Alcohol and Glucose Counterregulation During Acute Insulin-Induced Hypoglycemia in Type 2 Diabetic Subjects

Birthe M. Rasmussen, Lotte Ørskov, Ole Schmitz, and Kjeld Hermansen

To investigate the influence of alcohol on glucose counterregulation and recovery during acute insulin-induced hypoglycemia in type 2 diabetic subjects, 8 diet-treated type 2 diabetic subjects were examined twice after an overnight fast. A graded hyperinsulinemic (1 mU/kg/min, 60 to 195 minutes) euglycemic/hypoglycemic clamp was performed with concomitant infusion of 3-^{3}H-glucose to assess glucose turnover. After a euglycemic baseline period (150 to 180 minutes), 200 mL of water was taken either alone or with alcohol (0.4 g/kg body weight). Hypoglycemia (plasma glucose nadir, 2.8 mmol/L) was subsequently induced, and the recovery period followed after discontinuation of insulin and the variable glucose infusion. On both study days, circulating concentrations of insulin and glucose were comparable. Alcohol intake markedly increased plasma lactate (area under the curve [AUC], recovery period) (244 ± 30 *v* 12 ± 4 mmol/L × 240 minutes; *P* = .00009) and suppressed plasma nonesterified fatty acids (NEFA) (AUC, recovery period) (95 ± 13 *v* 161 ± 18 mmol/L × 240 minutes; *P* = .0008). No differences were found in the counterregulatory response of catecholamines, cortisol, and growth hormone (GH). However, alcohol intake decreased peak glucagon significantly (155 ± 12 *v* 200 ± 17 pg/mL; *P* = .038). In diet-treated, mild type 2 diabetic subjects, alcohol does not modify recovery from insulin-induced hypoglycemia.

HYPOGLYCEMIA IS A common and feared complication in type 1 diabetes.[1-3] Being a potent inhibitor of gluconeogenesis through its effect on the hepatic redox state,[4] alcohol intake highly increases the risk of hypoglycemia. The oxidation of ethanol to acetaldehyde and then to acetate requires nicotinamide adenine dinucleotide (NAD) as a cofactor with the subsequent formation of dihydronicotinamide adenine dinucleotide (NADH), thus decreasing the availability of NAD for the conversion of gluconeogenic precursors to glucose.[5] Alcohol-induced hypoglycemia occurs especially when hepatic glycogen stores are depleted,[6] ie, in relationship to inadequate food intake or exercise. Type 1 diabetic subjects are predisposed to hypoglycemia by a defective glucose counterregulation. A deficient glucagon response is acquired during the early course of type 1 diabetes, being almost universally present after 5 years of diabetes duration.[7] After 5 to 10 years, a decreased epinephrine response follows, and eventually impairment of growth hormone and cortisol secretion may occur.[8]

In type 2 diabetes, plasma glucose levels seem to be less influenced by alcohol than in type 1 diabetes,[9-11] and obese nondiabetic subjects seem refractory to alcohol-induced hypoglycemia.[12-14] However, alcohol may decrease counterregulatory hormone responses to hypoglycemia in normal subjects,[15,16] and defective glucoregulatory mechanisms have been observed in type 2 diabetic subjects.[17]

Alcohol intake impairs glucose counterregulation during insulin-induced hypoglycemia in type 1 diabetes and inhibits spontaneous recovery from hypoglycemia.[18] This raises the question whether alcohol during hypoglycemia also in type 2 diabetes may prevent or delay the recovery from hypoglycemia. The present study was performed to assess the effect of alcohol intake on glucose counterregulation and recovery in response to acute insulin-induced hypoglycemia in type 2 diabetic subjects.

SUBJECTS AND METHODS

Subjects

Eight type 2 diabetic subjects (5 men and 3 women) diagnosed by an oral glucose tolerance test according to the criteria of the World Health Organization (WHO)[19] participated. Average age was 55 ± 3 years (±SEM), body mass index (BMI), 26.8 ± 0.9 kg/m^2, and diabetes duration was 3 ± 1 years. They were well regulated with a glycosylated hemoglobin (HbA_{1c}) of 6.3% ± 0.2% and a fasting plasma glucose on the 2 different study days of 6.6 ± 0.4 and 6.7 ± 0.4 mmol/L (control and alcohol, respectively). All subjects were treated with diet only. Apart from background retinopathy, none of the subjects had any signs of diabetic complications or any history of heart disease, liver disease, or alcohol abuse. None were treated with beta blockers. All subjects were recruited from the outpatient clinic and were fully informed of the experimental nature of the investigation, which had been approved by the local ethics committee. Informed consent was obtained before the study began, and all subjects participated with full compliance to the protocol.

Experimental Protocol

After an overnight fast (10 hours), the subjects randomly participated on 2 study days with a minimum interval of 2 weeks. No major physical exercise, no alcohol intake, or medication was allowed on the day before the study, and the subjects consumed a weight maintenance diet containing at least 250 g carbohydrate. Each study day commenced at 8 AM; 2 catheters were placed, 1 in a heated dorsal hand vein for sampling of arterialized blood and 1 in the contralateral antecubital vein for infusions.

