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### Research Article

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# Plasma Acetate Turnover and Oxidation

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**ABSTRACT** Plasma acetate turnover and oxidation were determined in 11 healthy subjects by the constant infusion of a trace amount of [1-<sup>14</sup>C]acetate for 6 h. The subjects ages ranged from 22 to 57 yr. There was a positive correlation ( $P < 0.001$ ) between plasma acetate concentration and turnover rate, and a negative correlation ( $P < 0.001$ ) between turnover and age. The plasma acetate concentration in the subjects 22–28 yr old was 0.17 vs. 0.13 mM ( $P < 0.02$ ) in subjects 40–57 yr old. The plasma acetate turnover rate was also greater in the younger age group ( $8.23 \pm 0.66$  vs.  $4.98 \pm 0.64$   $\mu\text{mol}/\text{min} \cdot \text{kg}$ ,  $P < 0.01$ ). Approximately 90% of the plasma acetate turnover was immediately oxidized to CO<sub>2</sub> in both age groups, however,  $13.2 \pm 0.89\%$  of the CO<sub>2</sub> output in the younger group was derived from plasma acetate oxidation compared to  $7.9 \pm 0.94\%$  in the older group ( $P < 0.01$ ). The mean plasma acetate concentration, turnover, and oxidation in six cancer patients 47–63 yr old were similar to the values observed in the age-matched healthy subjects.

Uptake or output of acetate by various tissues was measured by arterial-venous plasma acetate concentration differences. In seven of eight subjects undergoing elective surgery, the arterial-portal venous concentration difference was negative, which indicated that the gastrointestinal tract can contribute to plasma acetate production. Uptake of plasma acetate by both the leg and liver appeared to be dictated by the arterial acetate concentration. Net production of acetate by both the leg and liver was most often observed at arterial plasma acetate concentrations  $< 0.08$  mM.

## INTRODUCTION

Acetate is a major source of energy in ruminant animals formed primarily from the microbial fermentation of cellulose and hemicellulose in the ruminant (1). The acetate so produced is absorbed rapidly resulting in arterial plasma concentrations of 1.0–2.0 mM (1, 2). Plasma acetate oxidation alone is estimated to fulfill 50% of the caloric requirement of sheep (3, 4). In con-

trast, blood acetate concentrations in man are normally  $< 0.2$  mM (5) except after ethanol consumption, in which case arterial plasma acetate levels are elevated by as much as 20-fold (6). It was during ethanol infusion and at  $\approx 1.5$  mM plasma acetate that Lundquist (7) estimated that the maximum utilization rate of acetate in man was 300 mmol/h. To our knowledge, however, plasma acetate production and utilization have not been measured directly in man.

It was the purpose of this investigation to determine in the human the turnover and oxidation of acetate at normal plasma acetate concentrations and to measure either net uptake or output of acetate by various tissues of the body.

## METHODS

Table I summarizes the clinical status of the subjects in whom plasma acetate turnover and oxidation were determined. One of the subjects (G.G.) had a history of breast cancer and another (S.B.) colon cancer, but at the time of the study both were considered disease free. Subjects G.T. through E.S. had progressive cancer and were losing weight at the time of the study. The subjects were requested not to consume alcoholic beverages for one day before the study. Each subject gave his written voluntary consent after informed of the nature, purpose, and potential risks of the procedure.

After an overnight fast, the subjects were studied at rest in a ventilated room. A polyethylene catheter was inserted into an antecubital vein of one arm for isotope infusion and in the other for the withdrawal of blood. Plasma acetate turnover and oxidation were determined by the primed-continuous infusion of 75–100  $\mu\text{Ci}$  of [1-<sup>14</sup>C]acetate, 2.5 mCi/mmol sp act (Amersham Corp., Arlington Heights, Ill.) over 6 h. The ratio of prime rate to infusion rate ( $\mu\text{Ci}/\text{min}$ ) was 50. Samples of heparinized blood and expired air were taken hourly. The expired air was collected in Douglas bags as described by Issekutz et al. (8).

The O<sub>2</sub> and CO<sub>2</sub> content of the expired air was measured with a calibrated Noyons diaferometer (9) and the specific activity of the expired [<sup>14</sup>C]O<sub>2</sub> was determined according to Fredrickson and Ono (10). The CO<sub>2</sub> specific activity was corrected (8) to account for the slow exchange between the [<sup>14</sup>C]O<sub>2</sub> produced from the oxidation of [1-<sup>14</sup>C]acetate with the large unlabeled bicarbonate pool.

