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The Tailgate Study: Differing metabolic effects of a bout of excessive eating and drinking

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ABSTRACT

Introduction: Excess energy intake by spectators at a sporting event (i.e., a tailgate) might cause acute negative health effects. However, limited data exist regarding the effects of overeating and alcohol consumption on lipid metabolism and the potential to gain intrahepatic triacylglycerols (IHTG). We tested the hypothesis that overconsumption of food and alcohol would significantly increase both hepatic *de novo* lipogenesis (DNL) and IHTG.

Methods: Eighteen males (mean ± SD, age: 31.4 ± 7.3 years, BMI: 32.1 ± 5.9 kg/m^2) were given alcoholic drinks to elevate blood alcohol for 5 h, while highly palatable food was presented. Blood samples were collected and DNL in TG-rich lipoproteins (TRL) was measured by GC/MS, IHTG was measured via MRS (n = 15), and substrate oxidation was measured via indirect calorimetry.

Results: Subjects consumed 5087 ± 149 kcal (191 ± 25% excess of total daily energy needs including 171 ± 24 g alcohol), which increased plasma insulin, glucose, TG, and decreased NEFA (ANOVA $p \leq 0.003$ for all). Both DNL and TRL-TG increased ($p < 0.001$), while IHTG did not change in the group as a whole ($p = 0.229$). Individual subject data revealed remarkably differing responses for IHTG (nine increased, five decreased, one did not change). Despite maintaining equal breath alcohol levels, subjects with IHTG elevations exhibited higher DNL, consumed 90% less alcohol ($p = 0.048$), tended to consume more carbohydrates, and exhibited lower whole-body fat oxidation (not significant) compared to those whose IHTG was reduced.

Discussion: This study demonstrates that acute excess energy intake may have differing effects on an individual's DNL and IHTG, and dietary carbohydrate may influence DNL more than alcohol.

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Introduction

The consumption of excess nutrients is a frequent practice of spectators at sporting events. In addition to major macronutrients (carbohydrates, fats, and proteins), alcohol is commonly consumed during such 'tailgate parties' (Neal & Fromme, 2007; Neighbors, Oster-Aaland, Bergstrom, & Lewis, 2006). World soccer and U.S. college football game tailgating can be associated not just with drinking, but with heavy or excess drinking that in some cases leads to acute adverse health consequences (Glassman, Werch, Jobli, & Bian, 2007; Leavens, Croff, Feddor, & Olson, 2019; Neal & Fromme, 2007). For many years, two aspects of alcohol have been the focus of research – the negative social consequences of alcohol intake (Glassman et al., 2007; Leavens et al., 2019), and its impact on blood lipids and cardiovascular risk (Chung et al., 2003; Fuhrman, Lavy, & Aviram, 1995; Hampton, Isherwood, Kirkpatrick,

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Lynne-Smith, & Griffin, 2010; Hendriks, Veenstra, van Tol, Groener, & Schaafsma, 1998; van Tol, van der Gaag, Scheek, van Gent, & Hendriks, 1998). A smaller number of studies have investigated the acute effects of meals and alcohol combined on metabolism, in particular, the effects of red wine (Fuhrman et al., 1995; Hendriks et al., 1998; van der Gaag et al., 2000; van Tol et al., 1998) or vodka (Fielding et al., 2000; Hampton et al., 2010) with dinner. However, no studies have determined the magnitude of acute alcohol effects on intrahepatic triacylglycerols (IHTG) and the biochemical pathway of *de novo* lipogenesis (DNL), when combined with excess food intake. With regard to the accrual of IHTG, past research studies, conducted both in animals (Sprague Dawley rats) and humans, have tested the effects of alcohol alone (Barson et al., 2009; Brodie, Butler, Horning, Maickel, & Maling, 1961; Chang et al., 2007; Oliva et al., 2008; Siler, Neese, Parks, & Hellerstein, 1998; Wiebe, Lundquist, & Belfrage, 1971), and normal meals consumed with or without alcohol (Chung et al., 2003; Clevidence et al., 1995; Fielding et al., 2000; Fuhrman et al., 1995; Gin, Morlat, Ragnaud, & Aubertin, 1992; Hampton et al., 2010; Hendriks et al., 1998; Rubin & Lieber, 1968; van der Gaag et al., 2000; van Tol et al., 1998). Acutely, a bolus of alcohol in rats increased plasma triacylglycerols (TG) by 55% (Chang et al., 2007), and animals chronically treated with alcohol increased plasma TG, accumulated IHTG (Brodie et al., 1961), and developed microvesicular steatosis, necrosis, and fibrosis (Barson et al., 2009; Oliva et al., 2008). In a classic study, Wiebe and co-workers fed healthy men a bolus of alcohol (3.0 g/kg) in combination with a meal, followed by pre- and post-liver biopsies, and observed significant increases in plasma liver enzymes (aspartate aminotransferase [AST] and alanine transaminase [ALT]) and IHTG – both of which resolved over 24 h (Wiebe et al., 1971). Siler et al. (1998, 1999) administered an oral bolus of alcohol (vodka, 40% alcohol by volume) without food to healthy men, which led to an increase in plasma TG of 25–43% over 6 h, and a 30-fold increase in DNL in TG-rich lipoproteins (TRL). Past research on hepatic DNL has focused on the stimulatory effect of positive energy balance over a period of 5 days (Schwarz, Neese, Turner, Dare, & Hellerstein, 1995) and diets high in sugars (Chong, Fielding, & Frayn, 2007; Hudgins et al., 2000; Parks, 2001; Timlin & Parks, 2005). These data have shown that the process of DNL is a key contributor to excess IHTG (Donnelly et al., 2005; Lambert, Ramos-Roman, Browning, & Parks, 2014), and excess consumption of food and alcohol together has the potential to stimulate DNL in a manner that would increase IHTG, especially in individuals at risk for insulin resistance who are susceptible to fatty liver (Schwarz, Linfoot, Dare, & Aghajanian, 2003). Thus, although key information is available on the acute effects of alcohol on blood lipids and sugar's effects on hepatic DNL, previous study designs were not reflective of the combined intake of excess alcohol and food. The present project was designed to investigate liver metabolism in a translational manner that is characteristic of a tailgate event. Surprisingly, we found that in overweight males, after an extended duration of eating and drinking, metabolic responses were not uniform and revealed significant individual variation in the ability to protect the liver from nutrient toxicity. These findings underscore the inter-relationships between hepatic and peripheral metabolism that can work in concert to protect individuals from the metabolic challenges brought on by over-consumption of nutrients.

Materials and methods

The study was approved by the University of Missouri (MU) Institutional Review Board (IRB#1211233) and registered at ClinicalTrials.gov (NCT02141880). As shown in Fig. 1A, 54 subjects were screened via telephone to determine preliminary eligibility. Thirty-two subjects signed the informed consent to screen in person and a total of 18 subjects completed the in-patient protocol.