The subjects were examined during a graded hyperinsulinemic hypoglycemic clamp lasting 420 minutes. Insulin (Insulin Actrapid human; Novo Nordisk, Copenhagen, Denmark) was infused at a rate of 2.0 mU/kg/min (0 to 30 minutes), 1.5 mU/kg/min (30 to 60 minutes), and 1.0 mU/kg/min (60 to 195 minutes). At 195 minutes, insulin

From the Department of Endocrinology and Metabolism, Aarhus Amtssygehus, Aarhus University Hospital, Aarhus; and the Medical Department M (Endocrinology and Diabetes), Aarhus Kommunehospital, Aarhus University Hospital, Aarhus, Denmark.

Submitted June 26, 2000; accepted October 19, 2000.

Supported by grants from the National Board of Health (Board of Alcohol Policy), The Novo Nordic Foundation, and the Mogens and Jenny Vissing Foundation.

Address reprint requests to Kjeld Hermansen, DMSc, Aarhus University Hospital, Aarhus Amtssygehus, Tage-Hansens Gade 2, DK-8000 Aarhus C, Denmark.

0026-0495/01/5004-0014$35.00/0

doi:10.1053/meta.2001.21697

infusion was discontinued. Throughout the study, 3-^{3}H-glucose (Du Pont New-England Nuclear, Boston, MA) was infused in a primed (17 μCi) continuous (0.17 μCi/min) manner, as well as in a variable manner (170 μCi/1,000 mL 20% glucose) according to the principles of hot ginf,[20] all to assess glucose metabolism. Two hours were allowed for appropriate tracer equilibration. During the initial 150 minutes of the study, plasma glucose was decreased to 5 mmol/L. After a euglycemic baseline period (150 to 180 minutes) 200 mL of water was taken either alone or with alcohol 0.4 g/kg body weight (195 minutes). Hypoglycemia, aiming at a plasma glucose nadir of 2.8 mmol/L, was then acutely induced by reducing the variable glucose infusion. The recovery period was followed after discontinuation of the insulin infusion (Fig 1).

Analyses were performed for HbA_{1c}, glucose, glucose-specific activity, insulin, lactate, ethanol, nonesterified fatty acids (NEFA), triglycerides (TG), glucagon, growth hormone (GH), cortisol and catecholamines. Samples for plasma glucose were drawn every 5 minutes; the other samples with intervals as shown in the figures. Samples for plasma glucose were mixed with fluoride and analyzed in duplicate immediately. Samples for lactate and glucose-specific activity were mixed with fluoride, samples for glucagon with aprotinin, samples for HbA_{1c} with EDTA before being stored at −20°C for later analyses. Serum for the other samples was stored at −20°C, and samples for catecholamines were mixed with glutathion-EDTA and stored at −80°C for later analyses.

Analytical Methods

Plasma glucose was analyzed on a glucose analyzer (Beckman Instruments, Palo Alto, CA). Serum insulin was measured by an enzyme-linked immunosorbent assay using a commercial kit (DAKO Diagnostics, Cambridgeshire, UK).[21] Plasma lactate was measured by an oxidase method using a YSI Model 2300 Stat Plus lactate analyzer (Yellow Springs, OH).[22] HbA_{1c} was measured by a commercial kit with a range of 3.5% to 5.5% (Bio-Rad, Richmond, CA). Serum ethanol was measured by gas chromatography using the head space technique.[23] Serum TG and NEFA levels were measured by standard enzymatic colorimetric assays using commercial kits (Boehringer, Mannheim, Germany and Wako Chemicals, Neuss, Germany). Plasma glucagon was determined by radioimmunoassay as previously described with the modification that polyethylen glycol (PGE) was used for separation before determination and that plasma was extracted with ethanol.[24] Serum GH and serum cortisol were measured using an immunofluorometric sandwich assay with 2 monoclonal antibodies (AutoDelfia hGH kit and AutoDelfia Cortisol kit; Wallace Oy, Turku, Finland). Plasma epinephrine and norepinephrine were determined by electrochemical detection after high performance liquid chromatography (HPLC).[25] For determination of tritiated glucose activity, plasma was deproteinized using 0.3 mol/L $Ba(OH)_2$ and 5% $ZnSo_4$, after which the supernatant was evaporated under vacuum, resuspended in distilled water, and counted by a liquid scintillation counter after the addition of 5 mL Aqualuma Plus (Lumbac, Schaesburg, the Netherlands).

Statistical Methods

The rate of total glucose appearance (Ra) and disappearance (Rd) were determined from tritiated glucose samples drawn from minutes 120 to 420. The values were calculated according to the nonsteady-state equations of Steele[26] as modified by De Bodo et al.[27] A distribution volume of 220 mL/kg and a pool fraction of 0.65 was used. Hepatic glucose production (HGP) was calculated by subtracting the glucose infusion rate (GIR) from Ra. The lactate, NEFA, and counterregulatory responses are expressed as the incremental area above the 180-minute level (end of the baseline period), the area under the curve, designated AUC (180 to 420). This is calculated according to the trapezoid rule, in which values below the 180-minute levels are ignored.[28] Glucose, glucose kinetics, insulin, and TG responses are expressed as means. The fasting values are the mean values of measurements at −5 and 0 minutes. The peak values are calculated as the means of the maximal response occurring after the administration of ethanol/placebo and the development of insulin-induced hypoglycemia. The nadir values are calculated as the means of the lowest value for each subject during the 7 hours.

