SUPPLEMENTARY DATA

**Supplementary Figure 1.** Flow diagram of the study design (a) and the clamp procedure (b). See also methods for details. (a) After run-in, subjects were randomly assigned to once daily dose of glargine either evening (22.00 h, N=5), or morning (10.00 h, N= 5). After 9 days of treatment, subject underwent a 24-h glucose clamp experiment after s.c injection of basal insulin glargine according to the dosing schedule followed. After wash-out period, subjects received the alternate treatment, and at the end of 9 days treatment they were studied again with the euglycemic clamp technique for 24 h.

(b) All clamps began after low dose insulin infusion (insulin feedback phase), at least 4 hrs before s.c. insulin injection (glargine 0.4 U/kg), given at 10:00 h or 22:00 h (time 0 of clamp). At -120 min a constant infusion of [6,6-2H2]-glucose was started and maintained throughout the experiment to determine glucose kinetics. A variable rate of 20% dextrose solution enriched to 4% with [6,6-2H2]-glucose was used to clamp the plasma glucose at the desired target of 100 mg/dl for 24 h. Blood for experimental parameters was sampled throughout the experiment at pre-specified interval