

Adiposity, Leptin Resistance, Hyperphagia, Hyperglycemia, Glucose Intolerance And Insulin Resistance In C57BL/6J Mice Fed High Fat Diets

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ABBOTT DIABETES

ABSTRACT



Male C57BL/6J mice approximately 6 wk old were housed 5 mice/cage, and were assigned to one of five diets, provided *ad libitum*: D12451 (45kcal%fat, lard, Research Diets, Inc., New Brunswick NJ), D12450B (10kcal%fat, lard control), D12330 (58kcal%fat, hydrogenated coconut oil/HCO), D12328 (11kcal%fat, HCO control), and Teklad 8604 (12kcal%fat, soy). The D12451 mice had increased mean body weight vs. the D12450B group as early as 1 wk after initiation of diet, but D12451 average body weight was similar to mice fed 8604 diet. Mice fed D12451 decreased g/d intake, and maintained kcal intake similar to D12450B, D12328 or 8604 diets for 9 wk (12-13 kcal/d). Mice on D12330 diet ate progressively more each wk for 9 wk on diet, at which time they were eating approx 27 kcal/d. The D12451 mice developed insulin resistance, 3 h fasting and fed hyperglycemia, glucose intolerance, hyperinsulinemia, hyperleptinemia after 5-7 wk on diet. Mice on D12330 gained weight more rapidly, were not insulin resistant or glucose intolerant at 5 wk on diet but were insulin resistant after 10 wk on diet. Mice maintained on high fat diets have greater abdominal and subcutaneous fat mass determined by MRI than mice on low fat diet, even in the absence of a difference in total body weight. Mice (n=10/diet) were individually housed after 10 wk on diet, and acclimated for 1 wk. Individual housing increased food intake with all diets. Leptin (10 mg/kg, i.p.) vs. vehicle (10 ml/kg, ip) was administered at the start of dark cycle daily for 3 d, and food intake monitored over 0-3, 0-6 and 0-24h. Except for mice fed D12330 diet, all groups treated with leptin had significantly decreased food intake relative to vehicle in the 0-3h interval. In summary, both high fat diets produced obesity. D12451 produced insulin resistance with moderate obesity, while D12330 produced greater obesity first without insulin resistance, followed by later development of insulin and leptin resistance.

Introduction

- Obesity and diabetes in man have been linked to excessive caloric intake as well as a high percentage of daily intake as fat.
- C57BL/6J mice on high fat diets for several weeks will develop obesity and prediabetes.
- We have evaluated several commercially available high fat diets in C57BL/6J mice, comparing blood glucose, plasma insulin and leptin, glucose and insulin tolerance, and response to exogenous leptin.

Methods

- C57BL/6J male mice obtained at 5 weeks age, from Jackson Laboratories.
- Randomly assigned mice to 5 dietary groups

Diet ID	Source	% kcal fat	fat form	fat type	n/group
D12451	RDI	45	lard	animal	150
D12450B	RDI	10	lard	animal	50
D12330	RDI	58	hydrogenated coconut oil	vegetable	50
D12328	RDI	10	HCO control	vegetable	50
8604	Teklad	12	soybean oil	vegetable	80

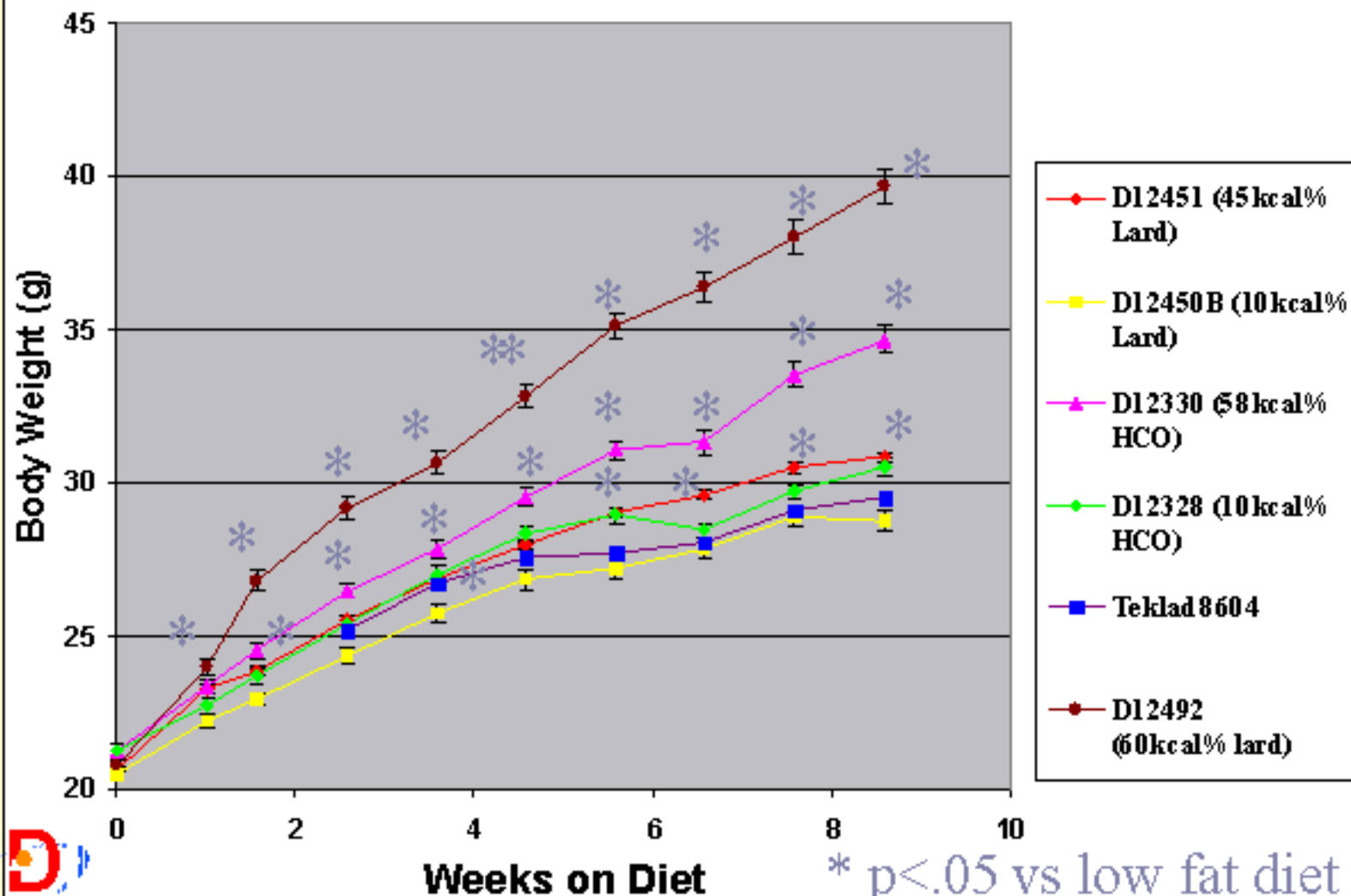
- RDI is Research Diets, Inc, New Brunswick, NJ
- Monitored:
 - Body weight and food consumption weekly.
 - Ad lib AM blood glucose, plasma insulin and leptin after 5 weeks on diets (11 weeks age).
 - 3 hr fasted glucose and insulin tolerance at 8-9 weeks on diet (13-14 weeks age).
 - Food intake response to leptin (10 mg/kg, I.p.) was evaluated at 11 weeks.