The TG data did not fulfill the assumption of normality before being transformed to natural logarithms, the other data fulfilled the assumption of normality and homogeneity of variance. A 2-way analysis of variance (ANOVA) for repeated measurements of 2 factors (time and treatment) was used a priori to test for changes with time and between treatments. If this test showed significant differences, it was followed by Student's *t* test for paired samples.[29] The statistical software used was SPSS (SPSS, Chicago, IL). Results are given as mean ± SEM (also for the transformed data for the sake of comparison) and a *P* level less than .05 is considered statistically significant.

RESULTS

Serum Insulin

Fasting serum insulin levels (34 ± 6 *v* 34 ± 7 pmol/L) and baseline insulin levels (control *v* alcohol) did not differ (377 ± 21 *v* 385 ± 15 pmol/L) (Fig 2A). After discontinuation of the insulin infusion (195 minutes), insulin levels rapidly decreased and reached the fasting level, 34 pmol/L at 224 minutes (calculated by interpolation).

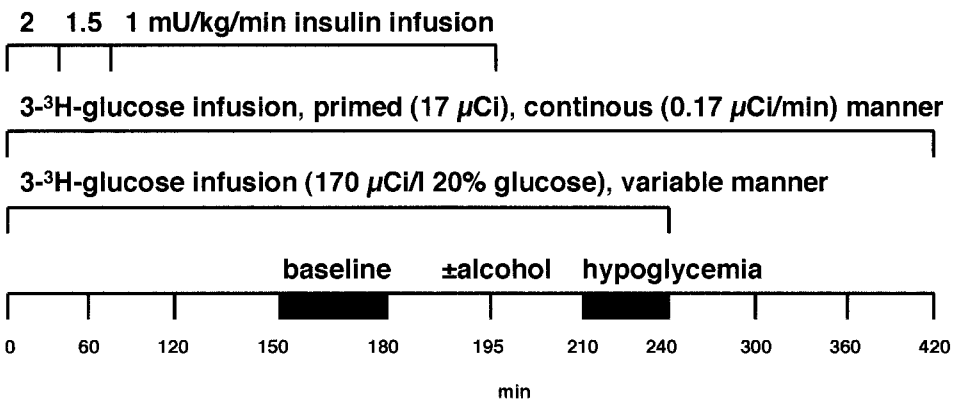

Fig 1. Study design.

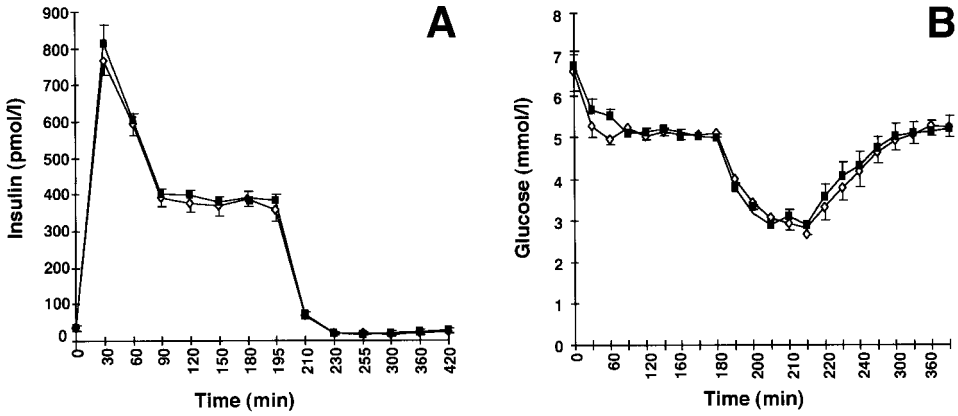

Fig 2. Serum insulin (A) and plasma glucose (B) levels during the clamp on the control day (◇—◇) and after alcohol (■—■). Data are means ± SE.

Plasma Glucose

No difference was seen in fasting plasma glucose levels on both days (6.6 ± 0.4 *v* 6.7 ± 0.4 mmol/L; control *v* alcohol) (Fig 2B). In response to insulin infusion, plasma glucose levels did not differ during baseline, 150 to 180 minutes (5.1 ± 0.1 *v* 5.1 ± 0.1 mmol/L), hypoglycemia, 205 to 215 minutes (2.9 ± 0.1 *v* 3.0 ± 0.2 mmol/L), or during the recovery period, 215 to 420 minutes (4.5 ± 0.6 *v* 4.6 ± 0.9 mmol/L; control *v* alcohol). Glucose nadirs did not differ (2.7 ± 0.1 *v* 2.9 ± 0.2 mmol/L; control *v* alcohol). The duration of hypoglycemia was initially planned to last for 30 minutes (minutes, 210 to 240), however, due to the efficiency of counterregulatory responses, it lasted for only 10 minutes (205 to 215 minutes). To observe the recovery period without the influence of hyperinsulinemia, insulin infusion was discontinued at 195 minutes, and hypoglycemia was sustained by decreasing the variable glucose infusion.

