VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Preliminary Data Report for Dr Ambati

C57BL6/J mice on a Chow or HF diet, or HF+AB Drug, or HF + XY Drug – twice daily ip injections

Vanderbilt MMPC

12/21/2018

Prepared by Louise Lantier, PhD

Please acknowledge the Vanderbilt MMPC in your publications (DK059637)
For indirect calorimetry please also acknowledge 1S10RR028101-01.

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Study Design

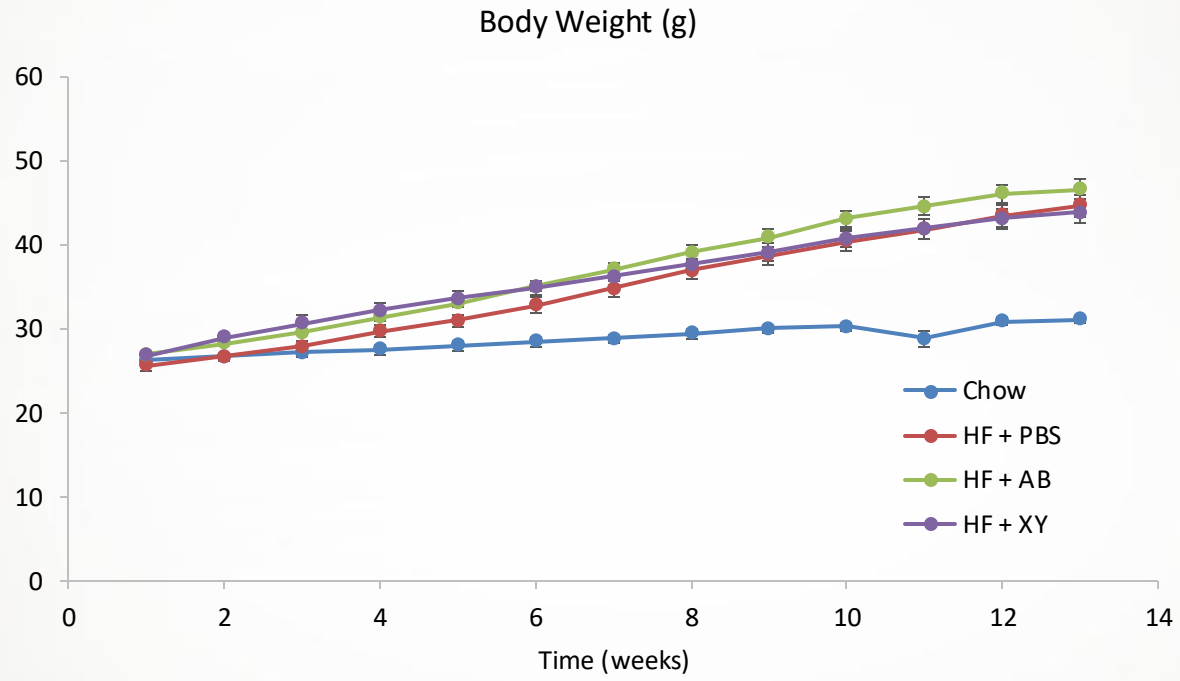
- C57BL6/J mice fed either a regular chow or a HFD (60% kcal from fat) for 12 wks.
- Mice on HF were treated twice daily by ip injection of either PBS, AB Drug, or XY Drug for 12 wks.
- Hyperinsulinemic-euglycemic clamp on 5-hour fasted mice.
  - Chow: n=10, HF+PBS: n=9, HF+AB: n=8, HF+XY: n=8.
  - Continuous infusion of 4mU/min/kg of Insulin.
  - Continuous infusion of ^{3}H-glucose to assess glucose turnover rates.
  - At 120min, bolus of ^{14}C-2-deoxyglucose to assess tissue-specific glucose uptake.
  - At 155min, pentobarbital anesthesia and tissue collection (flash freeze).
- Statistics:
  - The Chow vs HF+PBS groups showed the expected HF-induced changes and will not be commented further. Comments will focus on differences within the HF-fed mice (effect of drugs).
  - For convenience, Student's t-tests were run comparing each group to the HF controls (* $p<0.05$ by Student's t-test). However this test is not appropriate to use in this situation given the number of groups to compare.
  - Outlier test: data points outside of "Average -/+1.5*SD" were considered outliers and not included in the following graphs (full data provided in excel spreadsheets).