- **Another high fat diet became available after the first study had been started. Two additional groups of mice were placed on diets (D12492 and D12450B) at 5 weeks age, and events were monitored for effects of diet on blood glucose, plasma insulin and leptin, glucose and insulin tolerance at the same relative times as indicated above.**

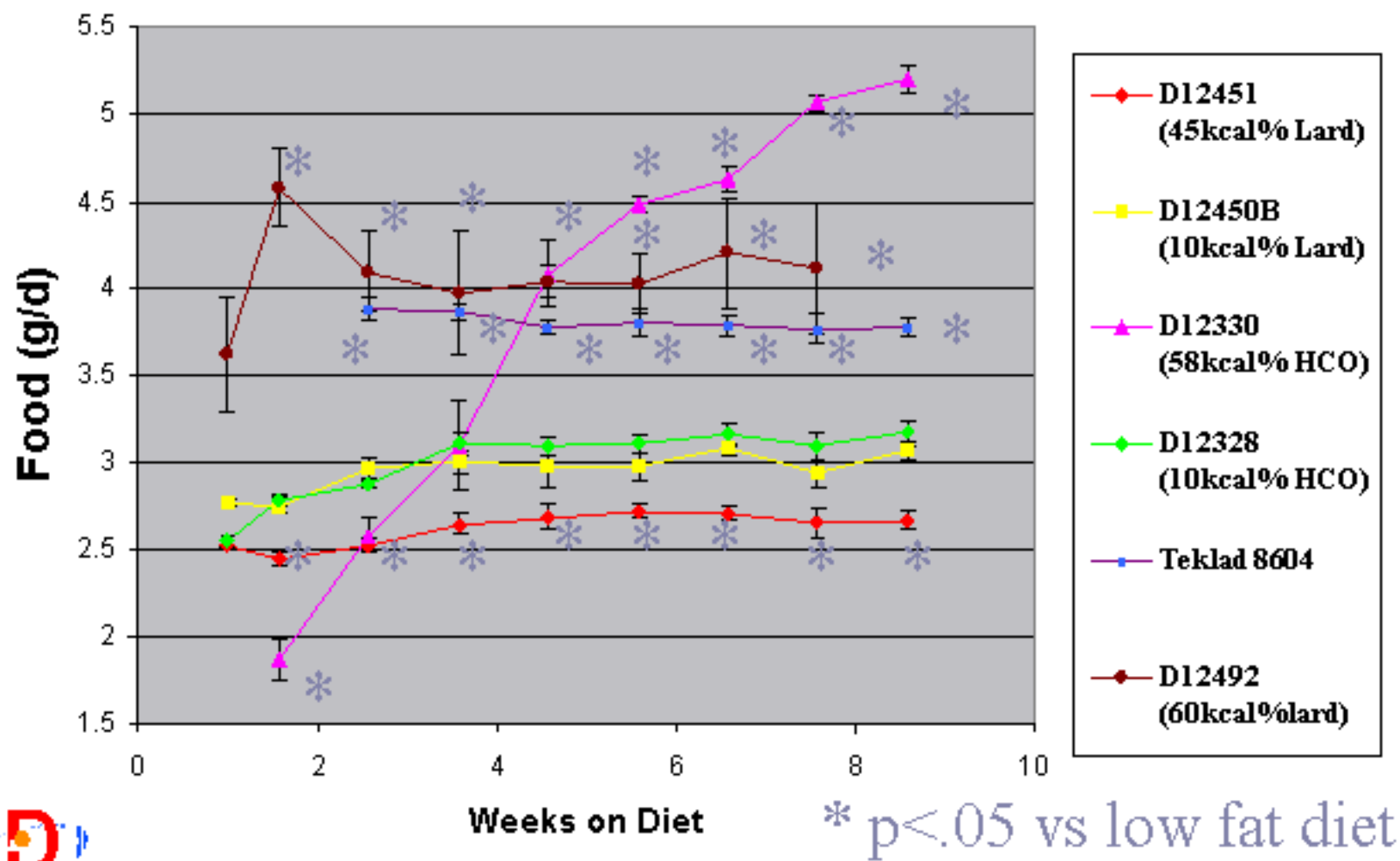
Diet ID	Source	% kcal fat	fat form	fat type	n/group
D12492	RDI	60	lard	animal	90

- **Statistics – Means were compared by one way ANOVA. Differences by ANOVA were further evaluated with Dunnet’s multiple range tests.**

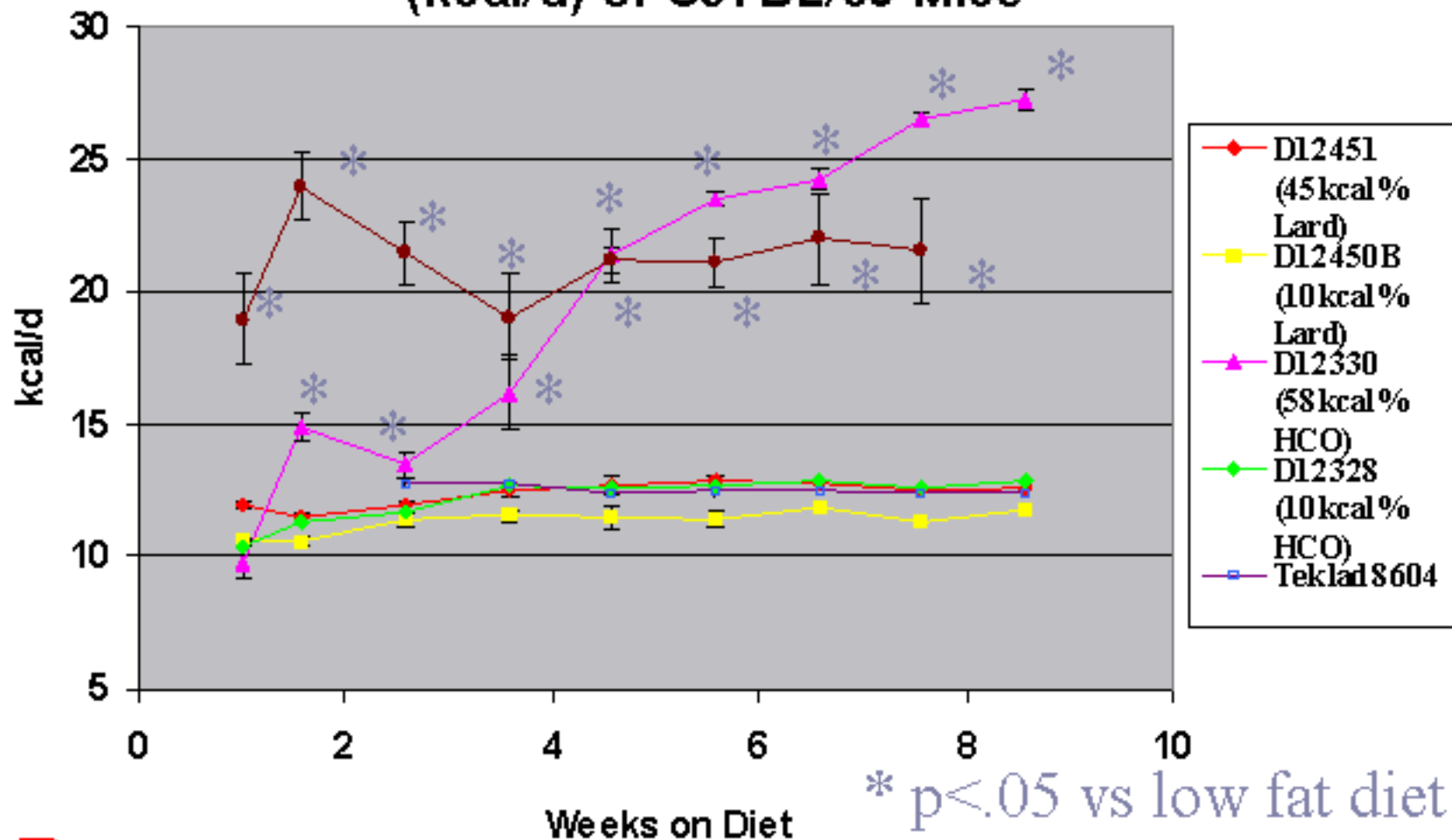
Effect of High Fat Diet on Body Weight of C57BL/6J Mice