Inclusion and exclusion criteria

The inclusion criteria included sedentary men who participated in less than 3 h of aerobic exercise/week, age 21–52 years, BMI 25.1–51.0 kg/m^2, and waist girth <55 inches, due to a limitation of the MRI scanner. The subjects were required to be nonsmoking with a fasting blood glucose <125 mg/dL (non-diabetic), ALT <40 U/L, plasma TG < 200 mg/dL, and free of metabolic disorders (thyroid and kidney conditions). To ensure the relative safety of the subjects as they ingested alcohol sufficient to maintain intoxication over a period of several hours, eligibility included greater than moderate alcohol consumption (regularly consuming alcohol in the last 12 months). A survey was used to assess typical alcohol consumption (National Institute on Alcohol Abuse and Alcoholism, 2003). Subjects were excluded whether they used any tobacco products, did not regularly consume alcohol or abstained from alcohol use, or consistently consumed more than 16 alcoholic drinks/week on average (one glass is approximately equivalent to a 12-oz. beer, 4-oz. glass of wine, or 1.5-oz. shot of distilled spirits). Given the repeated blood draws throughout the afternoon of the study (see below), subjects were also excluded if they had donated blood within the past 2 months or if they had metal in their body which precluded a magnetic resonance spectroscopy (MRS) scan. Eight of the subjects completed a 3-day food record (two weekdays and one weekend day) before the study (Tinker, Schneeman, & Willits, 1993), which revealed that they consumed 2748 ± 167 kcals/day under free-living conditions (Table 1).

Overall study design

As shown in Fig. 1B, 3 days prior to the study, subjects consumed, by mouth, deuterium oxide (70%, d_2O, 50 mL twice daily) to label the pathway of DNL *in vivo* (Santoro et al., 2015). All subjects were also advised to eat their *ad libitum* diet for these 3 days. The goal was to have subjects eat a diet of typical composition and avoid novel settings of eating (buffet meals, long periods of going without food, etc.) that may alter energy balance. Subjects were advised to not consume alcohol the night before the study and avoid any acute dietary patterns that would increase lipogenesis (foods high in sugar content). Subjects were in frequent communication with research staff during these 3 days to assess their adherence.

Alcohol dosing protocol

The doses of alcoholic beverages were specific for each subject (formula given below) and contained 80-proof Grey Goose® vodka (40% alcohol by volume) mixed with their choice of orange juice, peach juice, or fruit punch. For each subject, the level of breath alcohol (BrAC) was collected with an Alco-Sensor® IV (Intoximeters; St. Louis, Missouri, United States) starting 30 min post-alcohol consumption and then repeated every 30 min thereafter, with a goal of a BrAC of between 0.08 and 0.10 g/210 L. Before each BrAC measurement, the subject rinsed their mouths with water to ensure that no alcohol residue was left in the oral cavity, and a new mouthpiece was used for every measurement.

Attaining initial BrAC

For the first hour of this study, the initial dose was calculated based on the subject's height, weight, gender, and total body water (TBW) to achieve the BrAC level of 0.10 g/210 L. TBW was calculated

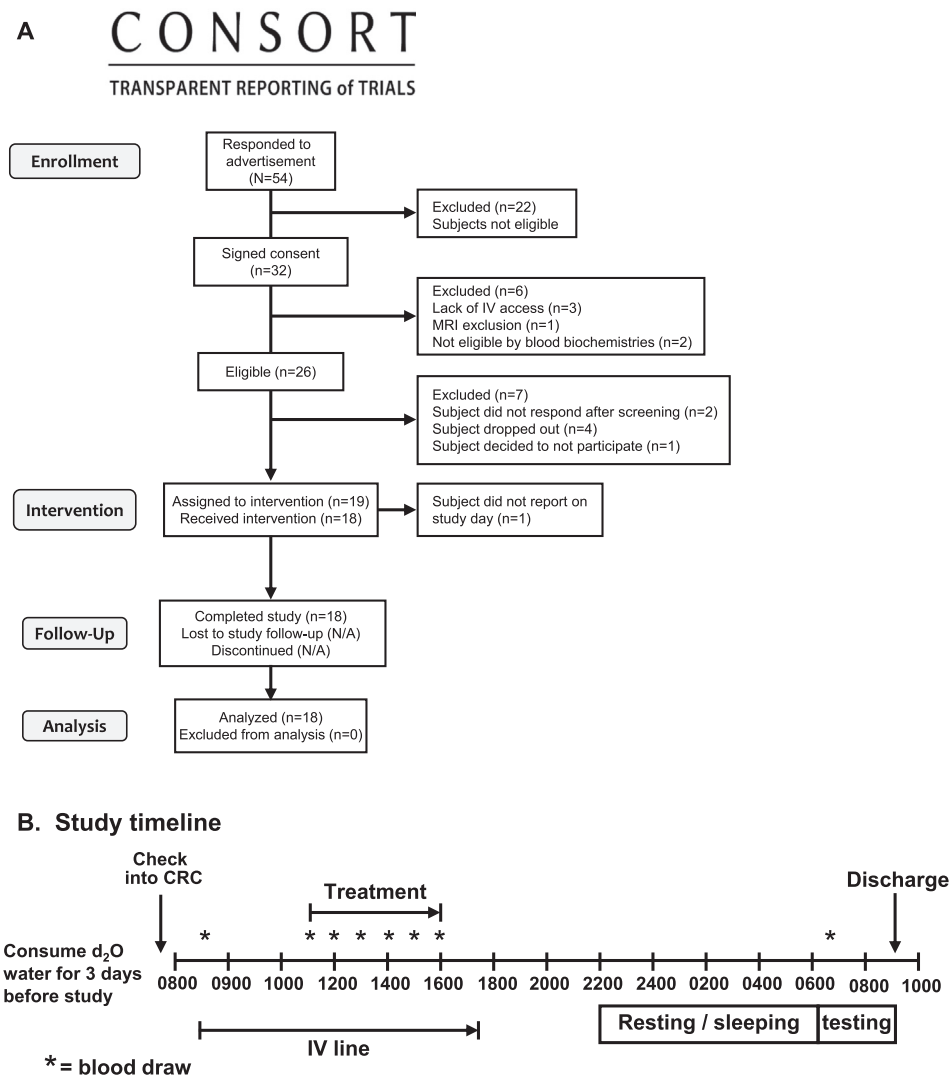

Fig. 1. Study consort flow diagram and protocol timeline. Fig. 1A presents the Consort flow diagram describing recruitment and completion of the subjects. Fig. 1B displays the study protocol description of activities. Abbreviations: CRC, clinical research center; d_2O, deuterated water.

