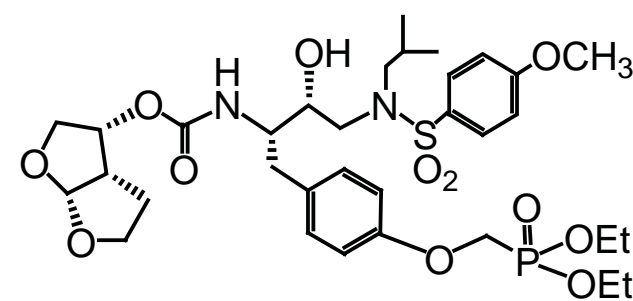


GS-8374, a Novel HIV Protease Inhibitor, Does Not Alter
Peripheral Glucose Disposal in a Healthy Rodent Model SystemC Callebaut,¹ Q Yan,² J Koster,² L Tsai,¹ T Cihlar,¹ and P Hruz^{2,3}¹Gilead Sciences, Inc. Foster City, CA; ²Department of Pediatrics; ³Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO

Introduction

- GS-8374 is a novel HIV-1 protease inhibitor (PI) with a unique diethylphosphonate motif incorporated into its molecule (Fig. 1). We previously showed that GS-8374 is a potent and selective PI, and that its phosphonate moiety favorably affects its resistance profile.^{1,2}
- The clinical use of HIV PIs in combination with other antiretroviral drugs dramatically reduced the morbidity and mortality of HIV infection.³ However, adverse metabolic effects induced by antiretroviral therapies remain one of the major factors often limiting their long-term clinical benefit.^{4,5} Notably, many of the first generation HIV PIs are known to contribute to the development of lipodystrophy and peripheral insulin resistance.⁶
- In vitro* studies have demonstrated that some PIs can inhibit lipogenesis and adipocyte differentiation.⁷ Furthermore, it has been shown that PIs such as ritonavir and lopinavir induce insulin resistance *in vitro* by reducing glucose transport mediated by the facilitative glucose transporter GLUT4;⁸ conversely, atazanavir has been shown to have minimal effects on insulin sensitivity *in vitro*,⁹ and these profiles were respectively confirmed *in vivo*.^{10,11}
- This presentation summarizes the *in vitro* effects of GS-8374 on adipocyte functions and characterizes the *in vivo* potential of GS-8374 to affect glucose disposal in a rat model, in comparison with key marketed PIs.