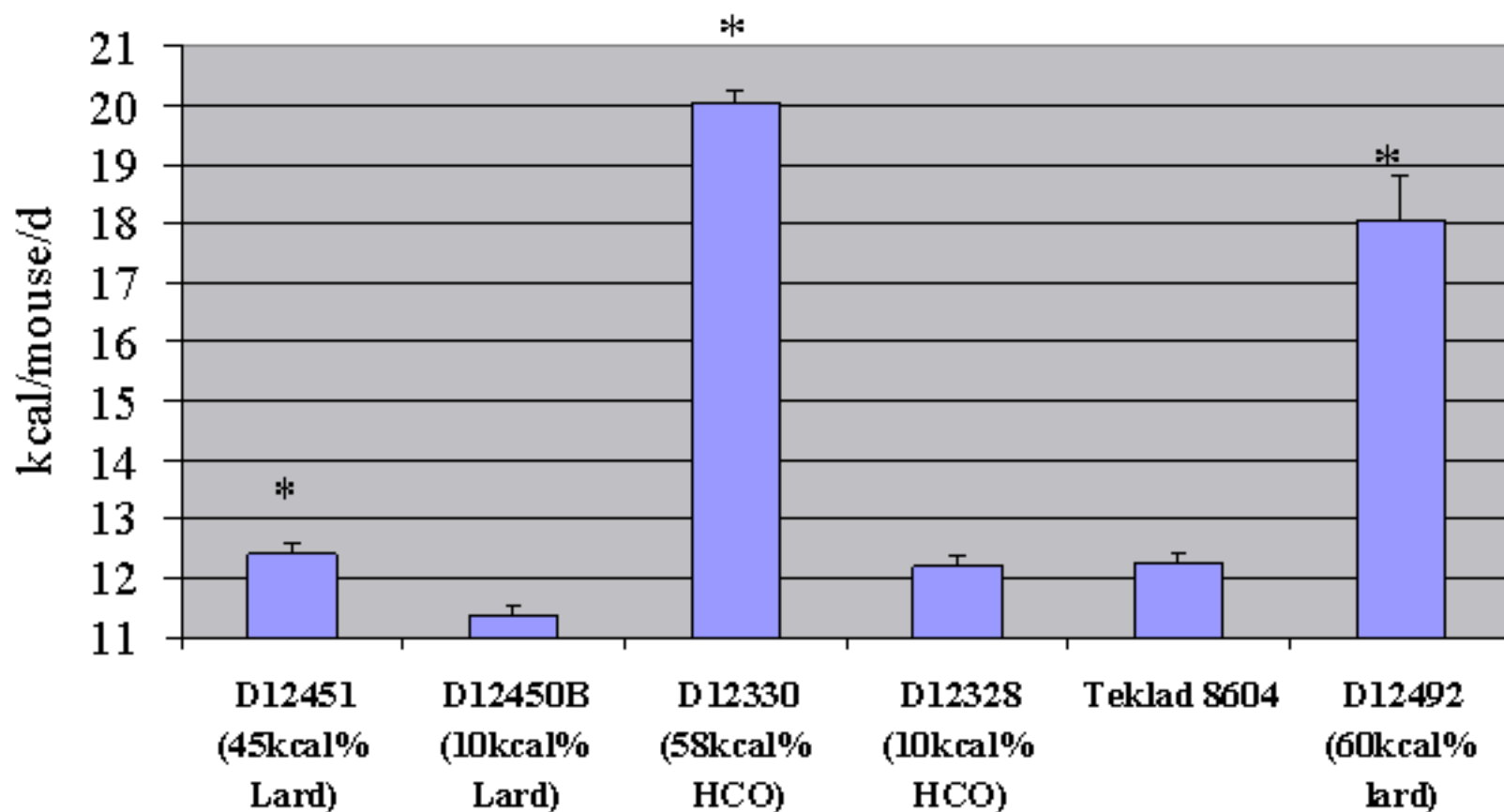
Effect of High Fat Diet on Food Consumption (g/d) of C57BL/6J Mice



