Effect of antecedent GABA A receptor activation on counterregulatory responses to exercise in healthy man.

Maka S. Hedrington, MD, MBA1, Donna B. Tate, MS1, Lisa M. Younk, BS1 and Stephen N. Davis, MBBS, FRCP, FACP1

1Department of Medicine, University of Maryland

Please address all correspondence to:

Stephen N. Davis, MBBS, FRCP, FACP
Chairman, Department of Medicine
University of Maryland School of Medicine
22 S. Greene Street, Room N3W42
Baltimore, MD 21201
P: 410-328-2488
F: 410-328-8688
sdavis@medicine.umaryland.edu

Word count:

Tables: 2

Figures: 5
ABSTRACT

The aim of this study was to determine if antecedent stimulation of GABA A receptors with the benzodiazepine alprazolam can blunt physiologic responses during next day moderate (90 min) exercise in healthy man. Thirty-one healthy individuals (16M/15F, 28±1 yr, BMI 23±3 kg/m²) were studied during separate, 2 day protocols. Day 1 consisted of morning and afternoon 2 hr hyperinsulinemic euglycemic or hypoglycemic clamps with or without 1 mg alprazolam given 30 min before a clamp. Day 2 consisted of 90 min euglycemic cycling exercise at 50% VO₂ max. Despite similar euglycemia (5.3±0.1 mmol/L) and insulinemia (46±6 pmol/L) during day 2 exercise studies, GABA A activation with alprazolam during day 1 euglycemia resulted in significant blunting of plasma epinephrine, norepinephrine, glucagon, cortisol and growth hormone responses. Lipolysis (glycerol, non-esterified fatty acids) and endogenous glucose production during exercise were also reduced and glucose infusion rates were increased following prior euglycemia with alprazolam. Prior hypoglycemia with alprazolam resulted in further reduction of glucagon and cortisol responses during exercise. We conclude that prior activation of GABA A pathways can play a significant role in blunting key ANS, neuroendocrine and metabolic physiologic responses during next day exercise in healthy man.

Keywords: GABA, hypoglycemia, epinephrine, glucagon, exercise.
INTRODUCTION

Exercise is a cornerstone of diabetes management, improving insulin sensitivity and reducing the risk of cardiovascular disease, and is beneficial for weight management (1-4). Nevertheless, exercise remains a significant risk and cause of hypoglycemia in individuals receiving insulin secretagogues and/or insulin (5,6). During exercise, homeostatic (counterregulatory) responses are activated to provide glucose and fat to the working muscles and maintain normal plasma glucose levels. These counterregulatory responses include: inhibition of insulin release and enhanced catecholamine (ANS) and neuroendocrine hormone (glucagon) secretion (7). The result is increased hepatic glucose production via glycogenolysis and gluconeogenesis (8). These mechanisms function so efficiently that healthy individuals do not become hypoglycemic during all but prolonged exercise.

Counterregulatory defenses against falling plasma glucose in exercise are similar to the ANS and neuroendocrine mechanisms activated during hypoglycemia (8,9). Studies in rats and primates have demonstrated that activation of GABA A receptors reduces major counterregulatory responses (epinephrine, glucagon) during hypoglycemia (10,11). Our laboratory has also reported similar effects in non-diabetic healthy human subjects (12) demonstrating that prior activation of GABA A receptors with the benzodiazepine alprazolam reduces autonomic, neuroendocrine and metabolic counterregulatory responses during next day hypoglycemia.

Studies investigating the integrated effects of activation of GABA A receptors on neuroendocrine, ANS and metabolic counterregulatory responses to subsequent exercise are lacking. The aim of the present study was to test the hypothesis that antecedent pharmacologic activation of GABA A receptors with alprazolam can result in neuroendocrine, ANS and/or metabolic counterregulatory failure during next-day moderate exercise in healthy humans.
RESEARCH DESIGN AND METHODS

**Subjects**

Thirty-one healthy individuals (16M/15F, 28±1 yr, BMI 23±3 kg/m\(^2\)) were studied. Subjects were non-smokers and had no family history of diabetes. Individuals participated in moderate recreational exercise but no elite athletes were studied (mean VO\(_2\) max: 39±2 ml/kg/min). All individuals had normal liver, renal and hematological parameters. Studies were approved by the Vanderbilt University human subjects institutional review board and all subjects gave informed written and verbal consent.

