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Accurate caloric compensation in rats for electively consumed ethanol–beer or ethanol–polycose mixtures

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Abstract

High elective intake of ethanol was achieved in rats by presenting ethanol in palatable vehicles. We simultaneously measured intake of food (chow) to assess the accuracy of caloric compensation for the energy in the alcoholic commodity. In the first study, we used beer; nonalcoholic beer was consumed in large amounts, and when 5% or 10% ethanol was added, intake amounted to ~10% of daily calories. In the second study, Polycose in either solution or a gel matrix was used as the palatable vehicle for ethanol. The intake of ethanol was even higher than in the beer study, particularly in the gel preparation. In all cases, both male and female rats showed accurate caloric compensation by a reduction in chow intake. In a final study, we showed that restricted time access to the Polycose–alcohol gel produced high elective intakes and substantial blood alcohol levels. Over 24 h, caloric compensation was again accurate. Thus, unlike some reports in humans, rats seem able to compensate accurately for alcohol calories and in particular when, as with most alcohol consumption by humans, these are presented in palatable vehicles.

Keywords: Caloric compensation; Alcohol; Rats

1. Introduction

It has been estimated that ethanol accounts for approximately 10% of the energy intake of individuals who drink alcohol on a regular basis (Block et al., 1985) but substantial long-term alcohol consumption by humans is not inevitably associated with excessive weight gain (Lieber, 1991; Prentice, 1995). This suggests either that alcohol decreases the overall efficiency of metabolism or that intake of food is reduced by an amount that compensates for alcohol calories. However, neither of these suggestions has strong empirical support: the effects of alcohol intake on overall metabolism are small (Murgatroyd et al., 1996) and caloric compensation for alcohol is poor (DeCastro and Orozco, 1990; Prentice, 1995; Tremblay et al., 1995). These observations are consistent with an assertion made over 50 years ago that "…Americans do not think of alcoholic beverages as a source of nutriment; they regard alcohol as a drug…that does not contribute directly to their diet" (Richter, 1953).

Well-controlled animal studies are suited to address the question of caloric compensation. Richter (1940, 1953) pioneered this line of research showing that rats that received alcoholic solutions (8–24% aqueous alcohol, beer, or wine) as their sole drinking fluid reduced intake of their stock (McCollum) diet by an amount that closely matched the calories consumed in the alcohol. The calories derived from ethanol in Richter's studies amounted to 20–40% of the total intake. In a recent study, male Sprague–Dawley rats were fed either a standard low fat chow or a high fat diet, and then either water or 5% ethanol as drinking fluid for 28 days (Cornier et al., 2002). Under these conditions, ethanol accounted for ~15% of daily caloric intake and there was

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almost perfect caloric compensation through reduced food intake in both diet groups. There was no effect of ethanol consumption on either energy expenditure or respiratory quotient in these rats.

Most strains of rats do not consume large amounts of ethanol on a voluntary basis, and protocols of forced consumption such as those described above thus involve a dimension of stress that is not present in the human drinker. An additional difference is that many rat studies use ethanol in water, whereas humans invariably consume ethanol in vehicles that contain carbohydrates and sometimes other macronutrients. The objective of the present study is to extend Richter's forced consumption studies to an elective protocol in rats by using alcoholic vehicles that resemble those consumed by humans, and to assess the effects of such elective intake on Chow intake. There may be sex differences in the effects of alcohol on metabolism in humans (Prentice, 1995) so both male and female rats were studied.

2. Material and methods

2.1. Animals and housing

Subjects were six male and five female Sprague–Dawley rats (Harlan). At the time of the study, the rats were about 6 months of age; males weighed ~500 g and females ~300 g. They were housed individually in stainless steel cages, with a mesh floor and front, suspended over absorbent paper bedding. These cages were chosen because they allow accurate measurement of 24 h intakes and spillage. The vivarium was maintained at 23±2 °C, with lights on from 2200 to 1000 h on a reverse cycle. Rat use was approved by the Institutional ACUC and was consistent with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Food and fluid measurements

Throughout the study, 5001 rodent Chow meal (Purina) was available ad libitum from a glass jar attached firmly inside the cage with a spring. Jars were weighed daily and refilled, and intakes corrected for spillage recovered underneath the cage. Data for the one rat that spilled significant amounts were not included in the analyses. The stated composition of Chow is 23% protein, 4.5% fat, 16.5% nonnutritive fiber, ash and minerals, and the balance (56%) carbohydrate. The caloric density of this diet is assumed to be 3.6 kcal/g.