The specific activity of plasma acetate was determined as follows. 1 vol of plasma was added to 2.5 vol of ice-cold 6% HClO<sub>4</sub> (wt/vol). The protein precipitate was washed twice with ice-cold 6% HClO<sub>4</sub> and the combined supernates were neutralized with 3 M KHCO<sub>3</sub>. [1-<sup>14</sup>C]acetate in 1.0 ml of the

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TABLE I  
Clinical Data of Subjects

Subject	Age	Sex	Weight	Height	Diagnosis
	yr		kg	cm	
G.B.	22	M	69.7	175	
R.R.	22	M	84.1	185	
M.M.	24	M	81.9	184	
N.M.	28	M	78.2	177	
P.G.	25	M	84.1	193	
W.C.	25	M	79.1	180	
E.M.	45	F	58.3	155	
J.S.	48	F	54.1	153	
W.H.	57	M	81.9	171	
G.G.	40	F	58.1	157	
S.B.	51	M	108.6	177	
G.T.	47	F	50.3	172	Breast carcinoma
A.A.	63	M	72.1	171	Colon carcinoma
R.S.	51	M	69.1	177	Prostate carcinoma
F.S.	54	M	57.9	169	Lymphoma
E.G.	60	M	55.9	173	Colon carcinoma
E.S.	61	F	49.3	165	Colon carcinoma

HClO<sub>4</sub> extract was isolated by the distillation-diffusion procedure of Bartley (11). An aliquot of the distillate was added to 15 ml of Bray's scintillation solution (National Diagnostics Inc., Parsippany, N. J.) and counted in a Packard tri-carb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.). Duplicate 1-ml aliquots of the neutralized extract were transferred to a spectrophotometer cuvette to assay acetate by a modification of the acetate kinase method of Ballard et al. (12). The reaction medium contained 120 mM Tris-HCl buffer, pH 7.8, 12 mM ATP, 24 mM MgCl<sub>2</sub>, 2.97 mM phosphoenolpyruvate, 0.575 mM NADH, and 25 μg/ml of pyruvate kinase, lactic dehydrogenase, and acetate kinase in a final vol of 2.085 ml. Before the addition of acetate kinase, the cuvettes were incubated at room temperature until a constant absorbance was established at 340 nm measured in an Eskalab alpha spectrophotometer (SmithKline Instruments, Inc., Sunnyvale, Calif.). The acetate kinase was then added and the incubation continued for 20 min at 37°C. Final absorbance readings were recorded immediately after the cuvettes stood for an additional 10 min at room temperature. Acetate standards and blanks were treated as plasma samples. The change in absorbance was directly related to the acetate concentration up to 0.086 μmol/ml of reaction medium. At a plasma acetate concentration <0.1 mM, the SD of the mean of a series of sample replications was 8.3% and the recovery of acetate added to plasma was 105%. In more recent determinations of plasma acetate, ultrafiltrates of plasma were prepared by ultrafiltration through membranes with a nominal molecular weight cutoff of 10,000 (Millipore Corp., Bedford, Mass.) thus eliminating the preparation of HClO<sub>4</sub> protein-free extracts. The ultrafiltrate was diluted with deionized water and an aliquot taken for the enzymatic analysis of acetate. [1-<sup>14</sup>C]acetate was isolated from 1 ml of ultrafiltrate by distillation-diffusion.

Within 1 h of the isotope infusion period, plasma acetate specific activity was constant, indicating that steady-state conditions were established. Plasma acetate turnover was calculated from the plasma acetate specific activity and the isotope infusion rate (13). The percentage of respiratory CO<sub>2</sub> output derived from plasma acetate and the rate of plasma acetate oxidation were calculated as described by Issekutz et al. (9).

The equations for these calculations are as follows:

(a) plasma acetate turnover rate (μmol/min·kg)

$$= \frac{[1-^{14}\text{C}]\text{acetate infusion rate } (\mu\text{Ci}/\text{min})}{\text{plasma acetate specific activity } (\mu\text{Ci}/\mu\text{mol}) \times \text{body wt (kg)}}$$

(b) % CO<sub>2</sub> from plasma acetate oxidation

$$= \frac{\text{CO}_2 \text{ specific activity} \times 2}{\text{plasma acetate specific activity}} \times 100$$

(c) plasma acetate oxidation rate (μmol/min·kg)

$$= \frac{\% \text{CO}_2 \text{ from plasma acetate oxidation} \times \text{CO}_2 \text{ output}}{2 \times \text{body wt (kg)}}$$

(d) % plasma acetate turnover oxidized

$$= \frac{\text{plasma acetate oxidation rate}}{\text{plasma acetate turnover rate}} \times 100$$

Net uptake or output of plasma acetate by various tissues was measured by arterial-venous concentration differences. Arterial, portal, and femoral venous blood samples were drawn during elective surgery and arterial, femoral, and hepatic venous blood samples were obtained during cardiac catheterization.