Table 1
Dietary intake in a subset of subjects' *ad libitum* days for comparison to the study day

Macronutrient	*Ad libitum*	Study day	*p* value
Total energy (kcal)	2748 ± 472	4875 ± 786	0.001
Carbohydrates			
Grams	320 ± 62	336 ± 58	0.688
Kcals	1281 ± 249	1342 ± 231	0.688
Percent	47 ± 7	28 ± 6	0.002
Proteins			
Grams	106 ± 23	116 ± 19	0.303
Kcals	390 ± 143	462 ± 75	0.208
Percent	14 ± 4	10 ± 3	0.042
Fat			
Grams	111 ± 24	180 ± 29	<0.001
Kcals	1002 ± 212	1618 ± 263	<0.001
Percent	36 ± 4	34 ± 7	0.133
Alcohol			
Grams	11 ± 18	207 ± 149	0.008
Kcals	75 ± 128	1452 ± 1041	0.008
Percent	3 ± 4	28 ± 15	0.003

Data from 3-day food records were collected and analyzed by NDSR®. Data are presented as mean ± SD. *p* value for paired *t* test, n = 8.

using the following formula (Jéquier, Acheson, & Schutz, 1987): TBW = (2.447 − 0.09156 × age [years]) + (0.1074 × height [cm]) + (0.3362 × weight [kg]). The first dose of alcohol was calculated using the following formula (Barrows & Parks, 2006):

$$\text{Alcohol dose (g)} = [(10 \times \text{BAL} \times \text{TBW})/0.8] + [10 \times \text{MR} \times (\text{DDP} + \text{TPB}) \times (\text{TBW}/0.8)]$$

where BAL is blood alcohol level target of 0.10, MR is alcohol metabolism rate (0.015 g/100 mL TBW/hour), DDP is duration of the drinking period [in this case, the dose was calculated for the first (1) hour], and TPB is the time to peak BAL target, with the first dose goal being achieved within 1 h. At the start of the study, the calculated first-hour dose was administered in two parts – the first half of the dose was consumed at the start of the study (average time to complete consumption of this first drink was 16.6 min) and the second half of the dose was consumed 30 min later.

Maintaining BrAC

After subjects had achieved the target BrAC, they were encouraged to drink in a manner to maintain this level. Considering an

estimated alcohol metabolism rate of 0.015 g/100 mL TBW/hour (Pineda et al., 2009), a subsequent dose of 40–50 mL was provided if BrAC was <0.10 g/210 L. If the subject's BrAC level was >0.10 g/210 L, no additional alcohol was added and BrAC was rechecked in 30 min.

Study day protocol

Subjects were studied in pairs, with entertainment (e.g., sports) shown on a television to increase social interactions. As shown in Fig. 1B, subjects were admitted to the MU Clinical Research Center at 8:00 AM. Vital signs were recorded and a fasting venous blood sample was immediately obtained through an intravenous line. A light breakfast was served, followed by measurement of body composition by dual-energy X-ray absorptiometry (Hologic A version 13.5.2; Marlborough, Massachusetts, United States). At ~11:00 AM, a blood sample was collected and the food and alcohol were served for the next 5 h. Aside from alcohol intake, food consumption was encouraged by the replenishment of an abundant buffet composed of highly palatable foods (hamburgers, chips, cupcakes, etc.). The buffet food that was presented was weighed periodically (to the 0.1 g) throughout the afternoon and plate waste was taken into account. For the first 10 subjects, each subject's food items were weighed separately, while for the last 8 subjects, the total food consumed by the 2 subjects combined was weighed/recorded and divided by 2 to estimate each subject's intake. The reason for this change was to reduce the likelihood of the subject feeling "under close scrutiny", which could influence his eating and drinking behavior. Nutrient intakes were calculated using the Nutrition Data System for Research (NDSR system 2019, University of Minnesota, Minneapolis, Minnesota, United States). During the 5 h of overfeeding (from here on, referred to as treatment), blood samples were collected every hour and BrAC was measured every 30 min using the breathalyzer. BrAC units are reported as the weight of alcohol in grams per volume of breath (210 L). Due to the timing and flow of measurements in the protocol, 14 of the 18 subjects underwent MRS (reproducibility: pooled standard deviation of 0.69%) (Pineda et al., 2009). The scanner was a Siemens 3T Skyra series 45839 and data were analyzed by LiverLab software (Siemens Healthcare GmbH, Erlangen, Germany; for accuracy, R^2 fits during the study were as follows: pre − 0.92 ± 0.13 post − 0.94 ± 0.09, mean ± SD). The subjects remained in the clinical unit overnight where they rested and slept, each in a private room. The next day in the morning, a fasting blood sample was collected followed by measurement of energy expenditure by indirect calorimetry in the fasting state (Jéquier et al., 1987). The morning BrAC was confirmed to be zero before the subject was fed breakfast and discharged from the unit.

Primary and secondary outcomes

The primary outcome of this study was the measurement of IHTG before and after 5 h of treatment in adult men. The secondary outcome was the measurement of DNL in plasma TG.

Analytical methods

Labeled water (d_2O) was purchased from Cambridge Isotope Laboratory, Inc. (Purity ≥99.5%, Catalog# DLM-4-70-0; Andover, Massachusetts), and final d_2O enrichments in plasma were measured by cavity ringdown spectroscopy using a liquid water isotope analyzer with automated injection system, version 2 upgrade (Los Gatos Research; Mountain View, California, United States) by Metabolic Solutions, Inc. (Nashua, New Hampshire, United States). This timing of d_2O consumption resulted in a body d_2O enrichment of 0.32 ± 0.07% (mean ± SD), which is similar to that achieved in past studies (Santoro et al., 2015). To maintain body d_2O enrichments during the study, deuterium oxide was also added to the alcoholic drinks. After treatment was concluded, plasma samples were immediately processed to isolate TRL using ultracentrifugation at 40,000 rpm at 15 °C in a 50.3Ti rotor (Beckman Instruments; Palo Alto, California) for 20 h (Barrows & Parks, 2006). The TRL-TG were separated via thin-layer chromatography and fatty acid methyl esters were prepared as described previously (Barrows & Parks, 2006). Labeled fatty acids were measured using a 6890N gas chromatograph coupled to a 5975 mass spectrophotometry detector (Agilent Technologies; Palo Alto, California), and DNL was calculated by mass isotopomer distribution analysis (Hellerstein & Neese, 1992). In the postprandial state, TRL particles include both chylomicrons and VLDL. Assuming intestinal DNL is minimal (Timlin & Parks, 2005), then the apparent fractional DNL in TRL may be lower than DNL analyzed only in VLDL. Absolute DNL is calculated by multiplying the fractional DNL in TG by the quantity of TG (Parks, Skokan, Timlin, & Dingfelder, 2008) and thus, absolute DNL reflects liver fatty acid synthesis and secretion (in mg/dL).

Plasma concentrations of TC, TG, LDLc, HDLc, AST, and ALT were measured by a CLIA-standardized laboratory (Quest Diagnostics; St. Louis, Missouri, License #26D0652092). The measurements of lipids were performed via auto-analyzer (Roche Cobas 8000 System, CV 0.6–0.9%; Indianapolis, Indiana, United States) using electro-chemiluminescent immunoassay. Liver enzymes were measured using UV Absorbance (Roche Cobas 8000 System, CV 0.5–3.2% for AST and 0.5–3.1% for ALT; Indianapolis, Indiana). Assay kits were used to measure the concentrations of plasma glucose (Wako #439-90901, CV 6.6%, Mountain View, California) and NEFA (Wako #991-34891, CV 6.9%, Mountain View, California). Plasma insulin was measured using an enzyme-linked immunosorbent assay (Human Insulin, EMD Millipore #EZHI-14K, CV 7.2%; Billerica, Massachusetts).

