Increased brain uptake and oxidation of acetate in heavy drinkers

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Introduction

Alcohol is the most used recreational substance and one of the most widely abused drugs in the world. Alcohol use is characterized by CNS intoxication symptoms, impaired brain activity, poor motor coordination, and behavioral changes. The impairments in CNS activities are due to alcohol’s effect on synthesis, release, and signaling of neuron transmitters, including glutamate, GABA, and other neurotransmitters (1–3). Alcohol use also affects insulin sensitivity that regulates protein, carbohydrate, and fat metabolism (4). Chronic abuse of alcohol can result in tolerance and physical dependence. Although significant advances in understanding of alcohol’s effects have been made over the past decades, the pathogenesis of alcohol use and abuse is not fully understood (5). Understanding the mechanisms that lead to tolerance and dependence may give valuable insight into alcohol addiction and vulnerability and ultimately result in effective therapeutic intervention to facilitate detoxification.

Alcohol is converted to acetate in the periphery, particularly in the liver (6, 7), and it is released to the blood (8). Administration of ethanol to humans elevates blood acetate (6, 9, 10) from <0.1 mM to 1 to 2 mM within minutes of the start of the administration (11, 12). Consumption of enough alcohol to achieve breath alcohol levels of even 50 mg% is sufficient for plasma acetate levels to approach 1–2 mM, beyond which the plasma acetate concentration does not rise (6, 9, 10, 12, 13). Acetate travels to other organs, including the brain, for use as an energy substrate (14) and in fatty acid and cholesterol biosynthesis (15, 16). The conversion of ethanol to acetate begins with metabolism to acetaldehyde. In people who consume alcohol at moderate levels or occasionally, the etha-

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always have elevated acetate. If acetate consumption by brain is higher, then adenosine effects can be expected to be enhanced. Faced with persistent elevation of adenosine, the brain likely adapts, and during withdrawal, the loss of adenosine may contribute to symptoms.

Our goal in this study was to test whether chronic heavy drinking can affect brain acetate uptake and metabolism. To this end, we used [2-13C]acetate as a metabolic substrate in combination with in vivo magnetic resonance spectroscopy (MRS) to compare brain acetate consumption in heavy drinkers and light drinkers.