IMPORTANT: The excel formula will kick out individual points, but we strongly encourage you to go through the data set and, based on # of outlier points within a mouse, either remove all the data from the mouse or include all the data from the mouse (blood glucose, GIR, EndoRa, Rd, Insulins). This ensures the data set is consistent. Please give us a call or email if you want additional information about why we recommend this.

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Body Weight over time

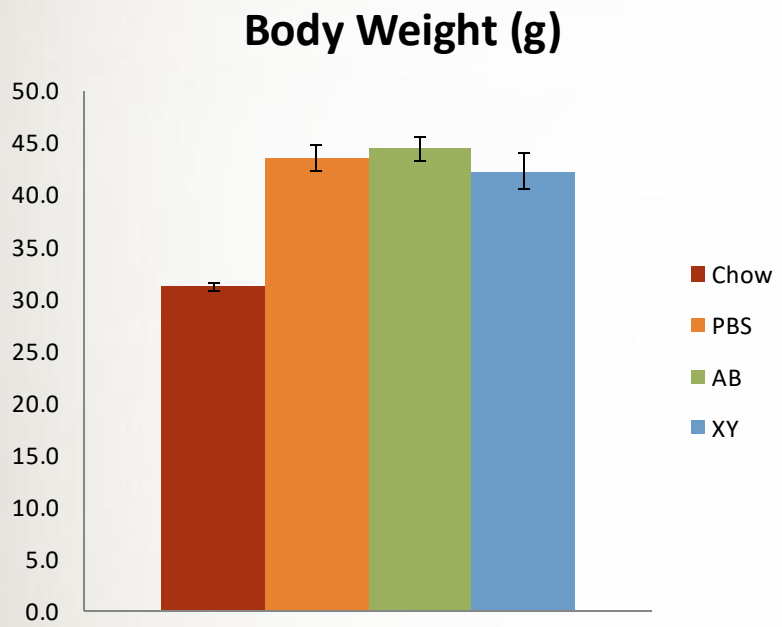


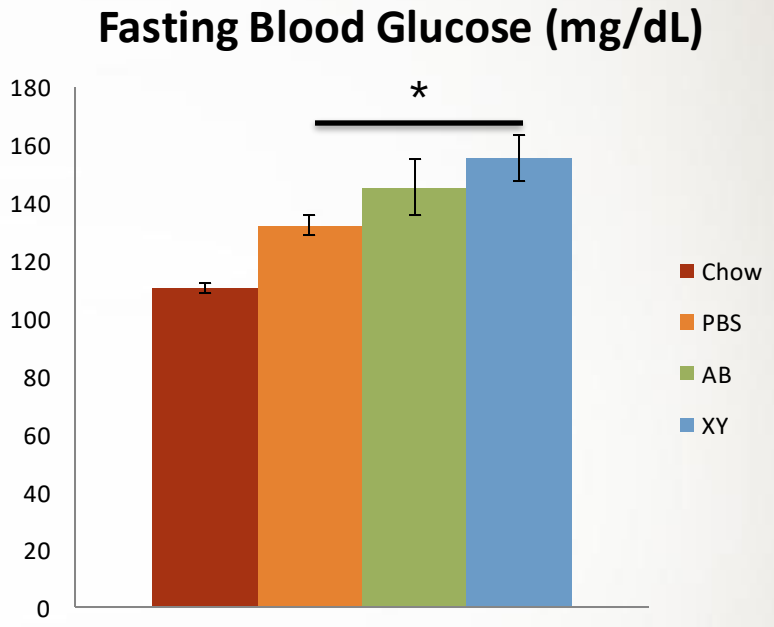
- HF-feeding increased body weight in all three treatment groups.
- Body weights were not different in HF-fed mice after 12 weeks of treatment.

