



Confirmation of Hypoglycemia in *Goat*^{-/-} Mice When Total Body Fat Falls Below 2% of Body Weight

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Abstract

Ghrelin is an octanoylated peptide hormone first identified in the stomach, with the octanoyl group being essential to its biological activity. The enzyme that attaches the octanoyl group to ghrelin is called Ghrelin-O-acyltransferase (GOAT). By studying mice that have the GOAT gene knocked out (GOAT KO mice), we have shown that these mice develop severe hypoglycemia under a 60% calorie restricted diet. In order for this hypoglycemia to occur, depletion of fat deposits is required. Specifically, GOAT knockout mice will not develop severe hypoglycemia until the total fat mass drops to 2% of the total body weight. These observations were made in 8-week-old mice with an average starting fat mass between 8-10% of total body weight. In our present work, we wanted to know whether we could reproduce the results using older mice with a higher percentage of fat mass. The mice used in this study were 32-34 week old male mice (wild type and GOAT knockout mice, n=8/group), and both groups had an average starting fat mass of 17% of total body weight. We then subjected these mice to a 60% calorie restriction and monitored their fat mass and blood glucose level every one or two days. For the first 7 days of calorie restriction, both wild type and GOAT knockout mice were able to maintain their blood glucose around 60 mg/dl. After that, the GOAT knockout mice start to develop hypoglycemia when their body fat mass dropped below 2% of the body weight. However, the wild type mice were able to maintain their blood glucose level above 40 mg/dl throughout the course even when their fat mass dropped below 2% of their body weight. The results here further confirm that in order to develop hypoglycemia in the GOAT knockout mice, the fat mass needs to be depleted from these mice during calorie restriction, even in older mice (32-34 weeks versus 8 weeks).

Introduction

Ghrelin

- Discovered by Kojima and Kangawa – 1999
- Peptide hormone - 28 amino acids
- Produced in stomach (1% of cells) as preproghrelin
- Modified by O-acylation with octanoate (8 carbon fatty acid)
- Octanoylation is essential for biologic activity
- Two forms in blood: Ghrelin & Desacyl Ghrelin
- Ghrelin, but not desacyl-Ghrelin, acts on receptors in brain to release Growth Hormone