**Results**

**Impact of heavy drinking on plasma acetate.** On a typical drinking day, the heavy drinkers consumed 8 ± 1 drinks (n = 7), and the light drinkers consumed 1 or 0 (i.e., some drank very rarely). For the heavy drinkers, the last drink was 3 ± 1 days earlier (n = 5, 2 did not answer that question), while for the light drinkers, one had a drink 7 days before, and the others consumed no alcohol for at least 20 days and in several cases over 2 months. Heavy drinkers tested sober, with breath alcohol of 0 on the test days, having drunk no fewer than 2 days before the study, and in one case one week before the study. The averaged longest interval without drinking for heavy drinkers was 5 ± 1 days. Heavy drinkers had significantly greater levels of plasma acetate before infusion (0.20 ± 0.13 mM, range: 0.09–0.48 mM) compared with those of light drinkers (0.09 ± 0.01 mM, range: 0.08–0.11 mM) (P = 0.05) (Figure 1A). Differences in acetate before infusion between the groups may reflect slower acetate clearance rates in the heavy drinkers. Plasma glucose showed no significant difference before and during the acetate infusion, with levels before infusion of 5.0 ± 0.5 mM for heavy drinkers and 4.8 ± 0.4 mM for light drinkers (P = 0.6). During the acetate infusion, subjects showed no significant plasma glucose changes (overall group effect, P = 0.61), with variations of less than 2% (Figure 1B). Plasma lactate and β-hydroxybutyrate enter the brain by the same monocarboxylic acid transporter as acetate, and so differences could potentially affect acetate entry to the brain by competitive inhibition. β-Hydroxybutyrate differed insignificantly between the 2 groups at baseline (0.17 ± 0.01 mM and 0.21 ± 0.11 mM for light and heavy drinkers, respectively, P = 0.13) and after 120 minutes (0.09 ± 0.03 mM and 0.61 ± 0.42 mM, respectively, P = 0.27). The rise in β-hydroxybutyrate during the infusion was significant in the heavy drinkers (P = 0.05), if no Bonferroni correction is applied to account for the multiple measures in plasma, but not in the light drinkers (P = 0.28). However, plasma lactate showed no significant differences between groups before and during the acetate infusion, with lactate levels before infusion of 0.95 ± 0.13 mM and 0.83 ± 0.10 mM for light and heavy drinkers, respectively (P = 0.46), rising slightly to 1.03 ± 0.12 mM and 0.84 ± 0.12 mM, respectively, by the end of the infusion (P = 0.30). The rise in lactate was also insignificant for each group (P = 0.93 and P = 0.56 for heavy and light drinkers, respectively) or for all data together (P = 0.63). Infusion of [2-13C] acetate increased the plasma acetate from baseline (~0.1 mM) to approximately 1 to 2 mM within 5 minutes (Figure 1C) among both groups. The steady-state plasma acetate concentrations between heavy drinkers and light drinkers were comparable (1.2 ± 0.3 mM for heavy drinkers and 1.3 ± 0.2 mM for light drinkers, P = 0.78; Figure 1C). Plasma 13C-acetate enrichments followed a similar pattern as plasma acetate concentrations, rising from 0% to 70%–80% within 5 minutes. No significant differences were seen between groups at steady state (72% ± 5% for heavy drinkers and 77% ± 5% for light drinkers, P = 0.5; Figure 1D).
Impact of heavy drinking on $^{13}$C labeling in the brain. Figure 2 shows representative $^{13}$C-MRS spectra during the steady-state portion of the $^{13}$C-acetate infusion in a heavy drinkers and light drinkers, normalized to the subjects’ own natural $^{13}$C abundance of N-acetylaspartate (NAA) C3 and C6 resonances. The $^1$H MRS measurements showed no significant differences between heavy drinkers and light drinkers with respect to any of the metabolites, respectively, including the ratio of NAA to water ($P = 0.10$), that of glutamate to water ($P = 0.49$), that of glutamine to water ($P = 0.62$), and that of GABA to water ($P = 0.4$). The most abundant $^{13}$C labeling occurred in the glutamate and glutamine C4 positions. Heavy drinkers had higher $^{13}$C labeling incorporation across the time course in glutamate C4 ($P = 0.01$) and glutamine C4 ($P = 0.021$) relative to that of light drinkers, including at the end point ($P = 0.0013$ and 0.012, respectively) (Figure 3, A and B). Glutamate and glutamine C3 are also labeled, which occurs as $^{13}$C is processed through multiple turns of the TCA cycle.

The time courses of $^{13}$C enrichments of glutamate and glutamine C4 and the steady-state $^{13}$C enrichments of glutamate and glutamine C3 were analyzed with a mathematical model of brain acetate metabolism in CWave software (33) to calculate the metabolic fluxes (Table 1 and Figure 4). Metabolic modeling of the individual time courses showed that heavy drinkers had a greater cerebral metabolic rate of acetate (CMR$_{ac}$) than light drinkers, with values of $0.069 \pm 0.008$ mmol/min/kg and $0.048 \pm 0.006$ mmol/min/kg, respectively ($P = 0.02$) (Figure 5A). $^{13}$C resonances of GABA, acetate, and other metabolites were small due to their low overall concentrations in the brain. $^{13}$C-GABA was undetectable in light drinkers but was detected at a level of $0.09 \pm 0.03$ mmol/kg in heavy drinkers, indicating that $^{13}$C-GABA labeling in heavy drinkers is also greater than that in light drinkers (Figure 2). In light drinkers, $^{13}$C-GABA was necessarily much lower than in heavy drinkers. Some idea of the limit can be drawn from the standard deviation of the spectral noise, $\pm 0.029$ mmol/kg. To detect the presence of labeled GABA across the group, signal-to-noise ratios of at least 1.5 would be needed, which have a detection limit of $0.044$ mmol/kg. The fraction of glutamate that resides in astroglia (FracGluA) was set to 0.10, calculated from data in ref. 34 for a similar voxel, but was tested also for a much lower value ($0.01)$.
Table 1

