Supplementary Data

Supplemental Table 1. Composition of Western and Mediterranean experimental diets[1,2]

Western diet		Mediterranean diet	
Ingredient	**g/kg**	**Ingredient**	**g/kg**
Casein, USP	85.0	Casein, USP	17.4
Whey protein – 895	85.0	Whey protein – 895	17.4
		Dried egg white	26.1
		Fishmeal (Menhaden)	26.1
		Walnuts	8.7
		Black bean flour	43.5
		Garbanzo bean flour	17.4
		Wheat flour (all purpose)	243.5
Dextrin	260.0	Dextrin	96.6
Sucrose	180.0	Sucrose	34.8
High fructose corn syrup 55	70.0	Banana	130.4
		Applesauce	38.2
		Tomato paste	17.4
Cellulose (Alphacel)[1]	79.4	Cellulose (Alphacel)[1]	94.8
Lard	41.5	Olive oil (Filippo Berio Extra Virgin)	61.7
Beef tallow	40.0	Menhaden oil (Omegapure)	8.7
Butter, lightly salted	12.5	Butter, lightly salted	8.7
Corn oil	35.0	Corn oil	10.4
Flaxseed oil	3.0	Flaxseed oil	1.7
Dried egg yolk	6.0	Dried egg yolk	14.8
Crystalline cholesterol	0.4		
Complete vitamin mix[3] (Teklad 85529)	25.0	Complete vitamin mix[3] (Teklad 85529)	21.7
Mineral mix[4] (without Ca, P, NaCl) (Teklad 140306)	50.0	Mineral mix[4] (without Ca, P, NaCl) (Teklad 140306)	43.5
Calcium carbonate	4.3	Calcium carbonate	3.7
Calcium phosphate, monobasic	7.5	Calcium phosphate, monobasic	6.5
NaCl (table salt)	16.0	NaCl (table salt)	6.3
Total	**1000**	**Total**	**1000**

[1]Total Fiber (% of diet): Western: 7.94, Mediterranean: 12.7
[2]Caloric density of diets: Western: 3.42 kcal/g, Mediterranean: 2.64 kcal/g
[3]Vitamin mix composition: Vitamin A palmitate (500,000 IU/g) (1.8 g/kg), vitamin E DL-alpha tocopheryl acetate (2.5 g/kg), inositol (5.0 g/kg), riboflavin (1.0 g/kg), vitamin K MSB complex (2.3 g/kg), p-aminobenzoic acid (5.0 g/kg), niacin (4.5 g/kg), pyriodoxine HCl (1.0 g/kg), thiamin (81%) (1.0 g/kg), calcium pantothenate (3.0 g/kg), vitamin B12 (0.1% in mannitol) (1.4 g/kg), biotin (0.02 g/kg), folic acid (0.1 g/kg), vitamin C, ascorbic acid, coated (97.5%) (90.0 g/kg), choline chloride (75.0 g/kg), vitamin D3, cholecalciferol (500,000 IU/g) (0.2 g/kg), dextrose monohydrate (806.3 g/kg)
[4]Mineral mix composition: Potassium carbonate (313.9 g/kg), magnesium sulfate heptahydrate (143.9 g/kg), dried ferrous sulfate (7.9 g/kg), manganese sulfate monohydrate (1.4 g/kg), zinc chloride (0.9 g/kg), cupric sulfate (0.3 g/kg), potassium iodide (0.08 g/kg), chromium acetate hydroxide (0.05 g/kg), sodium fluoride (0.02 g/kg), sodium selenite pentahydrate (0.004 g/kg), dextrin (531.6 g/kg)

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Supplemental Table 2. Macronutrient composition of Western and Mediterranean experimental diets

	Western	**Mediterranean**
% of Total Calories		
Protein	16	16
Carbohydrate	54	54
Fat	31	31
% of Total Fats		
Saturated	36	21
Monounsaturated	36	57
Polyunsaturated	26	20