Fluids were available at the front of the cage from graduated cylinders fitted with sipper spouts. Intakes were measured volumetrically to the nearest ml. In experiments with ethanol-containing fluids, two bottles were always available, one containing tap water and the other the alcoholic beverage. The left/right position of these tubes was alternated daily.

In the first experiment, we used non-alcoholic beer (Coors Brewing, Golden, CO) as a vehicle. This beer has a declared ethanol content <0.5% and caloric density of 0.21 kcal/ml (Table 1), presumably mostly carbohydrates. We then added either 5 or 10 ml absolute ethanol to 95 or 90 ml, respectively, of this non-alcoholic vehicle to give 5 and 10% ethanol by volume. Estimated caloric values and the percentage of calories as ethanol are shown in Table 1. Fresh decarbonated beer was presented each day at about noon. Bottles were opened in the morning, stirred vigorously in a beaker for 2–3 h, then alcohol was added prior to filling tubes.

In the second experiment, we used solutions of the oligosaccharide Polycose (Ross labs; 3.76 kcal/g) as vehicle. We used either 5% or 10% Polycose solutions to which 5% or 10% by volume absolute ethanol was added, as above. In another phase of this experiment, and in experiment 3, ethanol–Polycose mixture was presented in a solid gel matrix. Estimated caloric densities and the percentage of calories as ethanol are shown in Table 1. To prepare the gel, water was boiled and gelatin powder (Knox; 3 g/100 ml) added. Polycose was then added (5 or 10% by weight), and the solution allowed cooled to room temperature. The relevant volume of ethanol was then added, the solution poured into chilled screw-top glass gars (~60 ml capacity) and the jars were capped and placed in the refrigerator overnight. This yields a solid gel matrix. The cooling and capping procedure was designed to minimize evaporation of ethanol. To validate the effectiveness of the cooling and capping procedure, gels were made as above and 24 h later were melted under warm water and ethanol content determined using a NAD-ADH spectrophotometric assay (customized in the laboratory from the discontinued Sigma kit instructions). The ethanol content was within about 10% of the expected assay values. Gel was presented inside the home cage alongside the jar of chow; detectable spillage of the gel did not occur. Intake was measured gravimetrically. A small amount of evaporation (typically ~1 g/24 h) occurs from dummy jars of gel placed in the same room, but we did not correct the gel intakes by this amount because the solids left behind would be of correspondingly higher caloric density. Water was available ad libitum but its intake was not recorded in gel studies.

Table 1
Composition of alcoholic fluids and gels

	kcal/ml or g	Fraction of calories contained as EtOH
Nonalcoholic beer	0.21	<10
Nonalcoholic beer + 5% EtOH	0.45	57
Nonalcoholic beer + 10% EtOH	0.71	73
5% Polycose + 5% EtOH solution	0.45	58
10% Polycose + 10% EtOH solution	0.90	58
5% Polycose + 5% EtOH gel	0.56	46
10% Polycose + 10% EtOH gel	1.03	50

Nonalcoholic beer data from Coors; EtOH=5.24 kcal/ml (% shown refer to volume); Polycose=3.76 kcal/g; gel (3%w/v gelatin)=0.11 kcal/g.

2.3. Experiment 1: caloric compensation for alcohol with non-alcoholic beer as vehicle

In a preliminary study, we ascertained that elective intakes of 5% or 10% ethanol in water were very low (2–4 ml/day) but when non-alcoholic beer was the vehicle the intakes were 5–10 fold higher. Each phase of the main experiment lasted 4–5 days and, because we found no systematic change in intakes across days, intakes were averaged across the entire phase. In the first phase, rats received chow and water. This provided a baseline measure of chow and energy intake. In the next phase, rats received chow and a choice of non-alcoholic beer and water. In the third and fourth phases, 5% and 10% ethanol by volume was added to the non-alcoholic beer vehicle, again in a choice with water.

2.4. Experiment 2: caloric compensation for alcohol in polycose as vehicle

Beer is a complex mixture of nutrients and volatile molecules and, while it is suitable to model some aspects of human consumption, it lacks the rigorous control over content and composition that is desirable in dietary studies. In the second experiment, we examined the intake of ethanol and caloric compensation using a carbohydrate vehicle (Polycose) that is highly palatable but uses a different taste receptor than sucrose in rats (Ackroff et al., 1993). From both metabolic (glucose polymer vs. glucose–fructose disaccharide) and gustatory perspectives, Polycose differs considerably from sucrose solutions used in previous many studies (e.g. Samson, 1986) as a vehicle to boost alcohol consumption in rats.