Background

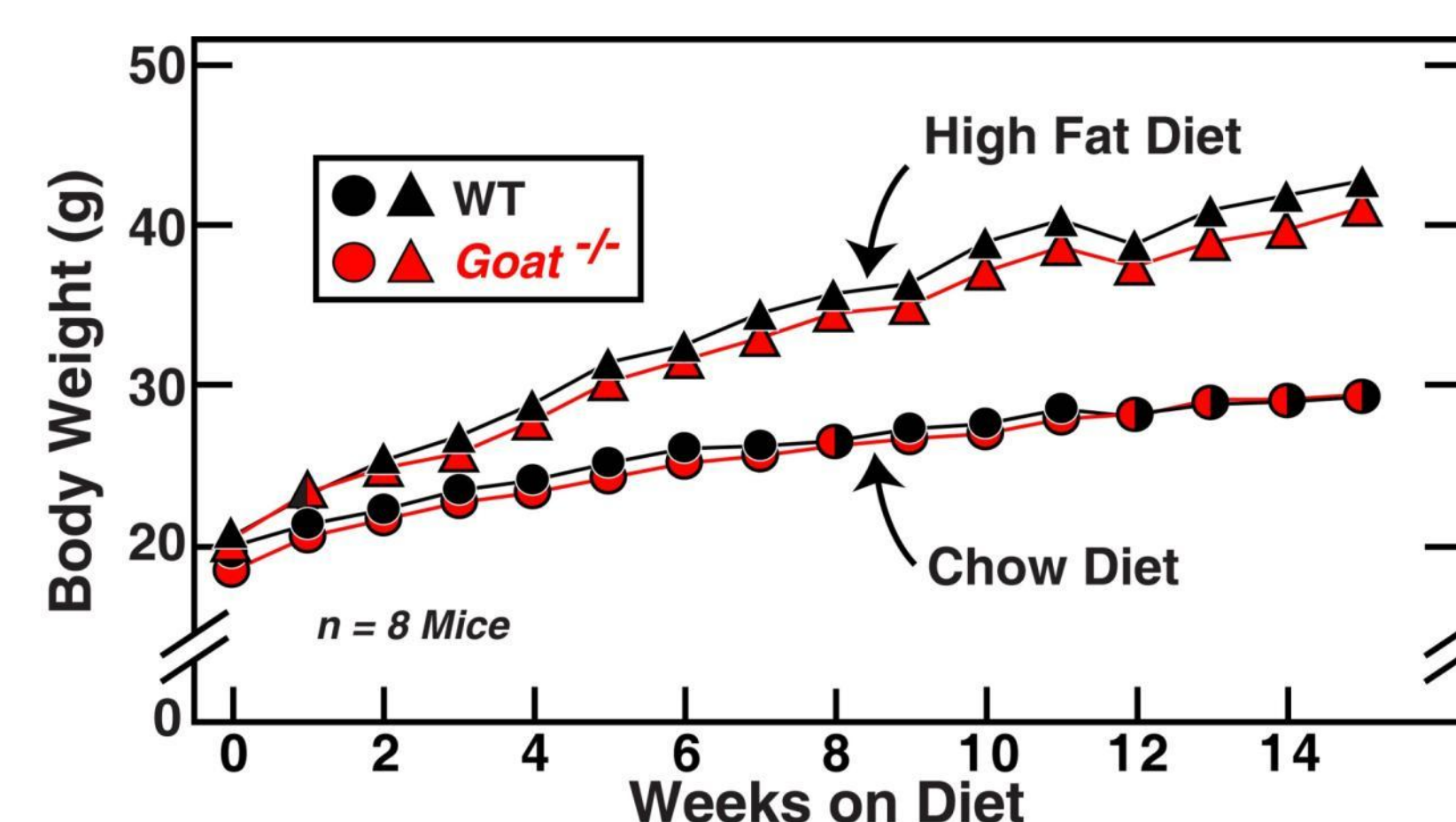


Fig. 1 No Difference between Body Weights of WT and *Goat*^{-/-} mice on either Chow or High Fat Diet.