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Body weight and fasting blood glucose



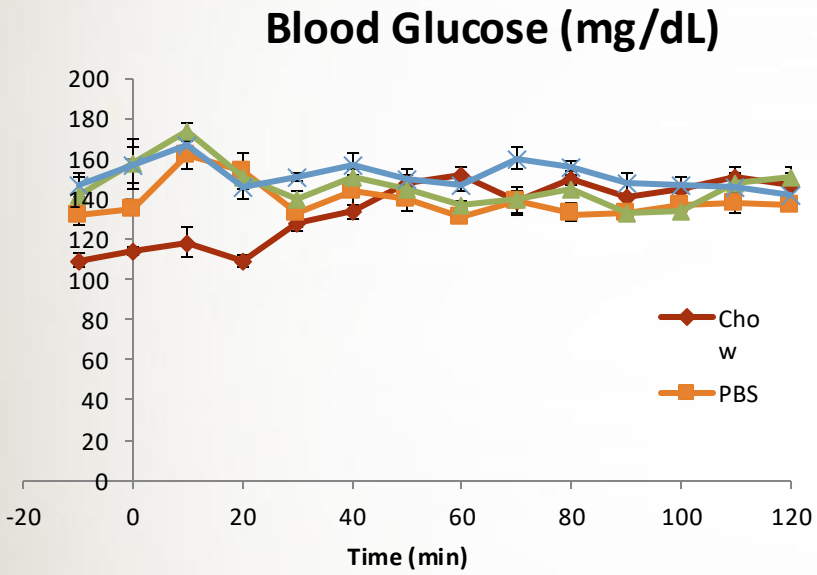


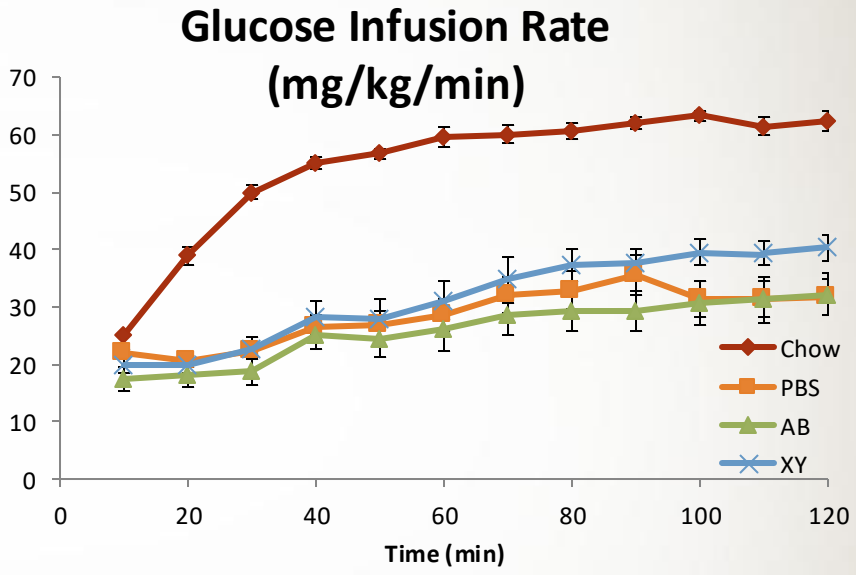
- Body weights were not different in HF-fed mice after 8 weeks of treatment.
- Fasting blood glucose was significantly increased by XY treatment relative to PBS.

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Blood glucose and Glucose Infusion Rate