Supplemental Table 3. Summary of respiration measurements in each SUIT protocol

Respiration Measurement	Description	Measurement recorded after additions of:
SUIT protocol with fatty acids		
FAO	Respiration mediated by fatty acid oxidation	octanoylcarnitine and malate
FAO + Complex I	Respiration mediated by fatty acid oxidation and complex I	octanoylcarnitine, malate, pyruvate, and glutamate
FAO + Complex I + II	Respiration mediated by fatty acid oxidation and complexes I and II	octanoylcarnitine, malate, pyruvate, glutamate, and succinate
FAO Max ETS Capacity	Maximum electron transport system capacity / maximum respiration	FCCP (titrations until maximum)
FAO ETS Rot Sensitive	Respiration sensitive to inhibition of complex I by rotenone	obtained by subtracting respiration after rotenone from max ETS capacity
FAO ETS Rot Insensitive	Respiration insensitive to inhibition of complex I by rotenone	rotenone
SUIT protocol without fatty acids		
Complex I	Respiration mediated by complex I	pyruvate, malate, and glutamate
Complex I + II	Respiration mediated by complexes I and II	pyruvate, malate, glutamate, and succinate
Max ETS Capacity	Maximum electron transport system capacity / maximum respiration	FCCP (titrations until maximum)
ETS Rot Sensitive	Respiration sensitive to inhibition of complex I by rotenone	obtained by subtracting respiration after rotenone from max ETS capacity
ETS Rot Insensitive	Respiration insensitive to inhibition of complex I by rotenone	rotenone

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Supplemental Table 4. Bioenergetic characteristics of female cynomolgus macaques fed either a Western or Mediterranean diet[1]

	Western Diet Group	**Mediterranean Diet Group**	***P*-value**
Respirometry of Permeabilized Muscle Fibers ***O_2 Flux (pmol · s^{-1} · mg $tissue^{-1}$)***			
FAO	5.4 ± 0.7 (2.9 – 10.0)	3.5 ± 0.6 (0.9 – 6.8)	0.05*
FAO + Complex I	14.8 ± 2.2 (8.6 – 31.7)	9.7 ± 1.0 (4.4 – 15.5)	0.05*
FAO + Complex I + Complex II	18.0 ± 2.2 (10.2 – 32.2)	13.4 ± 1.4 (6.6 – 19.8)	0.10
FAO Max ETS Capacity	20.1 ± 2.3 (11.6 – 34.9)	16.3 ± 1.2 (10.4 – 21.4)	0.16
FAO ETS Rotenone Sensitive	7.9 ± 1.2 (3.4 – 16.2)	6.0 ± 1.3 (3.2 – 16.4)	0.28
FAO ETS Rotenone Insensitive	12.2 ± 1.2 (7.6 – 18.7)	10.3 ± 1.1 (5.0 – 15.5)	0.26
Complex I	11.3 ± 0.9 (7.2 – 16.4)	8.7 ± 0.9 (4.2 – 12.0)	0.06
Complex I + Complex II	15.1 ± 1.0 (10.0 – 20.5)	11.6 ± 1.1 (5.9 – 15.3)	0.03*
Max ETS Capacity	23.7 ± 1.8 (16.3 – 33.0)	18.2 ± 1.7 (10.3 – 26.7)	0.03*
ETS Rotenone Sensitive	16.0 ± 1.4 (9.0 – 34.7)	12.2 ± 1.2 (6.5 – 19.5)	0.05*
ETS Rotenone Insensitive	7.7 ± 0.5 (4.4 – 11.5)	6.0 ± 0.8 (2.6 – 9.4)	0.08
Citrate Synthase Activity ***(μmol · min^{-1} · mg $protein^{-1}$)***	1.11 ± 0.10 (0.72 – 1.84)	0.98 ± 0.09 (0.46 – 1.47)	0.33

[1]Summary of all measurements obtained from respirometry of permeabilized muscle fibers with and without fatty acids and citrate synthase activity. Values are presented as mean ± SEM (range), *n* = 11 per group. Differences between groups were evaluated by unpaired two-tailed Student's t-tests and *P*-values are shown.

* Represents significant difference between groups with $P \leq 0.05$.