Statistical analysis and calculations

Subject characteristics are presented as mean ± SD, while the effects of the 5-h treatment over time are presented as mean ± SEM. Basal metabolic rate was calculated using the Harris Benedict equation (Harris & Benedict, 1918), and total energy needs were calculated by multiplying basal metabolic rate by the individual subject's physical activity factor (average physical activity factor was 1.3 ± 0.1). A paired sample *t* test was performed using StatView®, 5.0.1 software (v2008), regression analyses were performed using the Statistical Package for the Social Sciences (SPSS®, v24, 2016), and Pearson correlation analysis was performed using SPSS® (v24, 2016). HOMA-IR was calculated as [(glucose in mg/dL × insulin in μU/mL)/405]. The nonalcoholic fatty liver disease (NAFLD) fibrosis score was calculated using the formula (Angulo et al., 2007): NAFLD Fibrosis score = −1.675 + [0.037 × age (years)] + [0.094 × BMI (kg/m^2)] + [1.13 × hyperglycemia or diabetes (yes = 1, no = 0)] + [0.99 × AST/ALT ratio] − [0.013 × platelet ($\times 10^9$/L)] − [0.66 × albumin (g/dL)], and FIB-4 was calculated using the formula (Vallet-Pichard et al., 2007): FIB-4 = [age (years) × AST (U/L)]/[platelet (10^9/L) × $\sqrt{ALT\ (U/L)}$].

Results

Table 2 presents the subject characteristics and shows that the participants ranged from overweight to obese, and had healthy levels of glucose, lipids, and liver enzymes. According to the entry criteria, each subject was a habitual consumer of alcohol and had consumed more than six drinks in at least one setting within the past 30 days. The levels of IHTG ranged from very low to elevated

Table 2
Baseline subject characteristics

Age and alcohol intake	n = 18	Range
Age (years)	31.4 ± 7.3	[21–47]
Alcohol intake		
Maximum drinks in one occasion (30 days)	6.5 ± 5.3	[1.0–18.0]
Maximum drinks in one occasion (12 months)	10.1 ± 6.0	[2.0–20.0]
Average drinking days/week (3 months)	1.9 ± 1.3	[0.3–3.5]
Anthropometrics and blood pressure		
Height (m)	1.77 ± 0.08	[1.60–1.87]
Weight (kg)	100.7 ± 15.2	[75.1–129.0]
BMI (kg/m^2)	32.1 ± 5.9	[26.3–50.4]
Waist (cm)[a]	109.5 ± 13.1	[93.0–138.0]
Body composition		
Body fat (kg)	32.7 ± 8.8	[21.8–55.8]
Body fat (%)	34.2 ± 8.6	[24.0–62.5]
Trunk fat mass (kg)	22.7 ± 14.8	[9.5–64.1]
Trunk fat mass (%)	22.3 ± 13.3	[10.7–51.6]
Visceral adipose tissue (g)[b]	585 ± 209	[324–1058]
Lean mass (kg)	62.2 ± 12.3	[24.3–81.2]
Lean mass (%)	64.4 ± 4.9	[53.5–72.5]
Trunk lean mass (kg)	40.0 ± 6.3	[13.3–44.8]
Trunk lean mass (%)	39.6 ± 4.7	[17.7–37.4]
Blood pressure		
Systolic blood pressure (mmHg)[c]	127 ± 10	[104–148]
Diastolic blood pressure (mmHg)[c]	81 ± 10	[65–105]
Heart rate (bpm)[c]	70 ± 12	[58–100]
Biochemical measurements		
Plasma glucose (mg/dL)[c]	91 ± 10	[73–111]
Plasma insulin (U/L)[c]	10 ± 12	[1–51]
NEFA (mmol/L)[c]	0.47 ± 0.22	[0.18–0.85]
HOMA-IR[c]	2.1 ± 2.5	[0.2–10.7]
Lipids		
Total cholesterol (mg/dL)	173 ± 28	[135–255]
Plasma triacylglycerols (mg/dL)[c]	98 ± 32	[47–147]
HDL cholesterol (mg/dL)	46 ± 10	[28–66]
LDL cholesterol (mg/dL)	107 ± 25	[57–171]
Liver-related measurements		
IHTG (%)[d]	9.1 ± 6.9	[1.6–22.9]
AST (U/L)	24.9 ± 5.7	[17.0–40.0]
ALT (U/L)	29.5 ± 10.0	[15.0–50.0]
ALP (U/L)	69.5 ± 20.8	[32.0–127.0]
Albumin (g/dL)	4.7 ± 0.3	[4.1–5.0]
NAFLD Fibrosis Score	−2.6 ± 1.6	[−6.0 to −0.1]
FIB-4	0.65 ± 0.34	[0.22–1.63]

Data are reported as mean ± SD, n = 18 males unless otherwise noted (race categories: 14 white, 3 Black, and 1 other).
HOMA-IR was calculated using the formula: [plasma glucose (mg/dL) × Insulin (U/L)]/405.
Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NEFA, nonesterified fatty acids; HOMA-IR, homeostatic model assessment for insulin resistance; IHTG, intrahepatic triacylglycerols; AST, aspartate aminotransferase/serum glutamic-oxaloacetic transaminase; ALT, alanine aminotransferase/serum glutamic pyruvic transaminase; ALP, alkaline phosphatase.
[a] n = 14.
[b] n = 16.
[c] n = 17.
[d] n = 15.

(1.6–22.9%). During the 5 h of food and alcohol treatment, subjects consumed 5087 ± 632 kcal or 191 ± 25% of their calculated (Harris & Benedict, 1918) total daily energy needs (i.e., 2690 ± 369). The composition of the energy taken in during treatment was 32% from carbohydrates, 35% from fat, 10% from protein, and 23% from alcohol. The total alcohol intake was 171 ± 24 g, which resulted in an average BrAC level of 0.08 ± 0.00 (range 0.05–0.09) g/210 L (Fig. 2A) and subjective intoxication level of 3.9 ± 0.4 (range 1.6–4.6, Fig. 2B).

Biochemical measurements and de novo lipogenesis, and liver fat

As shown in Fig. 2C–F, treatment significantly increased plasma insulin from 10 ± 3 U/L at fasting to 71 ± 17 U/L after eating ($p = 0.003$), plasma glucose from 91 ± 3 mg/dL to 113 ± 5 mg/dL ($p < 0.001$), plasma TG from 98 ± 8 mg/dL to 358 ± 40 mg/dL ($p < 0.001$), and reduced NEFA concentrations from 0.47 ± 0.05 mmol/L to 0.40 ± 0.06 mmol/L ($p < 0.001$). The next morning, values returned to normal for plasma concentrations of glucose (94 ± 17 mg/dL, $p = 0.300$, Fig. 2D), NEFA (0.47 ± 0.05 mmol/L, $p = 0.828$, Fig. 2E), and TG (135 ± 20 mg/dL, $p = 0.053$, Fig. 2F). Fasting plasma insulin remained slightly higher (16 ± 4 U/L, $p = 0.011$, Fig. 2C) than it had been the previous morning. Shown in Fig. 3A–C are the significant increases observed for TRL-TG (60 ± 8 mg/dL to 208 ± 31 mg/dL, $p < 0.001$), and both percent DNL (11 ± 2% to 17 ± 2%, $p < 0.001$) and absolute DNL (7 ± 2 mg/dL to 34 ± 6 mg/dL, $p < 0.001$). As shown in Fig. 3D, for the group as a whole, no significant changes were observed in total