Glucose Kinetics

Mean HGP responses (control *v* alcohol) were comparable (1.89 ± 0.1 *v* 1.87 ± 0.08 mg/kg/min) as were peak values (3.56 ± 0.3 *v* 3.74 ± 0.2 mg/kg/min). No difference was found between glucose infusion rate (GIR) at baseline (7.11 ± 0.65 *v* 6.10 ± 0.95 mg/kg/min), mean glucose utilization (Rd) (3.40 ± 0.27 *v* 3.17 ± 0.33 mg/kg/min) or Ra (3.47 ± 0.26 *v* 3.24 ± 0.31 mg/kg/min) (control *v* alcohol).

Serum Lipids

Fasting (1.52 ± 0.33 *v* 1.67 ± 0.52 mmol/L) and mean (1.28 ± 0.31 *v* 1.46 ± 0.49 mmol/L) serum TG levels (control *v* alcohol) did not differ on the 2 study days. No difference was seen in fasting serum NEFA (Fig 3A) (control *v* alcohol) (0.70 ± 0.08 *v* 0.78 ± 0.10 mmol/L) and NEFA levels were equally and almost totally suppressed by the insulin infusion at baseline (0.06 ± 0.01 *v* 0.09 ± 0.03 mmol/L). After 210 minutes (serum insulin 70 pmol/L), NEFA levels increased rapidly on both days, however, the AUC (180 to 420) remained significantly lower (95 ± 13 *v* 161 ± 18 mmol/L × 240 minutes, $P = .0008$) when alcohol was taken.

Plasma Lactate

Fasting plasma lactate levels (Fig 3B) did not differ on the 2 days (1.42 ± 0.25 *v* 1.21 ± 0.13 mmol/L; control *v* alcohol), and alcohol intake significantly increased the plasma lactate AUC (180 to 420) (244 ± 30 *v* 12 ± 4 mmol/L × 240 minutes; $P = .00009$).

Counterregulatory Hormones

No difference was found in glucagon responses (Fig 4) AUC (180 to 420) on the 2 days (12,093 ± 957 *v* 9,953 ± 2,027 pg/mL × 240 minutes; $P = .38$) (control *v* alcohol). Interestingly, alcohol suppressed peak glucagon levels significantly

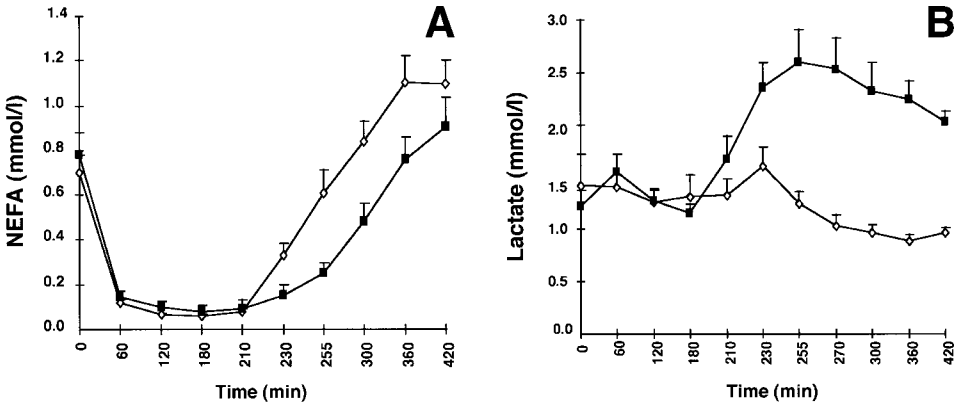

Fig 3. Serum NEFA (A) and plasma lactate (B) on the control day (◇—◇) and after alcohol (■—■). Data are means ± SE.

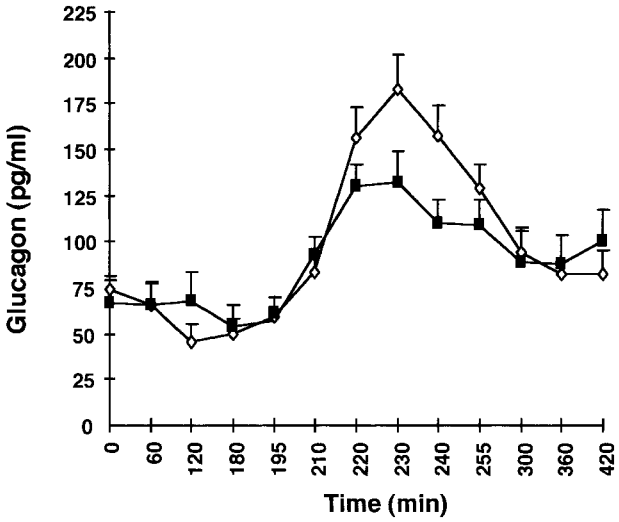

Fig 4. Plasma glucagon on the control day (◇—◇) and after alcohol (■—■). Data are means ± SE.