In this experiment, after a baseline phase of chow and water, the rats received chow and non-alcoholic beer in a choice with water, then 5% and 10% alcoholic beer phases. In the next two phases of the experiment, rats received 5% Polycose–5% ethanol followed by 10% Polycose–10% ethanol. Half the rats received solutions and half-received gel. In the final two phases of the experiment, the sequence was repeated but with the solution rats now receiving gel and vice-versa. The data were similar regardless of whether gel or solution was presented first, so these two phases have been combined in the data presentation. As in the first experiment, each phase lasted 4–5 days and we observed no systematic change in intakes across days, so averages were computed for each rat for the entire phase.

2.5. Experiment 3: restricted access to alcohol–polycose gel

In the second experiment, we found that alcohol in gel was consumed in high amounts over 24-h periods. A useful extension of this mode of administration for pharmacokinetic work would be to restrict the alcohol-gel presentation to shorter periods. In experiment 3, using only male rats, chow was present for the entire 24 h, but 10% Polycose–10% ethanol gel was given for successively shorter periods (24, 6, 3, and 1 h) with each phase lasting 6 days. In each case, the alcoholic gel was presented at the time of lights out (1000 h). Data for each rat were averaged for each phase, as before.

To verify that substantial blood ethanol concentration (BEC) was achieved, an alcohol-naive batch of 6 male rats was familiarized with 1 h access to 10%Polycose–10% ethanol gel for 4–5 days until intakes were stable. On the last day, at the end of the hour access, the rats were anesthetized rapidly by inhalation of isoflurane, and a blood sample taken by cardiac puncture. The sample was deproteinized and assayed for BEC as described earlier.

2.6. Statistical analyses

Chow intakes were compared between phases using within subjects ANOVA or *t*-tests with significance set at $P<0.05$. Ethanol intakes were compared similarly. To assess caloric compensation, intakes were converted to calories using the estimated values presented in Table 1, and reduction in chow intake compared to estimated energy intake from the alcoholic beverage or gel. Individual data averaged across the phase were used to construct linear correlations to assess further the accuracy of caloric compensation.

3. Results

3.1. Experiment 1: caloric compensation for alcohol in beer as vehicle

The means±S.E. baseline food intakes of males and females were 29.7±2.4 g (107±9 kcal) and 22.4±0.9 g (81±3 kcal), respectively. Non-alcoholic beer was highly preferred with mean intakes in excess of 100 ml/day while concurrent water intakes (not shown) were 20–40 ml. Beer intakes were much lower when ethanol was added (Table 2), and water intakes rose inversely. While the volume intakes of the 5% ethanol-added beer were approximately in the ratio of the body weights of males compared with females,

Table 2
Intakes of alcohol containing solutions or gels by rats in experiments 1–2

	% EtOH	Males	Females
Experiment 1			
Nonalcoholic beer (ml/24 h)	0	126±10	122±7
	5	16±2	11±3
	10	9±1	9±1
Experiment 2			
Polycose–EtOH solution (ml/24 h)	5	44±7	16±5
	10	18±3	9±3
Polycose–EtOH gel (g/24 h)	5	54±3	50±6
	10	38±5	28±3

Shown are means±S.E.M. for *N*s=5.

intakes of non-alcoholic and 10% ethanol-added beers did not differ by sex. Total caloric intake in each phase is shown in Fig. 1. Calories from chow varied significantly as a function of sex and phase of the experiment (Ps<0.001), but with a non-significant ANOVA interaction term. Chow intakes during the 0% beer phase were lower (Ps<0.05) than in any other phase, and chow intake in the 10% beer phase was lower than during the baseline phase. Fig. 1 shows that beer accounted for about 25% of the caloric intake during the 0% phase, and this was reasonably well compensated by a reduction in chow intake. Caloric intakes from 5% and 10% ethanol-added beer accounted for less than 10% of the total calories consumed.

