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human reproduction SUPPLEMENTARY DATA

Supplementary Material and Methods

Food intake

Food intake was measured at time 0, 1, 3, 6, 12, 24 and 36 months of treatment, for a period of 5–14 days. Animals are separated for their morning meal from 09:30 to 11:00 h, and again from 14:00 to 15:00 h, then allowed paired access from 15:00 to 17:00 h. Individual food intake from the morning was summed across pairs and combined with afternoon paired intake to give daily kcal/pair. Daily caloric intake per pair was averaged across the 5–14 days sampling period for each time point.

Hormone assays

Insulin concentrations in monkey plasma were determined using a chemiluminescence-based automatic clinical platform (Roche Diagnostics Cobas e411, Indianapolis, IN); this assay was used previously in nonhuman primates (Varlamov *et al*., 2010). The range of the insulin assay is 0.2–1000 uIU/ml. The intra- and inter-assay variations were <7%.

Leptin concentrations were measured by radioimmunoassay following the manufacturer's instructions (Millipore HL-81K, Billerica, MA). This assay was described previously for the nonhuman primate (Power *et al*., 2013). The assay range was 0.78–100 ng/ml and intra- and inter-assay variations were 13.9% and 24.7%, respectively.

Total adiponectin levels were measured by ELISA following the manufacturer's instructions (Alpco 80-ADPHY-E01, Salem, NH) and was previously validated in nonhuman primates (Swarbrick *et al*., 2009). The assay range was 0.075–4.8 ng/ml. Intra- and inter-assay variations for total adiponectin were 1.9% and 10.9%, respectively.

Plasma ghrelin levels were measured by RIA following the manufacturer's instructions (Phoenix RK-031-31, Burlingame, CA) and this assay was described previously in humans (Weigle *et al*., 2003). The assay range was 10–1280 pg/ml and intra- and inter-assay variations were 3.2% and 12.2%, respectively.

C-reactive protein was measured by ELISA following the manufacturer's instructions (Alpco 30–9710 s). This assay was described previously in humans (Harris *et al*., 2013), and the assay range was 1.9–150 ng/ml and intra- and inter-assay variations were 4.2% and 21.6%, respectively.

Supplementary Material References

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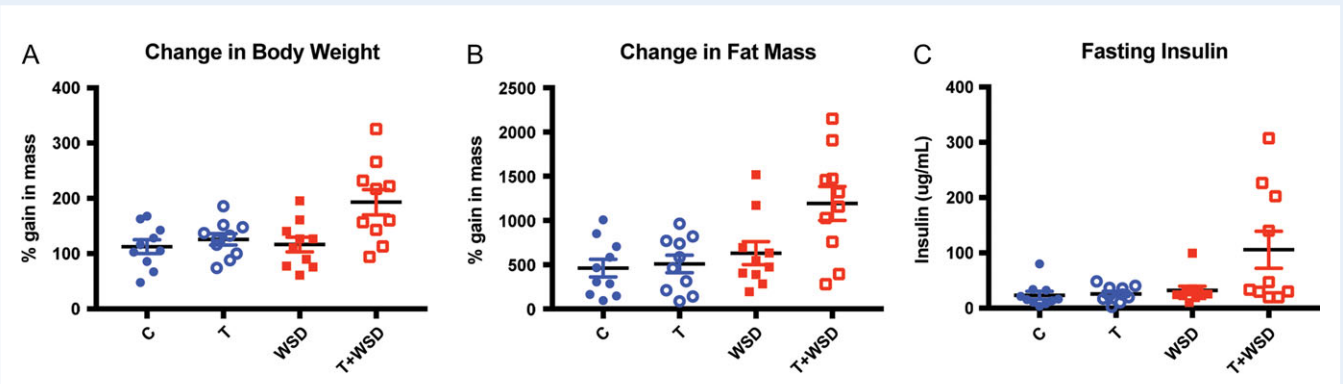

Supplementary Figure S1 Individual variability in metabolic measurements following Control (C), Testosterone (T), western-style diet (WSD), and T + WSD treatment. Horizontal lines represent the mean with $n = 10$/group. Change in body weight (**A**), fat mass (**B**) and fasting insulin (**C**) are shown for the 36-month treatment time point.