(155 ± 12 *v* 200 ± 17 pg/mL; $P = .038$), but despite this, the hepatic glucose production did not differ on the 2 days. We found no difference in cortisol responses (Fig 5A) AUC (180 to 420) or cortisol peak values on the 2 days (control *v* alcohol) (38,024 ± 8,457 *v* 33,606 ± 8,699 nmol/L × 240 minutes) and (595 ± 37 *v* 561 ± 55 nmol/L), respectively. Neither the plasma GH response (Fig 5B) AUC (180 to 420) nor the GH peak values differed on the 2 days (control *v* alcohol) (365 ± 87 *v* 378 ± 179 ng/mL × 240 minutes) and (7.28 ± 1.34 *v* 7.04 ± 2.42 ng/mL), respectively. The plasma epinephrine AUC (180 to 420) and peak values did not differ (control *v* alcohol) (22,078 ± 5,599 *v* 21,383 ± 7,272 pg/mL × 240 minutes) and 505 ± 83 *v* 513 ± 101 pg/mL); neither did the norepinephrine responses (Fig 5D) (435 ± 75 *v* 355 ± 47 pg/mL). The time course of the counterregulatory responses to acutely induced hypoglycemia was not changed by alcohol.

Ethanol

Serum ethanol peaked at 13.8 ± 1.5 mmol/L (230 minutes) on the alcohol day. Ethanol concentration at time of the glycemic nadir was 8.2 mmol/L. Fasting values on both study days and peak time values on the control day were undetectable.

DISCUSSION

This study shows that in diet-treated type 2 diabetic subjects a moderate alcohol intake corresponding to 2 to 3 drinks before acute insulin-induced hypoglycemia by a graded, hyperinsulinemic euglycemic/hypoglycemic clamp does not influence the recovery of plasma glucose. This is in contrast to the adverse effects on glucose counterregulation observed in type 1 diabetic subjects in whom alcohol inhibits spontaneous recovery from hypoglycemia.[18] The type 2 diabetic subjects investigated had mild diabetes with moderate overweight and relative insulin sensitivity. The counterregulatory hormone responses to hypoglycemia was found to be prominent in these subjects. Abnormal counterregulation might have been found at a diabetes duration of more than 3 years or at a more advanced stage of diabetes.

Glucagon plays a primary role in glucose counterregulation, stimulating both glycogenolysis and gluconeogenesis. Blunted glucagon responses to hypoglycemia may occur in patients with type 2 diabetes, although less severely than in type 1 diabetes.[8] The responsible mechanisms are unknown. They cannot be ascribed to autonomic neuropathy or to changes in the beta cell function,[30] and they do not improve after intensi-

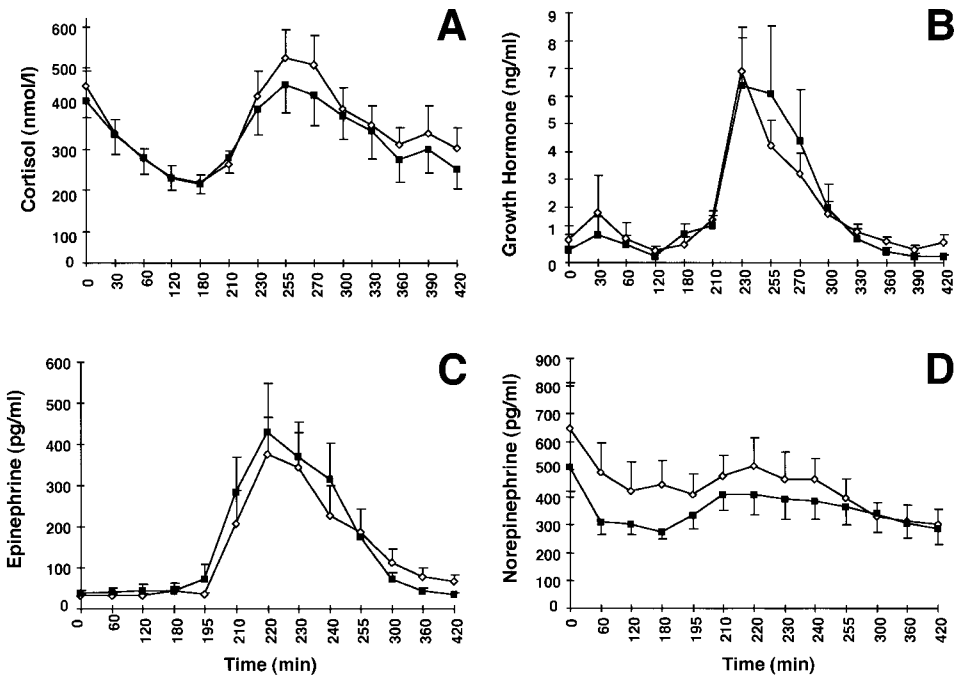

Fig 5. Serum cortisol (A), serum GH (B), plasma epinephrine (C), and plasma norepinephrine (D) on the control day (◇—◇) and after alcohol (■—■). Data are means ± SE.

fied glucoregulation.[31] Bolli et al[32] found a defective glucose counterregulation after subcutaneous insulin in type 2 diabetic subjects. The presence of alcohol and its metabolites are able to enhance alpha cell secretion in vitro.[33] Palmer and Ensinck[34] showed that a small decrease in extracellular glucose, combined with relative insulin deficiency in the presence of alcohol, resulted in exaggerated glucagon release in normal subjects. Avogaro et al[18] showed that alcohol significantly increases plasma glucagon during hypoglycemia in both normal and type 1 diabetic subjects. However, despite the increased glucagon response, an increased glucose production did not occur. On the other hand Yki-Järvinen et al[35] reported that alcohol administration during a euglycemic, hyperinsulinemic clamp did not influence the glucagon response in normal subjects.

