Ethanol-induced Activation of Adenine Nucleotide Turnover

Evidence for a Role of Acetate

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Abstract. Consumption of alcohol causes hyperuricemia by decreasing urate excretion and increasing its production. Our previous studies indicate that ethanol administration increases uric acid production by increasing ATP degradation to uric acid precursors. To test the hypothesis that ethanol-induced increased urate production results from acetate metabolism and enhanced adenosine triphosphate turnover, we gave intravenous sodium acetate, sodium chloride and ethanol (0.1 mmol/kg per min for 1 h) to five normal subjects.

Acetate plasma levels increased from 0.04±0.01 mM (mean±SE) to peak values of 0.35±0.07 mM and to 0.08±0.01 mM during acetate and ethanol infusions, respectively. Urinary oxypurines increased to 223±13% and 316±44% of the base-line values during acetate and ethanol infusions, respectively. Urinary radioactivity from the adenine nucleotide pool labeled with [8-^{14}C]adenine increased to 171±27% and to 128±8% of the base-line values after acetate and ethanol infusions.

These data indicate that both ethanol and acetate increase purine nucleotide degradation by enhancing the turnover of the adenine nucleotide pool. They support the hypothesis that acetate metabolism contributes to the increased production of urate associated with ethanol intake.

Introduction

Alcohol consumption has been traditionally associated with hyperuricemia and is a common precipitating cause of acute gouty arthritis (1). Ethanol-induced hyperuricemia has been associated with both a decreased renal excretion of uric acid (2, 3) that is secondary to increased blood lactate level (4–6) and an elevated production of urate that is secondary to enhanced turnover of adenine nucleotides (7). The increased synthesis of urate is hypothesized to be related to the utilization of acetate formed during ethanol oxidation (7).

In the present study we have reexamined ethanol-induced alterations of uric acid metabolism. Our objective was to test the hypothesis that ethanol-induced activation of adenine nucleotide turnover results from the further metabolism of acetate formed from ethanol oxidation (7). Our studies provide evidence for increased synthesis of uric acid precursors during acetate and ethanol metabolism.

Methods

Adenosine, inosine, hypoxanthine, xanthine, uricase, xanthine oxidase, adenosine deaminase, and purine nucleoside phosphorylase were purchased from Sigma Chemical Co., St. Louis, MO. Erythro-9-(2-hydroxy-3-nonyl)adenine was purchased from Burroughs Wellcome Co., Research Triangle Park, NC. From Boehringer Mannheim Biochemicals, Indianapolis, IN, we purchased acetate kinase. [8-^{14}C]adenine and aqueous counting scintillant (ACS) were obtained from Amersham Corp., Arlington Heights, IL. In-line 0.22-μm cathivex filter units were obtained from Millipore Corp., Bedford, MA. All other reagents were of the highest commercial quality.

Our previous studies demonstrated that ethanol administration leads to increased oxypurine (hypoxanthine and xanthine) excretion and elevated urinary radioactivity originating from degradation of ATP (7). These observations indicate that ethanol increased purine nucleotide degradation from accelerated turnover of ATP (7). We hypothesized that since ethanol increases the synthesis of acetate, the subsequent conversion of acetate to acetyl-CoA converts ATP to adenosine monophosphate (AMP). Some of the AMP formed enters the purine nucleotide degradation pathway and leads to increased production of oxypurines (hypoxanthine and xanthine) and uric acid. To provide evidence to support this hypothesis requires the demonstration that acetate elevates urinary oxypurine and radioactivity originating from degradation of ATP. The current study was designed to examine this possibility.

To assess the rate of intravenous sodium acetate infusion that would increase plasma acetate levels and urinary oxypurine excretion, a preliminary dose-response study was performed in four normal ambulatory subjects. The subjects were on a regular diet. The amount of sodium acetate (8–10% solution) infused in the four studies was

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0.01 mmol/kg per min for 2 h, 0.02 mmol/kg per min for 2 h, 0.04 mmol/kg per min for 2 h, and 0.10 mmol/kg per min for 1 h.