**VO\(_2\) max testing**

An estimate of physical fitness and maximal oxygen consumption (VO\(_2\)max) was obtained 1-3 weeks prior to the initial study, using a graded maximal exercise test on a bicycle ergometer. Rates of ventilation, oxygen consumption (VO\(_2\)) and CO\(_2\) production (VCO\(_2\)) were continuously monitored using a computerized, open-circuit indirect calorimetry cart (Parvo Medics, Sandy, UT) with a mouthpiece and nose clip system. Heart rate and surface electrocardiogram were monitored continuously before, during, and after exercise via surface ECG electrodes placed on the anterior chest. Blood pressure was monitored every 2-3 minutes using a manual cuff.

**Experimental Design**

Individuals participated in four separate, single-blind, 2-day experiments with differing day 1 protocols, separated by at least 2 months (Figure 1). All subjects were instructed to avoid intense exercise and alcohol and to consume their usual weight-maintaining diet for 3 days before each study. Each subject was admitted to the Vanderbilt University Clinical Research Center (CRC) the evening before an experiment. The next morning, after an overnight 10 hr
fast, subjects had intravenous cannulae placed into the arm under local 1% lidocaine anesthesia. One cannula was placed in a retrograde fashion into a vein in the back of the hand. This hand was placed in a heated box (55-60°C) so that arterialized blood could be obtained (13). The other cannula was placed in the arm for infusions of dextrose solution, insulin, potassium chloride, and labeled glucose. As a safety measure, an ECG was recorded continuously throughout all 2 hr hyperinsulinememic clamps and day 2 exercise studies.

Day 1 consisted of four different antecedent challenges (Figure 1). Protocol 1 consisted of day 1 morning and afternoon hyperinsulinememic euglycemic clamps (n=20). Protocol 2 involved day 1 morning and afternoon hyperinsulinememic euglycemic clamps with 1 mg alprazolam administered 30 minutes before each clamp (n=14). Protocol 3 consisted of day 1 morning and afternoon hyperinsulinememic hypoglycemia (2.9±0.1 mmol/L) (n=16). Protocol 4 involved morning and afternoon hyperinsulinememic hypoglycemia (2.9±0.1 mmol/L) with alprazolam (n=10). Four individuals participated in three studies, twenty-one individuals participated in two studies, and six individuals participated in one study. Some of the individuals had participated in and provided data in a previous study (14).

Day 1 began with a baseline period (0 to 120 min) followed by a 2 hr hyperinsulinememic experimental clamp period (120 to 240 min). At the start of the experimental period, a primed continuous infusion of insulin (Eli Lilly, Indianapolis, IN) was administered at a rate of 9 pmol/kg/min for 120 min. Potassium chloride (5 mmol/hr) was also infused during the clamp period to reduce insulin-induced hypokalemia. Plasma glucose levels were measured every 5 min, and a variable infusion of 20% dextrose was adjusted so that plasma glucose levels were held constant in the euglycemia studies (15). During hypoglycemia, the rate of fall of glucose was controlled (≈0.08 mmol/min) and the hypoglycemic nadir (2.9±0.1 mmol/L) was achieved and held constant using a modification of the glucose clamp technique (16). After completion of
the initial 2 hr clamp period, the insulin infusion was stopped and a 2 hr period of euglycemia was maintained using 20% dextrose infusion. At that point, insulin was restarted, and a second hyperinsulinemic euglycemic clamp, or hyperinsulinemic hypoglycemic clamp (identical to the morning study), was performed (Figure 1). At completion of the second glucose clamp, subjects consumed a standardized meal, a bedtime snack and remained in the CRC.