In our study, the type 2 diabetic subjects had a reduced peak glucagon response to hypoglycemia after alcohol administration, although no difference in the overall glucagon response was seen on the study days. This finding is consistent with Kolaczynski et al[15] who found reduced responses of glucagon, cortisol, and GH after alcohol in response to hypoglycemia in normal subjects, and Lecavalier et al[16] who showed that alcohol reduced the initial, but not the overall, response of glucagon in normal subjects. The inconsistent findings may be due to eg, differences in study subjects, differences in duration and degree of alcohol intoxication, differences in rate of fall, duration, and degree of hypoglycemia,[36] degree of insulin resistance, and insulin levels at the time of hypoglycemia. Thus, high insulin concentrations prevent the stimulation of glucagon release by glucopenia.[37] Also to be considered is the ability of NEFAs to inhibit[38] and of the gluconeogenic precursor, alanine, to stimulate[39] glucagon secretion. Due to the shift in redox potential and an inhibition of lipolysis by acetate, both the alanine levels and the NEFA levels decrease after alcohol intake.[40,41] We found a significantly lower peak glucagon after alcohol in our study with similar insulin and glucose levels on the 2 days, and the NEFA levels being equally and almost totally suppressed by insulin at baseline.

Cortisol and GH antagonize the effects of insulin, thus inhibiting glucose utilization and stimulating glucose production. These hormones are, in particular, important for recovery from prolonged hypoglycemia, as their effects require several hours to occur.[17] Alcohol has a direct effect on the hypothalamus-pituitary-adrenal axis probably based on stimulation of adrenocorticotropic hormone.[42] Bellet et al[43] found increased GH levels to alcohol in normal subjects, while Puhakainen et al[44] showed no effect of acute alcohol administration on cortisol or GH levels, confirming previous studies.[35,45] Others have shown that hypoglycemia decreased GH and cortisol responses to alcohol in normal[15,16] and in type 1 diabetic subjects.[18] Acetate, which is metabolized by the brain at hypoglycemia,[46] may inhibit the secretion of GH explaining a blunted response.[47] In our type 2 diabetic subjects, neither GH nor cortisol responses to hypoglycemia differed regardless of whether alcohol was taken or not.

Epinephrine stimulates glucose production, inhibits peripheral glucose uptake, and releases NEFA, lactate, glycerol, and alanine into the circulation. This is mediated directly through beta-adrenerg mechanisms and indirectly through an alfa-adrenergic inhibition of insulin secretion. In the absence of both glucagon and epinephrine, glucose recovery fails to occur.[48] Norepinephrine, released from sympathetic postganglionic neurons, exerts hyperglycemic effects through the same mechanisms.[49] Alcohol may cause a dose-dependent increase in plasma norepinephrine and may reduce norepinephrine clearance.[50] However, alcohol intake did not increase the catecholamine secretion in type 2 diabetic subjects,[44] and similar catecholamine responses to insulin-induced hypoglycemia were found irrespective of alcohol intake in normal subjects.[15]

In our study, NEFA levels were maximally suppressed by insulin infusion. The effect of insulin on lipolysis and very–low-density lipoprotein (VLDL) TG production vanes simultaneously with the increasing effect of serum ethanol. Our findings of decreased NEFA levels and similar TG levels after alcohol corroborates our previous studies.[9-11] This does not, however, exclude that long-term alcohol consumption or greater amounts of alcohol may elevate the circulating TG. NEFAs play a crucial role in the integrated response to hypoglycemic counterregulation,[51] and alcohol may interfere with glucose recovery not only because of its inhibition of gluconeogenesis, but also because of its ability to depress lipolysis. Thus, Avogaro et al[18] found impaired glucose recovery after alcohol in type 1 diabetic subjects, and when the alcohol-induced inhibition of lipolysis was counteracted by heparin and Intralipid infusions, glucose counterregulation to hypoglycemia was normalized.

As expected, we found an increased lactate response to alcohol. This may be due to either an increased hepatic lactate production or a decreased hepatic uptake of lactate derived from peripheral tissues or both.[52]

Interestingly, we found that alcohol did not influence plasma glucose levels, HGP, or glucose utilization during acute insulin-induced hypoglycemia in diet-treated, mild type 2 diabetic subjects. Thus, alcohol intake in these type 2 diabetic subjects does not influence spontaneous recovery from hypoglycemia despite suppression of NEFA levels and a diminished peak glucagon level. In this context, it is interesting that we recently showed that the combination of alcohol intake and exercise with or without a meal does not elicit acute hypoglycemia in type 2 diabetic subjects.[53] A plausible explanation for our finding may be that alcohol inhibits gluconeogenesis, but not glycogenolysis, and recovery from short-term hypoglycemia is largely a result of increased glycogenolysis. Our results do not exclude that type 2 diabetic subjects may experience a deficient alcohol-induced glucose recovery in the presence of depleted glycogen stores, at a more advanced stage of diabetes or, eg, after insulin or sulfonylurea treatment.