Effect of High Fat Diet on Food Consumption (kcal/d) of C57BL/6J Mice

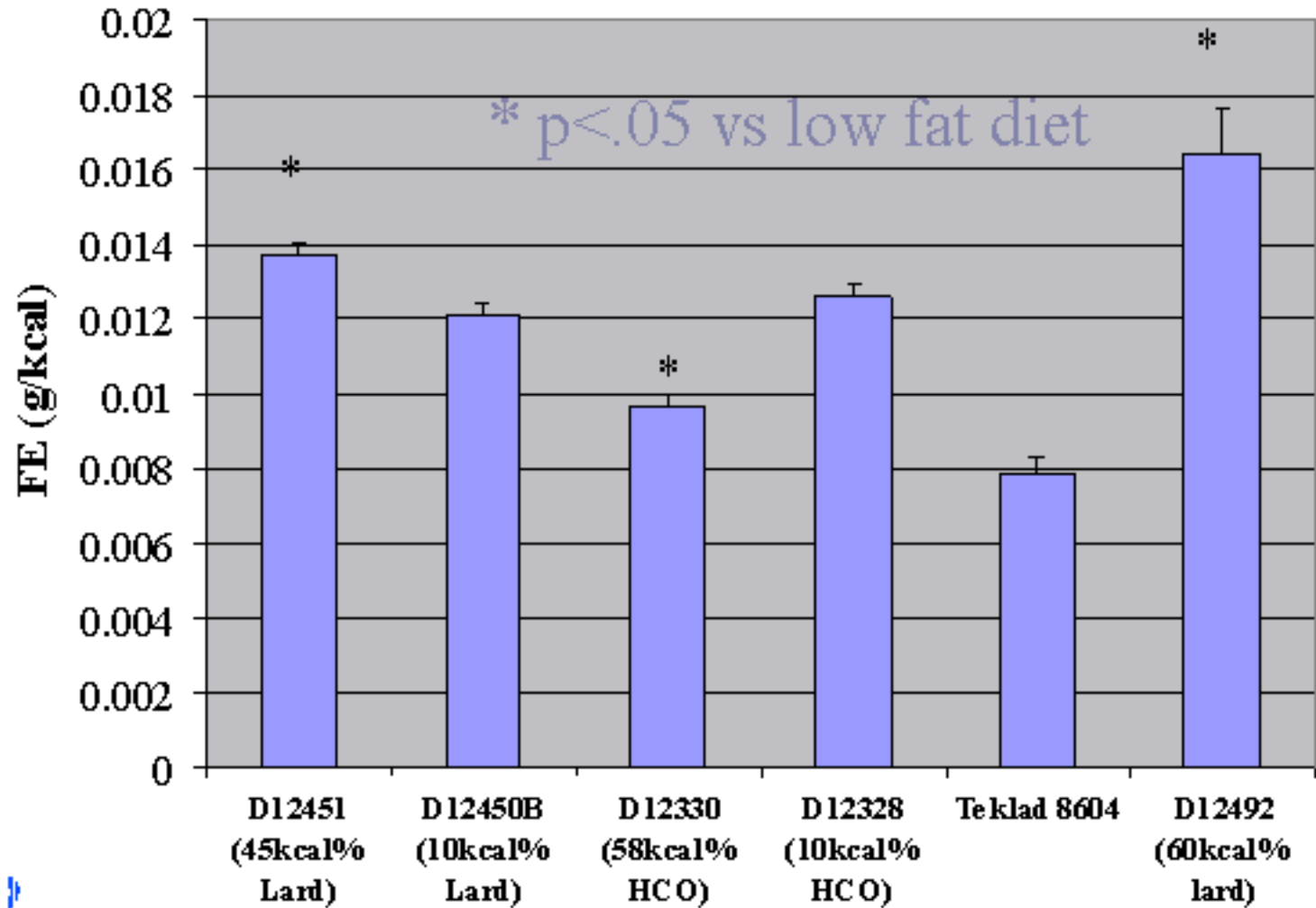


Average Dietary Energy Intake in C57BL6/J Mice over Duration of Study



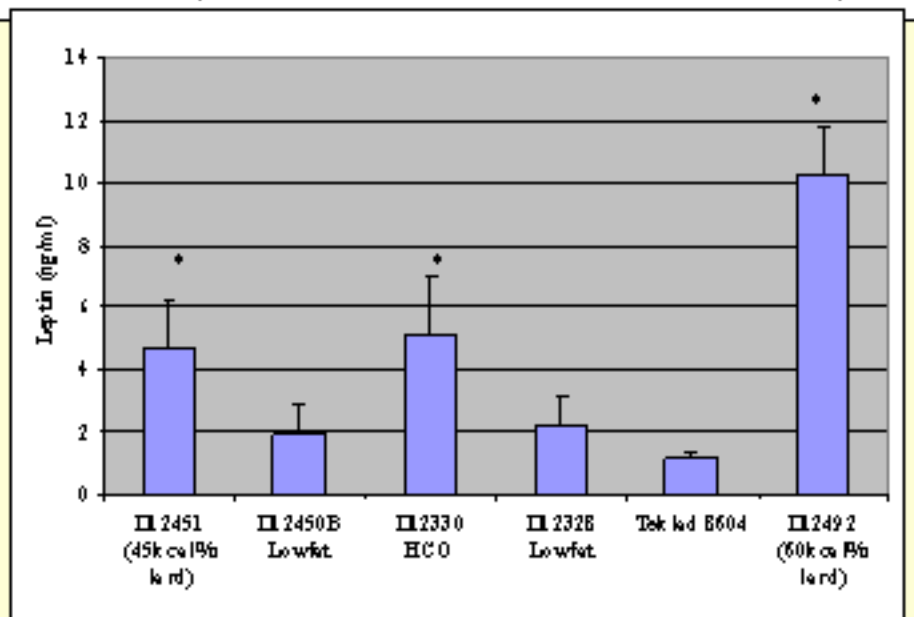
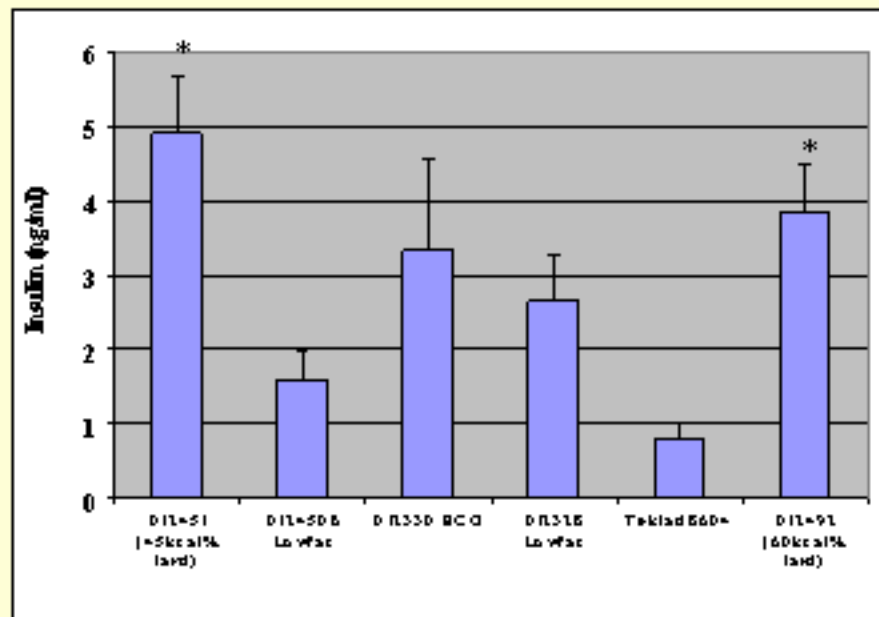
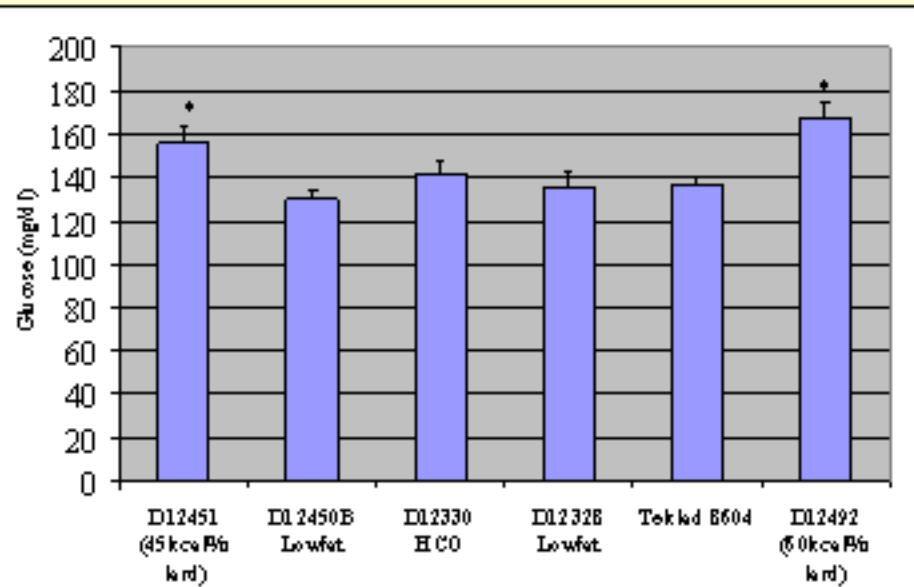
* $p < .05$ vs low fat diet

Feed Efficiency Based on Caloric Intake

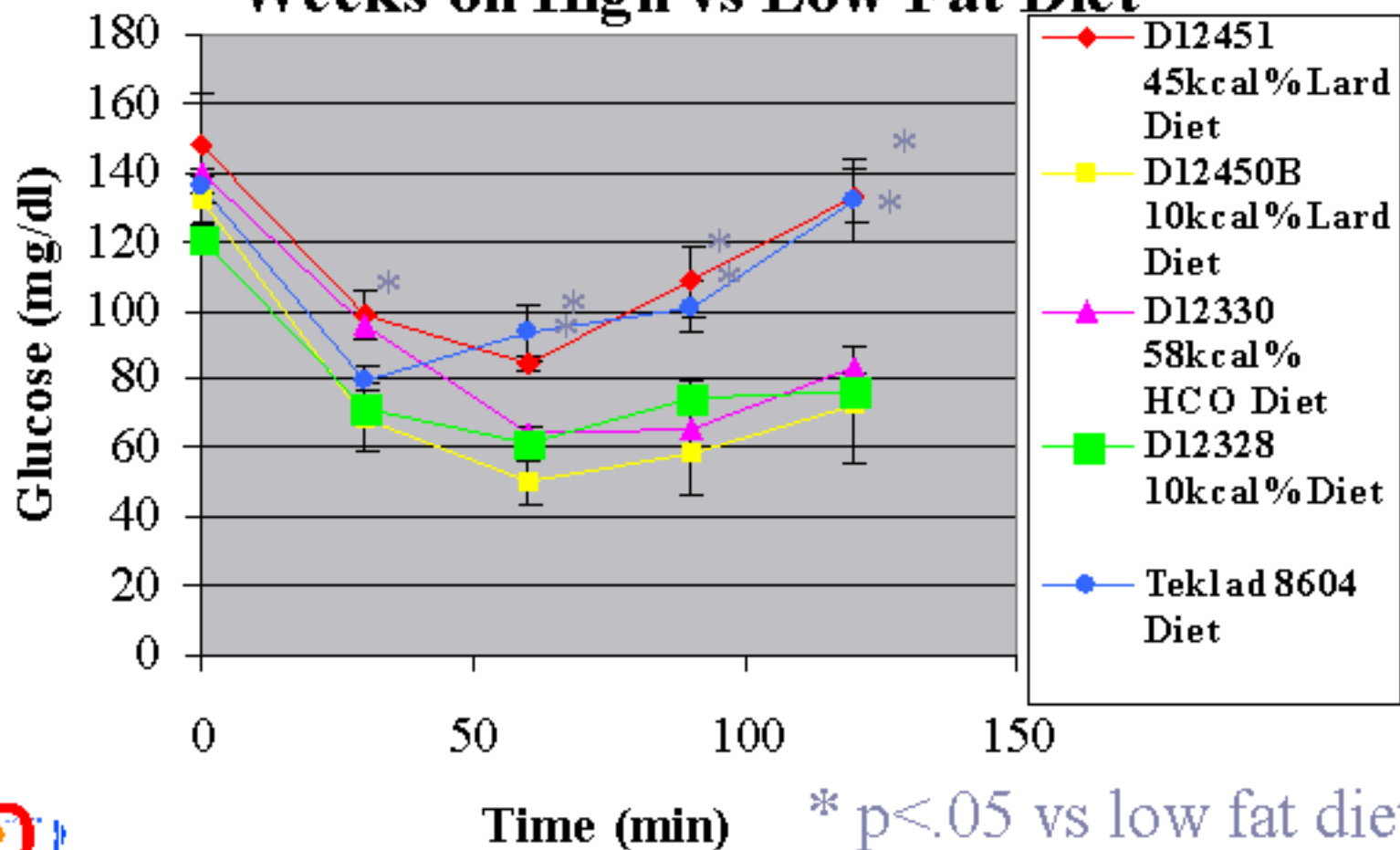


Postprandial Blood Glucose, Plasma Insulin and Leptin in C57BL/6J Mice after 5 Weeks on Diet