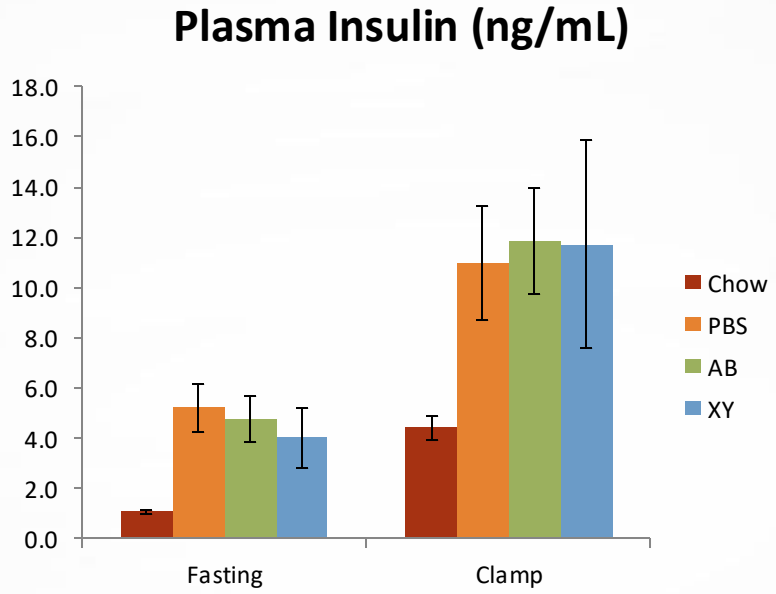


- Blood glucose was maintained at euglycemia throughout the clamp in all four groups.
- The Glucose Infusion Rate tended to be higher in XY mice compared to PBS and AB mice.
- Note: the automatic outlier test causes some distortion of the data, particularly for the GIR. See next slides for full data and a suggestion for handling outlier mice.

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Plasma Insulin Levels



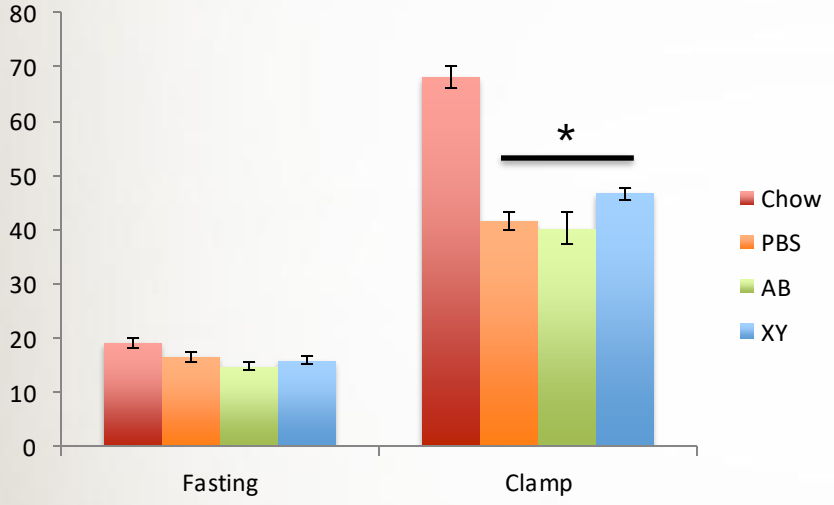
- Fasting plasma insulin levels were not different between treatments in HF-fed mice.
- Insulin levels were raised during the insulin clamp to a much higher level in HF-fed mice compared to Chow-fed mice. this would be expected due to the difference in fasting insulin levels.
- Clamp insulin levels were unchanged between treatment groups on HF diet.

VANDERBILT UNIVERSITY MEDICAL CENTER

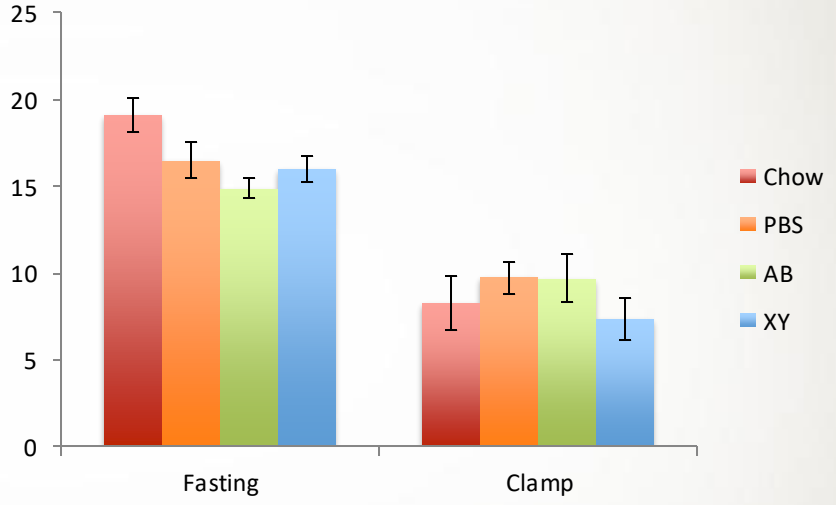
MMPC National Mouse Metabolic Phenotyping Centers