**Day 2 Exercise**

Day 2 was identical for all four protocols and was started after an overnight 10 hr fast. Each study consisted of a tracer equilibration period (0 to 120 min) and a 90 min experimental period (120 to 210 min). A primed (18 µCi) continuous infusion (0.18 µCi/min) of HPLC purified [3-3H] glucose (Perkin Elmer Life Sciences, Boston, MA; 11.5 mCi/mmol/L) was administered starting at 0 min and continued throughout the study to measure glucose kinetics. Exercise consisted of 90 min continuous cycling (at 60–70 rpm) on a bicycle ergometer at a relative work intensity of 50% of the individual’s VO$_2$ max. 20% dextrose was infused with a variable rate so that the euglycemic target (5.2-5.3 mmol/L) was held constant for the duration of the study.

**Tracer Calculations**

Endogenous glucose production (EGP) was calculated according to the method of Wall, et al. (17). EGP was calculated by determining the total rate of appearance (Ra; which comprises both EGP and any exogenous glucose infused to maintain the desired euglycemia) and subtracting from it the amount of exogenous glucose infused. It is now recognized that the original model described by Wall et al. and Steele et al. (18) is not fully quantitative, since underestimates of total Ra and rate of disappearance (Rd) can be obtained. The use of a highly purified tracer and taking measurements under steady-state conditions (i.e., constant specific activity) in the presence of low glucose flux eliminates most, if not all, of the problems. In order
to minimize changes in specific activity, the tracer infusion rate was gradually doubled during the first 30 min of exercise. During the remaining 60 min of exercise, proportional, additional increases of the tracer infusion rate were made commensurate with the changes of the exogenous glucose infusion rate.

**Analytical Methods**

The collection and processing of blood samples have been previously described (19). Plasma glucose concentrations were measured in triplicate using the glucose oxidase method with a glucose analyzer (Beckman, Fullerton, CA). Glucagon was measured according to the method of Aguilar-Parada et al. (20) with an interassay coefficient of variation (CV) of 12%. Insulin was measured as previously described (21) with an interassay CV of 11%. Catecholamines were determined by high-pressure liquid chromatography (HPLC) (22) with an interassay CV of 12% for epinephrine and 8% for norepinephrine. We made two modifications to the procedure for catecholamine determination: 1) we used a three-point rather than one-point standard calibration curve; and 2) we spiked the initial and final samples of plasma with known amounts of epinephrine and norepinephrine so that accurate identification of the relevant catecholamine peaks could be made. Growth hormone (23) (interassay CV=8%), cortisol (Clinical Assays Gamma Coat Radioimmunoassay Kit, interassay CV=6%), and pancreatic polypeptide (24) (interassay CV=8%) were measured using radioimmunoassay techniques. Lactate and glycerol were measured from deproteinized whole blood, using the method of Lloyd, et al. (25). Non-esterified fatty acids (NEFA) were measured using a kit from Wako Diagnostics (26).
Cardiovascular Parameters

Heart rate and systolic, diastolic and mean arterial blood pressures were measured noninvasively by a Dinamap vitals monitor (Critikon, Tampa, FL) every 10 min.

Statistical Analysis

Data are expressed as mean ± SE and were analyzed using standard, parametric, one- and two-way analysis of variance with repeated measures where appropriate (Graph Pad Software, Inc., San Diego, CA). Tukey’s post hoc analysis was used to delineate statistical significance within each group. A p-value of <0.05 was accepted as statistically significant. Changes (responses) from baseline to the end of exercise on day 2 were compared. Baseline data were calculated as an average of two time points (110 and 120 min) and final 15 min data represent an average of two measurements taken during time 195 and 210 min.

