AZP-3404, a Peptide Analog of IGFBP-2, Induces Weight Loss and Improves Glucose Metabolism in Leptin-Resistant db/db Mice

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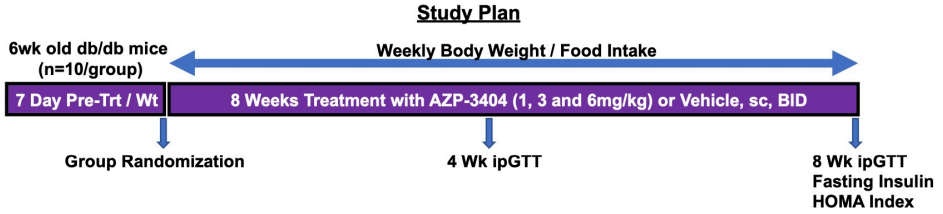
INTRODUCTION: Insulin-like growth factor binding protein-2 (IGFBP-2) has been demonstrated to be a key mediator of the peripheral metabolic actions of leptin. In animal models of metabolic dysregulation, IGFBP-2 has been demonstrated to reverse insulin resistance and diabetes, and to prevent development of obesity (1, 2). In clinical studies, circulating IGFBP-2 levels are positively correlated with protection from development or worsening of insulin resistance, obesity and diabetes (3-6). The metabolic activity of IGFBP-2 is independent of IGF1 binding, and can be localized to a unique heparin-binding domain (HBD-1) within its structure (7-9). AZP-3404 is a 9-amino acid analog of the IGFBP-2 HBD-1 that reproduces the activity of IGFBP-2 on adipocyte and osteoblast differentiation. In addition, AZP-3404 has been demonstrated to increase glucose uptake by differentiated mouse myotubes in vitro, and to increase glucose disposal following an intraperitoneal glucose tolerance test (IPGTT) in leptin-deficient ob/ob mice (10). In the present study, we hypothesized that AZP-3404 should be able to improve metabolic regulation in the db/db mouse, which is leptin-resistant due to a mutation in the leptin receptor, and, as a consequence, is also IGFBP-2 deficient.



METHODS: Six-week old male db/db mice (BKS(DB/DB): Janvier Labs, France) were treated with vehicle (10mM glutamic acid/NaOH buffer with 5% w/w mannitol, pH 4.8) for 1 week to allow acclimatization and to establish baseline weights. Mice were maintained on a 12h light cycle and fed a standard diet (SAFE, Ref: U8400G10R) and tap water ad libitum for the entire study. Following acclimatization, the mice were randomly distributed among 4 treatment groups (n=10/group) to receive either vehicle or AZP-3404 at doses of 1, 3 or 6mg/kg, sc, bid for 8 weeks. Body weight and food intake were measured weekly. To assess the impact of AZP-3404 treatment on glucose disposal, after both 4 and 8 weeks of treatment, and following an overnight fast, the mice were administered an intraperitoneal glucose tolerance test (ipGTT: tail vein blood collected and glucose measured by colorimetric assay at 0, 30, 60, 90, 120 and 240 minutes post-ip injection of 1g glucose/kg). Fasting insulin was also measured after 8 weeks of treatment and the HOMA measure of insulin resistance was determined. The study was conducted at the laboratories of Physiogenex, Escalquens, France.

Fig. 1

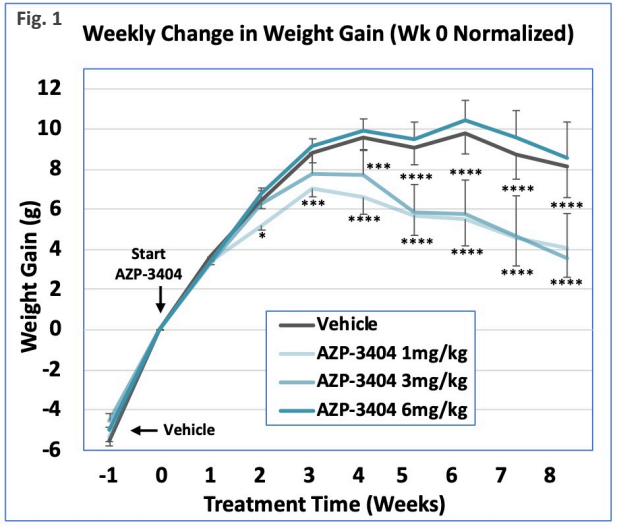


FIG 1. At the initiation of treatment, the mice weighed an average of 39.72 g and, after 8-weeks, vehicle-treated mice had gained 8.52 grams of body weight. Mice treated with either 1 or 3mg/kg AZP-3404 displayed a progressive decrease in weight gain that began after 2 weeks of treatment and by 8 weeks was 52.1% and 58.2% less than controls, respectively, and without a noticeable change in food intake. For reasons unknown, an only 5.2% decrease in weight gain from the vehicle-treated mice was observed with the 6mg/kg treatment. *p<0.05, ***p<0.001, ****p<0.0001 versus vehicle.

