respectively. The oxidation (Oxi) was expressed in μmol·kg⁻¹·min⁻¹ and was

$$Oxi = (ECO₂VCO₂)/(E_pCM)$$

where ECO₂ was the isotopic enrichment of expired CO₂ in APE, VCO₂ was the expired rate of CO₂ (μmol/min), C was the recovery coefficient (0.78) for [l-¹³C]acetate in the postabsorptive state (20), and M was the weight of the volunteer.

The percent oxidized (%Oxi) was determined as

$$%Oxi = 100(Oxi/R_a)$$

where Rₐ was the rate of disappearance of acetate. The percentage of VCO₂ from oxidation of acetate (%VCO₂) was

$$%VCO₂ = 100VCO₂/(E_pC)$$

The acetate energy contribution (Cₐₑ, in %) was calculated with the equation as below

$$Cₐₑ = (100E_a)/E_t$$

where Eₐ was the energy derived from acetate (1 mol of oxidized acetate = 879 kJ) (5), and Eₜ was the total energy (in kJ/min).

**Statistical Analysis**

Data are reported as means ± SE unless otherwise stated. Correlation studies were realized among acetate rate, glucose rate, and insulin sensitivity. Statistical analysis was performed with the use of Instat Statistical software (GraphPad, San Diego, CA). Student’s t-test was used to compare clinical and kinetic data of the subjects. Correlation analyses were performed with the use of Spearman’s coefficient analysis. A two-tailed probability level of 0.05 was accepted as statistically significant.

**RESULTS**

At the end of the clamp study, all the IS subjects had a glucose consumption >7.5 mg·kg⁻¹·min⁻¹ and was 9.6 ± 1.1 for the IS group vs. 4.9 ± 0.5 mg·kg⁻¹·min⁻¹ for the IR group (P < 0.01) (Table 2). In the IR group, BMI and the height/hip ratio were significantly higher for the IR group compared with the IS group: 35.0 ± 1.1 versus 25.6 ± 1.2 kg/m² (P < 0.01) and 1.0 ± 0.01 versus 0.9 ± 0.02 (P < 0.01). Glycemia and Hb A₁c were also higher in the IR group compared with the IS group: 7.4 ± 1.2 mmol/l and 6.2 ± 0.4% vs. 5.4 ± 0.1 mmol/l and 5.4 ± 0.1%, respectively (P = 0.11 and 0.17). In the IS group, only one subject (subject 5) was obese, but all subjects showed a normal glucose tolerance (Table 1). In the IR group, all subjects were obese, three were diabetic (subjects 11–13), and one showed glucose intolerance (subject 10). During and at the end of the protocol, mean glycemia and insulinemia were similar in the two groups: 5.2 ± 0.06 mmol/l and 103.7 ± 19.4 pmol/l in the IS group vs. 5.3 ± 0.08 mmol/l and 143 ± 11.3 pmol/l in the IR group (Table 2).

Free fatty acid plasma concentrations were higher in the IR group (0.6 ± 0.04 mmol/l) than in the IS group (0.4 ± 0.05 mmol/l) at baseline but decreased and became similar at the end of the clamp (IR: 0.07 ± 0.01 mmol/l, IS: 0.06 ± 0.01 mmol/l). No significant difference in baseline acetate concentrations was found between the two groups (Table 3).

In the basal state, the constant infusion of [6,6-²H₂]glucose led to similar glucose turnover in the two groups: 8.8 ± 0.4 vs. 8.8 ± 0.2 mmol·kg⁻¹·min⁻¹ (P = 0.6). During the hot clamp treatment, the glucose turn-

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**Table 2. Basal insulin, insulin at clamp plateau, and glucose utilization at end of clamp in IS and IR subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>IS</th>
<th>Values</th>
<th>IR</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Basal insulin, μU/ml</td>
<td>5.6</td>
<td>6.5</td>
<td>13.9</td>
<td>4.6</td>
</tr>
<tr>
<td>Clamp insulin, μU/ml</td>
<td>103.6</td>
<td>103.6</td>
<td>146.1</td>
<td>116.1</td>
</tr>
<tr>
<td>Glucose utilization, mg·kg⁻¹·min⁻¹</td>
<td>8.2</td>
<td>7.6</td>
<td>7.6</td>
<td>12.6</td>
</tr>
</tbody>
</table>

Values are means ± SE.

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**Table 3. Glucose turnover rates, acetate turnover rates, and plasma acetate concentration at basal state and after the clamp in IS and IR groups**

<table>
<thead>
<tr>
<th></th>
<th>Glucose Turnover, mmol·kg⁻¹·min⁻¹</th>
<th>Acetate Concentration, mmol·kg⁻¹·min⁻¹</th>
<th>Acetate Turnover, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>8.8 ± 0.4</td>
<td>127.1±11.2</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>IR</td>
<td>8.8 ± 0.2</td>
<td>129.2±10.9</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>Clamp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IS</td>
<td>45.1±3.8</td>
<td>112.8±7.9</td>
<td>8.1±0.8</td>
</tr>
<tr>
<td>IR</td>
<td>15.9±2.2*</td>
<td>102.8±5.0</td>
<td>5.5±0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.001, IR vs. IS group during clamp.
over rate increased and was higher in the IS group: 45.1 ± 3.8 vs. 15.9 ± 2.2 μmol·kg⁻¹·min⁻¹ (P < 0.001) in the IR group (Table 3).