RESULTS

Day 1 Glucose and Insulin

Plasma glucose levels were similar in the morning and afternoon during all day 1 euglycemic studies (5.3±0.1 mmol/L) (Figure 2). Plasma glucose levels during day 1 morning and afternoon hypoglycemia studies were similar with and without alprazolam (2.9±0.05 mmol/L) (Figure 2). Plasma insulin levels during all hyperinsulinemic day 1 studies were similar among all groups (500±48 pmol/L).
Day 2 Exercise

Glucose and Insulin

During day 2 exercise, plasma glucose was similar (5.3±0.09 mmol/L) in all four groups (Figure 2). Plasma insulin was suppressed by a greater amount during exercise following day 1 euglycemia (20±5 pmol/L) compared to day 1 hypoglycemia (33±4 pmol/L) and day 1 alprazolam studies (euglycemia: 35±4 pmol/L; hypoglycemia: 34±6 pmol/L) (p<0.02) (Figure 2).

Autonomic Nervous System Responses

Baseline values of autonomic nervous system mediated hormones were similar at the start of all day 2 exercise studies (Table 1).

Increases in day 2 plasma epinephrine responses (Figure 3) were significantly lower (p<0.005) during day 2 exercise following day 1 euglycemia and alprazolam (Δ 183±37 pmol/L), day 1 hypoglycemia (Δ 277±58 pmol/L) and day 1 hypoglycemia and alprazolam (Δ 225±53 pmol/L) as compared to day 1 euglycemia (Δ 769±213 pmol/L).

Day 2 norepinephrine responses (Figure 3) were also significantly lower (p<0.0001) following day 1 euglycemia and alprazolam (Δ 1.3±0.3 nmol/L) and day 1 hypoglycemia and alprazolam (Δ 1.8±0.4 nmol/L) as compared with day 1 euglycemia (Δ 4.8±0.6 nmol/L).

Day 2 pancreatic polypeptide (Figure 3) responses were lower (p<0.003) following day 1 euglycemia and alprazolam (Δ 43.6±18 ng/L), day 1 hypoglycemia (Δ 53±15 ng/L) and day 1 hypoglycemia and alprazolam (Δ 17.3±15 ng/L) as compared to day 1 euglycemia (Δ 109±16 ng/L).
Neuroendocrine Counterregulatory Hormones

Baseline values of neuroendocrine counterregulatory hormones were similar at the start of all day 2 exercise studies (Table 1).

Increases in plasma glucagon levels on day 2 (Figure 3) were significantly blunted (p<0.0001) following day 1 euglycemia and alprazolam (Δ 4.3±2.2 ng/L) and day 1 hypoglycemia (Δ 4.2±1.4 ng/L) as compared to day 1 euglycemia (Δ 13±1.6 ng/L). Day 1 hypoglycemia and alprazolam resulted in a greater reduction (p<0.05) in day 2 glucagon during exercise responses (Δ -4.9±4.3 ng/L) as compared to the other groups.

Day 2 growth hormone responses (Figure 4) were also blunted (p<0.006) following day 1 euglycemia and alprazolam (Δ 0.7±1.7 µg/L), day 1 hypoglycemia (Δ 3.2±1.0 µg/L) and day 1 hypoglycemia and alprazolam (Δ 1.2±3.0 µg/L) as compared to day 1 euglycemia (Δ 10.2±2.2 µg/L).

Day 2 plasma cortisol responses (Figure 5) were also lower (p<0.002) following day 1 euglycemia and alprazolam (Δ -76±37 nmol/L) and day 1 hypoglycemia and alprazolam (Δ -32.6±34.8 nmol/L) as compared to day 1 euglycemia (Δ 195±48 nmol/L) and day 1 hypoglycemia (Δ 131.0±53.6 nmol/L).