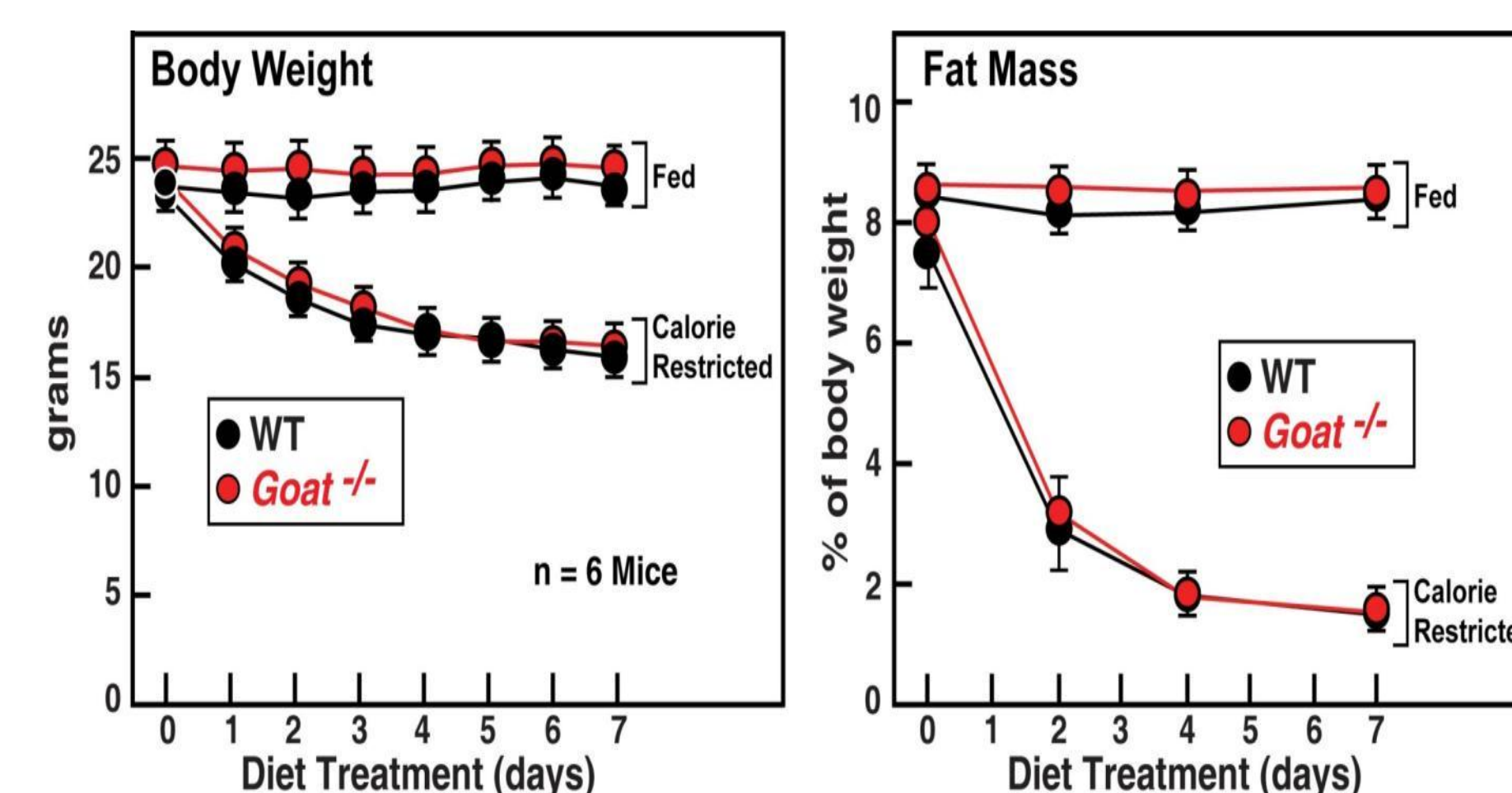


Fig. 2 Changes in Body Weight and Fat Mass in WT and *Goat*^{-/-} Mice during 60% Calorie Restriction