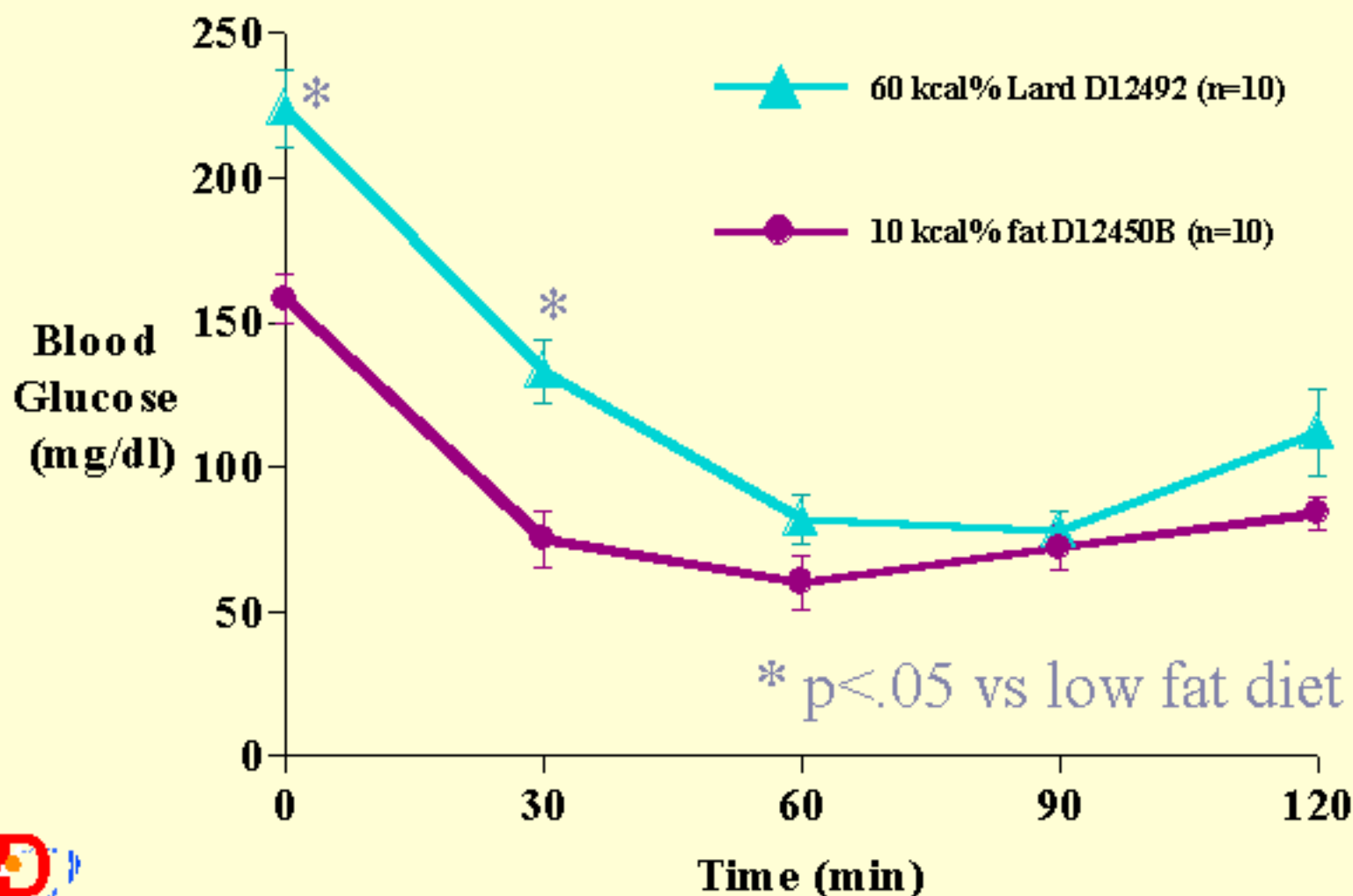
* $p < .05$ vs low fat diet



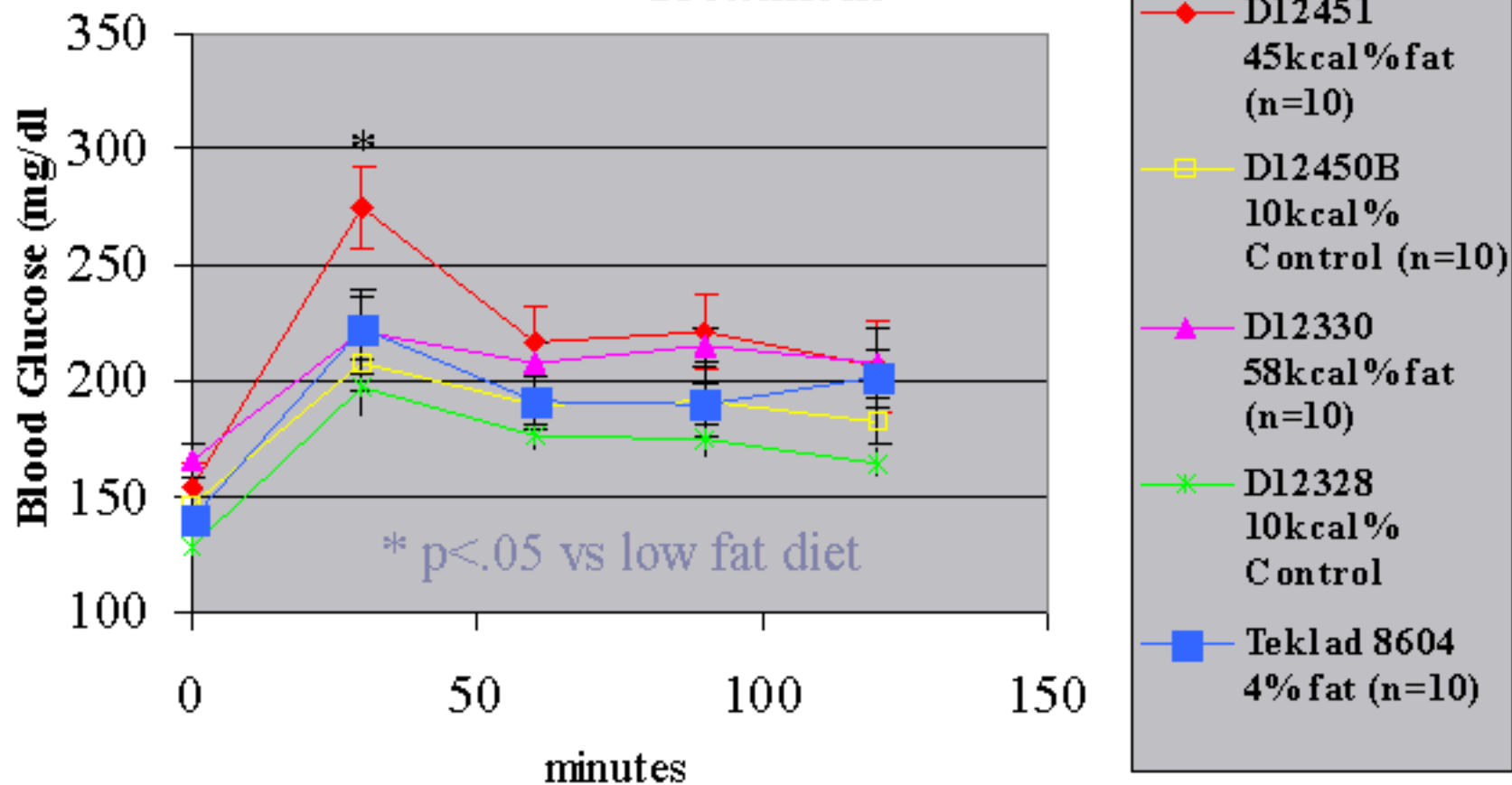
Insulin Tolerance Test (0.5U/kg, i.p.) in 3 Hour Fasted C57BL/6J Mice after 6 Weeks on High vs Low Fat Diet