Glucose Kinetics

Baseline rates of glucose kinetics were similar at the start of all day 2 exercise studies (Table 1). Rates of EGP were reduced (p<0.0001) during the final 15 min of day 2 exercise following day 1 euglycemia and alprazolam (13±1.5 µmol/kg/min), day 1 hypoglycemia (10.7±1.5 µmol/kg/min) and day 1 hypoglycemia and alprazolam (Δ 14.6±2.2 µmol/kg/min) as compared to day 1 euglycemia (20±1.1 µmol/kg/min) (Figure 5). Glucose infusion rates were increased (p<0.0001) during the final 15 min of day 2 exercise following day 1 euglycemia and
alprazolam (9.6±1.9 µmol/kg/min), day 1 hypoglycemia and alprazolam (11.8±2.0 µmol/kg/min) and day 1 hypoglycemia (7.1±1.5 µmol/kg/min) as compared to 1.2±0.4 µmol/kg/min following day 1 euglycemia. Rates of glucose disposal were similar among the four groups (Figure 5).

**Intermediary Metabolism**

Baseline levels of lactate, glycerol and NEFA were similar at the start of all day 2 exercise studies (Table 1). Blood lactate responses were significantly reduced (p<0.008) during day 2 following day 1 euglycemia and alprazolam (Δ 0.18±0.1 mmol/L), day 1 hypoglycemia (Δ 0.11±0.09 mmol/L) and day 1 hypoglycemia and alprazolam (Δ 0.07±0.06 mmol/L) as compared to day 1 euglycemia (Δ 0.7±0.2 mmol/L) (Figure 4). Day 2 plasma NEFA responses were also reduced (p<0.003) following both day 1 alprazolam groups (Δ 123±23 and 119±28 µmol/L) as compared to day 1 euglycemia (Δ 319±48 µmol/L) (Figure 4).

Day 2 glycerol responses (Figure 4) were also significantly lower (p<0.05) following day 1 euglycemia and alprazolam (Δ 66.3±15.6 µmol/L), and day 1 hypoglycemia and alprazolam (Δ 74.5±12.7 µmol/L) as compared with day 1 euglycemia (Δ 141±13 µmol/L).

**Cardiovascular Responses**

There were similar changes in blood pressure (systolic, diastolic and mean arterial pressure) and heart rate in all groups (Table 2).

**DISCUSSION**

The present study has determined the effects of prior GABA A activation with the benzodiazepine alprazolam on a background of euglycemia or hypoglycemia on homeostatic (counterregulatory) responses during next day exercise. The euglycemic clamp technique was used to maintain identical glycemia during all day 2 exercise studies. Our results demonstrate
that day 1 activation of GABA A receptors can blunt a wide spectrum of key neuroendocrine (glucagon, cortisol, growth hormone), ANS (epinephrine, norepinephrine) and metabolic (endogenous glucose production, lipolysis, glycogenolysis) counterregulatory responses during 90 minutes of next day moderate exercise in healthy humans.

In addition to being an important homeostatic neuromodulator, GABA is a major inhibitory neurotransmitter. Activation of the GABA A receptor subtype occurs commonly following benzodiazepine and alcohol use (27, 28). Despite the importance and frequent activation of GABA A receptors, very little is known about the effects of stimulation of these receptors on subsequent exercise. Only three studies appear to have addressed this question (29-31). All of the above studies were performed during maximal exercise and reported that alprazolam could blunt catecholamine (29) or pituitary/adrenal (ACTH, cortisol) (30,31) responses in healthy humans. However, the effects of prior GABA A activation on integrated ANS, neuroendocrine and metabolic counterregulatory responses during clamped euglycemia under moderately prolonged submaximal exercise conditions appears to be unknown. An important feature of the present experimental design was the use of the glucose clamp technique. This allowed equivalent day 1 euglycemia or hypoglycemia to be created and also maintain identical glucose levels during day 2 exercise studies. This is relevant as during exercise only small reductions in fasting glucose can amplify, whereas increases in glycemia can inhibit neuroendocrine responses (32).

