Progress report:

This progress report summarizes the results of euglycemic/hyperinsulinemic clamp studies in mice (C57Bl6J) to measure in vivo insulin sensitivity. We have used two different catheterization methods: 1) clamp protocol with jugular vein infusions and tail vein sampling in Study A; 2) double catheterization with jugular vein infusions and carotid artery sampling in Study B.

Study A: Effect of CB1 receptors antagonism on insulin action. To study the effect of CB1 antagonism on body composition and insulin action independent of food intake, we have treated diet-induced obese (DIO) mice with daily ip injections of Rimonabant, a selective inhibitor of CB1 receptors, or of vehicle. A third group was treated with ip vehicle and pair-fed to the daily caloric intake of the Rimonabant group. After 1 month, the Rimo group had lost significantly fat mass as compared to both vehicle and pair-fed groups (fig 1)

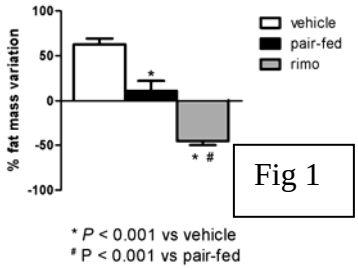

Fig 1

* *P* < 0.001 vs vehicle
# P < 0.001 vs pair-fed

*Euglycemic/hyperinsulinemic clamp studies: jugular infusions/tail vein sampling***.** After 1 month of treatment, we assessed in vivo insulin action. All mice were anesthetized and indwelling catheters inserted in the right internal jugular vein, as previously described. Euglycemic-hyperinsulinemic clamps were performed after complete recovery (4-6 days) and lasted 90 minutes (Fig 2). A solution of glucose (20%) was infused at a variable rate as required to maintain euglycemia (~6 mM). Mice received primed-constant infusions of HPLC-purified [^{3}H] glucose (0.1 mCi/min; New England Nuclear, Boston, MA), and insulin (5 mU/min/kg BW). Thereafter, plasma samples were collected from tail vein to determine glucose levels (at t=10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes) as well as [^{3}H]glucose specific activity (at t = 40, 50, 60, 70, 80, and 90 minutes). Samples were collected from tail vein for the assessment of plasma insulin, FFA or other plasma components. At the end of the *in vivo* studies, mice were anesthetized (pentobarbital 60 mg/kg BW i.v.), the abdomen quickly opened, and liver and other tissues are quickly frozen in liquid nitrogen. Tissue samples are stored at -80C for further analysis. To evaluate the insulin-dependent uptake of glucose in specific tissues we inject 10 uCi of ^{14}C-DOG as a bolus at time= 45 of the clamp study.

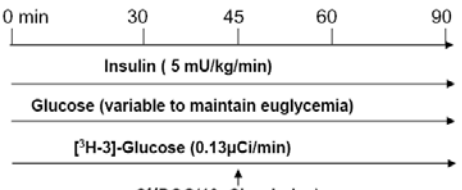

Fig 2

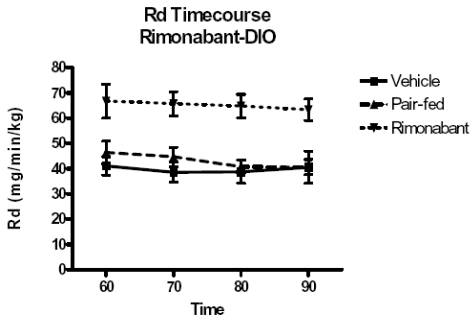

Fig 3

Results: Plasma glucose in all animals was clamped at ~180 mg/dl. The specific activity of [^{3}H] glucose and the rate of glucose disposal (Rd) were stable during the last 30 minutes of the clamp studies in all groups (figure 3). Using the measurements obtained in this steady-state period, we found that the glucose infusion rate (GIR) required to maintain

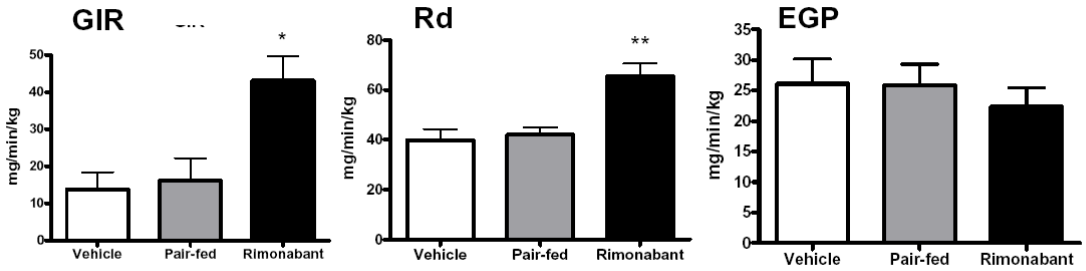

Fig 4

euglycemia was significantly elevated only in the Rimo group (figure 4), indicating that CB1 antagonisms improves insulin sensitivity independent of its effects on feeding behavior. Rimonabant treatment significantly improves the rate of glucose disposal, while it does not affect the rate of glucose production. Thus, these experiments show that the insulin sensitizing effect of Rimonabant is mostly secondary to an improvement in insulin-dependent glucose utilization, but does not significantly improve hepatic insulin sensitivity in this DIO mouse model.

Study B: Acute effect of a 5-HT_{2c} receptors angonist on insulin action. In collaboration with Lora Heisler (Cambridge, UK), we have tested the ability of mCPP (an agonist of 5-HT_{2c} receptors) to improve insulin sensitivity in insulin resistant DIO mice. For this study we have used double catheterization with jugular vein infusions and carotid artery sampling as outlined in figure 5. Our preliminary studies show that we are able to achieve stable tracer infusions using this technique as illustrated in figure 6.

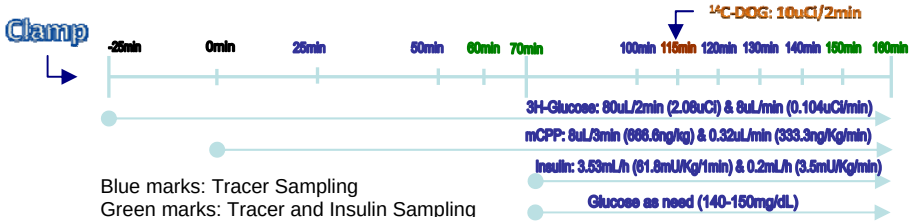

Figure 5

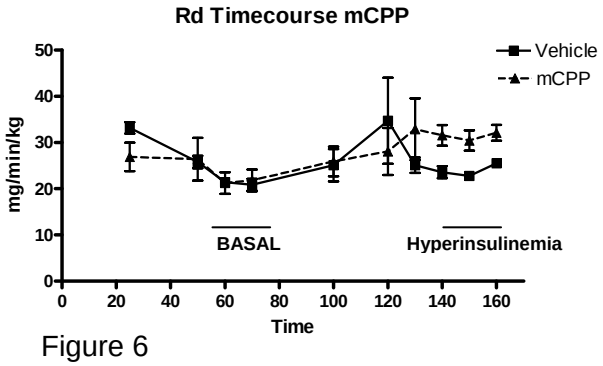

Figure 6