3.2. Experiment 2: caloric compensation for alcohol in polycose as vehicle

The intakes of the 5% and 10% Polycose–ethanol solutions and gels are shown in Table 2. During the solution phases, male rats consumed at least twofold more than females, a ration that is greater than their body weight differential. In both sexes the volume intake was about twofold higher in the 5% compared with the 10% phases. Thus, calories derived from solutions were similar at the two concentrations (~18 kcal in males, 8 kcal in females). During gel phases, intakes of males were slightly but not significantly higher than females. The intakes of gels were higher than same concentration solutions, especially in females.

Total caloric intake in each phase is shown in Fig. 2. During the solution phases, the chow intake of males was lower than baseline, and that during the 10% phase was lower than at 5%. In contrast, chow intake of females was not different between baseline, 5% and 10% solution phases. The

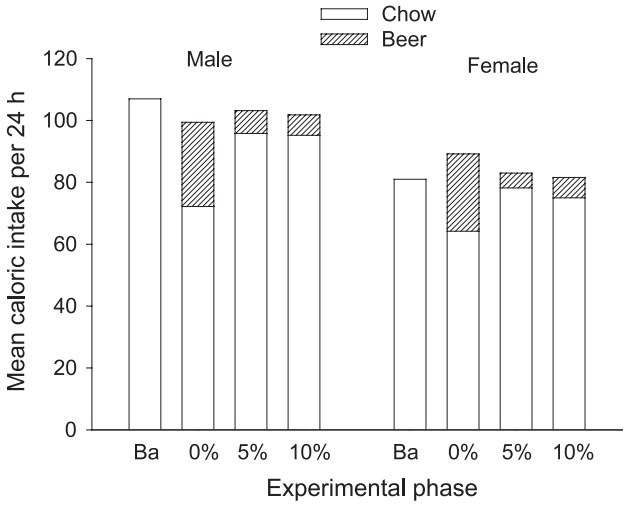

Fig. 1. Mean total caloric intake from chow (white portion of bars) and beer (shaded portion) under a no beer baseline (Ba) condition, and when the beer presented was either non-alcoholic (0%), or had 5% or 10% ethanol added.

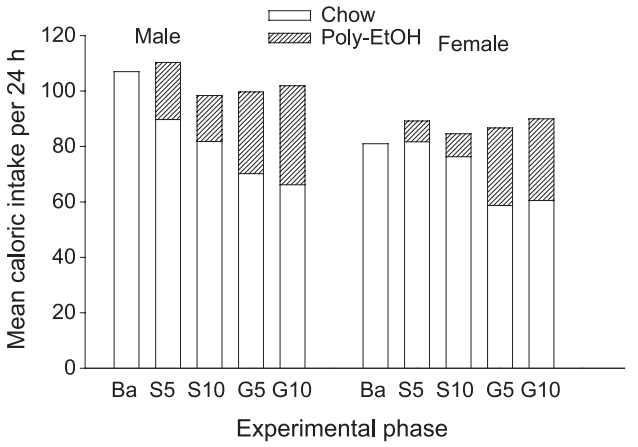

Fig. 2. Mean total caloric intake from chow (white portion of bars) and Polycose–EtOH (shaded portion) under a no beer baseline (Ba) condition, and when Polycose–EtOH was presented as 5% or 10% each in solution (S5, S10), or 5% or 10% each in gel (G5, G10).

sex by phase ANOVA interaction was highly significant (P<0.01).

During both 5% and 10% gel phases, and in both males and females, the chow intakes were lower than during the

