Sex-differences in Hippocampal CA1 Excitability and Systemic Responses in a High-Energy Diet Induced Prediabetic Rat Model

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Introduction

Increased dietary fat intake leads to drastic alteration of systemic metabolism, obesity, insulin resistance, and type II diabetes, all potentially altering brain function and cognitive performance. Successful consolidation of many learning tasks reduces Ca^{2+} dependent afterhyperpolarizations (AHPs) of hippocampal CA1 pyramidal neurons, increasing their intrinsic excitability. Since insulin has been shown to enhance memory retention, we investigated the effects of a chronic high-energy diet on systemic markers of metabolic dysfunction, on intrinsic excitability of CA1 hippocampal neurons, and on neural insulin sensitivity. Sex-dependent differences were also tested since estrogen effects both AHPs and insulin receptors.

Methods

Subjects: Experiments were performed using behaviorally naive male & female Long Evans rats. Rats were bred use and maintained in our animal facility under conditions approved by the UT Dallas ACUC on a 12hr/12hr light/dark schedule with ad libitum access to food and water. Littermates were distributed across all treatment conditions to reduce variance, and males and females were socially housed.

Diet: Subjects in the Control diet cohort received a standard rat pellet diet with kcal energy contributions of: 20.7% protein, 35.8% fat, and 35% carbohydrate. Subjects in the HE pilot cohort received a diet of standard rat pellet augmented to achieve an energy contribution of: 15.6% protein, 57.6% fat, and 26.8% carbohydrate. Subjects received only their assigned diet from weaning (21 d) for approximately 15 weeks.

Slice preparation: Rats were anesthetized with isoflurane and decapitated. The brain was quickly hemisected and immersed in cooled sucrose-aCSF [in mM: 124 sucrose; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose]. After the brain chilled for 3-4 min, it was blocked and 400 µm slices cut using vibratomes then placed in room temperature (25°C) aCSF [in mM, 124 NaCl; 3 KCl; 1.3 $MgSO_4$; 1.24 NaH_2PO_4; 2.4 $CaCl_2$; 26 $NaHCO_3$; 10 d-glucose]. Both aCSFs were continuously oxygenated (95% O_2: 5% CO_2; ,pH 7.4).

Current-clamp recordings: Sharp electrodes were prepared from borosilicate glass filled with 3 M KCl (30-80 MΩ), and intracellular recordings made (using an AxoClamp-2B amplifier and National Instrument LabView interface) from submerged slices (31°C) . Measures of excitability included AHP peak amplitude, and duration, as well as measurements of AHP amplitude at varying intervals post-burst (to assess mAHP and sAHP components).

Insulin Perfusion: After baseline recordings, brain slices were perfused with the most effective dose of insulin (12.5 nM), as previously determined via dose response curve and recordings were repeated.

Glucose Tolerance Testing & Insulin Tolerance Testing (GTT/ITT): Rats were fasted for 8 hours and handled for 1 hour prior to testing. Basal plasma glucose levels (mg/dL) were assessed via glucometer immetiately prior to injecting either glucose (2g/kg)or insulin (1U/kg) or saline. Plasma glucose was then sampled every 15 min for 120min.

Systemic Measures: Blood samples were collected during decapitation and plasma was frozen until use. Insulin was assessed using rat insulin ELISA kits (Abnova) and cortisol was assessed using CSCI ELISA kits (Abcam), using a ELx800 plate reader (BioTek) abd Gen5 software. Glycated hemoglobin levels were assessed with a DCA2000x analyzer (Bayar Laboratories) and DCA Systems Hemoglobin A1c test kits (Seimens).

High-Energy Diet Alters Intrinsic Excitability: Sex Differences

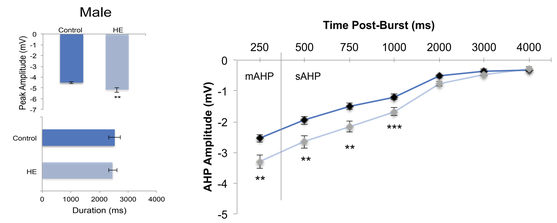


Figure 1: Effects of High-Energy Diet on Male CA1 Excitability. Male AHPs were significantly increased in peak amplitude ($p < 0.01$) but not duration by the HE diet. The HE diet significantly enhanced both mAHP ($p < 0.01$) and sAHP ($p < 0.01$) measures in males

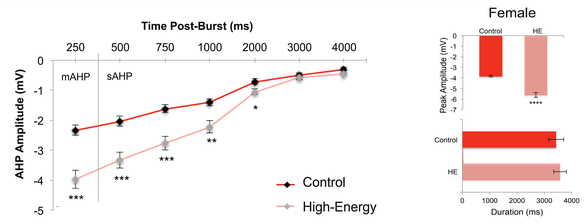


Figure 2: Effects of High-Energy Diet on Female CA1 Excitability. Female AHPs were significantly increased in peak amplitude ($p < 0.0001$) but not duration by the HE diet. The HE diet enhanced, more profoundly than males, both mAHP ($p < 0.001$) and sAHP ($p < 0.01$) measures in females.