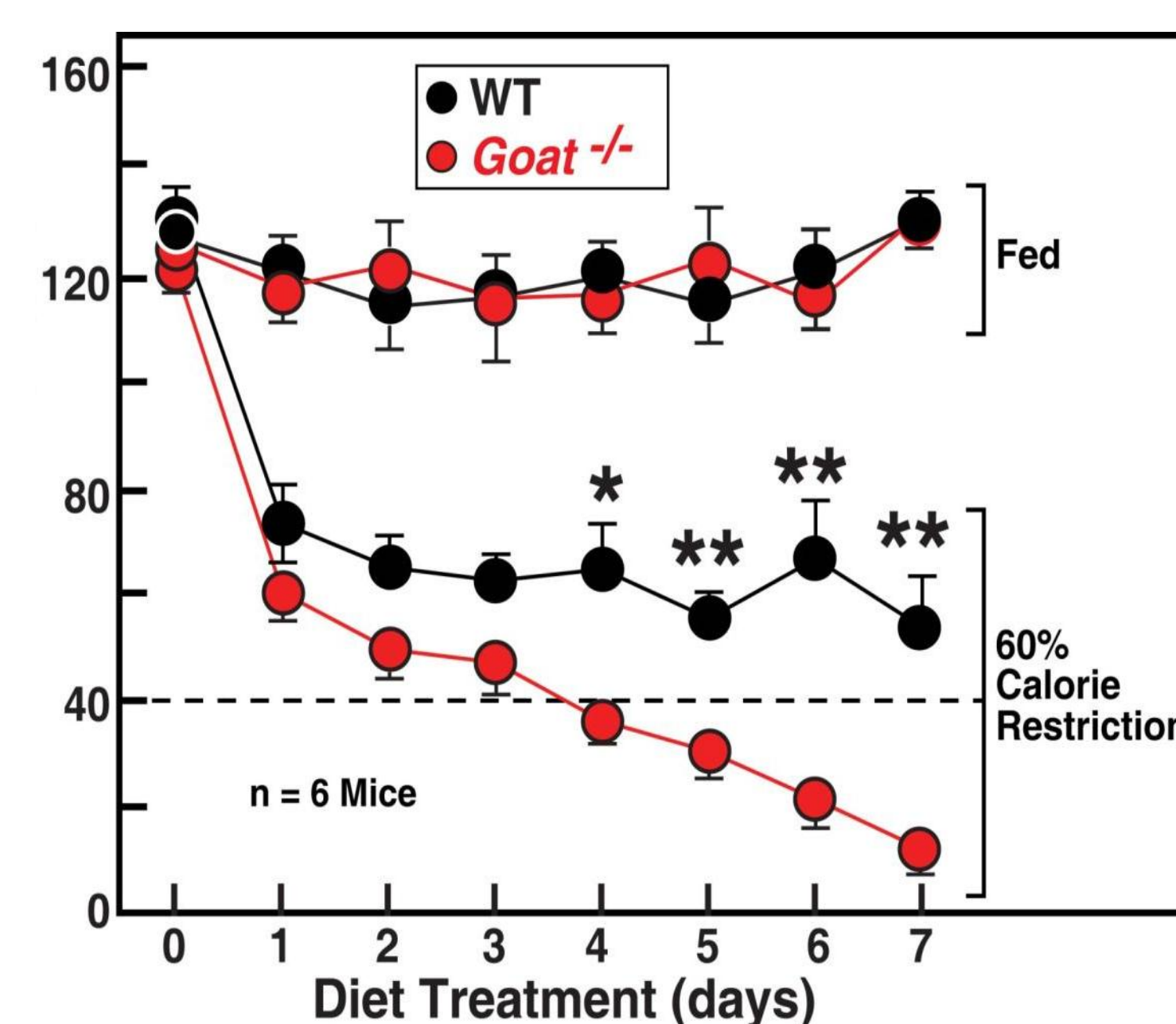


Fig. 3 Calorie-restricted *Goat*^{-/-} Mice Exhibit Severe Hypoglycemia Before Next Meal