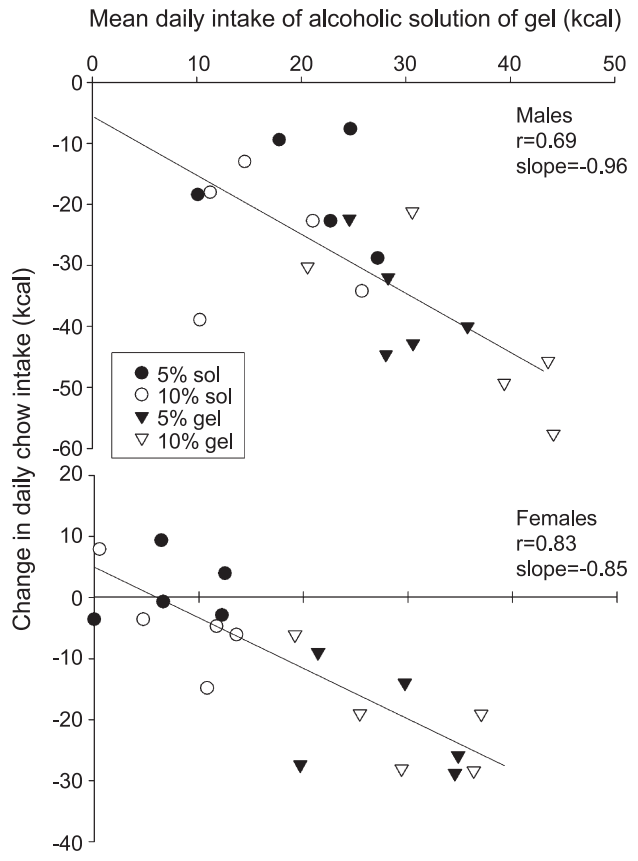

Fig. 3. Reduction in 24 h chow intake relative to baseline when rats also received Polycose–EtOH as 5% or 10% solutions or 5% or 10% gels. Shown are 4-day means for each of five male (upper panel) and five female (lower panel) rats that were studied in each condition. The y-intercepts and slopes of the linear regressions were: Intercept=−5.4 and +4.5; slope=−0.96 and −0.85 for males and females, respectively.

baseline period, but did not differ between 5% and 10% phases. The total caloric intake in males differed significantly as a function of phase ($P<0.001$), with intake during baseline and 5% solution phases significantly higher than during 10% solution and both 5% and 10% gel phases. In contrast, the intake in females was not different from baseline in any experimental phase, but their intake in the 5% gel phase was lower than in the 5% solution phase. Thus, both main effects of phase and sex (Ps<0.01) and their interaction ($P<0.02$) were significant.

Individual changes in chow intake were calculated and plotted in Fig. 3 as a function of calories consumed from either gel or solution. For males (top panel), the data for 5% and 10% lie on a linear regression with slope close to −1.00, which would be indicative of perfect caloric compensation. The y-intercept, theoretically zero, was in fact slightly negative. For females (bottom panel), the data were similar except that the slope was a little lower (−0.85) and the y-intercept was slightly positive. Caloric compensation was thus quite accurate.

3.3. Experiment 3: restricted access to alcohol–polycose gel

The mean intakes of 10% Polycose–ethanol gel at the four durations of access are shown in Table 3. As expected, intakes declined as access time was reduced, but the decrease was not proportionate. For example, rats ate half as much in 1 h as in 6 h of access. The corresponding daily chow intakes are shown in Table 3 and all were significantly reduced from the no gel baseline. These data were reanalyzed as decreases in chow intake as a function of gel intake (both in kcal) across all durations in Fig. 4. This relationship is highly linear ($r^2=0.81$, $P<0.01$) with a slope of −0.97 kcal chow/kcal gel, indicating nearly perfect caloric compensation.

The rats used for BEC assay consumed 2.6–10.2 g gel on the test day. These rats weighed ~500 g, so the approximate dosage of ethanol was 0.4–1.6 g/kg body weight. Their BEC ranged from 5 to 45 mg% with an approximately linear relationship to amount consumed (regression: BEC=−17±5.9(grams of gel); $r=0.94$). Most of the gel was consumed soon after presentation; it should be noted that these samples were taken 1 h after presentation by which time plasma concentrations may have declined from their peak (Roberts et al., 1999).

Table 3
Intakes of chow and polycose/alcohol gel as a function of duration of access to gel per day

Gel access (h/day)	Chow intake (g/24 h)	Gel intake (g)
No gel (baseline)	32.5±0.6[a]	n/a
1	26.9±1.0[b]	11.0±0.4
3	27.0±1.5[b]	13.7±1.2
6	25.1±0.7[b,c]	20.4±0.3
24	19.9±.7[b,c]	38.1±2.9

Shown are means±S.E. for five rats. For chow, different superscripts denote significant differences. For gel, intake at each duration is different.