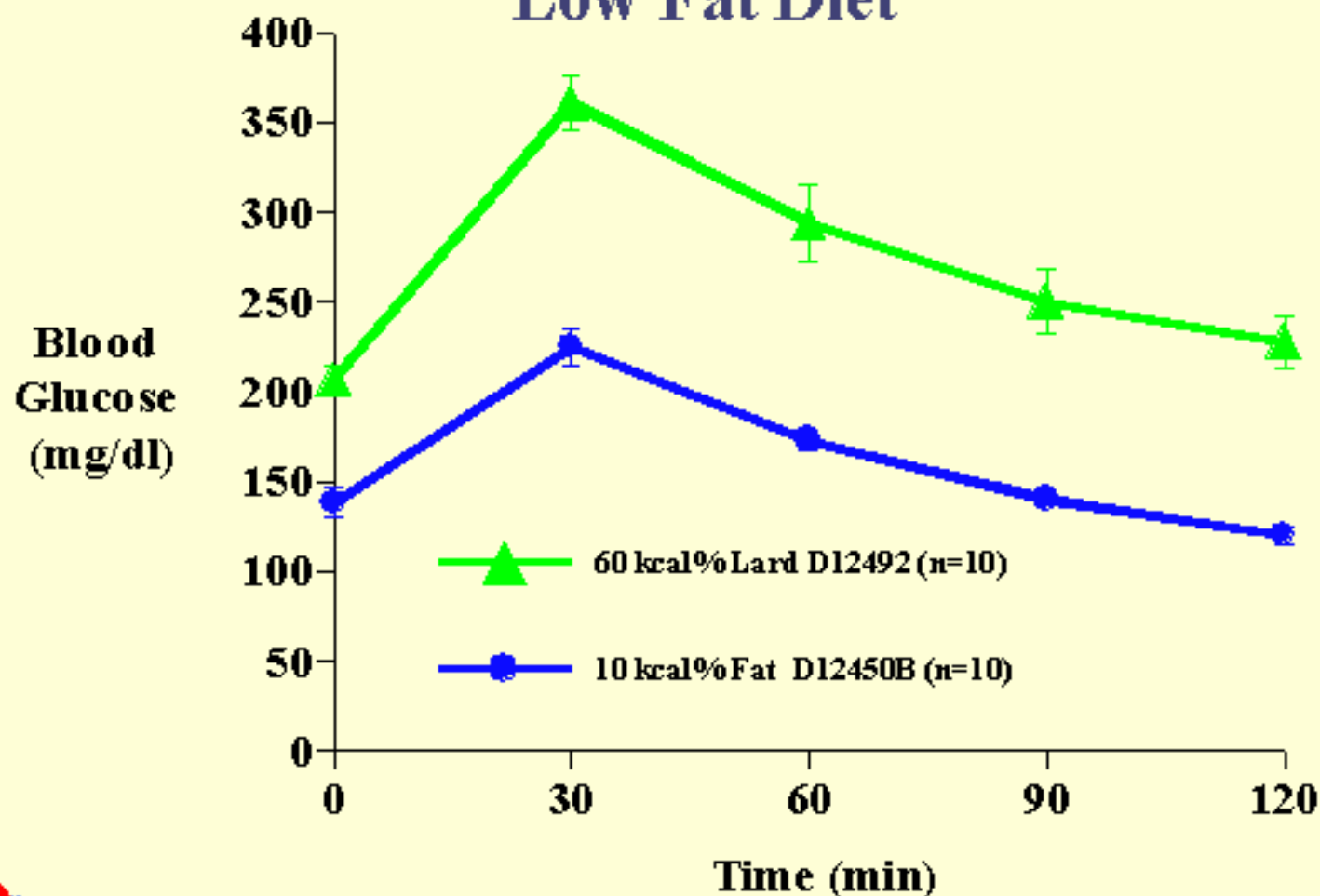
Insulin Tolerance Test (0.5U/kg) in 3 Hour Fasted C57BL6 Mice after 6 Weeks on High vs Low Fat Diet



Oral Glucose Tolerance Test (2 g/kg) in 3 Hour Fasted C57BL/6J Mice after 7 Weeks of Diet Treatment

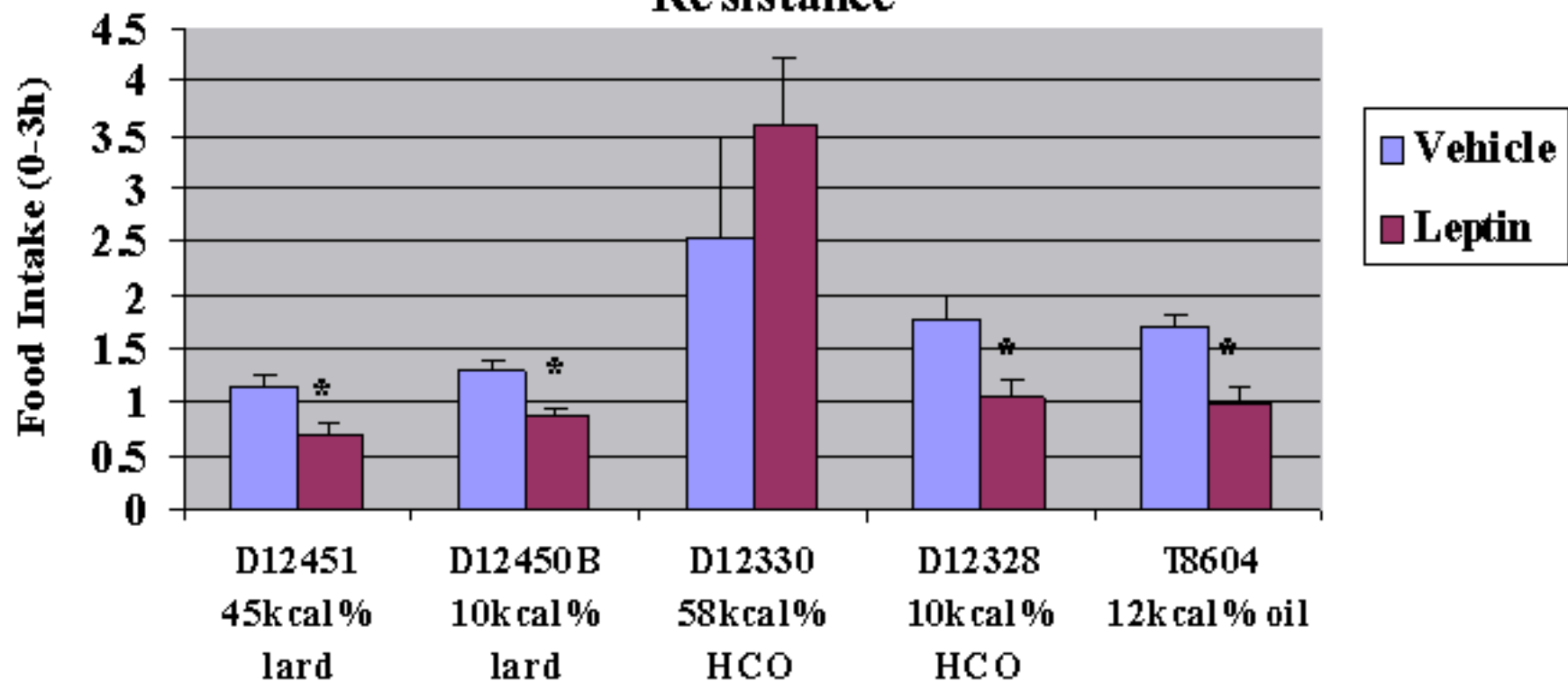


Oral Glucose Tolerance Test (2g/kg) in 3 Hour Fasted C57BL6 Mice after 6 Weeks of High vs Low Fat Diet