After the preliminary studies, five normal subjects with a mean age of 24 yr (range 21–32 yr) were admitted to the Clinical Research Center at the University Hospital. Informed consent was obtained from all individuals. The subjects were placed on a weight-maintenance, isocaloric, purine-free diet with a protein content of 10–12%. 3–4 d after hospital admission, 25 μCi of [8-^{14}C]adenine contained in 1.6 μmol of adenine was administered intravenously through a 0.22-μm cathivex filter. The filter was flushed with 40 ml of 0.9% sodium chloride to ensure delivery of the entire isotope dose. Urine samples were collected daily over the ensuing week. An aliquot of 0.5 ml of each urine sample was added to 5 ml of aqueous counting scintillant, mixed and counted in a model 3003 liquid scintillation spectrometer (Ambac; Packard Instrument Co., Inc., United Technologies, Downers Grove, IL) (8). The excretion of radioactivity reflects degradation of the adenine nucleotide pool (9). 3 d after the infusion of isotopic adenine each subject received an intravenous infusion of sodium acetate followed at least 2 d later by an infusion of sodium chloride and two days later by an infusion of ethanol with sodium chloride. Every subject received the three infusions in this sequence. The studies with sodium chloride and the addition of sodium chloride to the ethanol infusion were designed to control for the large quantities of sodium contained in the acetate.

Infusions of sodium acetate, sodium chloride, or ethanol were performed after an overnight fast. The subjects maintained an oral water intake of at least 750 ml. An intravenous line was inserted at 0800 h and 0.9% normal saline was run at the rate of 100 ml/h. The following sequence was used for each infusion: first, 3 h normal saline infusion; next, 1 h of the specific infusion (0.1 mmol/kg per min as an 8–10% solution); then, 3 h normal saline infusion. Ethanol was infused in a 5–10% solution (by weight) with an equal concentration of sodium chloride as contained in the previous infusions. Urine was collected at hourly intervals throughout the infusions to check levels of radioactivity, creatinine, uric acid, and oxypurines. Blood samples were obtained by a heparin lock in the arm opposite to the intravenous line. Beginning at the time of the basal periods, blood was drawn at intervals of 30 to 60 min for levels of urate, ethanol, acetate, lactate, pyruvate, urate, creatinine, adenosine, inosine, hypoxanthine, and xanthine. Blood samples for inosine, hypoxanthine, and xanthine (5 ml) were drawn into prechilled heparinized vacutainer tubes, cooled in ice and immediately spun at 1,900 *g* for 10 min (10). Plasma was separated into prechilled tubes and stored at −20°C until assayed. The same procedure was used to obtain blood (3 ml) for adenosine determinations but, in addition, the heparinized vacutainer tubes contained 75 μl of a solution composed of 8 mM sodium chloride, 0.01 mM erythro-9-(2-hydroxy-3-nonyl)adenine hydrochloride and 0.5 mM dipyridamole. The former is an inhibitor of adenosine deaminase and the latter inhibits adenosine transport across the cell membrane (11).

Serum urate, urine uric acid, and urinary oxypurines were quantitated by enzymatic spectrophotometric assays (12, 13). Blood lactate (14) and pyruvate (15) were measured by enzymatic methods by the Biochemistry Core Facility Laboratory of the Michigan Diabetes Research and Training Center. Alcohol determinations were performed on a DuPont Automatic Clinical Analyzer and creatinine levels were analyzed on an Astra (Beckman Instruments, Inc., Fullerton, CA) in the University Hospital Clinical Chemistry Laboratory. Urinary creatinine was measured by a modification of an automated Jaffe reaction by using an Auto-Analyzer technique (16). Plasma acetate was determined by a modification of the technique described by Holz and Bergmeyer (17). Plasma (1.0 ml) was deproteinized with 0.1 ml of 5.8 N perchloric acid and 0.5 ml of the supernatant neutralized with 25 μl of 5 M potassium bicarbonate. A 0.555-ml reaction mixture was incubated for 1 h at 37°C and contained the following: 0.1 M triethanolamine hydrochloride with 0.2 mM magnesium chloride adjusted to pH 7.4 with 4 N sodium hydroxide, 250 μl; 84 mM adenosine triphosphate, 100 μl; 4 M hydroxylamine hydrochloride neutralized before use with 4 N sodium hydroxide, 100 μl; standard solution or sample, 100 μl; 5 mg/ml acetate-kinase, 5 μl. The incubation was terminated by the addition of 1.0 ml of 0.6 M trichloracetic acid. Next, 0.5 ml of 60 mM ferric chloride was added. The acetohydroxamic acid formed was measured as a pink to pink-violet color at optical density 492 nm. The standard curve was linear up from 0.02 to 2.50 mM. Recoveries of different amounts of sodium acetate added to samples was between 90 and 102%.