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<th>Equations used in CWave software to calculate metabolic rates</th>
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<td>Mass balance</td>
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<td>$d\text{Glu}<em>u/dt = V</em>{cyt} + V_{sat} - (V_{cyt} + \text{Glu}_u) = 0$</td>
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| Isotope balance                                              |
| $d\text{Glu}_u/dt = V_{cyt}(\text{Glu}_u/\text{Glu}) + \text{Glu}_u(\alpha \text{KGA}_u/\alpha \text{KGA}) - (V_{cyt} + \text{Glu}_u)(\text{Glu}_u/\text{Glu})$ |
| $d\text{Glu}_u/dt = V_{cyt}(\text{Glu}_u/\text{Glu}) + \text{Glu}_u(\alpha \text{KGA}_u/\alpha \text{KGA}) - (V_{cyt} + \text{Glu}_u)(\text{Glu}_u/\text{Glu})$ |
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| $d\text{Glu}_u/dt = V_{cyt}(\text{Glu}_u/\text{Glu}) + \text{Glu}_u(\alpha \text{KGA}_u/\alpha \text{KGA}) - (V_{cyt} + \text{Glu}_u)(\text{Glu}_u/\text{Glu})$ |

| Values of rates                                              |
| $\text{CMR}_{ac}$; cerebral metabolic rate of acetate, iterated |
| $V_{glu}$; glutamate/glutamine neuron transmitter cycle, calculated as a ratio to $V_{glu}$ based on steady-state Glu and Gin enrichment $V_{glu}/V_{sat} = (\text{Glu}_u/\text{Glu})$ (27, 71) |
| $V_{sat}$; 0.77 mmol/g; distribution space of brain water (72, 73) |
| $V_{bl}$; 0.02 mmol/g/min; anaplerotic flux (74) |
| $V_{glu}$; $V_{sat}$; glutamine synthesis rate |
| $V_{glu}$; $V_{sat}$; glutamine efflux, balanced by $V_{sat}$ |
| $V_{glu}$; $V_{sat}$; brain pyruvate dehydrogenase rate |
| $V_{ble}$; $V_{sat}$; 0.73 mmol/kg/min; neuronal pyruvate dehydrogenase rate equal to neuronal TCA cycle flux set to 0.73 mmol/kg/min. Because acetate is metabolized only in astrocytes, there was insufficient sensitivity to calculate $V_{ble}$ (67, 75, 76) |
| $V_{sat}$; $V_{ble}$; CMR; astrocyte TCA cycle flux |
| $V_{sat}$; $V_{ble}$; astrocyte TCA cycle flux, free iterated parameter |
| $V_{sat}$; $V_{ble}$; astrocyte TCA cycle flux, iterated parameter |

| Values of pool concentrations                                |
| $\text{FracGlux} = 0.10$; fraction of total glutamate that resides in astroglia (34) |
| $\text{Glu}_u = 4.1 \text{ mmol/g}$; total brain glutamate concentration (77) |
| $\text{Glu}_u = 9.1 \text{ mmol/g}$; total brain glutamine concentration (77) |
| $\text{Glu}_u = \text{FracGlux} \times \text{Glu}_u$; astroglial glutamine concentration (78) |
| $\text{Glu}_u = \text{Glu}_u - \text{Glu}_u$; neuronal glutamate concentration |
| $\text{Oaa}_u$; astrocyte oxaloacetate concentration |
| $\text{Oaa}_u$; neuronal oxaloacetate concentration |
| $\alpha \text{KGA}_u$; astrocytic $\alpha$-ketoglutarate concentration |
| $\alpha \text{KGA}_u$; neuronal $\alpha$-ketoglutarate concentration |
| $\alpha \text{KGA}_u$; $\text{Oaa}_u = 0.1 \text{ mmol/kg}$ (76) |

which was found to have a negligible impact on the results. The rate of glutamate-glutamine cycling ($V_{pkl}$) was calculated relative to the neuronal TCA cycle ($V_{nkl}$), using the steady-state enrichments of glutamate and glutamine C4, as stated in Table 1. The value of $V_{pkl}$ was $0.18 \pm 0.03$ mmol/kg/min and $0.28 \pm 0.02$ mmol/kg/min in light and heavy drinkers, respectively ($P = 0.008$). Two other parameters, the astroglial rate of pyruvate dehydrogenase ($V_{pdhA}$) and the rate of glutamine synthesis ($V_{pkl}$), were defined from other rates, as defined in Table 1. $V_{pkl}$ was estimated to be $0.08 \pm 0.02$ mmol/kg/min and $0.04 \pm 0.02$ mmol/kg/min in light and heavy drinkers ($P = 0.13$), and the estimated value of $V_{pkl}$ was $0.22 \pm 0.03$ mmol/kg/min and $0.32 \pm 0.02$ mmol/kg/min in light and heavy drinkers ($P = 0.008$). It is important to note that lactate from the blood exchanges with lactate that is generated in the brain, and its effect is combined with that of glucose in the rate of flow through pyruvate dehydrogenase.