Glucose Fluxes

Total Glucose Flux (Rd) (mg/kg/min)



Endogenous Glucose Production (mg/kg/min)



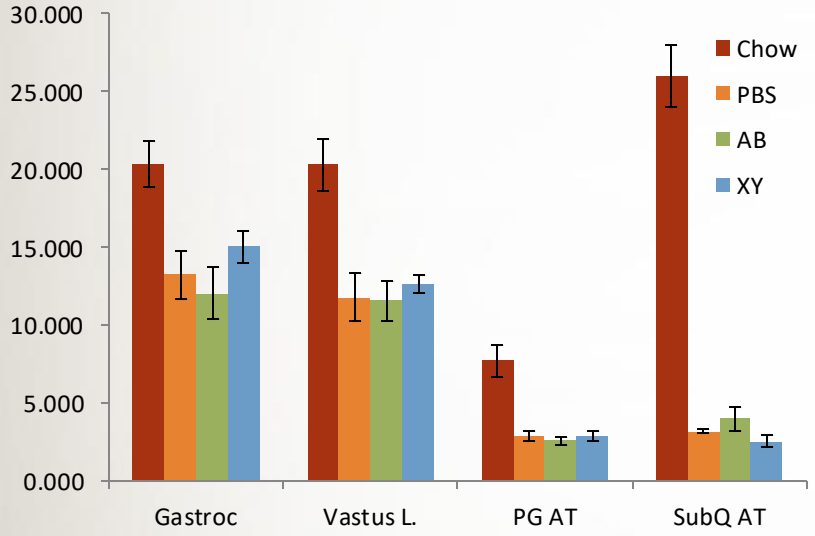
- Fasting glucose turnover rates were not different between groups.
- Rd (glucose rate of disappearance) was significantly higher in XY mice compared to PBS mice.
- Clamp Endogenous glucose production (endoRa, an index of hepatic glucose production) was unchanged between HF treatment groups.

VANDERBILT UNIVERSITY MEDICAL CENTER

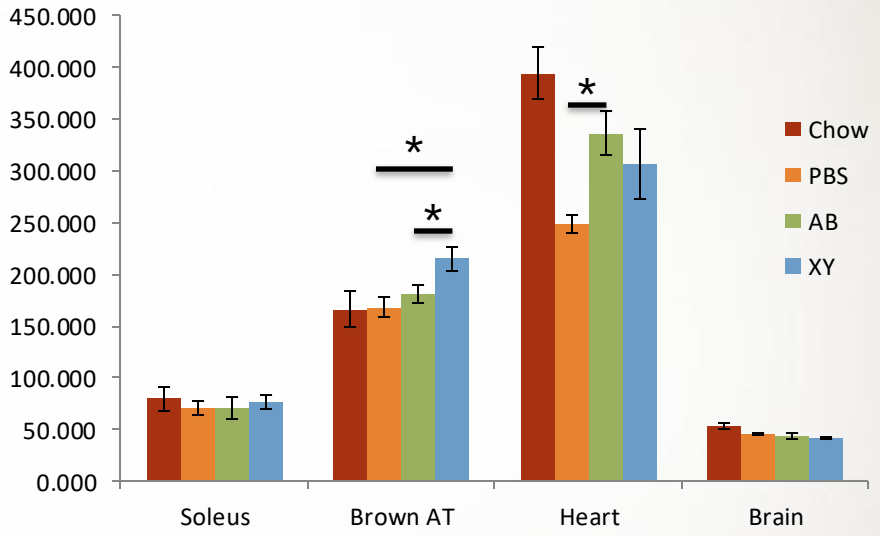
MMPC National Mouse Metabolic Phenotyping Centers

Tissue specific glucose uptake

Rg (umol/100g tissue/min)



Rg (umol/100g tissue/min)



- Tissue-specific glucose uptake was significantly increased in heart of AB-treated mice relative to PBS-treated mice.
- Tissue-specific glucose uptake was significantly increased in Brown Adipose Tissue of XY-treated mice relative to both PBS-treated and AB-treated mice.