Figure 1. Structure of GS-8374

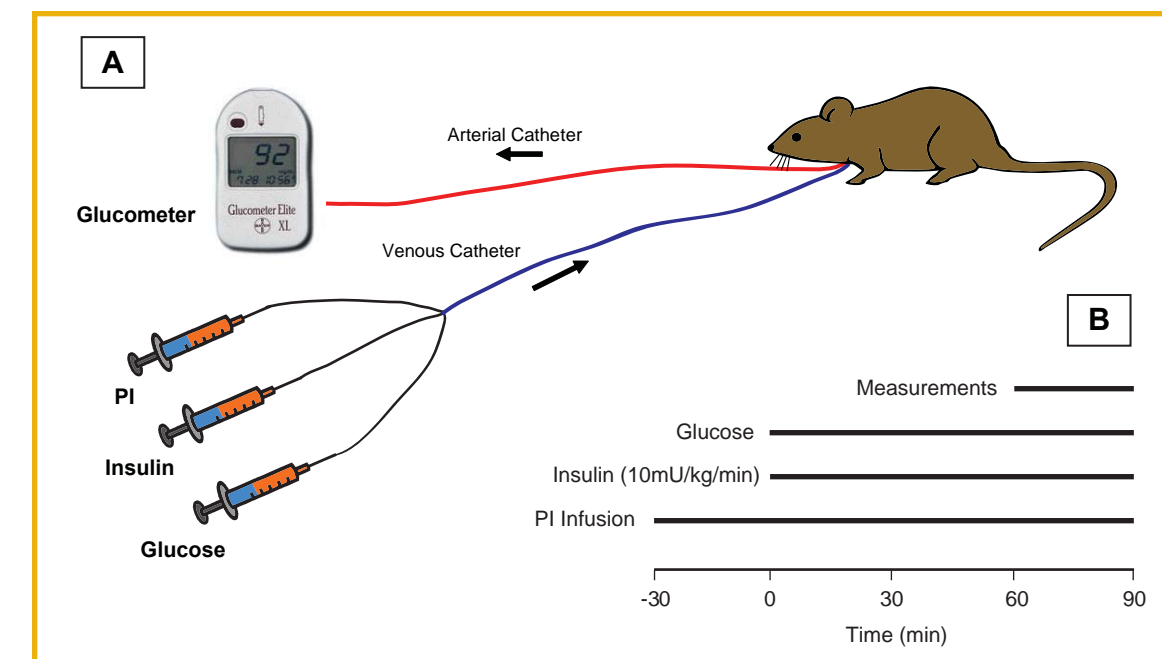


Methods

- Synthesis of GS-8374 and other protease inhibitors was performed at Gilead Sciences.
- For lipid accumulation assay, primary human adipocytes (Cambrex) were cultivated with 10 µg/mL insulin, 1 µM dexamethasone, 200 µM indomethacin, 500 µM isobutyl-methylxanthine in presence of tested compounds. After 9 days, AdipoRed reagent (Cambrex) was added to the cells and fluorescence was measured.
- For glucose uptake, mouse OP9 stromal cell-derived differentiated adipocytes (ATCC) were treated with insulin and tested compounds (10µM). After one hour, [³H]-deoxy-D-glucose (0.2 µM) was added for 10 min followed by cell washing and lysis. Deoxyglucose uptake was determined by scintillation counting.¹²

Methods (cont'd)

- For *in vivo* studies, euglycemic hyperinsulinemic clamp experiments were performed in chronically catheterized healthy lean male Wistar rats. (all animal procedures were approved by the animal studies committee at Washington University School of Medicine). Catheters were inserted into carotid arteries and jugular veins of ~200-g rats at least 5 days before the experiments. Intravenous infusion of the PIs was performed as previously described.¹³ Briefly, a constant infusion of PI was started through the venous catheter at a rate of 10 µL/min for the duration of the clamp experiment. After 30 min of drug infusion, insulin (10 mU · kg⁻¹ · min⁻¹) was infused through the venous catheter. At 5 or 10-min intervals, determination of blood glucose levels was performed. Dextrose (50%) was infused through the venous catheter at a rate sufficient to maintain a plasma glucose level of ~105 mg/dL. Insulin sensitivity was determined by the average infusion rate during the final 30 minutes of each 90 minute clamp experiment (Fig. 2).

Figure 2. Setup of *In Vivo* Glucose Disposal Experiments

Results

Table 1. *In Vitro* Effect of PIs on Insulin-stimulated Glucose Uptake and Lipid Accumulation in Adipocytes

Compounds ^a	Glucose uptake ^b	Lipid accumulation ^b
	% inhib. at 10 µM	EC ₅₀ [µM]
GS-8374	0 ± 0	>30
Atazanavir	0.5 ± 1	>30
Amprenavir	2 ± 2	>30
Darunavir	2 ± 4	>30
Nelfinavir	5 ± 10	8 ± 3
Indinavir	13 ± 14	>30
Saquinavir	13 ± 15	16 ± 4
Lopinavir	33 ± 12	16 ± 5
Ritonavir	50 ± 15	17 ± 8

a. Vehicle for all drugs was DMSO; no inhibition was observed in both assays.
b. Mean ± SD of 3 independent experiments.

Results (cont'd)