## RESULTS

Plasma acetate kinetics were determined in subjects whose plasma acetate levels ranged from 0.05 to 0.20 mM. There was a highly significant correlation ( $P < 0.001$ ,  $r = 0.70$ ) between plasma acetate concentration and turnover rate (Fig. 1), which varied from 2.83 to 10.65 μmol/min·kg. As shown in Fig. 2, plasma acetate turnover rate was also negatively correlated with age ( $P < 0.001$ ,  $r = 0.80$ ). Because of the observed correlation between age and plasma acetate turnover rate, we divided our subjects into two age groups.

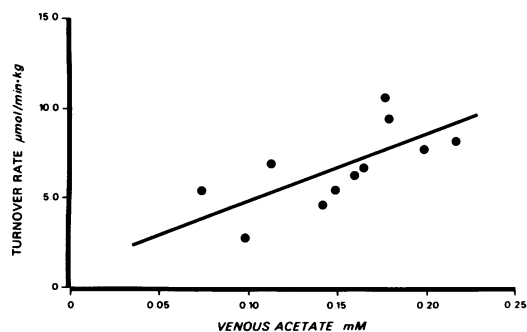


FIGURE 1 Correlation between venous plasma acetate concentration and plasma acetate turnover rate in healthy subjects. This correlation was described by the equation  $Y = 37.70X + 1.03$ , where  $Y$  = plasma acetate turnover in micromoles per minute per kilogram and  $X$  = millimolar plasma acetate.

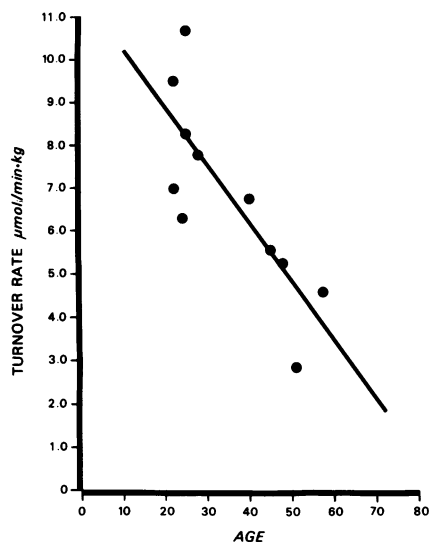


FIGURE 2 Correlation between age and plasma acetate turnover rate in healthy subjects. This correlation was described by the equation  $Y = -0.136X + 11.53$ , where  $Y$  = plasma acetate turnover in micromoles per minute per kilogram and  $X$  = age in years.

As shown in Table II, the mean plasma acetate concentration was significantly higher ( $P < 0.02$ ) in the group under 30 yr old than that in the middle-aged adults (45–57 yr old). Also, in the younger age group the mean plasma acetate turnover rate was  $\approx 1.5$  times higher than that observed in the middle-age group ( $P < 0.01$ ). In five of eight randomly selected subjects ranging in age from 65 to 79 yr old, the plasma acetate was too low to detect. The plasma acetate concentrations in the three other subjects whose ages were 66, 69, and 80 yr old were 0.037, 0.031, and 0.008 mM, respectively.

The mean basal caloric expenditure of both age groups

was essentially the same and was within normal limits (Table II). Of the plasma acetate produced in both age groups,  $\approx 90\%$  of it was immediately oxidized; however, the contribution of plasma acetate to respiratory  $\text{CO}_2$  output was significantly greater ( $P < 0.01$ ) in the younger age group due to their higher plasma acetate turnover rate.

Because a number of tumor cell types incubated in vitro produce appreciable quantities of acetate (14, 15), we measured plasma acetate turnover and oxidation rates in six middle-aged subjects who had progressive cancer (Table I). The mean plasma acetate concentration, turnover, and oxidation in the subjects with cancer were similar to their age-matched controls (Table II). Even though the mean plasma acetate turnover rate was higher in the subjects with cancer vs. their controls, the variation within the cancer group was too great for this difference to be significant.