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Summary

- Compared to HF+PBS mice, AB and XY-treated mice exhibit:
  - No difference in body weight, similar fasting insulin levels, and an increase in fasting blood glucose levels in XY mice only
  - Similar glucose infusion rate during the clamp, indicative of similar whole body insulin sensitivity,
  - In the XY mice only, a small increase in the rate of glucose disappearance during the clamp, indicative of slight increased peripheral glucose uptake,
  - No difference in clamp endogenous glucose production.
  - AB mice exhibited increased insulin-stimulated heart glucose uptake, while XY mice exhibited increased brown adipose tissue glucose uptake.
- AB and XY treatment for 12 weeks does not strongly affect insulin sensitivity. Subtle effects in glucose disappearance and glucose uptake are however observed with drug treatments.

- A note on outlier test: the excel spreadsheet contains these “outlier” graphs as well as the complete raw data. We want to emphasize that for clamp studies, best practices call that data from individual mice either be completely included, or completely excluded (this includes blood glucose, GIR, insulin, Rd and EndoRa).
- **See next slide for more details:** We noticed that a couple of the mice in this study had many outlier data points, so you may consider removing those mice entirely, and in contrast including all data from other mice. This will probably help to clarify the phenotype. I would be happy to assist in this process if you wish. Feel free to give me a call if you have any questions. **See next slide for more details**
- Louise Lantier, 615-936-4028; louise.lantier@vanderbilt.edu

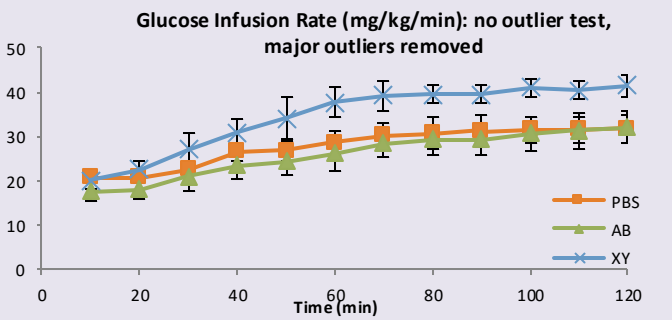
VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Comments

- We noticed that a few mice in this study had many outlier data points, so you may consider removing those mice entirely, and in contrast including all data from other mice. This will probably help to clarify the phenotype. I would be happy to assist in this process if you wish. Feel free to give me a call if you have any questions.

- Mice we identified as major outliers were the following:
  - Chow: MP-933
  - HF+PBS: MP-922
  - HF+XY: MP-916 and MP-940
  - The tab in the excel file labelled" Summary (outliers out)" has the data from those four mice completely removed.
  - When removing these 4 outlier mice (whole row), and including the data from all other mice, it appears that XY treatment sign ificantly improves insulin sensitivity through effects on peripheral glucose uptake, with a trend for improved suppression of hepatic glucose production. AB treatment has no effect relative to PBS treated mice.



- Let me know if you have any questions!
- Louise Lantier, 615-936-4028; louise.lantier@vanderbilt.edu

VANDERBILT UNIVERSITY MEDICAL CENTER

MMPC National Mouse Metabolic Phenotyping Centers

Thank you!

If you decide to remove a mouse from analysis, please remove all clamp data from the mouse, otherwise the clamp data will be inconsistent.

Please feel free to give me a call or email if you wish to discuss this further. I would be glad to go over the data with you.

Louise Lantier: Office 615-936-4028, louise.lantier@vanderbilt.edu.

Thank you for using the Vanderbilt MMPC!

Notes:
- Remaining tissues and plasma can be sent to you upon request. The MMPC will retain them at -80° C for two years after completion of the study.
- Please acknowledge the Vanderbilt MMPC in your publications (DK059637).
- Feel free to contact us if you have any questions.