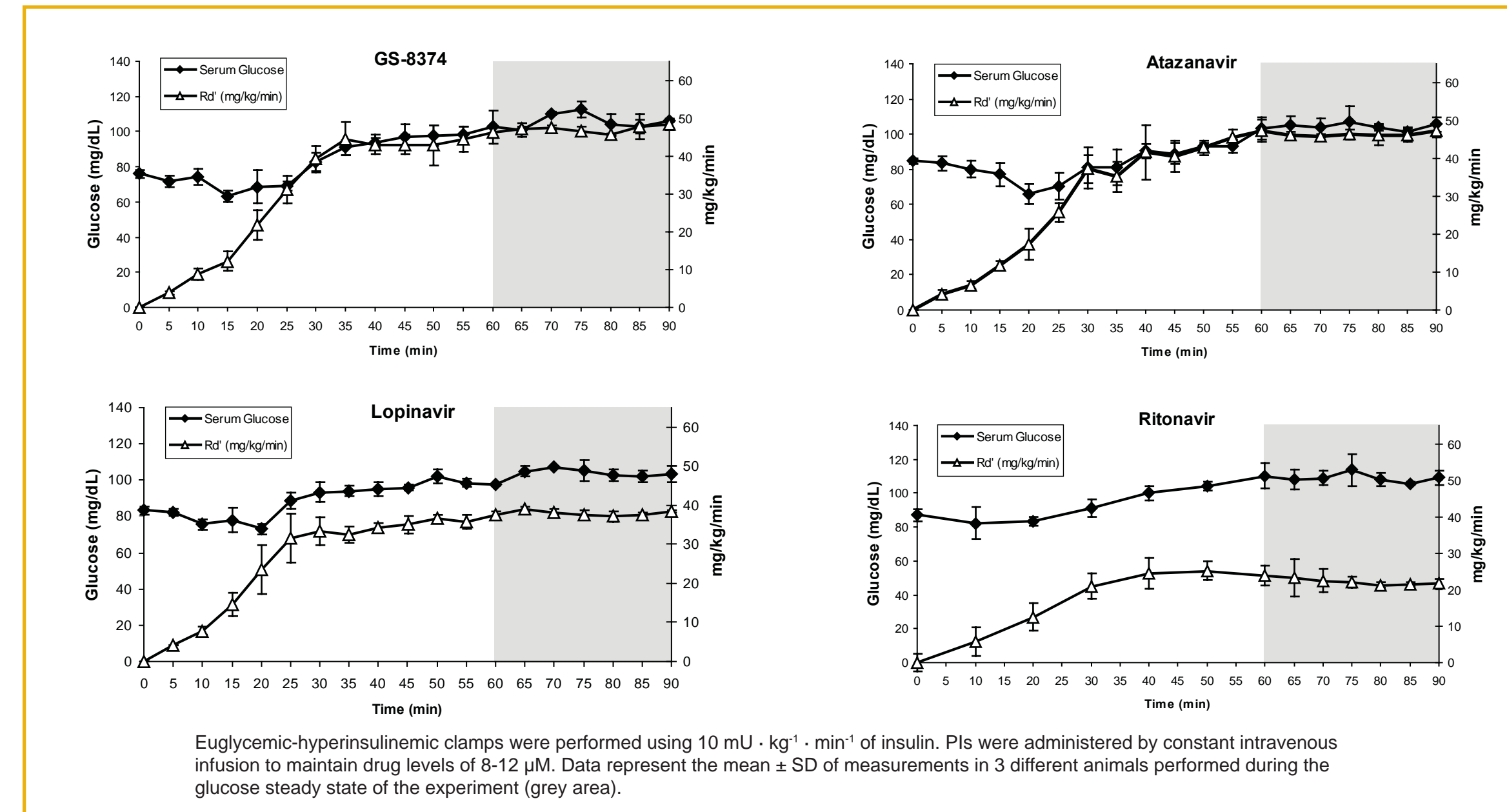
Figure 3. Acute *In Vivo* Effects of PIs on Peripheral Glucose Disposal