In conclusion, alcohol intake in diet-treated, mild type 2 diabetic subjects does not influence spontaneous recovery from hypoglycemia induced by a graded, hyperinsulinemic, euglycemic/hypoglycemic clamp. No alterations were seen in the counterregulatory hormone responses apart from a suppression of the peak glucagon response.

ACKNOWLEDGMENT

We appreciate the expert assistance of Anette Mengel, Inga Bisgaard, Tove Skrumsager, Kirsten Eriksen, Eva Pedersen, Aarhus University Hospital; Birthe Baumgarten, Institute of Pharmacology, Aarhus University; and Carsten Hansen, Department of Forensic Toxicology, Aarhus University.

REFERENCES

1. Lewis H, Kendall MJ: Alcohol and treatment of diabetes (review). J Clin Pharm Ther 13:321-328, 1988
2. McDonald J: Alcohol and diabetes. Diabetes Care 3:629-637, 1980
3. Connor H, Marks V: Alcohol and diabetes. Diabet Med 2:413-416, 1985
4. Lieber CS: Metabolism and metabolic effects of alcohol. Clin North Am 681:3-31, 1984
5. Marks V: Alcohol and carbohydrate metabolism. J Clin Endocrinol Metab 7:333-349, 1978
6. Nilsson LH, Hultman E: Liver glycogen in man—the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. Scan J Clin Lab Invest 32:325-330, 1973
7. Bolli G, DeFeo P, Compagnucci P: Abnormal glucose counterregulation in insulin-dependent diabetes mellitus: Interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion. Diabetes 32:134-141, 1983
8. Campbell PJ, Gerich JE: Mechanisms for prevention, development and reversal of hypoglycemia. Adv Intern Med 33:205-230, 1988
9. Christiansen C, Thomsen C, Rasmussen O, et al: Acute effects of graded alcohol intake on glucose, insulin and free fatty acid levels in non-insulin-dependent diabetic subjects. Eur J Clin Nutr 47:648-652, 1993
10. Christiansen C, Thomsen C, Rasmussen O, et al: Effect of alcohol on glucose, insulin, free fatty acid and triglyceride responses to a light meal in non-insulin dependent (NIDDM) diabetic subjects. Br J Nutr 71:449-454, 1994
11. Christiansen C, Thomsen C, Rasmussen O, et al: Wine for type 2 diabetic patients? Diabet Med 10:958-961, 1993
12. Nikkilä EA, Taskinen MR: Ethanol-induced alterations of glucose tolerance, postglucose hypoglycaemia and insulin secretion in normal, obese and diabetic subjects. Diabetes 24:933-943, 1975
13. Arky RA, Freinkel N: Alcohol hypoglycaemia. V. Alcohol infusion to test gluconeogenesis in starvation with special reference to obesity. N Engl J Med 274:426-433, 1966
14. Arky RA, Abramson AE, Freinkel N: Alcohol hypoglycaemia. VII. Further studies on the refractoriness of obese subjects. Metabolism 17:977-987, 1968
15. Kolaczynski JW, Ylikhari R, Härkonen M, et al: The acute effect of ethanol on counterregulatory response and recovery from insulin-induced hypoglycemia. J Clin Endocrinol Metab 67:384-388, 1988
16. Lecavalier L, Bolli G, Cryer P, et al: Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. Am J Physiol 256:E844-851, 1989
17. Cryer PE, Gerich JE: Glucose counterregulation, hypoglycaemia and intensive insulin therapy in diabetes mellitus. N Engl J Med 313:232-241, 1985
18. Avogaro A, Beltramello P, Gnudi L, et al: Alcohol intake impairs glucose counterregulation during acute insulin-induced hypoglycemia in IDDM patients. Evidence for a critical role of free fatty acids. Diabetes 42:1626-1634, 1993
19. World Health Organisation: Diabetes mellitus. Report of a WHO study group, Geneva, Switzerland, 1985
20. Molina JM, Baron AD, Edelman SV, et al: Use of variable tracer infusion method to determine glucose turnover in humans. Am J Physiol 258:E16-23, 1990
21. Andersen L, Dinesen B, Jørgensen PN, et al: Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chem 39:578-582, 1993
22. Christiansen T, Jakobsen KM: The slow penetration of enzyme-based biosensors into clinical chemistry analysis. Acta Anaesthesiol Scand 39:31-35, 1995 (suppl 104)
23. Machata G: The advantage of automated blood alcohol determination by head space analysis. Z Rechtsmedizin 75:229-234, 1975
24. Ørskov H, Thomsen HG, Yde H: Wick chromatography for rapid and reliable immunoassay of insulin, glucagon and growth hormone. Nature 219:193-195, 1968
25. Eriksson BM, Persson BA: Determination of catecholamines in rat heart tissue and plasma samples by liquid chromatography with electrochemical detection. J Chromatogr 228:143-154, 1982
26. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 82:420-424, 1959
27. De Bodo RC, Steele R, Altzuler N, et al: On the hormonal regulation of carbohydrate metabolism: Studies with C14 glucose. Recent Progr Horm Res 19:445-488, 1963
28. Wolever TMS, Jenkins DJA, Jenkins A, et al: The glycemic index: Methodology and clinical implications. Am J Clin Nutr 54:846-854, 1991
29. Armitage P, Berry G: Statistical Methods in Medical Research (ed 3). New York, NY, Blackwell Scientific, 1994
30. Polonsky K, Bergenstal R, Pons G: Relation of counterregulatory responses to hypoglycaemia in type 1 diabetics. N Engl J Med 307:1106-1112, 1982
31. Bolli G, DeFeo P, Decosmo S: Effects of long-term optimization and short-term deterioration of glycemic control on glucose counterregulation in type 1 diabetes mellitus. Diabetes 33:394-400, 1984
32. Bolli G, Tsalikian E, Haymond MW, et al: Defective glucose counterregulation after subcutaneous insulin in non insulin-dependent diabetes mellitus. J Clin Invest 75:1623-1631, 1985
33. Tiengo A, Valerio A, Molinari M, et al: Effects of ethanol, acetaldehyde, and acetate on insulin and glucagon secretion in the perfused rat pancreas. Diabetes 30:705-709, 1981
34. Palmer JP, Ensinck JW: Stimulation of glucagon secretion by ethanol-induced hypoglycemia in man. Diabetes 24:295-300, 1975
35. Yki-Järvinen H, Nikkilä EA: Ethanol decreases glucose utilization in healthy man. J Clin Endocrinol Metab 61:941-945, 1985
36. Gerich JE, Schneider V, Dippe SE, et al: Characterization of the glucagon response to hypoglycaemia in man. J Clin Endocrinol Metab 38:77-82, 1974
37. Lefebvre PJ, Pierre J, Unger RH: Glucagon, Molecular Physiology, Clinical and Therapeutic Implications. Tarrytown, NY, Pergamon, 1972, p 167
38. Luyckx AS, Lefebvre PJ: Arguments for a regulation of pancreatic glucagon secretion by circulating plasma free fatty acids. Proc Soc Exp Biol Med 133:524-528, 1970
39. Luyckx AS, Lefebvre PJ: Mechanisms involved in the exercise-induced increase in glucagon secretion in rats. Diabetes 23:81-93, 1974
40. Wilson NM, Brown PM, Juul SM, et al: Glucose turnover and metabolic and hormonal changes in ethanol-induced hypoglycaemia. Br Med J Clin Res Ed 282:849-853, 1981
41. Avogaro A, Tiengo A: Alcohol, glucose metabolism and diabetes. Diabetes Metab Rev 9:129-146, 1993
42. Rees LH, Jeffcoate WJ, Gouldie DJ, et al: Alcohol-induced pseudo-Cushing's syndrome. Lancet 1:726-728, 1977
43. Bellet S, Yoshimine N, DeCastro O, et al: Effect of alcohol ingestion on growth hormone levels: Their relation to II-hydroxycorticoid levels and serum FFA. Metabolism 20:762-763, 1971