Arterial-venous plasma acetate concentration differences were measured to determine uptake or output of acetate by various tissues. In seven of eight surgical subjects (Table III), the arterial-portal venous (A-PV)<sup>1</sup> difference was negative, which indicated net production of acetate across the gastrointestinal tract. The magnitude of the A-PV difference varied markedly between patients. Because the effects of bowel preparation before surgery and of the procedures during surgery on acetate metabolism by the gastrointestinal tract are not known, emphasis cannot be put on the magnitude of the difference, but rather that the A-PV was predominantly negative. Production of plasma acetate by both the leg and liver appeared to be dictated by the arterial acetate concentration. Net production of acetate

<sup>1</sup> Abbreviation used in this paper: A-PV, arterial-portal venous.

TABLE II  
Plasma Acetate Concentration, Turnover, and Oxidation in Young and Middle-Age Adults and in Middle-Age Adults with Cancer\*

Group†	Plasma acetate concentration mM	Plasma acetate turnover $\mu\text{mol}/\text{min} \cdot \text{kg}$	Turnover oxidized %	$\text{CO}_2$ output from acetate oxidation %	Caloric expenditure‡ kcal/24h · kg
A	$0.174 \pm 0.010$	$8.23 \pm 0.66$	$87.1 \pm 1.73$	$13.2 \pm 0.89$	$21.4 \pm 1.00$
B	$0.125 \pm 0.014^{\parallel}$	$4.98 \pm 0.64^{\parallel}$	$93.4 \pm 3.33$	$7.9 \pm 0.94^{\parallel}$	$23.2 \pm 1.42$
C	$0.121 \pm 0.014^{\parallel}$	$7.17 \pm 1.86$	$89.1 \pm 3.53$	$9.4 \pm 2.14$	$25.2 \pm 1.34$

\* Plasma acetate concentrations and turnover rates are expressed as the mean  $\pm$  SEM. Significant differences between group means were determined by Student's  $t$  test.

† A, six male subjects ranging from 22 to 28 yr old. B, two male and three female subjects ranging from 45 to 57 yr old. C, four male and two female subjects with progressive cancer ranging from 47 to 63 yr old.

‡ The caloric expenditure was estimated from the  $\text{O}_2$  uptake and the respiratory quotient.

<sup>||</sup> Significantly different from group A at  $P < 0.02$ .

<sup>¶</sup> Significantly different from group A at  $P < 0.01$ .

TABLE III  
A-PV Plasma Acetate Concentration Differences

Patient	Arterial	Venous	A-PV
<i>Plasma acetate mM</i>			
H.F.	0.067	0.092	-0.024
D.K.	0.000*	0.162	-0.162
D.G.	0.072	0.210	-0.138
R.L.	0.061	0.267	-0.206
C.G.	0.113	0.155	-0.042
E.W.	0.073	0.030	+0.043
M.K.	0.063	0.083	-0.020
B.B.	0.030	0.063	-0.033

\* The concentration of plasma acetate was too low to measure.

across the leg was observed in seven of nine patients at arterial acetate concentrations  $<0.08$  mM (Fig. 3). When the arterial acetate concentration exceeded 0.08 mM, the arterial-femoral venous difference was positive in 8 of 10 patients, which indicated net uptake. Similarly, the arterial-hepatic venous difference was negative at arterial concentrations below 0.08 mM and positive in five of eight patients whose arterial concentrations exceeded 0.08 mM (Fig. 4).

It is not known to what extent acetate as an end product of microbial fermentation in the gastrointestinal tract contributes to plasma acetate production. If plasma acetate originated primarily from such microbial metabo-

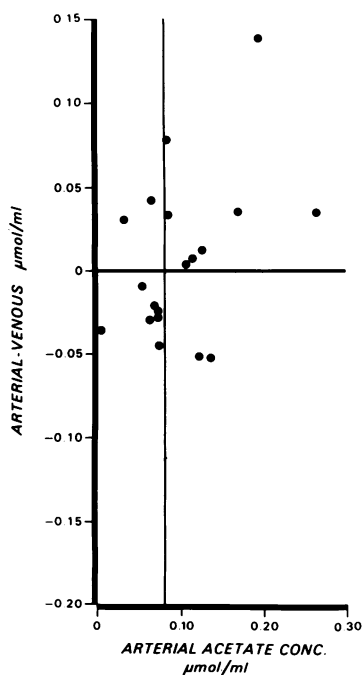


FIGURE 3 Arterial-femoral venous plasma acetate concentration differences in relationship to arterial plasma acetate concentration.