Figure 4. Comparable Levels of Tested PIs in Rat Serum During Infusion

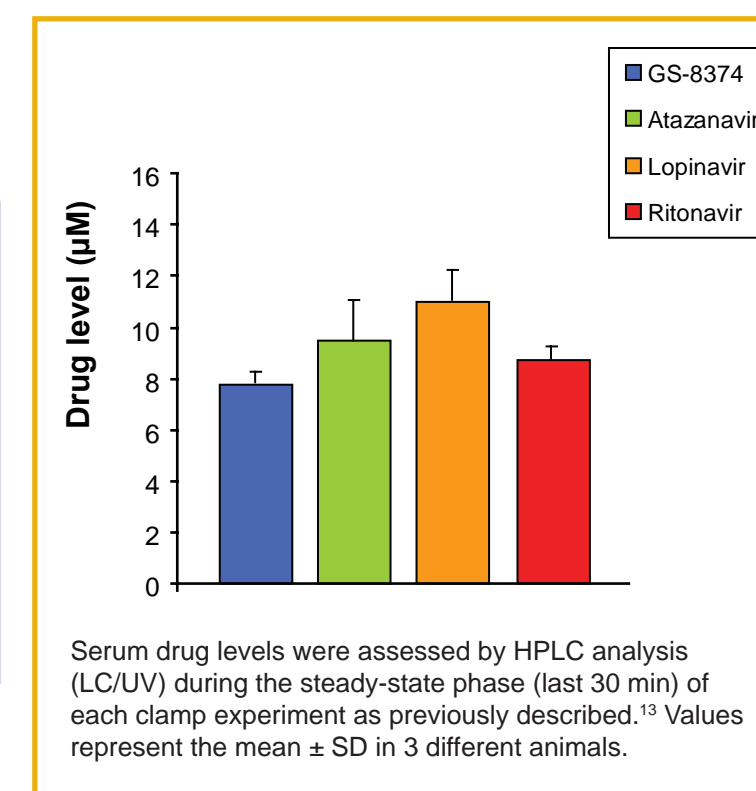


Figure 5. Comparable Serum Glucose Levels in Rats Treated with the Tested PIs

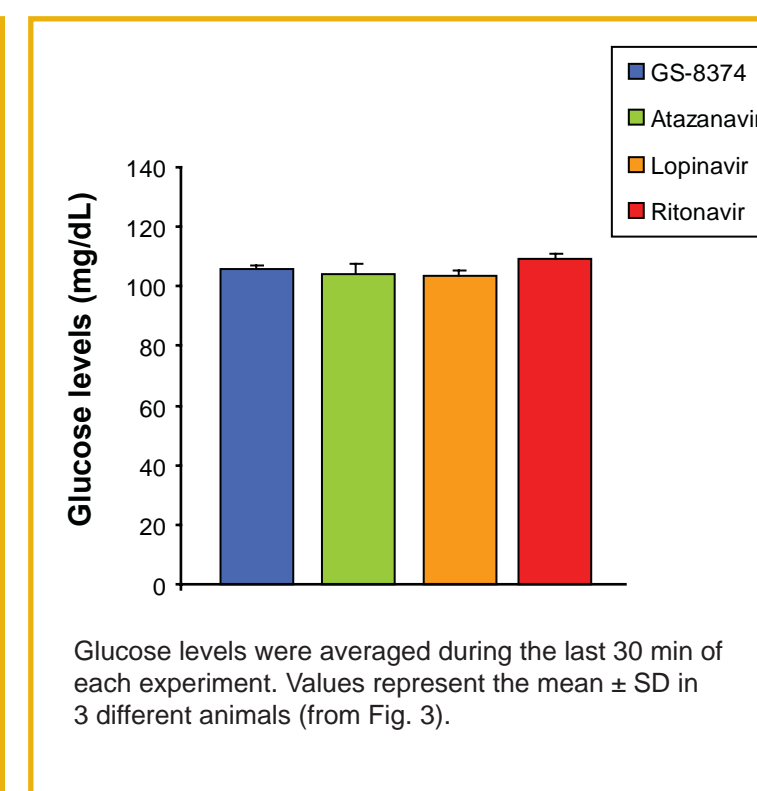
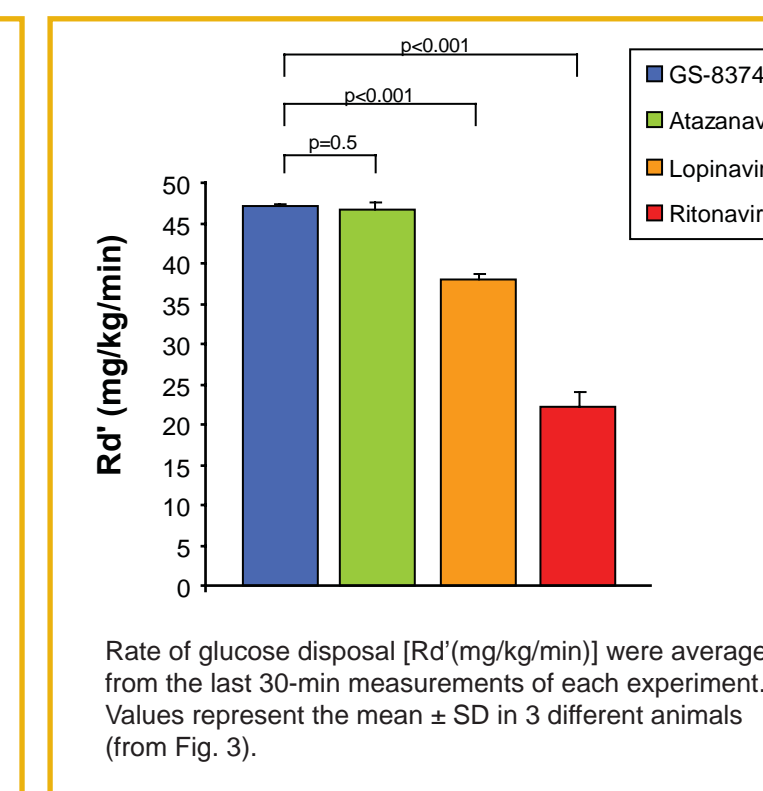


Figure 6. Similar Glucose Disposal in Animals Treated with GS-8374 and Atazanavir



Conclusions

- Treatment of human primary adipocytes with GS-8374 or atazanavir with concentrations up to 30 µM showed no effect on normal lipid accumulation; under the same conditions, ritonavir and lopinavir affected lipid accumulation with EC₅₀ values of 16 and 17 µM, respectively.
- Treatment of differentiated OP-9 mouse adipocytes with 10 µM GS-8374 or atazanavir showed no effect on the insulin-stimulated deoxyglucose uptake; ritonavir and lopinavir caused 50 and 33% reductions in deoxyglucose uptake, respectively, under the same conditions.
- In rats, sustained serum levels of 8 µM GS-8374 or atazanavir had no effect on peripheral glucose disposal. Consistent with previous studies, comparable serum levels of ritonavir or lopinavir produced acute reduction of glucose disposal.
- These results suggest a low potential of GS-8374 for metabolic adverse effects compared to some other PIs and confirm that these effects are not inherently linked to antiretroviral activity of PIs.

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