Following prior alprazolam or day 1 hypoglycemia, glucagon levels were substantially suppressed. Additionally, combined day 1 alprazolam and hypoglycemia further suppressed glucagon responses below baseline during day 2 exercise. Insulin levels fell during all day 2 exercise protocols. However, it is notable that the fall in insulin levels was blunted following day 1 alprazolam and prior hypoglycemia protocols compared to the day 1 euglycemic control
studies. Adrenal medullary (epinephrine), sympathetic neural (norepinephrine) and parasympathetic nervous system (pancreatic polypeptide) responses were also blunted during day 2 exercise by day 1 alprazolam and/or hypoglycemia. The diffuse blunting of autonomic nervous system responses following GABA _A_ receptor activation suggests reductions in central (CNS) sympathetic and parasympathetic outflow (33) and/or reductions of epinephrine, norepinephrine and pancreatic polypeptide release from the adrenal medulla and pancreas (34,35). Additionally, the reduced sympathetic nervous system drive (both direct and circulating catecholamines) may have contributed to the blunted glucagon and insulin physiologic responses during day 2 exercise.

Cortisol and growth hormone responses were also blunted during day 2 exercise. Studies in animals have determined that GABA _A_ receptors are present in the pituitary and the adrenal cortex (in addition to the adrenal medulla and pancreas) (36-38). Thus our present findings extend previous observations (29-31) and demonstrate the wide ranging effects of GABA _A_ on downregulating multiple key neuroendocrine responses during exercise.

As a result of the blunted neuroendocrine and ANS responses there were also significant reductions in key metabolic homeostatic responses during day 2 exercise. During submaximal exercise the hepatic sinusoidal glucagon : insulin ratio is a key regulator of glucose production (32). In the present study, glucagon responses were reduced and insulin levels were increased thus contributing to the substantially reduced glucose production rates during day 2 exercise. In fact, analysis of glucose kinetics during day 2 exercise demonstrated that the primary metabolic defect was indeed reduced endogenous glucose production as rates of peripheral glucose uptake were maintained. Thus the increased glucose infusion rates needed to maintain euglycemia during exercise following day 1 alprazolam and/or hypoglycemia were used to supplement the deficient endogenous (hepatic) glucose production and meet the needs of the working muscles.
In fact, it is notable that due to the severe blunting of neuroendocrine and ANS responses, almost the entire physiologic increment of EGP during exercise was blunted and had to be replaced by an exogenous glucose infusion.

The reduced neuroendocrine and ANS responses also blunted glycogenolytic (lactate) responses from liver and skeletal muscle and lipolytic (glycerol, NEFA) responses from adipose tissue. All of the above could have contributed to the deficient EGP response, as lactate and glycerol are important gluconeogenic substrates and NEFA produces energy to drive the process.

In addition to determining the effects of day 1 alprazolam on a background of euglycemia we also wanted to examine whether additional specific GABA A activation would alter the blunting effects of day 1 hypoglycemia on day 2 exercise. Most of the counterregulatory responses during exercise were not blunted further by the administration of alprazolam during day 1 hypoglycemia. The notable exceptions were glucagon and cortisol indicating an additive blunting effect of prior hypoglycemia and alprazolam on selective neuroendocrine responses during subsequent exercise.

The dose of alprazolam (1 mg before each of the day 1 glucose clamps) was chosen based on the average daily clinical dose of the drug, (1 to 4 mg/day). Thus we cannot comment on whether lower or higher doses of alprazolam would have had similar blunting effects on physiologic counterregulatory responses during exercise. Additionally, the half-life of alprazolam is 11.2 hours and the time from the last dose to exercise was about 21 hours. Therefore, ≈25% of the day 1 dose would still be pharmacologically active during day 2 exercise. Thus we cannot fully determine how much of the blunting effects of alprazolam were directly due to day 1 administration or additional lingering effects on day 2.

It is also notable that day 2 basal levels of neuroendocrine, ANS, and metabolic parameters were unaffected by prior GABA A activation. Thus it would appear that prior GABA A
stimulation is reducing the exercise induced stress response (i.e. ANS and neuroendocrine drive) rather than basal ANS and neuroendocrine constitutive tone.

We studied individuals with average fitness levels to avoid the possible confounding variables associated with the physiology of elite athletes. Submaximal moderate intensity exercise (50% VO2 max) of 90 min duration was chosen so that individuals could complete the exercise and produce large easily measurable neuroendocrine, ANS and metabolic counterregulatory signals. Additionally, this duration of exercise represents typical sporting activities (e.g. soccer, tennis) that are commonly played.