44. Puhakainen I, Koivisto VA, Yki-Järvinen H: No reduction in total hepatic glucose output by inhibition of gluconeogenesis with ethanol in NIDDM patients. Diabetes 40:1319-1327, 1991

45. Yki-Järvinen H, Koivisto VA, Ylikahri R, et al: Acute effects of ethanol and acetate on glucose kinetics in normal subjects. Am J Physiol 254:E175-180, 1988

46. Ørskov H, Hansen AP, Hansen HE, et al: Acetate: Inhibitor of growth hormone hypersecretion in diabetic and non-diabetic uremic subjects. Acta Endocrinol 99:551-558, 1982

47. Quabbe H, Trompke M, Luyckx AS: Influence of ketone body infusion on plasma growth hormone and glucagon in man. J Clin Endocrinol Metab 57:613-618, 1983

48. Cryer PE: Glucose counterregulation in man. Diabetes 30:261-264, 1981

49. Rizza RA, Cryer PE, Gerich JE: Role of glucagon, catecholamines and growth hormone in human glucose counterregulation. J Clin Invest 64:62-71, 1979

50. Eisenhofer G, Lambie DG, Johnson RH: Effects of ethanol on plasma catecholamines and norepinephrine clearance. Clin Pharmacol Ther 34:143-147, 1983

51. Caprio S, Gelfand RA, Tamborlane WV, et al: Oxidative fuel metabolism during mild hypoglycemia: Critical role of free fatty acids. Am J Physiol 256:E413-419, 1989

52. Kreisberg RA, Siegal AM, Owen WC: Glucose-lactate interrelationships: Effect of ethanol. J Clin Invest 50:175-185, 1971

53. Rasmussen BM, Christiansen C, Rasmussen OW, et al: Alcohol and postexercise metabolic responses in type 2 diabetic subjects. Metabolism 48:597-602, 1999