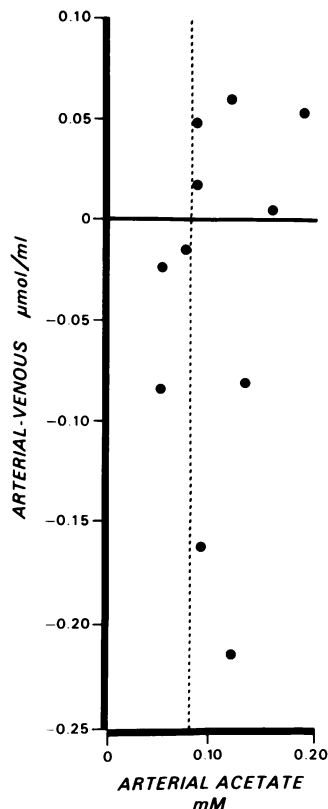


FIGURE 4 Arterial-hepatic venous plasma acetate concentration differences in relationship to arterial plasma acetate concentration.

lism, its plasma concentration may decrease during an extended fast. We did not observe, however, a significant difference in the mean plasma acetate concentration in six obese subjects after 12 h and 7 d of fast ( $0.116 \pm 0.010$  mM vs.  $0.143 \pm 0.124$  mM).

## DISCUSSION

In man, circulating levels of acetate are normally  $<0.02$  mM. The difficulty in measuring such low concentrations in tissues is the primary reason for the paucity of information on whole body metabolism of acetate in nonruminant animals and especially man. The body of information concerning acetate metabolism in man originates from studies after ethanol administration which show that ethanol is oxidized to acetate in the liver (6, 7, 16). The oxidation of acetate in the liver proceeds at a slower rate than its formation, thus a considerable quantity of acetate is released from the liver and metabolized by peripheral tissues. In these studies, the ethanol infusion rate was sufficient to establish arterial acetate concentrations of 1.0–1.5 mM. Under these conditions acetate is readily taken up by myocardial (17) and skeletal muscles (18), and brain (19). Although these studies demonstrate the ability of the

human to metabolize acetate, they are not direct measurements of plasma acetate production or utilization at normal plasma acetate concentrations in the absence of ethanol.

The plasma acetate concentrations in the subjects who participated in our studies ranged from 0.05 to 0.25 mM. Over this concentration range, the plasma acetate turnover rate was appreciable and linearly related to the plasma acetate concentration. Even though 90% of the plasma acetate turnover was immediately oxidized, plasma acetate accounted for only a small portion of the daily energy expenditure of our subjects. In contrast, in the ruminant animal in the fed state, plasma acetate oxidation contributes 30–60% of the total CO<sub>2</sub> output (3, 20, 21). After a 24-h fast, however, plasma acetate oxidation accounts for 10% or less of the CO<sub>2</sub> output (4, 22), which is similar to our observations in the overnight fasted human.

The decline in plasma acetate turnover with age is yet another metabolic change that occurs during aging in mammals. Accompanying the aging process in man, there is a decline in glucose disappearance rate after either oral or intravenous glucose that is not caused by differences in insulin or glucagon secretion (23) and a decline in lipid synthesis by adipose tissue in response to insulin (24). The age-related changes in plasma acetate turnover that we observed may well be related to alterations in both glucose and lipid metabolism that occur during the aging process, and suggest that appropriate age-matched controls be included in all studies of acetate metabolism in the human.

Various tumor cell types produce appreciable quantities of acetate (14, 15) suggesting there is increased plasma acetate production in subjects with cancer. In the limited number of subjects we studied, only E.G. and R.S. (Table I) had elevated plasma acetate turnover rates (15.63 and 8.50  $\mu\text{mol}/\text{min}\cdot\text{kg}$ , respectively) for their age. In the remainder of the subjects with progressive cancer the plasma acetate turnover rates were similar to those observed in healthy middle-age adults.

The predominantly negative A-PV concentration differences observed in subjects undergoing elective surgery indicated that the gastrointestinal tract can contribute to plasma acetate production in the human. Unlike the gastrointestinal tract, the production of plasma acetate by both the liver and leg appeared to be dictated by the arterial plasma acetate concentration. Acetate uptake by the rat hind quarter (25) has been found to be dependent on the level of acetate in the perfusion medium and, in addition, Buckley and Williamson (26) have shown that rat liver adds acetate to the hepatic venous blood when the PV concentration is below 0.2 mM. The effect of arterial concentration on the uptake or output of a metabolite by tissues is not unique to plasma acetate metabolism. The extrac-

tion of both plasma free fatty acids (27, 28) and glucose (29) by tissues in the human is dependent in part on their arterial concentrations.

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