

Early Time-Restricted Feeding Reduces Appetite and Increases Fat Oxidation but Does Not Affect Energy Expenditure in Humans

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METHODS

Participants: Individuals were excluded from the study if they had diabetes or significant gastrointestinal, cardiac, renal, liver, lung, or nervous system disease; regularly used antidiabetic medications, steroids, beta blockers, adrenergic-stimulating agents, laxatives, or any medications or supplements known to affect sleep, circadian rhythms, or metabolism; were pregnant or lactating; used Depo Provera, an IUD, or a hormonal patch for birth control; or changed their hormonal birth control dose within the last 3 months. They were also excluded if they performed overnight shift work; had irregular sleep and/or eating schedules; regularly fasted for more than 15 hours/day; smoked or used nicotine/tobacco products within the last 3 months; consumed an average of more than 3 servings of alcohol per day; or regularly engaged in competitive sport training. None of the enrolled participants were part of the first eTRF trial reported in (31).

Serum and Urine Chemistry. Leptin, active ghrelin, and PYY were measured by RIA Kits (EMD Millipore Corporation, Billerica, MA) on a gamma counter (Wizard 2470; PerkinElmer, Inc.; Waltham, MA). GLP-1 was measured using an ELISA kit (EMD Millipore Corporation, Billerica, MA) on a Bio Rad Microplate reader (Bio-Rad Laboratories, Inc.; Hercules, CA). Urine collected during the respiratory chamber testing was assayed for nitrogen by pyrochemiluminescence on an Antek 9000 Series Nitrogen & Sulfur Analyzer (Antek Instruments, Inc.; Houston, TX) to quantify protein oxidation.

Statistical Methods: Randomization was generated in SAS by the statistician (RAB). Randomization was concealed from participants until enrollment, and the enrollment and assignment to the intervention sequence were performed by the research coordinator. Although 11 participants completed the trial, respiratory chamber data were analyzed for only 10 completers due to technical problems with the CO₂ analyzer that led to data loss for one

participant. All other collected data were included in the analysis, with the exception that we excluded chamber data corresponding to excess participant motion during the measurement of resting energy expenditure in the morning; the exclusion was performed while being blinded to the treatment assignment.

SUPPLEMENTARY FIGURES

Supplementary Figure S1. Distribution of individual values for the difference in 24-hour energy expenditure (EE), which was calculated as the early time-restricted feeding (eTRF) value minus the control arm value. Although eTRF increased 24-hour energy expenditure in 8 out of 10 participants (median value of 24 kcal/day), the mean difference was not statistically or clinically significant ($\Delta=10\pm 16$ kcal/day; $p=0.55$).