In summary, the present study has demonstrated that prior activation of GABA A receptors with the benzodiazepine alprazolam can result in widespread blunting of homeostatic neuroendocrine (glucagon, cortisol, growth hormone, insulin), ANS (epinephrine, norepinephrine, pancreatic polypeptide) and metabolic (endogenous glucose production, lipolysis, glycogenolysis) responses during subsequent submaximal exercise in healthy humans.

We conclude that prior activation of GABA A receptors can inhibit a broad range of neuroendocrine (pituitary, adrenal, pancreas), ANS (both sympathetic and parasympathetic branches) and metabolic (liver, adipose tissue, muscle) counterregulatory responses aimed at preserving glucose levels during subsequent exercise in healthy humans.
M. H. performed studies, researched data and contributed to writing the manuscript. D. T. and L. Y. helped perform studies, researched data, and reviewed and edited the manuscript. S. D. devised the study, reviewed and edited data and contributed to writing the manuscript. All are affiliated with the University of Maryland, Baltimore.

Stephen Davis is the guarantor of this study and, as such, had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

There are no conflicts of interest to report.

**Acknowledgements**

We would like to thank Wanda Snead, Eric Allen and the Vanderbilt hormone assay core laboratory for their excellent technical assistance. We would also like to thank the nursing staff of the Vanderbilt clinical research center for their excellent care. This work was supported by the following NIH grants: P50 HL081009 NIH/NHLBI, RO1 DK069803 NIH/NIDDK, PO1 HL056693 NIH/NHLBI, Vanderbilt Diabetes Research and Training grant (DRTC) NIH/NIDDK P60 DK020593, Vanderbilt General Clinical Research Center NIH/NCRR TL1 TR000447.
REFERENCES

Table 1. Day 2 baseline neuroendocrine, endogenous glucose production (EGP) and intermediary metabolite values in overnight fasted healthy individuals following either day 1 euglycemia and alprazolam, day 1 euglycemia, day 1 hypoglycemia and alprazolam or day 1 hypoglycemia.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Prior eugly control (no alprazolam)</th>
<th>Prior eugly alprazolam</th>
<th>Prior hypo control (no alprazolam)</th>
<th>Prior hypo alprazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (pmol/L)</td>
<td>191±39</td>
<td>196±38</td>
<td>139±43</td>
<td>158±54</td>
</tr>
<tr>
<td>Norepinephrine (nmol/L)</td>
<td>1.4±0.2</td>
<td>1.6±0.1</td>
<td>1.4±0.2</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Glucagon (ng/L)</td>
<td>42±3</td>
<td>46±9</td>
<td>44±3</td>
<td>46±4</td>
</tr>
<tr>
<td>Growth Hormone (µg/L)</td>
<td>2.7±2</td>
<td>2.9±1</td>
<td>2.4±0.6</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>306±55</td>
<td>419±82</td>
<td>286±83</td>
<td>333±55</td>
</tr>
<tr>
<td>Pancreatic polypeptide (pmol/L)</td>
<td>18±3</td>
<td>21±1</td>
<td>19±2</td>
<td>21±6</td>
</tr>
<tr>
<td>EGP (µmol/kg/min)</td>
<td>10.4±0.5</td>
<td>9.3±1.6</td>
<td>11±0.5</td>
<td>8.8±0.5</td>
</tr>
<tr>
<td>NEFA (µmol/L)</td>
<td>332±50</td>
<td>325±62</td>
<td>337±57</td>
<td>273±52</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>1.07±0.1</td>
<td>1.01±0.1</td>
<td>1.0±0.2</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Glycerol (µmol/L)</td>
<td>66±10</td>
<td>76±13</td>
<td>51±12</td>
<td>54±7</td>
</tr>
</tbody>
</table>
Table 2. Day 2 cardiovascular parameters in overnight fasted healthy individuals following either day 1 euglycemia and alprazolam, day 1 euglycemia, day 1 hypoglycemia and alprazolam or day 1 hypoglycemia.

