Supporting Information

Mediterranean Versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates

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Methods

Subjects. This study was carried out in a well-established nonhuman primate (NHP) model, the omnivorous cynomolgus macaque (*Macaca fascicularis*, aka crab-eating or long-tailed macaque*)*.

Diet Storage. Diets were made in 10 kg batches, stored frozen at -20°C, and daily portions thawed under refrigeration just before feeding. Extra-virgin olive oil was stored under argon to prevent oxidation of polyphenols. Samples were kept from each batch, and samples from three separate batches were assayed for macronutrient and fatty acid content (one early, one midway, and one from the end of the study) to assure diet composition was stable over the course of the study.

CT Scans. Whole body CT scans acquired on a Toshiba Aquillion 32 Multislice Fast Whole Body CT Scanner. Scans were performed at 120 kVp and 300 mA and a helical pitch of 21, pitch factor=0.625, with 0.5 sec rotation speed. Contiguous 0.5 mm axial slices were then networked to an Aqnet1 thin-client PACS. MIMICS software was used to determine relative volumes of the different tissue compartments.

Caloric Content of the Experimental Diets. Caloric content of the experimental diets was calculated from the nutrition fact labels, material data safety sheets, or USDA Food Composition Databases for each ingredient; monkey chow calorie content was reported by the manufacturer.

Measurement of Food Consumption. Diet was weighed before and after the meal and calories consumed were calculated. This worked well for the WEST and MED diets which had the consistency of cookie dough. However, chow biscuit weights were variable due to absorbed moisture; thus weight-based caloric intakes could not be used. Instead we counted biscuits consumed, determined the average weight of a dry biscuit by weighing 165 biscuits, and used biscuit consumption as a measure of food intake during the Baseline phase. Since the chow and experimental diet consumption were measured differently they are graphed separately. At the end of treatment month 12, feeding was changed from 1 feeding/day for 2 hours to 2 feedings/day for 1 hour for each. WEST diet group intake declined after 6 months and flattened out after about a year. MED diet group intake increased steadily from the 3-6 month time point until the end of the study. No obvious change in intake occurred around the 12 month mark when the feeding strategy changed; consumption at time 7-10 months was not significantly different then consumption at 17-20 months (2 diet groups X 2 time points ANOVA, all p's>0.20).

Activity and Energy Expenditure. Four weeks prior to data collection, the monkeys were sedated (15 mg/kg ketamine HCl) and outfitted with nylon mesh jackets for two days to habituate them to the jackets. Thereafter, one day prior to data collection, the monkeys were sedated (15 mg/kg ketamine HCl) and outfitted with Actigraph GT3X monitors (Actigraph, Pensacola, FL, USA) in the pocket of the mesh jacket, and allowed to recover for 24 hours before recording. The monitor sensor in the Actigraph GT3X monitor detects acceleration in all directions, integrates speed and distance of acceleration producing an electrical current that varies in magnitude with change in acceleration which is stored as activity counts. (15). Preliminary analyses showed no

difference between treatment month 11 and 28 in activity or energy expenditure; thus treatment phase averages were used in analysis.

Glucose and Triglyceride Concentrations. Glucose and triglyceride concentrations were determined in plasma samples by colorimetric assay using reagents (ACE-GLU and ACE-TG) and instrumentation (ACE ALERA autoanalyzer) from Alfa Wasserman Diagnostic Technologies (West Caldwell, NJ; (1)). Insulin was determined by enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden).

Data Analysis. Analyses were conducted with STATISTICA release 13 (Dell, Aliso Viejo, CA). BMI, body fat, food intake, activity level, insulin responses, TG levels and hepatosteatosis were primary outcomes; BW and energy expenditure were secondary outcomes. Temporal changes should be interpreted with caution, since Treatment phase duration is naturally confounded by factors such as aging and season, and temporal changes may be exaggerated by regression to the mean (2). Thus, interpretations of temporal changes were kept to a minimum.

1. Adams MR, Golden DL, Williams JK, Franke AA, Register TC, Kaplan JR. Soy protein containing isoflavones reduces the size of atherosclerotic plaques without affecting coronary artery reactivity in adult male monkeys. The Journal of nutrition. 2005;135(12):2852-6. PubMed PMID: 16317131.

2. Torgerson DJ and Torgerson C. The Limitations of Before and After Designs. Designing Randomised Trials in Health, Education and the Social Sciences. London: Palgrave Macmillan; 2008. p. 9-16.

Figure S1

Sampling Schedule

BASELINE PHASE 7 MONTHS								EXPERIMENTAL PHASE 31 MONTHS																										
	1	2	3	3	5	6	7	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	31
BW/BMI							X						X								X						X				X			X			X
CT						X															X													X			
Food Intake					X					X	X	X	X	X	X	X	X							X	X	X	X							X	X	X	X
Activity/EE				X														X																	X		
ivGTT						X																											X				
TG					X																										X						

Figure S2