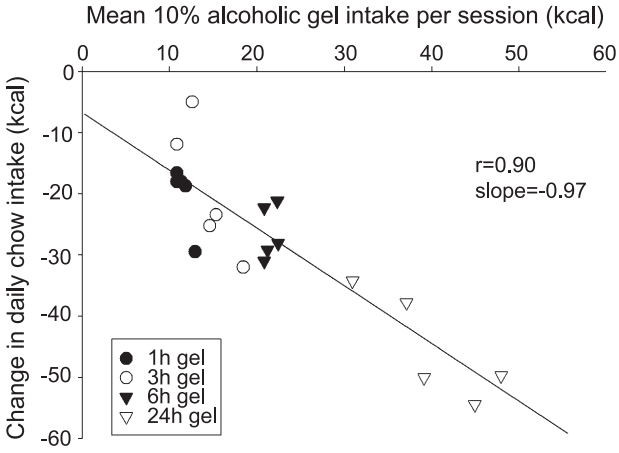

Fig. 4. Reduction in 24 h chow intake relative to baseline when rats also received a gel containing 10% Polycose plus 10% ethanol for either 1, 3, 6 or 24 h/day. Shown are 4-day means for each of five rats that were studied in each condition. The equation of the linear regression was: Change in chow intake=−6.5–0.97×energy content of gel.

4. Discussion

In each of the three experiments, both male and female rats showed accurate caloric compensation for alcoholic beverages or gels in which up to 50% of the caloric content was derived from ethanol. This conclusion, using elective consumption, is strikingly similar to those of Richter (1940, 1953) and Cornier et al. (2002) using forced consumption protocols.

In order to assess meaningfully the accuracy of caloric compensation, we tested palatable vehicles that boosted elective intake to levels that furnished an appreciable fraction of the daily calories. In preliminary work, the elective intake of 5% and 10% ethanol in plain water accounted for only 1–2 kcal/day. In the first experiment, the nonalcoholic beer vehicle was consumed avidly and accounted for ~25% daily calories. When ethanol was added, beer intake was much lower and accounted for only a modest fraction of caloric intake (Fig. 1), albeit much more than with plain water as vehicle. Although this result was encouraging, beer has at least two disadvantages as a routine experimental vehicle. First, the chemical constituents are complex, and most likely variable from brand to brand. Second, residual carbonation could lead to leakage from the tubes and overestimation of intake, although we verified that the present decarbonation procedure was adequate to avoid this problem.

To circumvent these objections, we used Polycose, a glucose oligomer. Polycose is highly preferred and seems to use a different taste receptor than sucrose (Ackroff et al., 1993) in rats. Because there is a physiological link between sweet and alcohol preferences (Kampov-Polevoy et al., 1999), we chose to use Polycose rather than mono-

or disaccharide vehicle. The intakes from Polycose–ethanol in solution were 1–3 times higher than in Experiment 1 at the same concentration of alcohol added to the non-alcoholic beer vehicle. To further boost ethanol intakes, we devised a gel matrix with the intent that swallowing an unpalatable substance in a semisolid should result in poorer access to the taste buds in the oral cavity. Intakes of gel were even higher than equivalent solutions, approaching 50% of daily caloric intake (or about 25% of calories as ethanol). In each case, compensation for the calories in the beverage by reduced chow intake was strikingly accurate (Fig. 3). Any contribution of gelatin (2.5% by weight, all protein) to this effect would be small compared to that of ethanol, which comprised about 50% of the calories in these gels (Table 1). It is also unlikely that the suppression in intake was due to Polycose alone; in that case the slopes in Fig. 3 would have been ~0.5. Further, our data suggest that males and females compensate similarly to alcohol calories.

In the final experiment, we determined that concentrated (10%) Polycose–ethanol gel is consumed in substantial amounts in short periods of time by non-deprived rats. Rats then compensate by reducing their chow intake throughout the next 24 h. Our BEC study was designed to show that pharmacologically relevant amounts of alcohol (~1 g/kg; e.g. Gill et al., 1986; Samson, 1986) are reached in this elective consumption protocol with minimal training. Because the presence of carbohydrate may produce slightly lower BEC than plain ethanol (Roberts et al., 1999), a more complete documentation of the time course of the rise and fall of BEC after gel consumption will be needed.

Under conditions of access to palatable diets, rats can develop hyperphagia and obesity, so the present data showing accurate caloric compensation over short periods of several days are quite striking and similar to short-term (Cornier et al., 2002) and long-term (Richter, 1940, 1953) forced consumption studies. Further studies are warranted to investigate the long-term effects on non-alcoholic compared with alcoholic commodities on intake and weight gain in these protocols. In humans, the compensation for alcohol calories seems to be poor (DeCastro and Orozco, 1990; Prentice, 1995; Tremblay et al., 1995), but this may reflect long-term or learned adaptations rather than short-term effects rather than a species difference. Internal signals of caloric intake and balance may be only one of many factors in the control of intake in the complex and varied alimentary world of humans.

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