<table>
<thead>
<tr>
<th></th>
<th>Prior eugly control (no alprazolam)</th>
<th>Prior eugly alprazolam</th>
<th>Prior hypo control (no alprazolam)</th>
<th>Prior hypo alprazolam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>final</td>
<td>baseline</td>
<td>final</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>111±7</td>
<td>145±15*</td>
<td>107±5</td>
<td>137±7*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>65±7</td>
<td>73±3*</td>
<td>66±4</td>
<td>75±9*</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>80±3</td>
<td>96±10*</td>
<td>79±3</td>
<td>96±7*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>63±5</td>
<td>139±7*</td>
<td>64±3</td>
<td>132±2*</td>
</tr>
</tbody>
</table>

*p<0.006-0.0001 significantly increased compared to baseline value.
Figure 1.  Diagram of study procedures.

Figure 2.  Plasma glucose (means ± SE) levels during day 1 and day 2 studies and plasma insulin levels (means ± SE) during day 2 studies.

* p<0.001 compared to prior eugly/eugly alprazolam, prior hypo/hypo control and prior hypo/hypo alprazolam

Figure 3.  Day 2 epinephrine, norepinephrine, glucagon and pancreatic polypeptide (change from baseline to final 15 min of day 2 clamps) in overnight fasted healthy individuals following either day 1 euglycemia, day 1 euglycemia and alprazolam, day 1 hypoglycemia or day 1 hypoglycemia and alprazolam.

* p<0.006 - 0.001 compared to eugly/eugly control (no alprazolam)
# p<0.04 compared to eugly/eugly alprazolam
& p<0.02 compared to hypo/hypo control (no alprazolam)

Figure 4.  Day 2 growth hormone, cortisol, lactate, non-esterified fatty acids (NEFA) and glycerol (change from baseline to final 15 min of day 2 clamps) in overnight fasted healthy individuals following either day 1 euglycemia, day 1 euglycemia and alprazolam, day 1 hypoglycemia or day 1 hypoglycemia and alprazolam.

* p<0.02-0.0009 compared to prior eugly/eugly control (no alprazolam)
# p<0.05-0.006 compared to prior hypo/hypo control (no alprazolam)

Figure 5.  Day 2 endogenous glucose production (EGP), glucose infusion rate (GIR) and rate of glucose disposal (Rd) (final 15 min of day 2 clamps) in overnight fasted healthy individuals following either day 1 euglycemia, day 1 euglycemia and alprazolam, day 1 hypoglycemia or day 1 hypoglycemia and alprazolam.

* p<0.04-0.0001 compared to eugly/eugly control (no alprazolam)
**Figure 2.**

**Day 1 Plasma Glucose**

**Day 2 Plasma Glucose**

**Day 2 Insulin**

* p<0.001 compared to prior eugly/eugly alprazolam, prior hypo/hypo control and prior hypo/hypo alprazolam
Figure 3.

Day 2 Epinephrine

Day 2 Norepinephrine

Day 2 Glucagon

Day 2 Pancreatic Polypeptide

* p<0.006 - 0.001 compared to eugly/eugly control (no alprazolam)
# p<0.04 compared to eugly/eugly alprazolam
& p<0.02 compared to hypo/hypo control (no alprazolam)
Figure 4.

Day 2 Growth Hormone

Day 2 Cortisol

Day 2 Lactate

Day 2 NEFA

Day 2 Glycerol

- prior eugly/eugly control (no alprazolam)
- prior eugly/eugly alprazolam
- prior hypo/hypo control (no alprazolam)
- prior hypo/hypo alprazolam

* p<0.02-0.0009 compared to prior eugly/eugly control (no alprazolam)
# p<0.05-0.006 compared to prior hypo/hypo control (no alprazolam)
* p<0.04-0.0001 compared to eugly/eugly control (no alprazolam)