The baseline acetate turnover rates were not significantly different among both groups (Fig. 2). After the clamp, mean acetate turnover of the two groups increased and was higher in the IS group: 8.1 ± 0.7 vs. 5.5 ± 0.5 μmol·kg⁻¹·min⁻¹ (P < 0.001) (Table 3). No significant correlation was found between acetate turnover or acetate concentration and glucose rate of infusion. No significant correlations were found between acetate turnover and glucose turnover or plasma free fatty acid concentration. The oxidation of acetate increased significantly with hyperinsulinemia in both groups and was higher in the IS group: 5.7 ± 0.7 μmol·kg⁻¹·min⁻¹ for IS group vs. 4.4 ± 0.3 μmol·kg⁻¹·min⁻¹ for IR group (P < 0.001). In basal conditions, the percentage of oxidized acetate calculated from acetate production was similar in both groups 67.7 ± 3.4% (IS group) vs. 70.0 ± 5.4% (IR group). After the clamp, it was 76.0 ± 4.3% vs. 76.9 ± 3.7%, respectively (Fig. 3). The percentage of expired CO₂ from VCO₂ and Cee were also similar, ~3%, in both groups under basal conditions and increased significantly (VCO₂ 70% for IS vs. 35% for IR; Cee 80% for IS vs. 70% for IR) after insulin administration (IS: P < 0.001, IR: P < 0.05).

**DISCUSSION**

In this study, the effect of insulin on acetate metabolism was analyzed. Under basal conditions, acetate turnover rate and plasma acetate concentration were similar in patients with normal or impaired insulin resistance. Acetate concentrations decreased with hyperinsulinemia in both groups but tended to be lower in the group with insulin resistance. The acetate turnover increased with insulin infusion, suggesting a concomitant increase in acetate production and utilization, but this effect was significantly lower in IR patients.

Akanji et al. (3) investigated changes in acetate concentration induced by a hyperinsulinemic euglycemic clamp in 13 nondiabetic individuals, including 7 obese patients, and in 9 diabetic patients. Acetate concentrations decreased in both groups during insulin administration. In the nondiabetic subjects, acetate concentrations returned to baseline during the second hour of the clamp. It was suggested that the lack of increase in acetate concentration of the diabetic patients was not related to insulin resistance, but rather to a difference in postreceptor glucose metabolism toward glycogenesis or glycolysis (1, 3). In diabetic patients, decreased glycolysis may explain the decreased production of acetyl-CoA and therefore of acetate. Some studies (2, 8, 18) have already found a correlation between plasma acetate and glucose, thus supporting a conversion between acetate and glucose via acetyl-CoA.

Insulin induced an increase in acetate turnover and a decrease in acetate concentrations, but this effect was lower in patients with insulin resistance. On the one hand, the results indicated a reduction in acetate production in subjects with insulin resistance. On the other hand, insulin accelerated the catabolism of acetate, which is dependent on glucose availability (7). Consequently, decreased glucose uptake associated with insulin resistance may result in a decrease in acetate utilization. This permissive effect of glucose may have led to the increase in acetate flux seen during the hyperinsulinemic clamp because glucose turnover was increased, not only by the administration of insulin but also by the exogenous glucose supply needed to maintain blood glucose concentrations within the normal range. However, in our study, acetate turnover was correlated neither to glucose flux nor to fasting blood glucose concentrations, suggesting that acetate metabolism is sensitive to insulin rather than to glucose.

Insulin increased the rate of acetate catabolism, as shown by the increase in acetate metabolic clearance during the clamp. However, this effect was less marked in patients with insulin resistance. Mechanisms that
may explain this decreased acetate catabolism associated with insulin resistance include decreased activity of the enzyme ACAS, decreased glucose utilization, and decreased acetate oxidation.

ACAS, which is involved in the conversion of acetate to acetyl-CoA, is sensitive to insulin action (13). The decrease in acetate utilization that results from a decrease in ACAS activity is induced by insulin resistance. This mechanism has been recently documented in rats (17). A decrease in acetate oxidation via acetyl-CoA may also explain the decrease in acetate utilization. In both groups, 65–68% of the acetate underwent oxidation. These percentages were similar to those found by our group in healthy volunteers (11). Acetate oxidation was more marked in subjects with normal sensitivity to insulin, both under basal conditions and with insulin stimulation. However, the contribution of acetate to energy expenditures was similar in the two groups under basal conditions, ~3%, and increased twofold with insulin administration, indicating that acetate oxidation was proportional to acetate utilization. Insulin probably acts on the enzyme ACAS, which regulates acetate homeostasis and not oxidation. Insulin probably acts on the enzyme ACAS, which is involved in the conversion of acetate to acetyl-CoA. Insulin decreases the oxidation of free fatty acids and total lipids (9). Thus acetate behaves differently compared with other fatty acids. Conversely, insulin increases glucose oxidation and nonoxidative glucose utilization (9). Furthermore, the alteration in insulin effects associated with insulin resistance results in a decrease in glucose utilization. Our hypothesis supports the sensitivity of acetate to insulin action. Acetate metabolism is similar in this respect to glucose metabolism. Insulin resistance seems to contribute to the alteration in acetate metabolism associated with diabetes or obesity. Several authors have suggested that short-chain triglyceride solutions, such as triacetin, may be useful parenteral nutrients (4). In case of insulin resistance, acetate should be used cautiously to avoid possible adverse effects as previously reported with acetate dialysis treatment (21).

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DISCLOSURES

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REFERENCES