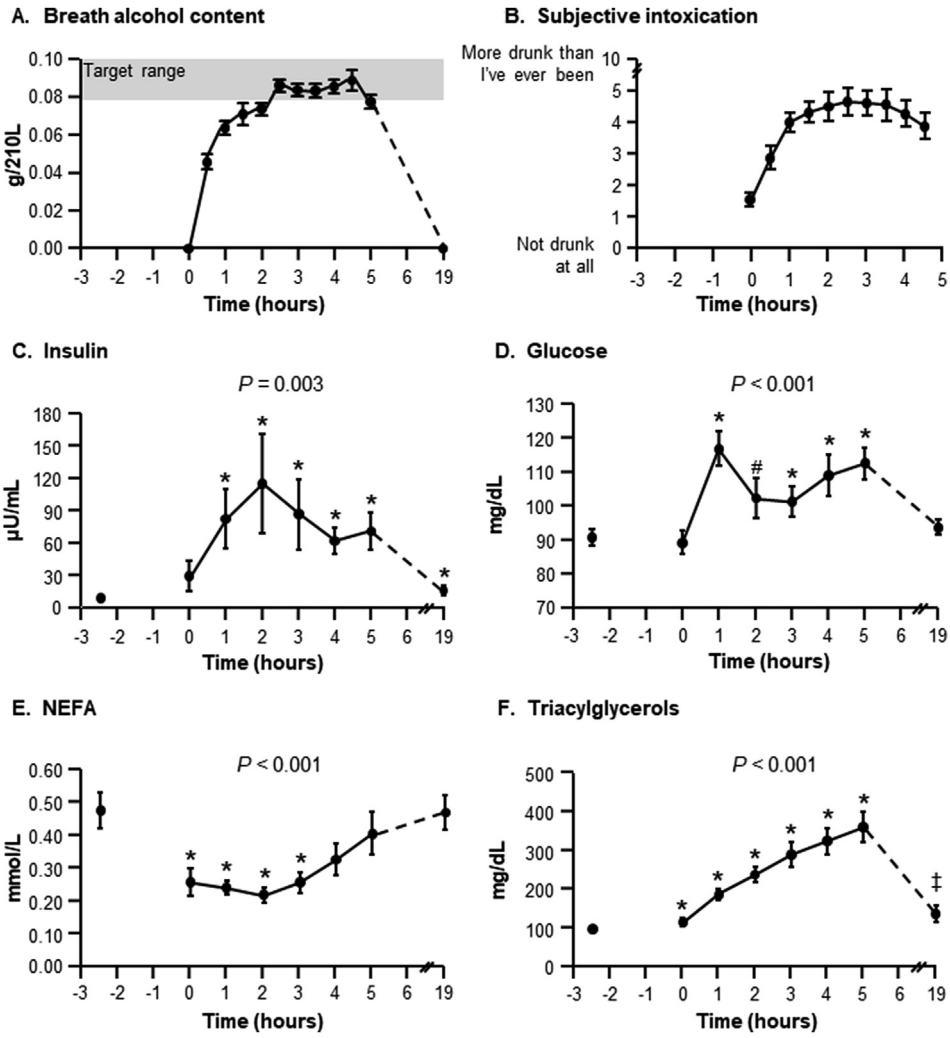

Fig. 2. Breath alcohol concentrations, subjective intoxication, plasma insulin, and metabolites. Fig. 2A presents the breath alcohol concentrations and in Fig. 2B, the participants' subjective levels of intoxication, and the plasma concentrations of insulin (Fig. 2C), glucose (Fig. 2D), NEFA (Fig. 2E), and triacylglycerols (Fig. 2F). Data are reported in mean ± SEM. p value for ANOVA effect of time, and $^{*}p \leq 0.05$, $^{\#}p = 0.08$, $^{\ddagger}p = 0.053$ compared to fasting. In the figures, time zero represents the value immediately before treatment began; please see methods for the alcohol-dosing regimen. The 19-h time point represents a measurement performed in the fasted state the next morning.

IHTG after treatment (9.1 ± 1.8% to 9.3 ± 1.9%, $p = 0.670$). However, when the absolute change in IHTG is plotted individually for the 15 subjects who underwent MRS (Fig. 3E), varying responses were observed: nine subjects increased IHTG, five subjects decreased, and one did not change IHTG. Correlation analysis (Fig. 4A) revealed a significant positive relationship between the absolute increase in IHTG and an increase in the fraction of TRL-TG originating from DNL ($R^2 = 0.381$, $p = 0.014$).

Group differences

When subjects were divided into two groups based on their change in IHTG, DNL was the only predictor of the differences between the groups when considered as either fractional lipogenesis (Fig. 4A, $p = 0.006$ for the entire effect of time, or Fig. 4B, $p = 0.018$, when comparing just the 5-h time point) or as absolute DNL (Fig. 4C). The change in TRL-TG was not different between the groups (Fig. 4D), and no significant differences were observed for the total amount of energy consumed by each group (Fig. 4E), although the group with reduced IHTG tended to consume less energy from non-alcohol sources (i.e., 15.7% fewer calories, $p = 0.076$, from carbohydrate, fats, and proteins) and needed significantly more alcohol to achieve BrAC of 0.08 g/210 L. Note that the study design aimed to have all subjects attain a BrAC of less than 0.10 g/210 L, and this was achieved equally in both groups (Fig. 4F). However, the amount of alcohol needed to reach steady state BrAC was 2.50 g/kg alcohol in those whose IHTG decreased and 1.32 g/kg alcohol in those whose IHTG increased ($p = 0.048$, data not shown). As shown in Fig. 5, other variables that were tested and failed to predict the change in IHTG included plasma concentrations of insulin, NEFA, and TG. Glucose tended to be higher during treatment in the group that reduced IHTG (Fig. 5D; the higher the glucose the more IHTG was reduced, $R^2 = 0.225$, $p = 0.074$, data not shown). No relationship was found for baseline IHTG, even when correlation analysis was performed for all subjects (data not shown). The groups were not different with respect to free-living, *ad libitum* alcohol intake (data not shown). Lastly, as shown in Fig. 5E, although the whole-body fat oxidation rates measured the next morning were 354% higher in the group with reduced IHTG, no significant differences were observed between the groups due to high variability (Fig. 5F, $p = 0.259$).