$p < .05$ significant differences at all timepoints

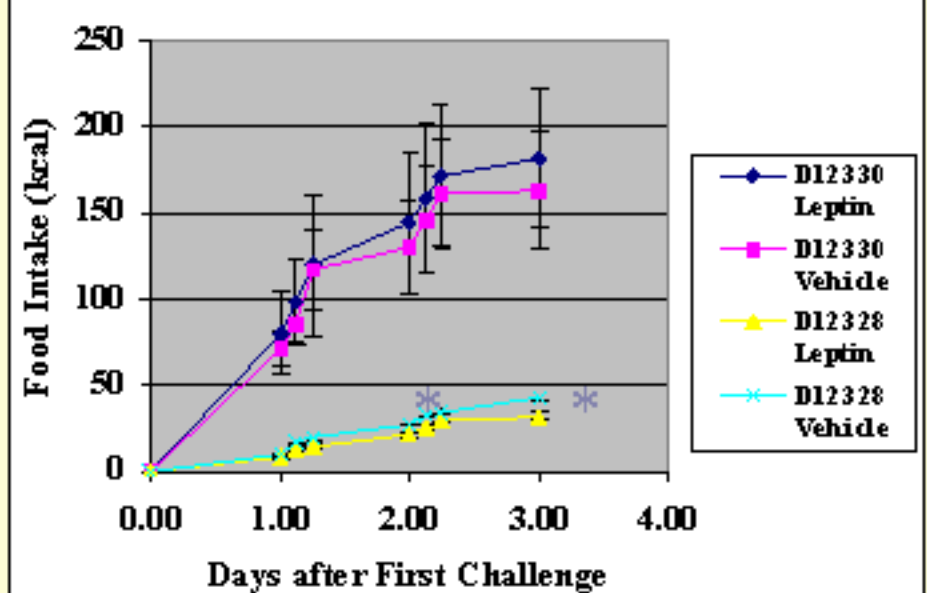
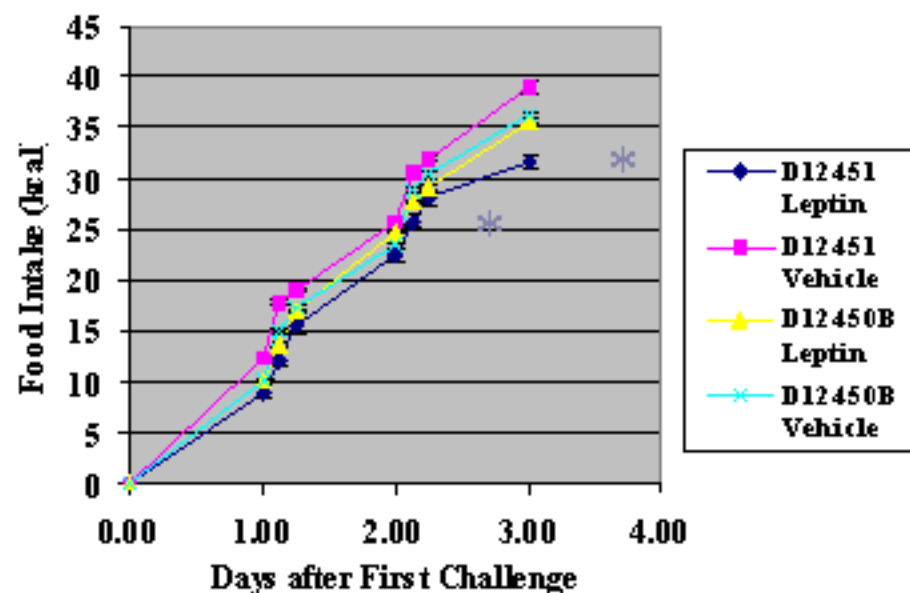
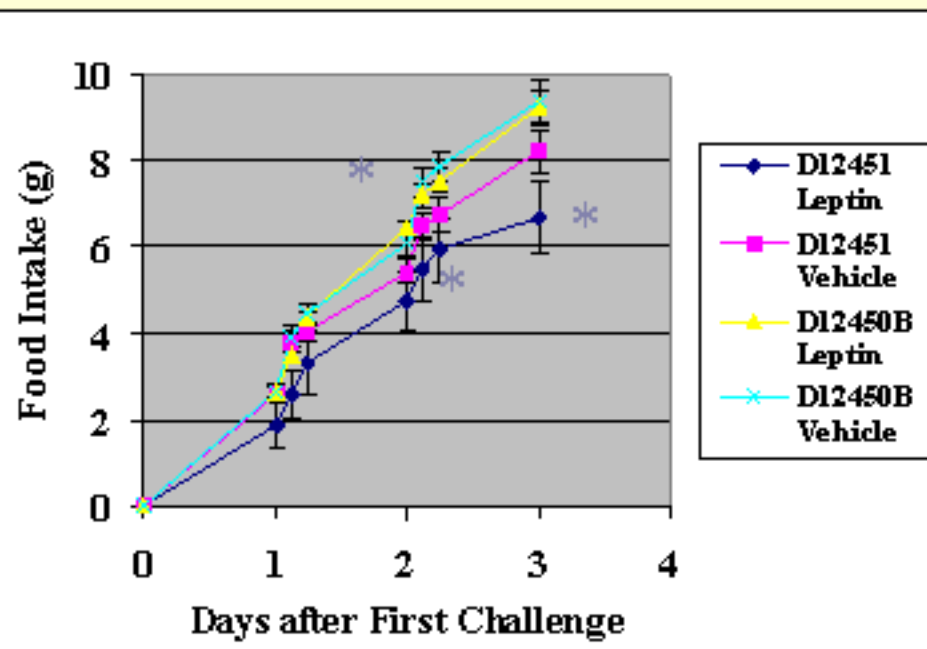
Leptin (10 mg/kg, i.p.) Decreases Food Intake in C57BL/6J Mice, and D12330 Diet Produces Leptin Resistance



* $p < .05$ vs low fat diet

Effect of Leptin (10 mg/kg, ip) on Cumulative Food Intake in C57BL/6J Mice, after 11 Weeks on Diets

* $p < .05$ vs low fat diet



MRI Methods for Fat Imaging in Mice

Magnetic Resonance Images (MRI) of mice on high fat (D12451) and low fat (12450B) diets were collected on a small animal imaging system, the console from Varian instruments (Inova) interfaced to a 4.7T/40 cm magnet/gradient coils from Magnex Scientific. Mice were euthanized before being placed in a quadrature rf coil (40 mm diameter) in the magnet and 11 scout sagittal images, 1mm each, were collected through the abdominal region of the mouse. The sagittal slices, in which the kidneys were viewed, were used to position transverse slices through the abdominal region of the mice with the caudal tip of the most caudal kidney in the 12th slice. In some instances the tip of the kidney was in the 13th slice. The transverse slices through the abdominal region of the mice were collected using Varian's SATSEMS imaging acquisition program. Briefly, the water resonance is saturated using a rf frequency selective pulse (gaussian) before each element of the image acquisition. During the course of the image acquisition, 18 transverse slices, each 1mm thick separated by 1mm, were collected from the middle of the most caudal kidney to the inguinal region of the mouse. The fat in the images was also enhanced by collecting the images with a short repetition time (TR = 0.7 s) and a short echo time (20 ms). The data matrix collection for each slice was 256 x 128 which was zero-filled to 256 x 256. The field of view was 4 cm x 4 cm which gives an in-slice pixel resolution of 156 microns. There were four acquisitions for each image so the total imaging time for each mouse was approximately 6 minutes.

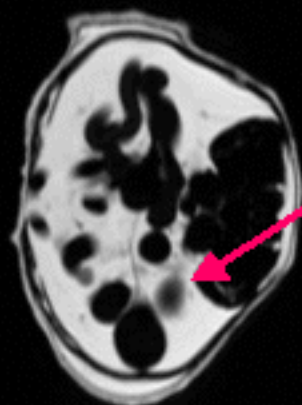
MRI Data Analysis

The imaging protocol generated fat enhanced (i.e., water suppressed) images which were analyzed for the fat content in each slice. Software (IDL) was written in the MRI lab for determining the amount of fat in each slice. First, the total amount of fat is determined in each slice by setting a threshold in each image which is only fat. After completing this procedure for each mouse, it is repeated, except this time only highlighting the fat within the abdominal cavity in each slice. The abdominal fat in each slice is then subtracted from the corresponding total fat to determine the subcutaneous fat in each slice. From the above procedure one can determine the total, abdominal and subcutaneous fat in each slice for each animal. The slice in which the caudal tip of the lower kidney was identified for each animal. In most instances the tip of the kidney is in slice 12. In cases where the caudal tip is not slice 12, the slices are renumbered so the tip of the caudal kidney becomes slice 12. Since the slices are then registered to the same anatomical location for each mouse, the fat (total, abdominal and subcutaneous) for each mouse can be combined within groups. The individual slices of all the mice in each treatment group can then be combined and statistics run on the resulting data.