The metabolic fitting required that the rate of exchange between astrocytic $\alpha$-ketoglutarate and glutamate ($V_{kgn}$) have an assumed value (Table 1). We fitted the data for Figure 5, A and B, with a value of the exchange rate that was 10-fold greater than the astrocytic TCA cycle rate ($V_{nkl}$). To test the sensitivity of the model to the value of $V_{kgn}$, fitting was repeated with $V_{kgn} = V_{nkl}$. The sensitivity test of $V_{kgn}$ showed that for the minimum value of $V_{kgn}$, which is equal to $V_{nkl}$, CMR was $0.103 \pm 0.013$ mmol/kg/min and $0.061 \pm 0.008$ mmol/kg/min for heavy drinkers and light drinkers, respectively, while for $V_{kgn} = 10 \times V_{nkl}$, CMR was $0.069 \pm 0.008$ mmol/kg/min for heavy drinkers and $0.048 \pm 0.006$ mmol/kg/min for light drinkers. In neither case did the value of $V_{kgn}$, which was $0.08-0.13$ mmol/kg/min (Figure 5, B and D), differ significantly between heavy drinkers and light drinkers, although in both cases heavy drinkers showed significantly higher CMR than that of light drinkers ($P = 0.02$) (Figure 5, A and C).

Impact of heavy drinking on brain acetate concentrations. Brain 13C-acetate concentrations during the steady-state portion of the infusion of [2-13C]acetate were 80% higher in heavy drinkers (0.071 ± 0.014 mmol/kg) than in light drinkers (0.039 ± 0.007 mmol/kg) (P = 0.06; Figure 6A). The ratios of brain/blood 13C-acetate concentrations in heavy drinkers were 84% greater (0.049 ± 0.007) than those in light drinkers (0.026 ± 0.006) (P = 0.02; Figure 6B). Because brain acetate levels and brain/blood ratios were higher, while at the same time the brain was consuming more acetate, heavy drinkers must have had elevated blood-brain transport of acetate compared with that of light drinkers.

Relationship of acetate uptake to recent drinking history. Steady-state glutamate C4 13C enrichments were significantly correlated with the number of drinks consumed in the past 30 days ($P = 0.0005, r^2 = 0.8$; Figure 7A) and past 60 days ($P = 0.0006, r^2 = 0.8$). Steady-state glutamine C4 13C enrichments were correlated with the number of drinking days in the past month ($P = 0.03, r^2 = 0.6$; Figure 7B), although the latter comparison did not survive a Bonferroni correction. The relationship suggests that the increase in the metabolite labeling is an adaptation of the brain to obtain more energy from acetate during chronic heavy drinking.
In this study, we have shown that chronic heavy drinking increases brain acetate utilization and blood-brain transport of acetate. In the presence of similar levels of plasma acetate, heavy drinkers were able to use more acetate as an alternative energy fuel to support brain metabolism. Acetate could potentially promote continued heavy drinking with a reward in the form of caloric benefit or adenosinergic adaptation.

Glutamate-glutamine cycling and GABA synthesis. A finding of potential clinical significance was the increased ratio of glutamate-glutamine cycling ($V_{\text{cycle}}$) relative to the neuronal TCA cycle rate ($V_{\text{tcaN}}$) in heavy drinkers, although in this nondependent group, the finding was unexpected. The change could occur if $V_{\text{cycle}}$ was greater or if $V_{\text{tcaN}}$ was lower or some combination of both. For the case of elevated $V_{\text{cycle}}$, one possibility is advanced liver disease, in which hepatic encephalopathy could increase conversion of glutamate to glutamine (35), but these subjects had normal liver function tests. Another explanation for elevated $V_{\text{cycle}}$ is that after at least 2 days without alcohol, there was increased glutamatergic tone that stimulated greater astroglial uptake and conversion to glutamine. Yet another possibility is that the $V_{\text{cycle}}/V_{\text{tcaN}}$ ratio was higher due to a slower $V_{\text{tcaN}}$. Most brain energy consumption is neuronal (36) and supplied by glucose. Brain glucose uptake has been reported to be 12% lower in the occipital cortex in recently abstinent alcohol-dependent subjects (37) or not different (38), so one expects at most a 12% reduction in $V_{\text{tcaN}}$ and therefore little impact on the estimated values of $V_{\text{cycle}}$ and $CMR_{\text{ac}}$ compared with the differences between the groups. Of note is the recently proposed theory that the ratio of glutamine/glutamate concentrations might reflect glutamate-glutamine cycling (39), but in this case, $V_{\text{cycle}}$ differed without differences in the overall ($^{13}\text{C}+^{12}\text{C}$) levels of glutamate and glutamine.