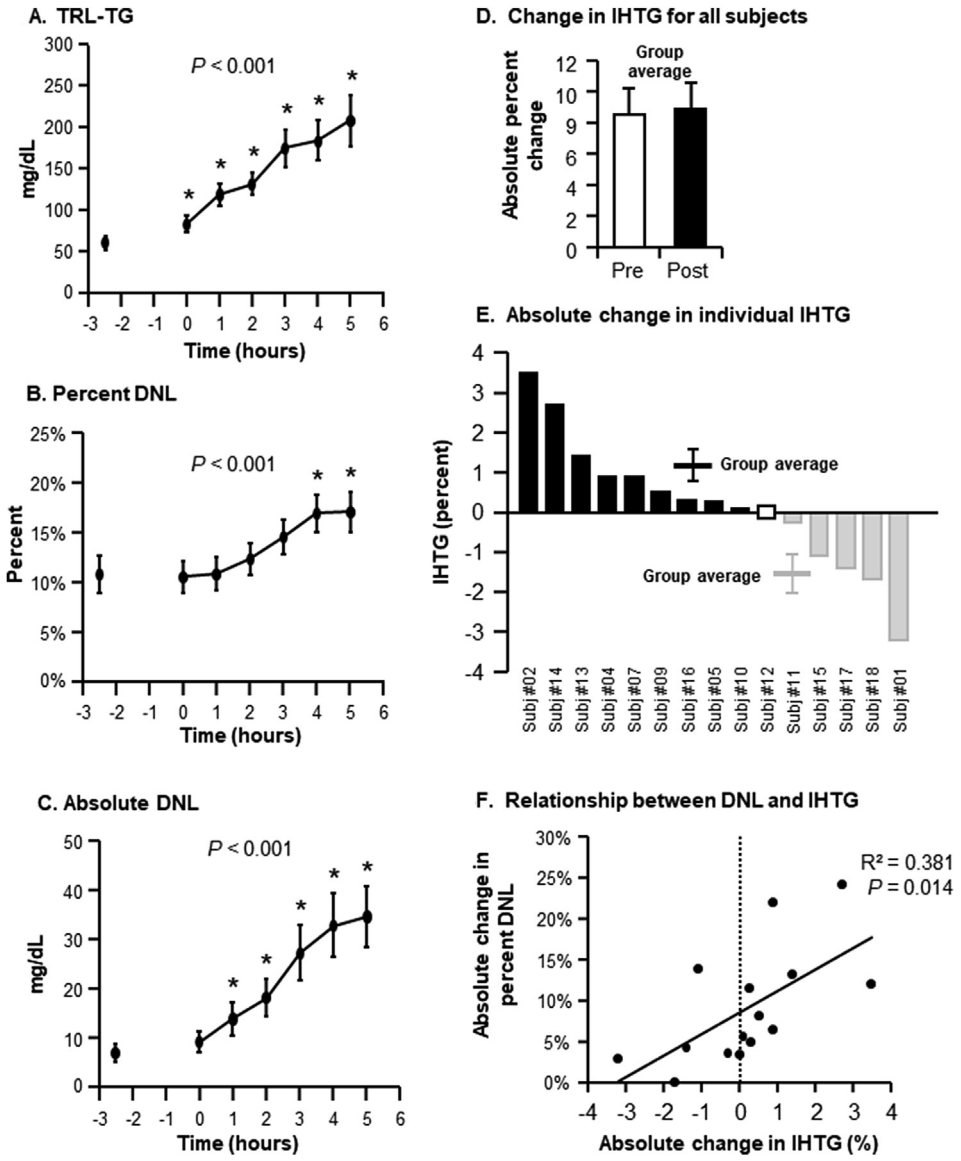

Fig. 3. Changes in DNL and liver fat. Data are mean ± SEM, *p* value for ANOVA effect of time. *$p \leq 0.05$ compared to fasting. Time zero represents the value immediately before treatment began. Fig. 3A presents the concentrations of TRL-TG, Fig. 3B, the fraction lipogenesis in TRL-TG, and Fig. 3C, the absolute level of lipogenesis in TRL-TG. In Fig. 3D, Pre and Post represent the timing of the liver MRS immediately before and after treatment. Fig. 3E shows individual data on the change in IHTG due to treatment. Of the 15 subjects who underwent repeat MRS, nine increased IHTG (black-filled bars), one showed no change (unfilled bar), and five subjects decreased IHTG (grey-filled bars). The horizontal lines represent the group mean±SEM (P=0.001). Fig. 3F shows the relationship between the change in lipogenesis and the change in IHTFG. Abbreviations: TRL, triacylglycerol-rich lipoprotein; DNL, *de novo* lipogenesis; IHTG, intrahepatic TG.

Discussion

The goal of the present study was to determine the metabolic impact of excess energy consumption that included both food and alcohol intake in a situation that mimicked celebrations associated with sporting events. The primary outcome of the study was the measurement of IHTG before and after this treatment in adult men. Contrary to our hypothesis, 5 h of eating and drinking alcohol did not lead to liver fat accumulation when data from all subjects were combined. These results are not consistent with previous literature conducted in both animals and humans (Brodie et al., 1961; Wiebe et al., 1971), most likely because the present subjects consumed a lower amount of ethanol, 1.7 ± 0.2 g/kg over a 5-h period, compared to past research. In Sprague–Dawley rats, Brodie et al. (1961) reported a significant increase in IHTG peaking 15–20 h after a 4.8 g/kg bolus of alcohol was given. Similar outcomes were observed in the study conducted by Wiebe and co-workers using repeat liver biopsies over a 24-h period in adult men after they had consumed ethanol (3 g/kg, which we estimate equaled a total of 210 g) and a 300 kcal meal (Wiebe et al., 1971). The protocol of this present study maintained BrAC less than 0.10 g/210 L (final values were 0.08 ± 0.00 g/210 L), while in the study of Wiebe et al. (1971), subject blood alcohol concentration was 1.3–1.8 parts per thousand (equivalent to BrAC of 0.13–0.18 g/210 L) within 6 h. Additionally, unlike the previous studies (Brodie et al., 1961; Wiebe et al., 1971), the subjects' energy consumption levels were very high, including 3890 ± 142 kcals from non-alcoholic sources. In a study by Rubin and Lieber (1968), subjects consumed both food and alcohol, and the multi-day protocol incorporated energy from alcohol being either substituted isocalorically (68–130 g/day) for carbohydrates or added to their standard diet (180–270 g/day). In either case, subjects increased IHTG by 2–4 times, and this occurred as early as