Plasma inosine, adenosine, hypoxanthine and xanthine concentrations were determined by high pressure liquid chromatography. Plasma samples were deproteinized by passage through ultrafiltration cones, type CF 25 (Amicon Corp., Lexington, MA) and final preparation was accomplished by filtration through nitrocellulose membranes 0.45 μm (Amicon Corp., Lexington, MA). The liquid-chromatographic system (Waters Associates, Inc., Milford, MA) consisted of a model M-45 solvent-delivery pump, a model U6K sample injector, a model 440 wavelength ultraviolet detector operated at 254 nm and a model 730 Data Module recording integrator. The 3.9 mm ID × 30 cm μBondapack C_{18} column (Waters Associates) was operated at room temperature. The separation was accomplished under isocratic conditions with 50 mM ammonium phosphate, pH 6.0, containing 1% methanol (vol/vol) at a flow rate of 1.3 ml/min. Plasma adenosine was determined under similar conditions but with 50 mM potassium phosphate, pH 4.5, containing 10% methanol (vol/vol) at a flow rate of 2.0 ml/min. 50 μl of a reference standard or sample were injected into the column and the sensitivity set at 0.01 *A*, full scale. Adenosine, hypoxanthine, xanthine, and inosine were identified by the retention times of standards and by the enzymatic peak-shift technique described elsewhere (18, 19).

Statistical calculations were carried out on a Hewlett-Packard 9825B (Hewlett-Packard Co., Palo Alto, CA) with the paired two-tailed *t* test, using the General Statistical Volume I program.

Results

Before performing the full study comparing the metabolic response with ethanol and acetate, it was necessary to establish an optimal and safe dose of acetate that would activate purine nucleotide degradation.

Acetate dose response. There appeared to be a direct relationship between the quantity of acetate administered per unit time, the increase of plasma acetate levels, and the increase in urinary oxypurine excretion in these preliminary studies (Fig. 1). A sodium acetate infusion rate of 0.1 mmol/kg per min for 1 h increased acetate plasma levels to 0.34 mM and maximally increased oxypurine excretion. This dose did not cause significant changes in the pulse rate, blood pressure, or blood pH. A lower dose of sodium acetate, 0.04 mmol/kg per min for 2 h, produced a blood level of 0.14 mM acetate and

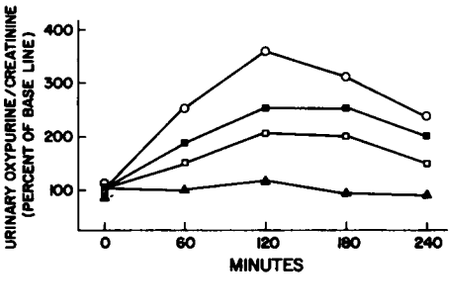


Figure 1. Oxypurine excretion with intravenous acetate infusion. Sodium acetate was infused in four normal subjects at a rate of 0.01 mmol/kg per min for 2 h (▲), 0.02 mmol/kg per min for 2 h (□), 0.04 mmol/kg per min for 2 h (■), and 0.10 mmol/kg per min for 1 h (○). The data are expressed as percentages of mean base-line values. Mean base-line values ranged from 0.050 to 0.084 mmol/g of creatinine. The dose-response relationship between acetate administration and oxypurine excretion is evident.

caused a substantial increase in urine oxypurine excretion, which was less than the larger rate of acetate infusion.

Properties of ethanol and acetate infusion. As expected from the preliminary studies, plasma acetate significantly increased during sodium acetate infusion from a mean base-line value (±SE) of 0.04±0.01 mM to 0.35±0.07 mM ($P < 0.0005$) (Fig. 2). Plasma acetate levels remained stable throughout hypertonic saline infusion. Ethanol infusion in equimolar quantities promoted an elevation of plasma acetate concentrations from a base-line value of 0.04±0.01 to 0.08±0.01 mM ($P < 0.01$). Plasma ethanol increased to a peak mean value of 17.1±1.4 mg/dl and was not correlated with plasma acetate.