A finding that is potentially related to the increased rate $V_{\text{cycle}}$ was that $^{13}\text{C}$ GABA was detected in heavy drinkers but not in light drinkers. In this study, if the light drinkers consumed half as much acetate as the heavy drinkers, then the light drinkers would be expected to have a concentration of $^{13}\text{C}$-GABA equal to 0.045 mmol/kg, which is right at the lowest reasonable limit of detection given the spectral noise. Therefore, although it is possible that $^{13}\text{C}$-GABA was increased by higher glutamate-glutamine cycling, we cannot differentiate between that possibility and simple detectability limits.

Increased availability of acetate for brain metabolism. Chronic heavy drinkers spend a large fraction of time with elevated blood acetate, and the greater brain acetate metabolism can potentially be induced by the habitual generation of acetic acid in the blood from alcohol, as observed previously in humans and other animals (40, 41) Furthermore, chronic heavy drinking without eating can induce episodes of hypoglycemia (42), which have been shown to increase blood-brain monocarboxylic acid transport and therefore increase brain availability of acetate (28). In the present case, the baseline levels of plasma acetate in heavy drinkers were more than twice those in the light drinkers, despite the study-required 48-hour abstinence from alcohol. The levels of brain acetate in light and heavy drinkers, respectively, were 0.039 mmol/kg and

Figure 4
Metabolic pathways showing brain uptake of [2-$^{13}\text{C}$]acetate and transfer of the $^{13}\text{C}$ labeling to glutamine and glutamate in astroglia and neurons. Astroglia consume acetate, while neurons and astroglia both consume glucose (Glc). The acetate labeled at the methyl group, whose carbon is designated 2 (Ac$_2$), enters the astroglia and the TCA cycle to form C2-labeled acetyl CoA (Ac$_2$CoA). In the first turn of the Krebs cycle, it labels the C4 of astroglial $\alpha$-ketoglutarate ($\alpha$-KGA$_4$), which exchanges to form glutamate C4 (Glu$_N$). Astroglia convert glutamate to glutamine, forming glutamine C4 (Gln$_4$), which is transferred to neurons, converted, and mixed with the large neuronal pool of glutamate (Glu$_N$). Some of the glutamate is released as part of glutamate-glutamine cycling, and some exchanges to form neuronal $\alpha$-ketoglutarate ($\alpha$-KGN$_4$). In both compartments, the carbon continues through the cycle and labels oxaloacetate (OAA) and labels glutamate and glutamine at C3 (data not shown) but does not return to the C4 of glutamate and glutamine. Meanwhile, the vast majority of glucose remains unlabeled and dilutes the pool of acetyl CoA and the Krebs cycle intermediates in neurons and astroglia. AcCoA, acetyl-CoA; Lac, lactate; Pyr, pyruvate.
0.071 mmol/kg (Figure 6). Given an acetate distribution space of 0.32 ml/g (43), the respective acetate concentrations in solution were 0.12–0.22 mM, close enough to the $K_M$ of 0.17 mM for utilization (43). There must have been a nearly exclusive unidirectional inflow of acetate from blood to brain, and the increased transport could double the rate of oxidation. It is therefore possible that the elevation of brain acetate via transport is sufficient to drive its oxidation. Possible impacts on transport were slightly higher levels of $\beta$-hydroxybutyrate in heavy drinkers, although that would compete with acetate for transport, so any increased transport in heavy drinkers is large enough to overcome whatever differences in ketone bodies might exist. The 10%-20% lower levels of lactate in the drinkers would be expected to increase acetate transport slightly through reduced competition for transport but not enough to explain the much greater metabolism seen here. Such impact of transport, facilitating some conversion from glucose to acetate consumption, would be consistent with recent data that show that the rat brain decreases uptake and phosphorylation of glucose when the acetate levels are higher in the blood (31). Human results also show decreased glucose uptake and higher acetate consumption in the presence of alcohol (30). An important but challenging measurement to make in these populations would be blood-brain transport of acetate.