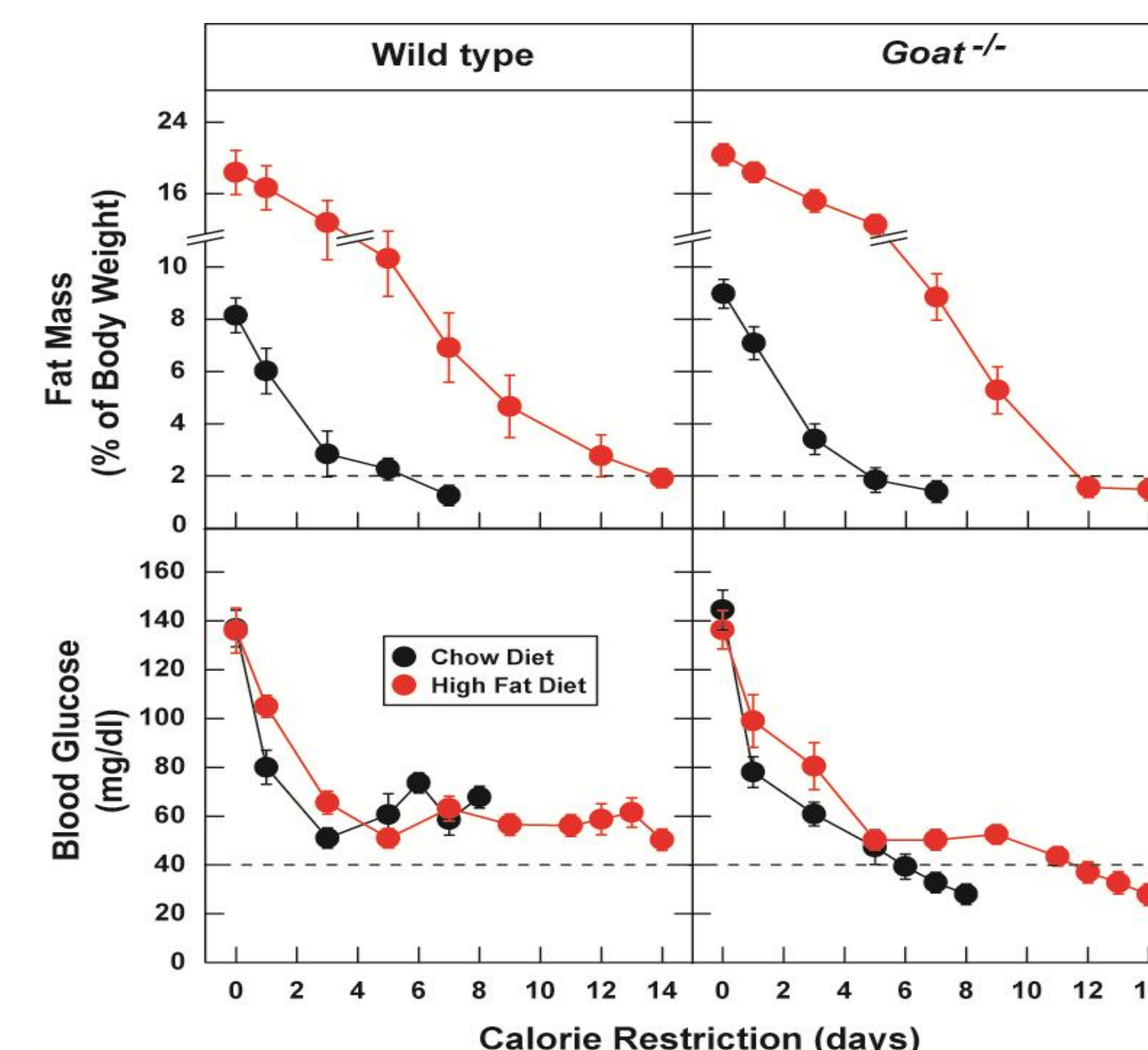


Fig. 4 Hypoglycemia in *GOAT*^{-/-} Mice Occurs Only When Total Body Fat Falls Below 2% of Body Weight

Methods and Materials

- 32-34 week old WT and *Goat*^{-/-} mice (8 in each group)
- Mice were housed individually
- Week 1, measure basal food intake
- Week 2, start 60% calorie restriction by feeding each mouse 40% of its basal food intake at 5:30 pm each day
- Monitor fat mass (nuclear magnetic resonance) and blood glucose (blood glucose meter) every 1-2 days right before feeding