There was evidence for increased purine nucleotide degradation during acetate and ethanol infusions (Fig. 3). Urinary oxypurines increased after acetate and ethanol infusions as compared with saline infusion. The oxypurine-to-creatinine ratio increased to peak values of 223±13% and 316±44% from a base-line value of 118±8 μmol/g of creatinine after acetate and ethanol infusions, respectively. The values for ethanol and acetate were not significantly different from each other. Although saline infusion promoted a modest elevation of oxypurine excretion, this increase was significantly less than that induced by acetate or ethanol. Urinary radioactivity increased to 171±28% from an acetate base-line value of 412±50 × 10^3 cpm/g of creatinine and to 128±8% from an ethanol base-line value of 401±36 × 10^3 cpm per gram of creatinine and remained significantly elevated for three successive hours. Saline infusion did not augment radioactivity excretion, which remained stable throughout the study. The ratio of urinary uric acid to creatinine, expressed as a percentage, increased to 132±16% of the preinfusion base-line values of 0.30±0.04 mg/mg of creatinine after ethanol infusion and remained elevated during successive hours. The moderate increases of the urinary uric acid to creatinine ratio induced by saline and acetate infusions were not statistically significant. Similar changes were seen in the ratio of the uric acid clearance to the creatinine

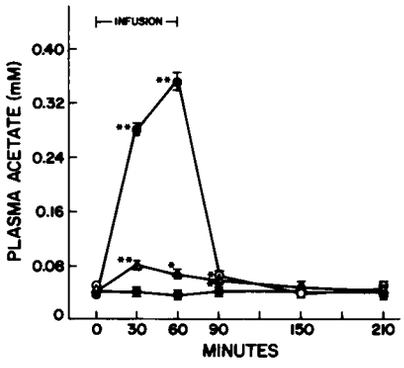


Figure 2. Plasma acetate levels during sodium acetate, sodium chloride, and ethanol infusion. Five normal subjects were infused with 0.1 mmol/kg per min for 1 h of sodium acetate (○), sodium chloride (●), and ethanol plus sodium chloride (△). A single asterisk denotes significance at $P < 0.05$ level with respect to base-line values. A double asterisk denotes significance at $P < 0.01$ level with respect to base-line values. A dot inside ○ or △ denotes significance at $P < 0.01$ level with respect to saline and ethanol infusions, respectively.

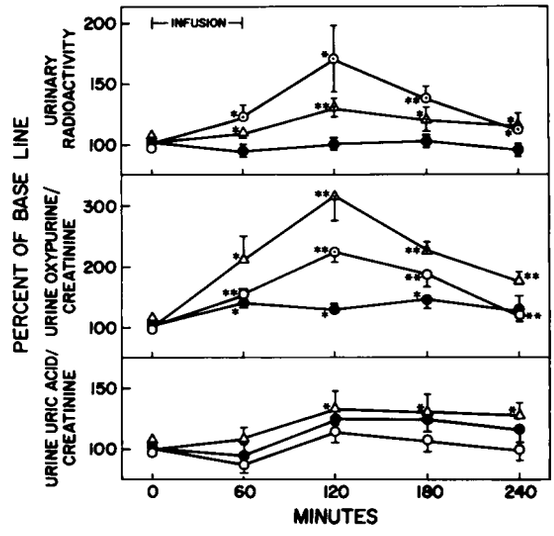


Figure 3. Alterations of purine degradation during sodium acetate, sodium chloride, and ethanol infusion. Sodium acetate (○), sodium chloride (●), and ethanol with sodium chloride (△) were infused at a rate of 0.1 mmol/kg per min for 1 h. The data are expressed as percentages of mean base-line values±SE. Mean base-line values are as follows: urinary radioactivity (cpm × 10^3/g of creatinine), 427±62; urinary oxypurines (μmol/g of creatinine), 118±18; urinary uric acid (mg/mg of creatinine), 0.30±0.04. A single asterisk denotes significance at $P < 0.05$ level with respect to base-line values. A double asterisk denotes significance at $P < 0.01$ level with respect to base-line values. A dot inside ○ or △ denotes significance at $P < 0.01$ level with respect to saline infusion.

clearance. These results indicate that both ethanol and acetate markedly increase the excretion of uric acid precursors and labeled degradation products derived from the adenine nucleotide pool (20). The enhanced uric acid and oxypurine excretion promoted by saline may be related to the well-known properties of increased urinary flow rate (21), rather than to an elevation in the rate of uric acid synthesis.