**Figure 5**

Metabolic rates calculated based on individual $^{13}$C time courses of Glu4 and Gln4 and the steady state of Glu3 and Gln3. The value of $V_{a,k}$, which is the rate of exchange between astroglial $\alpha$-ketoglutarate and glutamate, has not been determined. The kinetics was therefore tested over a range of values of $V_{a,k}$. Its minimum possible value is equal to the rate of the TCA cycle $V_{tca,k}$, and, for values above $10 \times V_{tca,k}$, there is negligible difference in the kinetic impact compared with infinity (70), so $V_{a,k} = 10 \times V_{tca,k}$ was selected as the maximum of the range. (A) CMR$_{ac}$ was calculated assuming $V_{a,k} = 10 \times V_{tca,k}$. CMR$_{ac}$ was significantly greater in the heavy drinking group ($P = 0.02$). (B) Astroglial TCA cycle ($V_{tca,k}$) rates did not differ ($P = 0.58$) when $V_{a,k} = 10 \times V_{tca,k}$. (C) CMR$_{ac}$ was calculated assuming $V_{a,k} = V_{tca,k}$, showing significant differences between heavy drinkers and light drinkers ($P = 0.01$). (D) $V_{tca,k}$ showed no difference between heavy drinker and light drinker groups when $V_{a,k} = V_{tca,k}$ ($P = 0.99$). Values with error bars represent group mean ± SEM. Symbols represent individual concentrations; horizontal bars indicate the mean.

**Figure 6**

Brain acetate concentrations during steady-state $[2-^{13}$C$]$acetate infusion, showing heavy drinkers have increased $[2-^{13}$C$]$acetate in brain. (A) Steady-state brain $[2-^{13}$C$]$ acetate concentrations during the $[2-^{13}$C$]$acetate infusion. (B) Ratios of brain/plasma acetate concentrations at steady-state $[2-^{13}$C$]$acetate infusion. Values with error bars represent group average ± SEM. Symbols represent individual concentrations; horizontal bars indicate the mean.
infusions were performed under euglycemia, acetate metabolism in heavy drinkers showed a profile that was similar to what has been seen in patients with type 1 diabetes who have experienced antecedent recurrent hypoglycemia (28). Heavy drinking promotes a wide range of health problems related to hypoglycemia, such as impairment of gluconeogenesis, malnutrition, and confounding conditions like diabetes (44). Binge drinking itself decreases levels of NADH, thereby decreasing pyruvate levels and reducing gluconeogenesis (45). Compounding hypoglycemic conditions, there is evidence that some alcoholics have depressed counterregulatory responses to hypoglycemia (46). Alcohol caused a small reduction in plasma glucose concentration, from 5.1 to 4.7 mM, when infused to achieve a blood alcohol level of 60 mg% (47), which demonstrates that even without causing full-blown episodes of hypoglycemia, alcohol has the capacity to reduce levels of glucose, which is the brain’s primary energy supply. In fact, the largest triggers of alcohol-induced hypoglycemia appear to be binge drinking and failure to eat. Although few heavy drinkers experience hypoglycemia at any given moment (48), our results in patients with type 1 diabetes show that intermittent hypoglycemia is sufficient to double acetate transport and consumption (28). Therefore, the adaptation of monocarboxylic acid transport and metabolism seen in heavy drinkers may reflect adaptations similar to those seen in type 1 diabetes with antecedent recurrent hypoglycemia.

Because acetate is consumed exclusively in astroglia (49), such an energy supplement is expected to have its primary impact on glia. When glucose is in short supply, elevated acetate consumption can potentially support a large fraction of astrocytic energy needs and leave more glucose available to supply neurons (27). In this study’s population, no additional energy was needed for brain function in heavy drinkers, as evidenced by the fact that $V_{\text{tot}}$ was constant between heavy drinkers and light drinkers and that the increase in CMR$_{\text{ac}}$ in heavy drinkers was accompanied by decreased $V_{\text{pahA}}$, as predicted by the observations of Pawlosky and colleagues (31). However, in an abusing or dependent population that is malnourished, the additional fuel could provide some reward in the form of energetic benefit. Given the ability of ethanol to cause acute drops in blood glucose (26, 45, 47), the acetate formed from ethanol has the potential to provide a compensatory reward in the form of calories, thereby encouraging continued drinking.