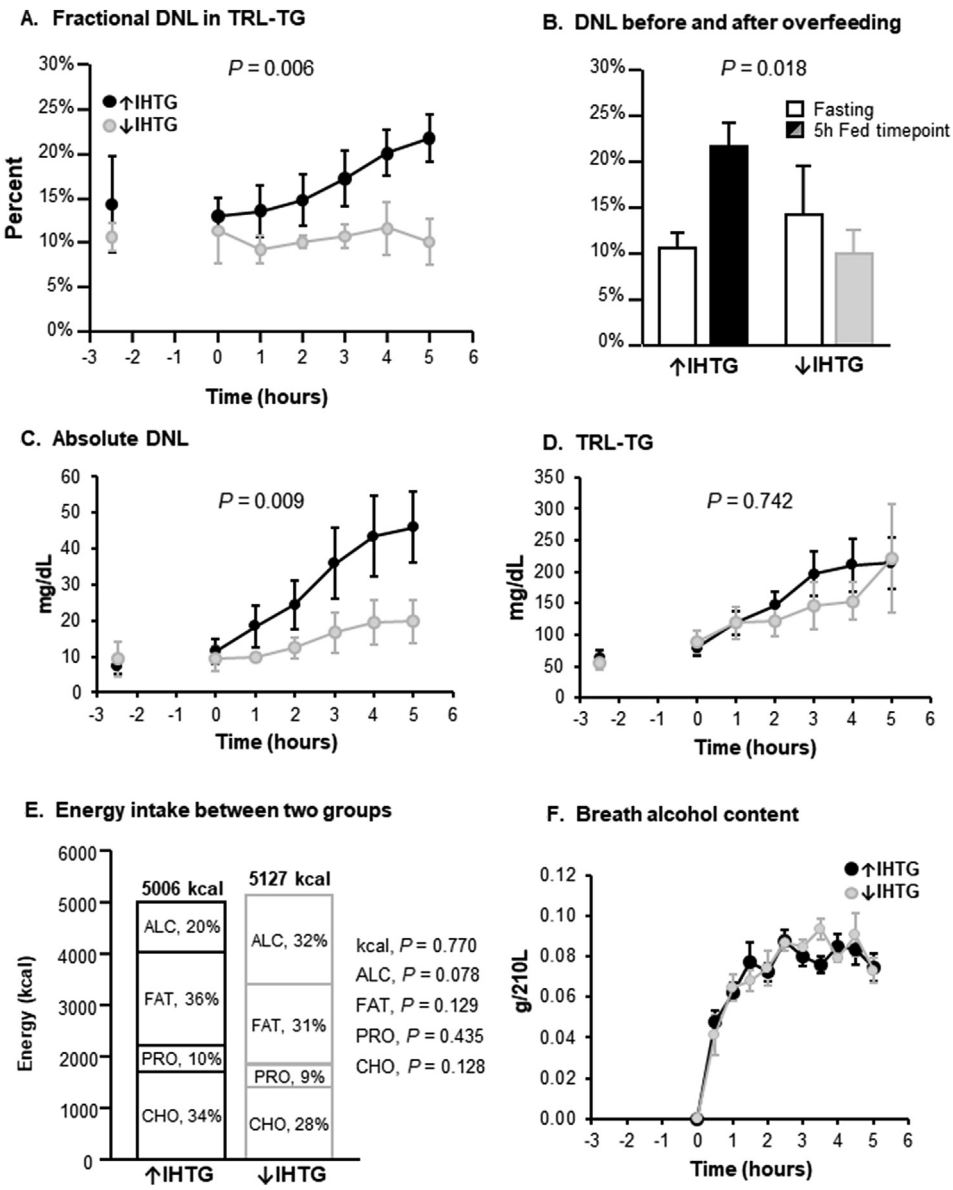

Fig. 4. Group differences in DNL, TRL-TG, food intake, and breath alcohol concentrations. Data are reported in mean ± SEM for the nine individuals who showed increased IHTG after 5 h after treatment (black symbols) and the five individuals who showed decreased IHTG 5 h after treatment (gray symbols). Fig. 4A displays the fractional lipogenesis in the two groups, Fig. 4B compares the fasted and fed state fractional DNL of the groups, and Fig. 4C the absolute levels of lipogenesis. Fig. 4D presents the TRL-TG concentfrations of the two groups and Fig. 4E shows food and alcohol intake during the 5 h treatment as analyzed by NDSR®. In Fig. 4F, no differences were found in the rates at which the groups achieved a steady-state of breath alcohol content. Abbreviations: DNL, *de novo* lipogenesis; IHTG, intrahepatic triacylglycerols.

following 2 days of alcohol administration. Thus, although unlikely, the lack of change in IHTG in a subset of the present subjects who consumed 171 ± 24 g of alcohol may have been due to an insufficient time for these subjects to accumulate IHTG.

The secondary goal of this study was to measure changes in DNL in response to 5 h of treatment. Increased DNL is a key distinguishing characteristic in patients with NAFLD (Lambert et al., 2014); however, no studies in the past have investigated the effects on DNL of food and alcohol consumed together. In a previous set of studies, Siler et al. (1998, 1999) tested the isolated effects of 24 g and 48 g of alcohol (no food) administered in four doses over 2 h (Siler et al., 1998) or two doses over 30 min (Siler, Neese, & Hellerstein, 1999). The increases in VLDL-TG DNL, peaking at 37 ± 7% (Siler et al., 1998) and 30 ± 8% (Siler et al., 1999), were higher than the increase observed here (20 ± 8%) after we administered a greater quantity of alcohol (171 ± 24 g) over 5 h. Lower observed fractional DNL in the present study results from DNL being measured in the TRL fraction, which contains both VLDL and chylomicrons, instead of VLDL alone (Siler et al., 1998, 1999). Because chylomicron-TG primarily carries dietary sources of fatty acids, DNL fatty acids measured in TRL are diluted by unlabeled chylomicron-TG. Interestingly, in the present group as a whole, only the amount of alcohol consumed during 5 h was found to be significantly related to the increase in percent DNL ($R^2 = 0.539$, $p = 0.038$, data not shown).

With regard to biochemical changes, as expected, significant increases were observed for plasma glucose and TG while NEFA concentrations decreased significantly. These changes are consistent with previous studies in which alcohol was consumed alone (Siler et al., 1998) or with meals (Chung et al., 2003; Fielding et al.,