Serum urate and whole blood lactate and pyruvate levels did not significantly change during the three infusions (Table I). Plasma inosine and adenosine did not show substantial changes either from the base-line values or in comparison with the other infusions. Peak plasma hypoxanthine levels increased significantly ($P < 0.05$) during acetate and ethanol infusions but remained stable throughout saline infusion. These observations together with the increased urinary oxypurine excretion suggest increased production of hypoxanthine during acetate and ethanol administration.

Discussion

Alcohol ingestion may cause hyperuricemia by both decreasing the renal clearance of uric acid and increasing urate production (2–7). We have previously demonstrated that the ethanol-induced elevation of urate production was secondary to accelerated turnover of ATP (7). We hypothesized that accelerated ATP turnover was triggered by the conversion of acetate, formed from ethanol oxidation, to acetyl-CoA as follows:

$$\text{Acetate} + \text{ATP} \rightarrow \text{PP}_i + \text{Acetyl-AMP}$$

$$\text{Acetyl-AMP} + \text{CoASH} \rightarrow \text{Acetyl-CoA} + \text{AMP}.$$

Although most of the AMP formed would be resynthesized to ATP, a small percentage of AMP could enter the pathway of purine nucleotide degradation and lead to increased urate synthesis (7). If this hypothesis is correct then the infusion of ethanol may elevate blood acetate levels and the infusion of acetate should promote similar changes of purine nucleotide degradation to those observed during ethanol administration. Measurement of plasma acetate during ethanol infusion allowed assessment of the first possibility. The second possibility was examined by measurement of plasma and urinary purines. ATP turnover was assayed by prelabeling the adenine nucleotide pool with radioactive adenine and quantitating urinary radioactivity (8, 9).

*Table I. Plasma Levels in Five Normal Subjects during Intravenous Administration of Sodium Acetate, Sodium Chloride, and Ethanol**

	Base-line‡	Acetate	Saline	Ethanol
Alcohol (*mg/dl*)	<0.1	<0.1	<0.1	17.1±1.4§
Lactate (*mM*)	0.5±0.1	0.6±0.1	0.5±0.1	0.7±0.1
Pyruvate (*mM*)	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1
Acetate (*mM*)	0.04±0.01	0.35±0.16§	0.04±0.01	0.08±0.01‖
Urate (*mg/dl*)	4.0±0.3	4.3±0.3	3.8±0.3	4.1±0.3
Hypoxanthine (*μM*)	2.0±0.2	2.7±0.3¶	2.1±0.4	2.8±0.4¶
Xanthine (*μM*)	0.3±0.1	0.5±0.3	0.4±0.2	0.9±0.3¶
Inosine (*μM*)	1.6±0.1	1.5±0.3	2.6±0.3	2.1±0.5
Adenosine (*μM*)	0.2±0.1	0.2±0.1	0.2±0.1	0.4±0.1

* Peak values expressed as mean±SE.
‡ Refers to the base-line values of the three infusions.
§ $P < 0.0005$ with respect to base-line and peak saline values.
‖ $P < 0.01$ with respect to base-line and peak saline values.
¶ $P < 0.05$ with respect to base-line and peak saline values.

The major metabolic pathway of ethanol metabolism involves the formation of free acetate in the liver (22, 23). Ethanol intake increases blood acetate levels in normal subjects (Table I) (24, 25). The acetate formed from ethanol may be oxidized in the liver or peripheral tissues at a rate of 300 mmol/h (22–28). Since hepatic metabolism of ethanol is capable of forming acetate at a rate greater than the rate of hepatic acetate oxidation (22–26, 28), increasing ethanol loads will provide larger quantities of acetate to peripheral tissues. As much as two-thirds of acetate formed from ethanol may be utilized by tissues outside of the gastrointestinal tract (22). It is this variability of the site for acetate metabolism, depending upon the ethanol load, that provides an explanation for the different plasma levels of acetate produced by equimolar quantities of ethanol and acetate in our study. The smaller quantitites of acetate in the venous circulation after ethanol infusion as compared with acetate infusion indicate predominant hepatic metabolism of acetate produced from ethanol in our experiments. This is related to the relatively low rates of ethanol administration in our study compared with previous reports (22–25).