Potential for adenosinergic impact of acetate consumption. Intracellular adenosine is produced by dephosphorylation of AMP (50) and other chemicals, including cAMP, ADP, and ATP (51), that involve signal transduction and metabolism sensing. Oxidation of acetate generates adenosine (52), and ethanol itself raises extracellular adenosine (42), which is sedating, with properties similar to those of alcohol intoxication (45, 46). The ethanol-based elevation of blood acetate persists for up to 24 hours (53), so heavy drinkers are exposed to high levels of acetate for sustained periods of time. It was not feasible with MRS to measure extracellular adenosine, but the elevated acetate oxidation suggests that adenosinergic effects from ethanol-derived acetate are likely to be enhanced in heavy drinkers. Persistent elevation of intracerebral adenosine has the potential to induce adenosinergic adaptations that contribute to continue heavy drinking. As drinkers become accustomed to chronic elevations of adenosine, drinking cessation may lead to discomfort with the decreased adenosine, and restoration of the adenosine by heavy drinking could be rewarding (20, 54).

Pitfalls and limitations. Although patients were instructed not to drink alcohol for 48 hours before the study, they could only be tested with a breathalyzer the morning of the measurement, which showed a value of 0 in every case. If someone drank less than 48 hours before the study, they could have cleared the alcohol but still have elevated acetate. That might explain the elevated levels of blood acetate before infusion, but during the infusion the acetate levels did not differ between the groups. A potential limitation is the apparent complexity of the metabolic model, given the small population of subjects. However, although this model contains many elements, it is subject to mass balance constraints that greatly reduce the number of free parameters and follows the generally accepted scenario of astroglial oxidation of acetate and glutamate-glutamine neurotransmitter cycling and has been applied in animals and humans in a variety of metabolic conditions (25, 55–58). If $^{13}$C-glucose infusions were performed to boost the sensitivity of the measurements to the value of the neuronal TCA cycle rate, that would decrease the number of assumptions to be made.

Conclusions. In summary, we have identified cerebral metabolic changes that are associated with heavy drinking. The changes suggest that chronic heavy drinking can induce upregulation of blood-brain monocarboxylate transport and increase the capacity of the brain to oxidize acetate. The results of increased ethanol-derived acetate consumption have the potential to increase extracellular adenosine levels, thereby adding an adenosinergic effect to the potential caloric benefit that acetate provides with continued heavy drinking. Our findings in heavy drinkers provide potentially useful information in treatment of alcohol-dependent subjects by suggesting the provision of acetate and/or adenosinergic support during detoxification to alleviate withdrawal symptoms.
Methods

Subjects. Healthy subjects, age 21–46, without alcohol dependence were recruited from the community via local advertisement. Healthy drinkers and light drinkers were age 29.7 ± 9.6 and 28.6 ± 7.7 years, respectively (P = 0.81). Pregnant women (evaluated by urine pregnancy tests) and people with a history of neurological disorders or contraindications for MRI were excluded. Individuals were also excluded on the basis of a structured diagnostic interview (SCID-II) that revealed a psychoactive substance abuse history other than tobacco smoking or heavy drinking in the previous 6 months (59). Other exclusion criteria included a lifetime history of posttraumatic stress disorder, bipolar disorder, or schizophrenia in self or first-degree family members and unwillingness to remain alcohol-free for the 48 hours before test days. Men who drank more than 14 drinks per week or women who drank more than 8 drinks per week were recruited as heavy drinkers, and people who drank fewer than 2 drinks per week were recruited as light drinkers. Alcohol consumption in the month prior to the testing date was determined using the timeline follow-back method (60). Subjects were asked to refrain from alcohol for 48 hours and fast for 10 hours before the acetate infusion in order to minimize the acute effect of ethanol and glucose on acetate metabolism. We enrolled 8 heavy drinkers (2 female, 6 male) and 8 light drinkers (2 female, 6 male). For the metabolic kinetic analysis, 1 light drinker was excluded due to extreme exercise and diet conditions, which have the potential to alter monocarboxylic acid transport and metabolism (61, 62), and 1 heavy drinker was excluded due to elevated 13C enrichment of β-hydroxybutyrate in the blood. For purposes of screening and acclimation to the scanner environment before undergoing the acetate infusion, we offered to perform a 1H MRS scan on subjects before the infusion day. Five out of seven heavy drinkers and six out of seven light drinkers included in the study underwent 1H MRS.