Sex-/Diet-dependent Alteration of Systemic Responses

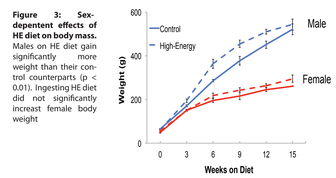


Figure 3: Sex-depentent effects of HE diet on body mass. Males on HE diet gain significantly more weight than their control counterparts ($p < 0.01$). Ingesting HE diet did not significantly increast female body weight

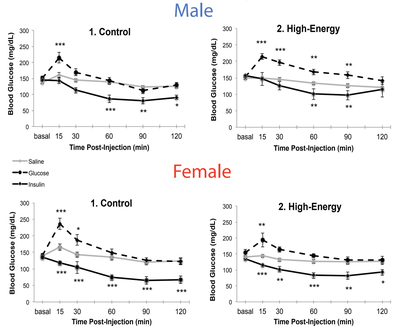


Figure 5: Sex-dependent effects of HE diet on GTT/ITT. HE fed male rats showed a prolonged elevation of blood glucose following glicose bolus consistent with type II diabetes. ITT in males also indicated a change in insulin sensitivity with a faster return to baseline levels. In contrast, females exhibited relatively little diet-depented alteration in GTT or ITT, i.e. failed to demonstrate clinical characteristics of T2D.

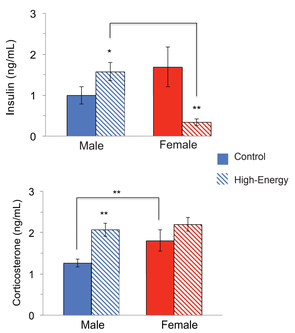


Figure 6: Sex-dependent effects of HE diet on systemic measures. In males, HE diet increased circulating insulin compared to controls ($p < 0.05$). However, in females the HE diet significantly reduced circulating insulin ($p < 0.01$). Cortisol levels were lower in control males compared to control females ($p < 0.05$). HE diet increases cortisol in males ($p < 0.001$) but not in females.

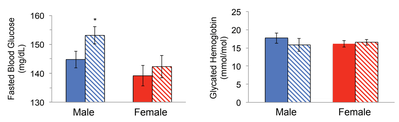


Figure 4: Sex-depentent effects of HE diet on glucose regulation. Male HE rats had significantly elevated fasting blood glucose compared to both control males and to HE females ($p < 0.05$), while no differences were observed between female dietary groups. Glycated hemoglobin (HbA1c) was not significantly altered between sex or diet groups.

Sex-/Diet-dependent Insulin-Sensitivity

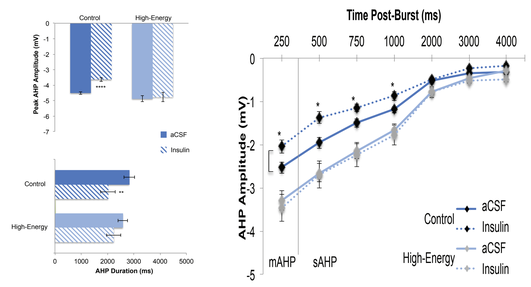


Figure 7: Diet-dependent responses to insulin in males. Insulin significantly reduced peak AHP amplitudes ($p < 0.0001$), AHP duration ($p < 0.01$), mAHP and sAHP in control fed but not HE fed neurons. Neurons from HE diet fed males were insulin insensitive across all measures of excitability

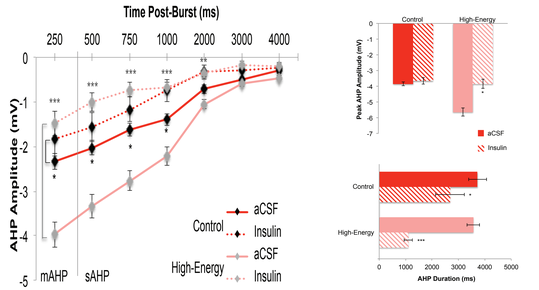


Figure 8: Diet-dependent responses to insulin in females. Unlike in males, while neurons from both diet groups were insulin-sensitive on multiple measures, insulin -sensitivity of AHPs actually increased in neurons from HE diet femlae rats. Insulin did not significantly alter peak AHP in control fed neurons but did reduce peak AHP in HE fed neurons ($p < 0.05$). mAHP and sAHP measures were reduced by insulin in control fed females, and still even more effectively reduced in HE fed females. WHile AHP durations were significantly reduced in control diet neurons ($p < 0.05$), much greater reductions were observed in HE diet neurons ($p < 0.001$)

Discussion

- After 15 wk on chronic HE diet, **intrinsic excitability** of male and female CA1 neurons were significantly **reduced**. HE diet female neurons had significantly larger peak amplitudes, mAHPs and sAHPs, and longer durations than those from HE males.

- **Fasted blood glucose** was significantly **elevated** in males (but not females) after 15 wk on the HE diet.

- **Glucose Tolerance Tests** (GTTs) were significantly **blunted** after 15 wk on the HE diet in males (but not females).

- **Circulating inulin** was significantly **increased** in HE diet males BUT were significantly **decreased** in HE diet fed females.

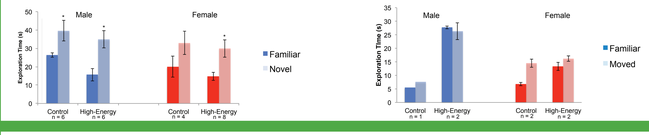
- **Cortisol** was significantly **elevated** in HE diet males (but not females).

- **Insulin** perfucion **enhanced CA1 hippocampal excitability in** both male and female **control** fed rats.

- **Male HE diet neurons** became **insulin-insensitive**
- **Female HE diet neurons** NOT ONLY remained **insulin-sensitive**, they became **MORE insulin-sensitive** than female control neurons

Future Directions

- Assess effects of sex and HE diet on AKT phosphorlyation, GLUT4 translocation, and hippocampal IGF-1.
- Assess effects of HE diet on brain insulin / hippocampal IR.
- Determine effects of HE diet/sex on memory retention on memory tasks (NOR, SOR, spontaneous alternation & Morris water maze).



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