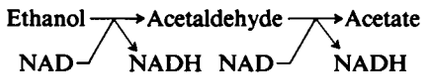
Acetyl-CoA is synthesized from acetate in either the cytoplasmic or mitochondrial cell compartments of hepatic or extrahepatic tissues (22–28). The energy for this reaction is provided by ATP. 2 mol of high energy phosphate are consumed for each mole of acetate metabolized. In fact, ~30% of the total mitochondrial pool of adenine nucleotides may be converted to AMP when acetate is oxidized (26). The release of ATP degradation products, such as hypoxanthine or adenosine, during infusions with acetate (27, 29) supports the concept of acetate induced ATP degradation.

Both acetate and ethanol infusions caused alterations consistent with increased adenine nucleotide degradation. Urinary oxypurine excretion increased markedly during acetate and ethanol infusion. Plasma hypoxanthine levels increased significantly during both these infusions. In addition, both infusions significantly accelerated the urinary excretion of radioactively labeled purine degradation products derived from the ATP. The exact quantitative response of urinary oxypurines and urinary radioactivity after acetate and ethanol administration was different. This most likely reflects the proposed different sites of metabolism of infused ethanol and acetate in our experiment.

Although our studies indicate that both ethanol and acetate administration increase ATP degradation, what is the evidence

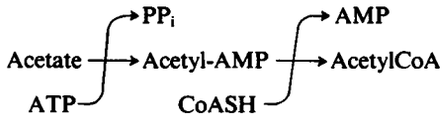
that this property of ethanol is mediated by acetate metabolism? There are two possible ways by which ethanol could increase adenine nucleotide turnover.

(*a*) By initial steps of ethanol metabolism:



It seems unlikely that the generation of reducing equivalents in the liver would cause accelerated breakdown of ATP. In fact, the elevated reducing equivalents would be expected to increase ATP synthesis by oxidative phosphorylation (30).

(*b*) By metabolism of acetate:



Each mole of acetate will consume 2 mol of high-energy phosphate for each mole of acetyl-CoA formed. Virtually all ethanol is converted to acetyl-CoA and then oxidized to form carbon dioxide and water. Therefore, 2 mol of high-energy phosphate are consumed for each mole of ethanol metabolized. The infusion of 400 mmol of ethanol during 1 h in our study will use 800 mmol of high energy phosphate during ethanol metabolism by this pathway.

Therefore, the extensive state of knowledge concerning ethanol and acetate metabolism imposes limits upon the possible interpretation of our experimental data. Since it is known that virtually all ethanol is converted to acetate (22, 23), then it is highly likely that a metabolic property common to both ethanol and acetate could explain this property of ethanol. This is especially the case when the other steps in the conversion of ethanol to acetate do not provide a likely mechanism for accelerated ATP turnover. One proposed mechanism, that alpha-glycerol phosphate accumulation during ethanol intake depletes inorganic phosphate and ATP in liver cells (31), cannot be ruled out by our studies. It is noteworthy that despite the discussion of ATP utilization, increased ATP production appears to result from other steps in ethanol metabolism and one of the constraints on the rate of ethanol conversion to acetate appears to be the limitation of ATP production (30).

Our observations concerning ethanol and acetate metabolism are clinically relevant. The metabolic basis for increased uric acid production and gout with ethanol intake has been further elucidated by our studies (2–7). Furthermore, the ability of acetate administration to increase ATP degradation has additional practical implications. Sodium acetate is a commonly used buffer for hemodialysis (32–38). Acetate-intolerance, observed as dialysis-induced hypotension (35), has been reported in some patients with chronic renal failure in whom plasma acetate levels increase markedly (39). This activity has been attributed to the vasodilator properties of acetate, which may be related to its ability to increase tissue adenosine (27, 29), a well-known vasodilator compound (40–42). Infusion of acetate elevates myocardial adenosine levels and augments cardiac output and coronary blood flow (27) by first increasing tissue AMP concentrations (29, 43) and decreasing the ratio of ATP to ADP. By a similar mechanism, acetate may contribute to the hemodynamic effects of ethanol. It is clear that the complex metabolic properties of ethanol related to ATP turnover have important biological activities in humans.

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