1H MRS. 1H MRS studies were performed with a 4T magnet (Oxford Magnetic Technology) and a Bruker spectrometer (Bruker Instruments), as described previously (63). The head of the subject was comfortably secured to a platform with the region of interest in a 4-cm distributed acquisition surface coil tuned to 170 MHz. A T1-weighted multislice MRI was obtained for anatomical localization. From the image, a 3 × 1.5 × 3-cm region of interest was selected, centered on the midline of the occipital cortex, within the volume of the larger 13C MRS voxels. Automated first- and second-order shimming was applied in the volume of interest (64). GABA was acquired over 20 minutes using a J-editing pulse sequence (65), as this provides a very sensitive test and training for the subject to hold sufficiently still for MRS. The spectral acquisition parameters were as follows: repetition time, 2.5 seconds; echo time, 68 milliseconds; sweep width, 15,000 Hz; and acquisition time, 510 milliseconds. An unsuppressed, localized spectrum of water was acquired for use as an internal concentration standard. The acquisition procedure, with setup, lasted approximately 45 minutes. In addition to GABA, glutamate, glutamine, NAA, and other metabolites were detected and fitted in the spectral domain with a measured basis set of metabolites. The free-induction decays were zero filled to 32 K, processed with –2 Hz Lorentzian to-Gaussian conversion and 16-fold zero filling, followed by Fourier transformation. An in-house LC model-type approach was used to fit peak areas of 13C-labeled glutamate C4 and C3, glutamine C4 and C3, GABA C2 and C3, and NAA C3 and C6. A Monte-Carlo analysis was used to estimate the standard deviations of each metabolite’s labeling from the fitting program (69). Because NAA C3 and C6 were not enriched measurably by the 2-hour acetate infusion, the natural 13C abundance of NAA signals of NAA C3 and C6 were averaged over the duration of the study and used as an internal standard to estimate the 13C-labeled concentrations of glutamate and glutamine. For steady-state brain 13C-acetate concentrations, we were reporting the summation of the last 5 FIDs.

Plasma acetate concentrations and 13C enrichments were measured in samples using water-suppressed 1H-observed/13C-edited NMR spectroscopy on a 11.7 T Bruker AVANCE high-resolution NMR spectrometer by mixing 50 μl plasma with 50 μl 2.5 mM formic acid in 100 mM phosphate buffer (pH 7.4) and 40% D2O.

Metabolic modeling analysis. Metabolic rates were determined by fitting a 2-compartment model of astroglia and neuronal metabolism, using the time courses of 13C enrichment of glutamate and glutamine C4 and the steady-state enrichments of glutamate and glutamine C3. The time courses of plasma acetate 13C enrichments were used as inputs for the modeling analysis. Because the blood-brain transport kinetics for acetate in humans has not been measured, the modeling does not include kinetic parameters for transport through the BBB or for acetate utilization. Therefore, CMRac reflects both transport and utilization. However, because plasma acetate levels were similar in both groups, any observed differences in acetate metabolism reflect actual changes in kinetic parameters for acetate transport and/or utilization, rather than changes in acetate uptake due solely to changes in plasma acetate concentration. Mass and isotopic flows from [2-13C]acetate to brain glutamate and glutamine were expressed as coupled differential equations with CWave 3.0 (33) running in Matlab (Mathworks). The equations (Table 1) were solved using a first-order Runge-Kutta algorithm, and least-squares optimization was achieved using a Levenberg-Marquardt algorithm.

Statistics. Repeated-measures multivariate ANOVA was used to compare the time courses of 13C labeling of glutamate and glutamine, with post-hoc pairwise testing performed to assess differences between groups. A 2-sample t test was performed to assess differences in initial plasma acetate, steady-state brain acetate, and CMRac between the heavy drinkers and light drinkers. A 2-tailed P value of < 0.05 was considered statistically significant. All data are presented as mean ± SEM. ANOVA was used to compare the enrichments of glutamate C4, glutamine C4, and GABA C2 between the heavy drinkers and light drinkers. Post-hoc t tests for independent samples were performed to assess which measurements differed significantly between the groups.