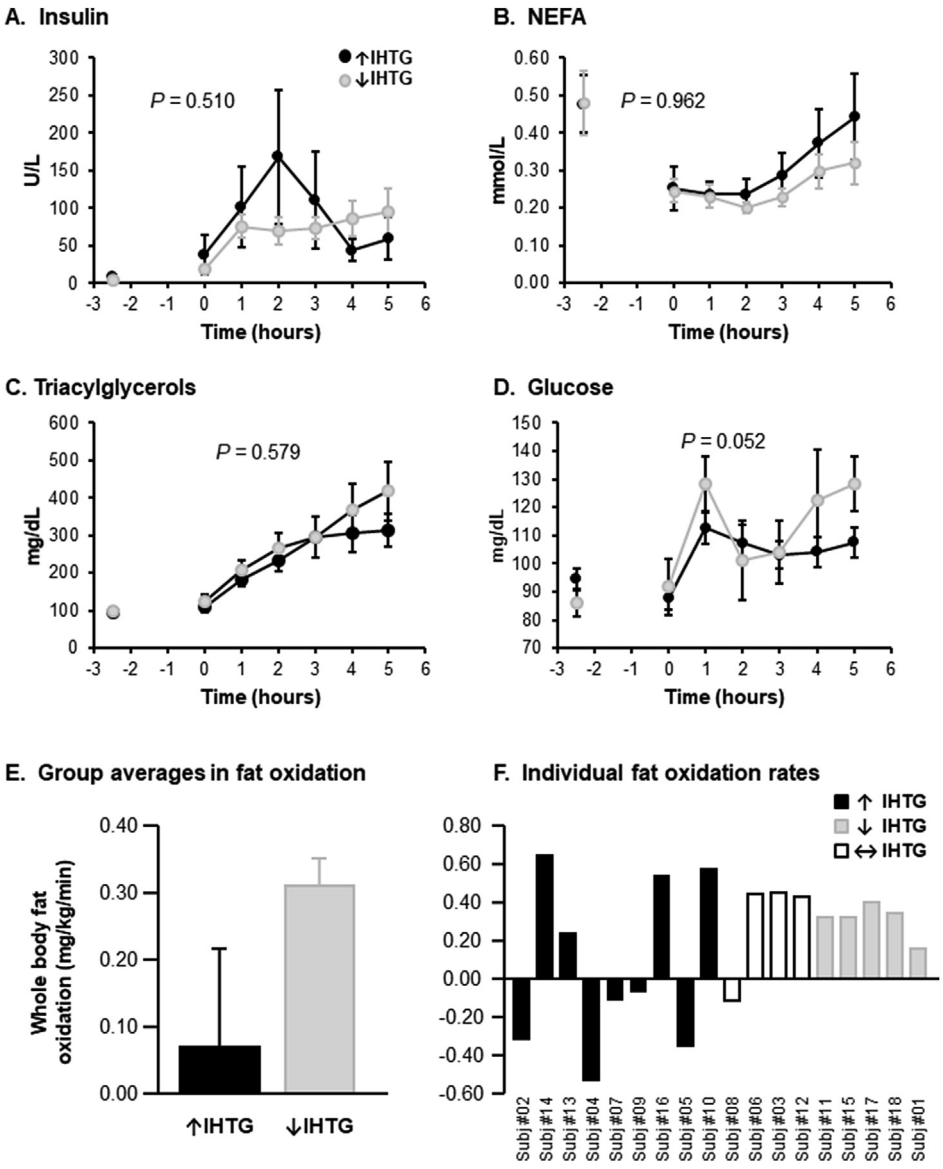

Fig. 5. Plasma insulin and blood metabolites, and fasting whole-body fat oxidation. Data are mean ± SEM, n = 18. Abbreviations: DNL, *de novo* lipogenesis; IHTG, intrahepatic triacylglycerols. Black symbols represent data from nine individuals who showed increased IHTG after 5 h after treatment; gray symbols represent data from five individuals who showed decreased IHTG 5 h after treatment. The patterns of plasma concentrations for the two groups are shown for insulin (Fig. 5A), NEFA (Fig. 4B), triacylglycerols (TG) (Fig. 5C), and glucose (Fig. 5D). Fig. 5E shows the fasting, whole-body fat oxidation data, which were assessed the morning after treatment. The t-test performed between the two groups showed no significant difference ($P = 0.259$). Fig. 5F shows that in 6 subjects, fat oxidation rates were calculated to be negative, which suggests that DNL was ongoing.

2000; Hampton et al., 2010; Hendriks et al., 1998; van der Gaag et al., 2000; van Tol et al., 1998). A study conducted by Fielding et al. (2000) in seven men consuming a bolus of vodka (47.5 g) with a meal high in carbohydrate reported a significant increase in large VLDL-TG particles (Svedberg flotation rate, S_f 60-400), but not small VLDL (S_f 20-60) and chylomicrons (S_f > 400). These data suggest that newly made fatty acids add to the size of the VLDL particle such that the significant increase in DNL observed here may have led to the secretion of larger VLDL particles and therefore higher TRL-TG concentrations.

Regarding changes in IHTG, Rubin and Lieber (1968) reported that three out of four subjects, in a group who received alcohol in addition to their study diet, failed to exhibit any change in the histological measures of steatosis, which suggests individual variability in response to a meal and alcohol. We also found a wide variation in the response of subjects after consuming food and alcohol. Of the 15 subjects who underwent liver scanning, nine increased IHTG, five subjects decreased IHTG, and one subject did not change. When subjects were categorized by IHTG response (increased IHTG, n = 9; and decreased IHTG, n = 5), changes in DNL were also significantly different between the groups. Consistent with our previous findings (Lambert et al., 2014), individuals with increased IHTG exhibited higher DNL, while those whose IHTG was reduced exhibited lower DNL values when expressed as AUC_{0-5} or postprandial average over 5 h. Subjects with increased IHTG also had lower plasma glucose concentrations, suggesting that glucose was readily utilized in the DNL pathway, although insulin levels were not different between groups (Fig. 5A). Interestingly, the group with increased IHTG consumed significantly less alcohol compared to the other group (1.32 g/kg vs. 2.50 g/kg, $p = 0.048$) and tended to consume 6% more energy from carbohydrates ($p = 0.128$). The BrAC was not different between the groups during treatment

(by design), which means that subjects with increased IHTG needed less alcohol to achieve BrAC of 0.08 g/210 L. We did not find a difference in *ad libitum* alcohol intake between the groups, but it is possible that the subjects who needed less alcohol to maintain BrAC were less able to oxidize, resulting in an accumulation of alcohol in the blood and in the liver, routing this substrate to storage. Thus, the protective nature of the group of individuals whose IHTG deceased could be partially attributed to higher whole-body fatty acid oxidation rates. Significance was not achieved between groups due to smaller sample sizes, which needs to be confirmed in future studies. Lastly, a potential explanation of these findings is that high carbohydrate consumption may have a greater impact on liver fat than alcohol in some subjects. Further studies are needed in a larger population to understand these differences between individuals in response to excess food and alcohol and their impact on DNL and IHTG.

The primary limitation of this study was the inclusion of only men as participants, which was due to a limit on the total number of subjects that could be studied using an intensive protocol and limited funding. In an early report by Taylor and co-workers, alcohol consumption was associated with increased serum-TG levels in males but not in females (Taylor et al., 1981), and the present data will be used to design future studies with a larger sample size to accommodate individual variability in response and sex as a biological variable. Further, the focus of the current study was to understand the liver's response to excess food and alcohol intake by conducting a protocol that mimicked real-life events, while maintaining scientific rigor. Although food intake was in vast excess above the subjects' daily needs, no data exist in the literature on the average food and beverage intake of spectators before and after sporting events, and there are undoubtedly some individuals who eat and drink in a manner similar to this study. Due to concerns for safety, the goal was for subjects to achieve a BrAC below 0.10 g/210 L, while under real-world tailgate conditions, some individuals drink more and some less. As a safety measure, plasma ALT was quantitated to assess liver health at the end of the 24-h study, and the values were similar to the previous day's fasting concentration. These data, and that from the literature (Rubin & Lieber, 1968; Wiebe et al., 1971), suggest that the livers of relatively young, healthy individuals may accommodate large excesses in nutrient intake, given time to recover.

Conclusions

Our previous cross-sectional observations of significant positive relationships between hepatic fatty acid synthesis and high liver fat (Donnelly et al., 2005; Lambert et al., 2014) are echoed in the results of the present study, even though these data were generated after a single bout of overeating. Overconsumption of food and alcohol significantly increased IHTG in some subjects, and although these individuals required less alcohol consumption to achieve the same BrAC, they tended to consume more dietary carbohydrate, and increased liver DNL to a greater extent. Given the high prevalence of overconsumption of food and alcohol in the U.S., further studies are warranted to better understand the interactions between personal consumption habits and individual metabolic variation in handling excess nutrients.

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Disclosure statement

The authors have nothing to disclose.

Author contributions

EJP and BDB designed the study. EJP, MMS-A, MJ-S, QH, AHG, NCW, NTL, JMM, and JMA collected and analyzed data. CM, GL, and AAJ provided medical oversight and interpretation of research findings. EJP and MMS-A drafted the paper, and all authors had input into the final manuscript.

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