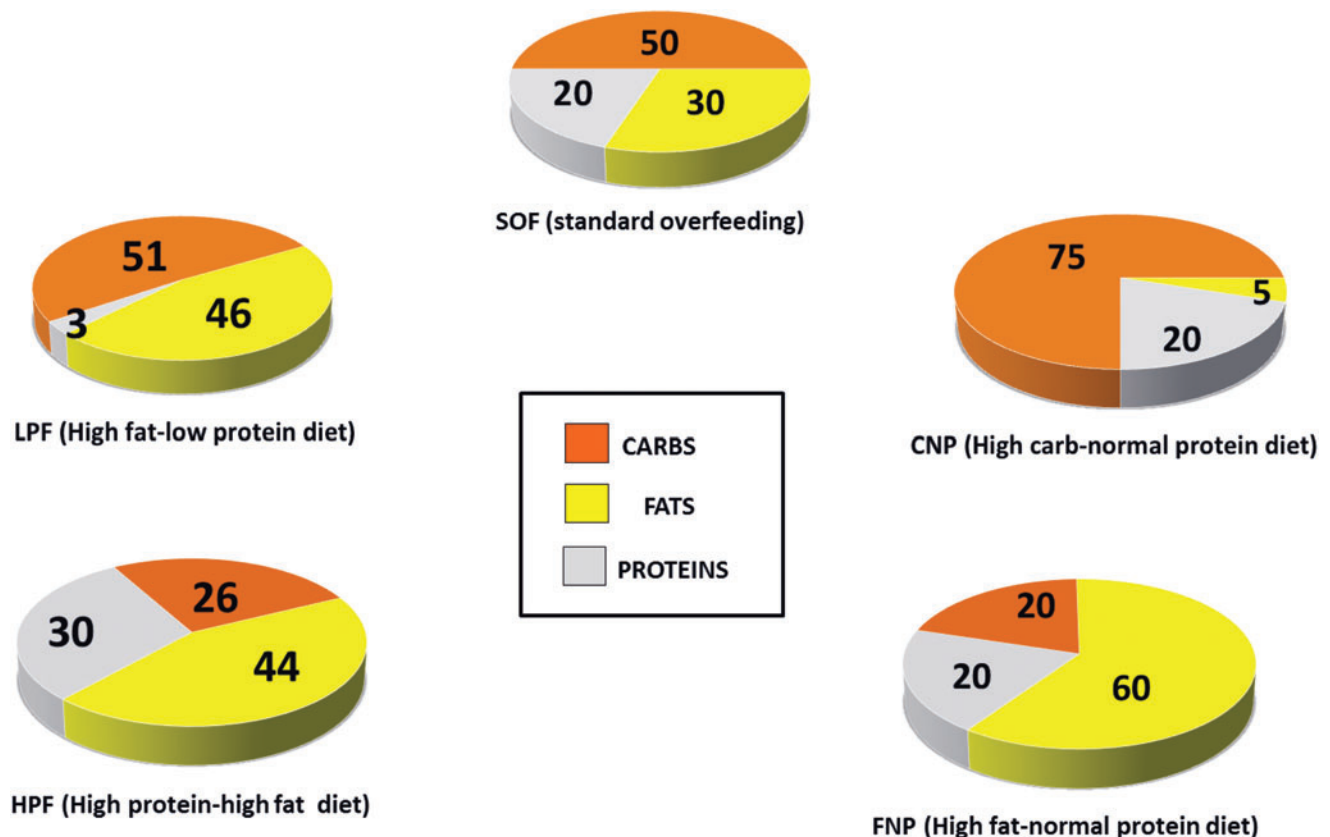


**SUPPLEMENTARY FIG. S2.** Study design. After admission, the volunteers were fed a WMD for 4 days. The subjects had two 24-hour EE assessments in the chamber for energy balance evaluation. Energy intake during the first eucaloric chamber (EB0) was calculated as 80% the WMD to account for decreased physical activity in the calorimeter. To achieve an energy balance close to zero during the measurement of 24-hour EE inside the calorimeter, the energy intake for the second eucaloric assessment (EB) was equal to the EE measurement of the first chamber session (EB0). The 24-hour EE from this second eucaloric assessment (EB) was doubled to determine the number of kilocalories given for the subsequent overfeeding diets and was used as the baseline comparator. Subsequently, each volunteer completed six EE measurements during the dietary interventions, five different overfeeding diets and fasting, done in random order and with 3 days period of WMD between interventions. Blood for measurements of plasma fT3, fT4, and FGF21 concentrations was collected in the morning after overnight fasting both before and upon exit the whole-room indirect calorimeter during each dietary intervention. CNP, high-carbohydrate normal-protein overfeeding with 75% carbohydrate, 5% fat, and 20% protein; DXA, dual-energy X-ray absorptiometry; EB0 and EB, energy balance diet with 50% carbohydrate, 30% fat, and 20% protein; EE, energy expenditure; FGF21, fibroblast growth factor 21; FNP, high-fat normal-protein overfeeding with 60% fat, 20% carbohydrate, and 20% protein; FST, 24-hour fasting; fT3, free triiodothyronine; fT4, free thyroxine; HPF, high-protein high-fat overfeeding with 26% carbohydrate, 44% fat, and 30% protein; LPF, low-protein high-fat overfeeding with 51% carbohydrate, 46% fat, and 3% protein; OGTT, oral glucose tolerance test; RQ, respiratory quotient; SOF, standard overfeeding with 50% carbohydrate, 30% fat, and 20% protein; WMD, weight-maintaining diet.



**SUPPLEMENTARY FIG. S3.** Macronutrient composition of overfeeding diets. The pie charts represent each overfeeding diet with the orange portion showing carbohydrate (CARBS), the yellow the fat (FATS), and light gray the protein (PROTEINS) content expressed as percentage of total calories given during the 24 hours.