Fig. 2

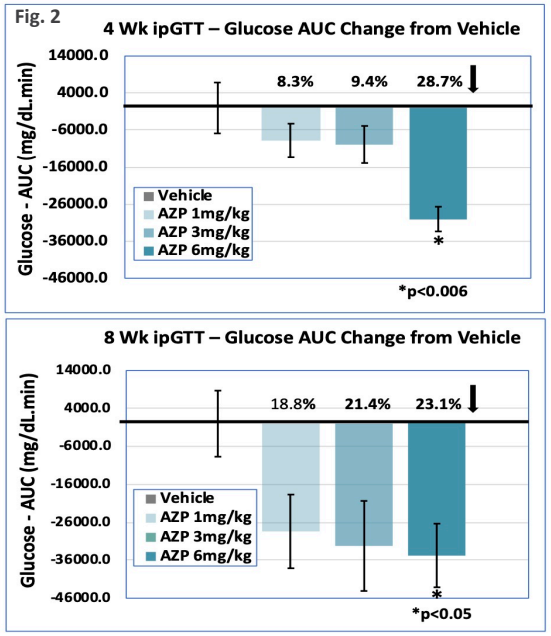


FIG 2. After 4 weeks of treatment, a significant increase in glucose disposal was observed in mice treated with the 6 mg/kg dose of AZP-3404 (AUC glucose decreased by 28.7%) versus vehicle-treated controls. By 8 weeks of treatment, all three doses produced similar increases in glucose disposal (AUC glucose decreased by 18.8, 21.4 and 23.1% with 1, 3 and 6 mg/kg AZP-3404, respectively) versus vehicle controls.

FIG 3 and 4. After 8 weeks of treatment with 1, 3 and 6 mg/kg AZP-3404, fasting plasma insulin was decreased by 54, 48 and 52%, and the HOMA measure of insulin resistance was decreased by 54, 52 and 67%, respectively, as compared with vehicle-treated mice.

Fig. 3

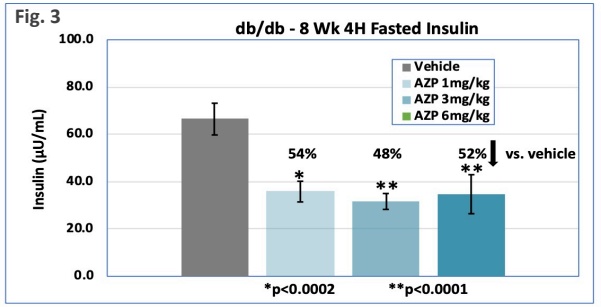
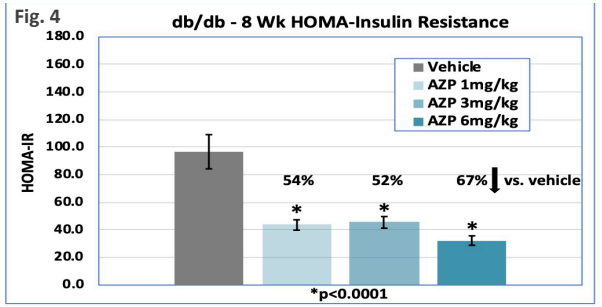


Fig. 4



SUMMARY: The present study demonstrates both decreased body weight gain and improved glucose metabolism following treatment of the leptin-resistant db/db mouse with the IGFBP-2-derived peptide, AZP-3404. The difference in the dose-related effects of AZP-3404 on these two parameters suggest that they may be regulated by independent IGFBP-2 mechanisms. These results further confirm the ability of AZP-3404 to reproduce the metabolic activity of IGFBP-2, and support the development of AZP-3404 as a novel therapy for disease states characterized by insulin resistance and/or obesity, and especially in leptin-resistant conditions.

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