Results

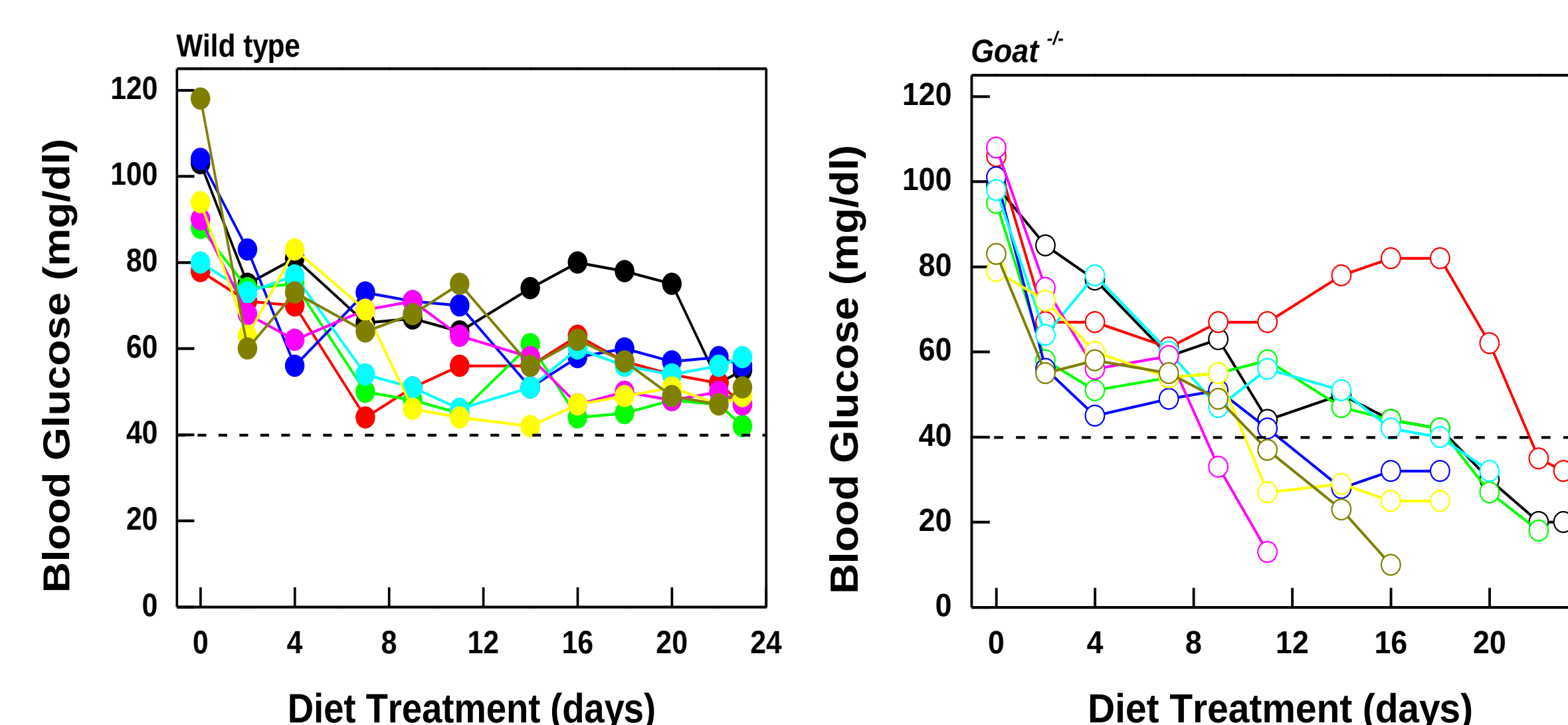


Fig. 5 Changes in Blood Glucose of Calorie-Restricted 32-34 week old mice

Results

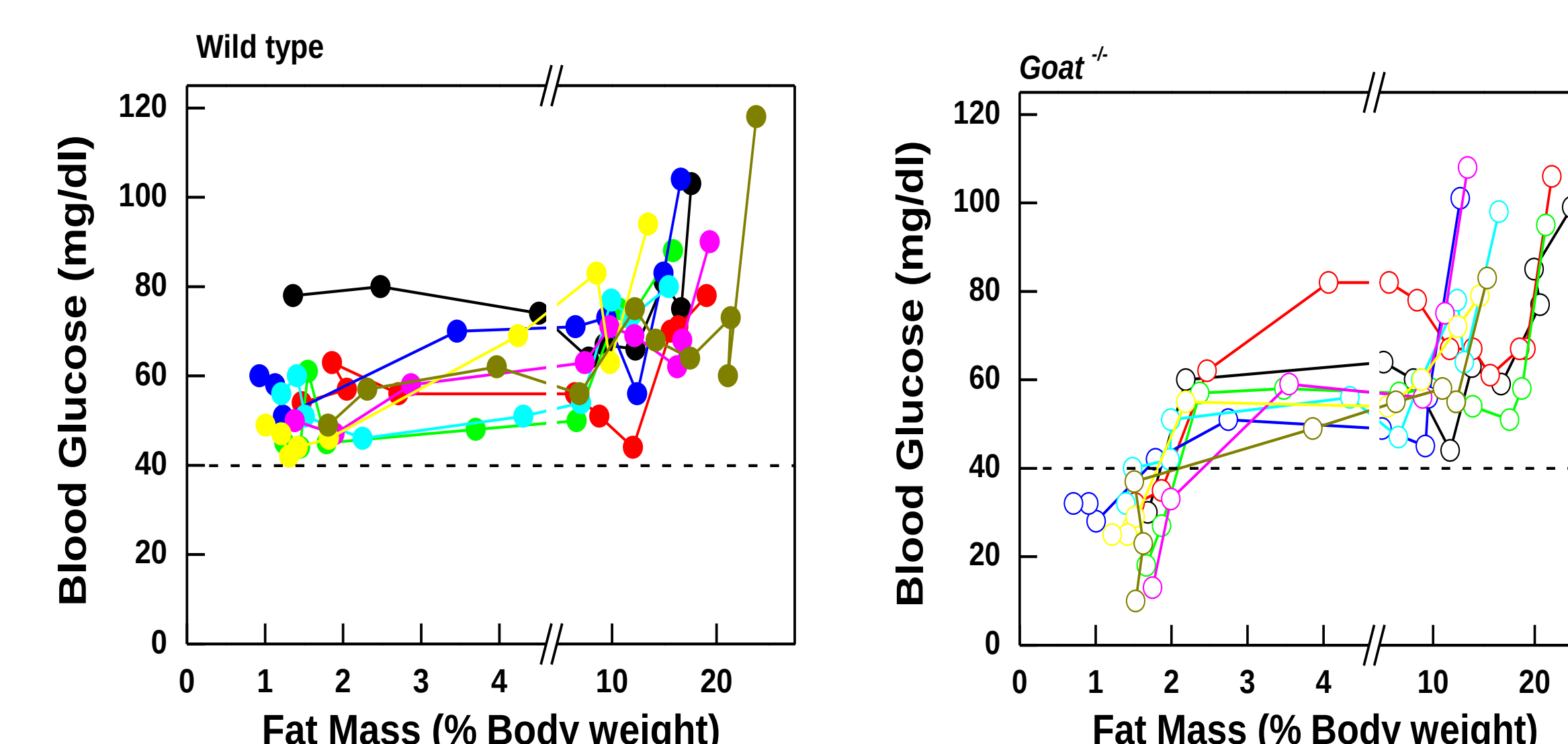


Fig. 6 Blood Glucose vs Fat Mass of Calorie-Restricted 32-34 week old mice during 60% Calorie Restriction

Conclusion

- For the first 7 days of calorie restriction, both wild type and GOAT knockout mice were able to maintain their blood glucose around 60 mg/dl.
- GOAT KO mice started to develop hypoglycemia when their body fat mass dropped below 2% of the body weight.
- Wild type mice were able to maintain their blood glucose level above 40 mg/dl throughout the course even when their fat mass dropped below 2% of their body weight.
- Results here further confirm that in order to develop hypoglycemia in the GOAT knockout mice, the fat mass needs to be depleted (less than 2% of body weight) from these mice during calorie restriction
- Assumption shown to be true in older mice (32-34 weeks vs. 8 weeks old)