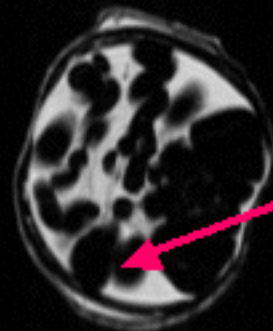
**32g Mouse on 45 kcal%
Lard Diet (D12451)**

**32g Mouse on 10 kcal%
Fat Diet (D12450B)**

Slice 12

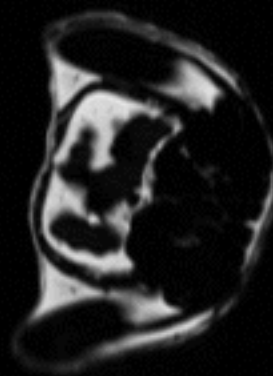
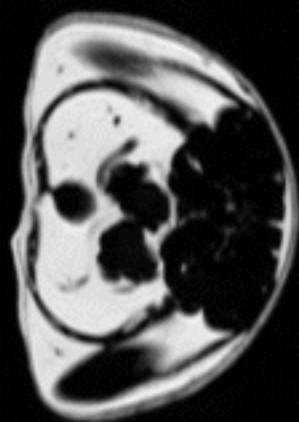


Kidney

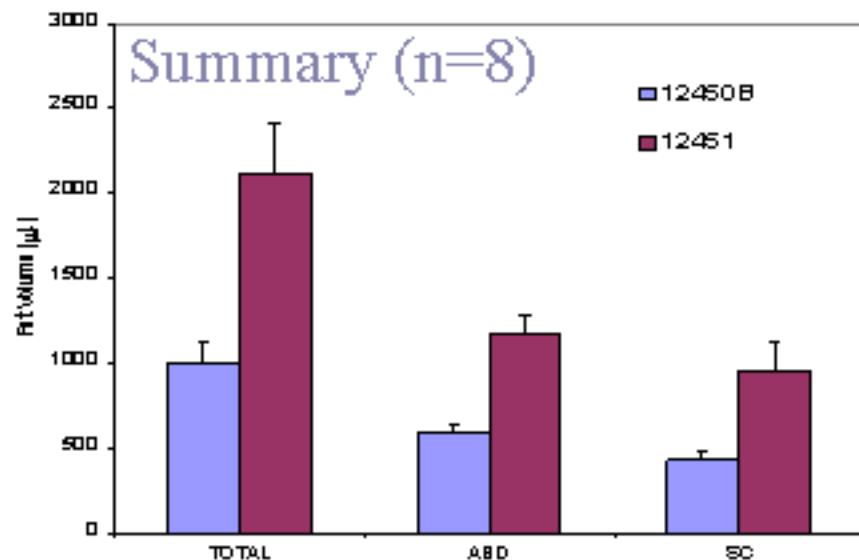
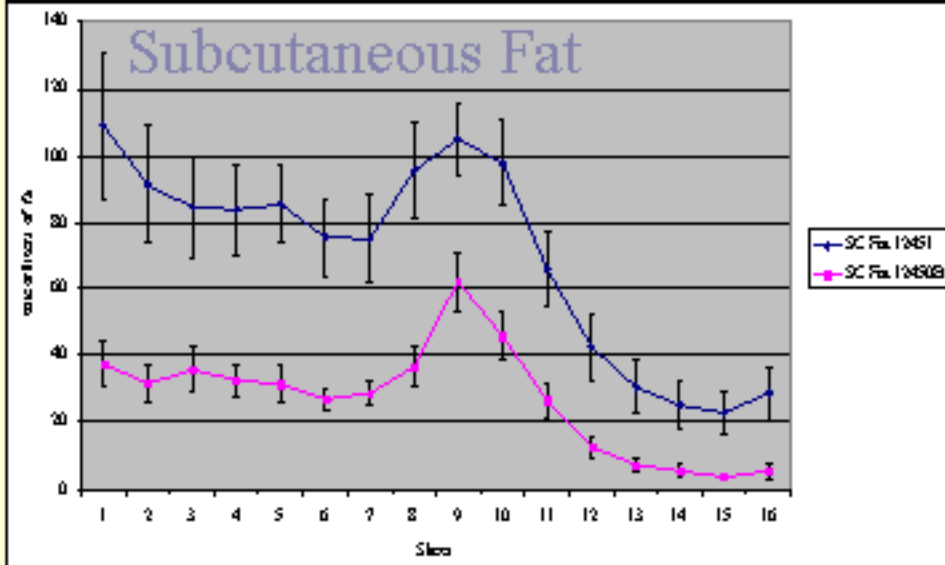
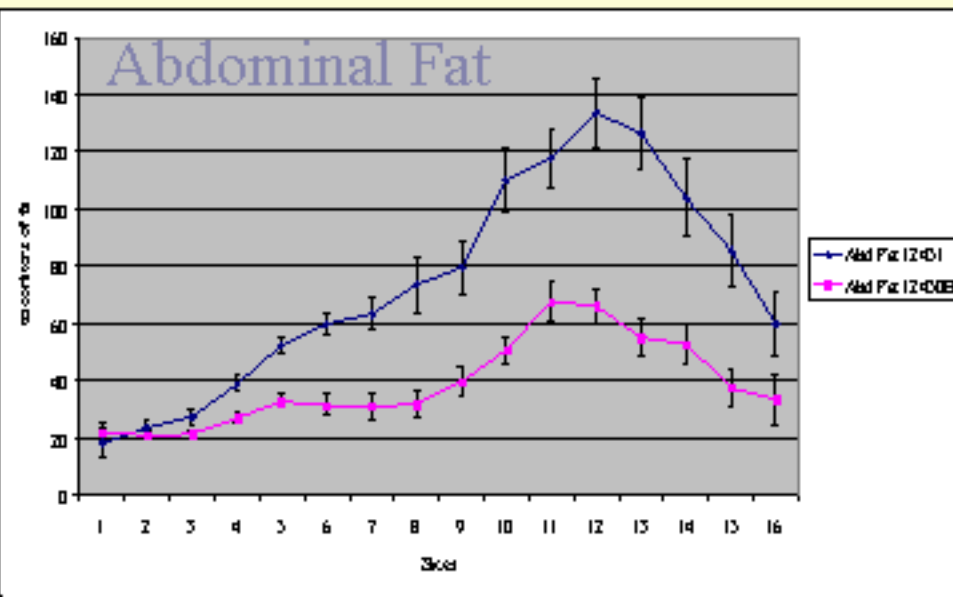
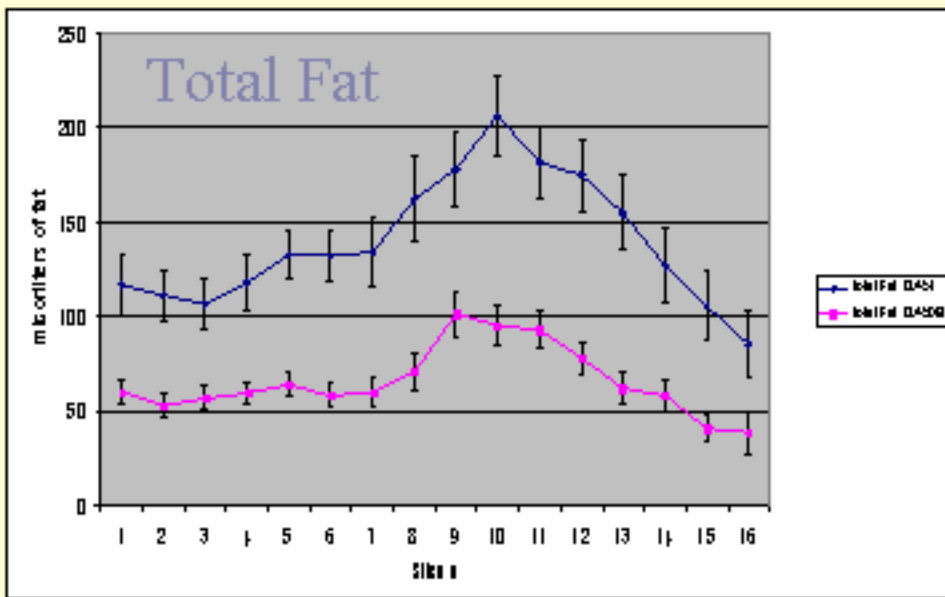


Kidney

Slice 8



Fat Mass in 32g Mice on High or Low Fat Diets for 13 Weeks



Summary

- C57BL/6J mice developed prediabetes and obesity on high fat diets, but all of the high fat diets tested were not equivalent.
- The 45 kcal% lard diet (D12451) produced hyperglycemia, insulin resistance, glucose intolerance and moderate obesity, all signs of prediabetes.
- The 58 kcal% hydrogenated coconut oil (D12330) produced greater obesity than 45 kcal% lard, but fewer signs of prediabetes. This may be related to fat composition (vegetable vs animal fat) and fatty acid chain length (predominantly C12 vs C18).
- 60 kcal% lard resulted in rapid and dramatic weight gain, associated with high feed efficiency. These mice had hyperglycemia, hyperinsulinemia, hyper-leptinemia and glucose intolerance, but were less insulin resistant than mice on 45 kcal% lard diet.
- Leptin treatment decreased food intake in mice fed 45 kcal% lard, but not mice fed 58 kcal% HCO. Leptin responsiveness has not yet been determined in mice fed 60 kcal% lard.