Supplemental Table 5. Correlations between carbohydrate metabolism phenotypes and respirometry of permeabilized muscle fibers of all female cynomolgus macaques in the study[1]

	FAO	**FAO + Complex I**	**FAO + Complex I + II**	**FAO Max ETS Capacity**	**FAO ETS Rot Sensitive**	**FAO ETS Rot Insensitive**	**Complex I**	**Complex I + II**	**Max ETS Capacity**	**ETS Rot Sensitive**	**ETS Rot Insensitive**
Pearson											
HOMA-IR	0.47*	0.56**	0.59**	0.57**	0.32	0.60**	0.06	0.02	0.14	0.27	-0.18
Fasting Blood Glucose	0.08	-0.17	-0.13	0.08	0.09	-0.08	-0.06	-0.11	0.03	0.09	-0.11
Fasting Blood Insulin	0.30	0.40	0.44*	0.40	0.23	0.46*	0.03	-0.03	0.13	0.27	-0.19
Glucose AUC	0.18	0.07	0.10	0.10	0.06	0.06	-0.27	-0.18	-0.02	0.06	-0.17
Insulin AUC	0.27	0.41	0.44*	0.29	0.26	0.34	-0.11	0.03	-0.12	-0.10	-0.11
Partial for Age + Weight											
HOMA-IR	0.48*	0.58**	0.61**	0.57**	0.27**	0.67**	0.05	-0.01	0.12	0.26	-0.20
Fasting Blood Glucose	0.12	-0.17	-0.09	0.09	0.11	-0.06	-0.17	-0.19	-0.05	0.02	-0.18
Fasting Blood Insulin	0.30	0.41	0.44	0.39	0.39	0.50*	0.01	-0.05	0.11	0.25	-0.20
Glucose AUC	0.11	-0.02	-0.01	0.07	0.07	0.04	-0.10	-0.11	0.14	0.25	-0.13
Insulin AUC	0.26	0.48*	0.48*	0.36	0.36	0.44	0.14	0.20	0.02	0.05	-0.03

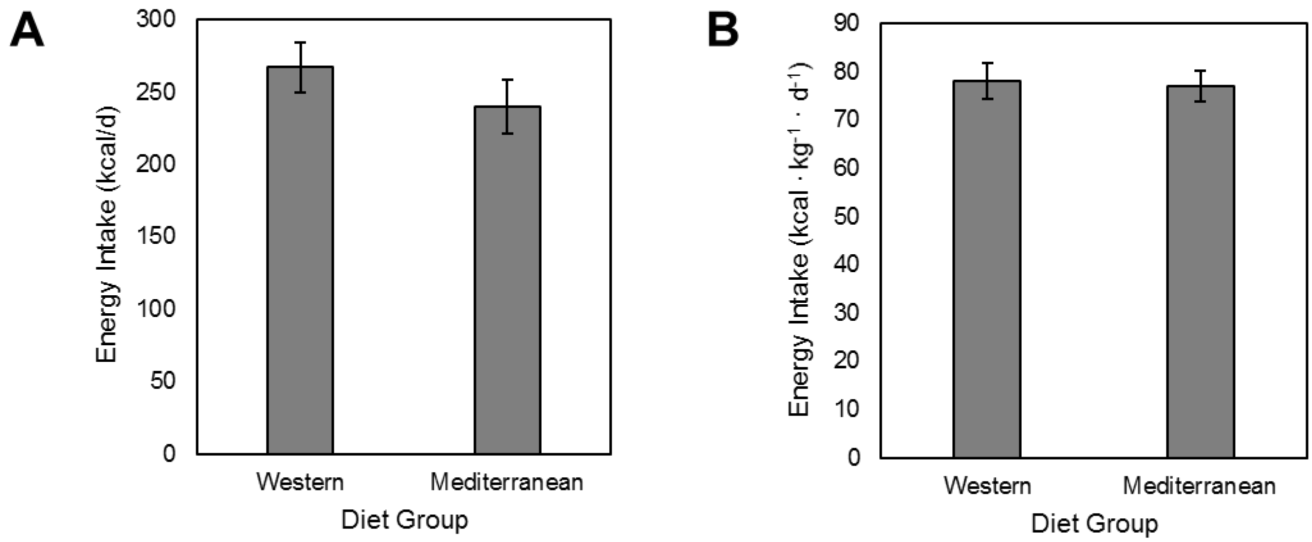
[1]Values are Pearson correlation coefficients (*R*) for relationships between measures of carbohydrate metabolism and measures of respiration of permeabilized skeletal muscle fibers. Partial correlations were controlled for age and weight at time of necropsy.

* Represents significant correlation with $P \leq 0.05$

** Represents significant correlation with $P \leq 0.01$

Supplementary Data

Supplemental Figure 1. Mean energy intake of female cynomolgus macaques fed either a Western or Mediterranean diet for 30 months

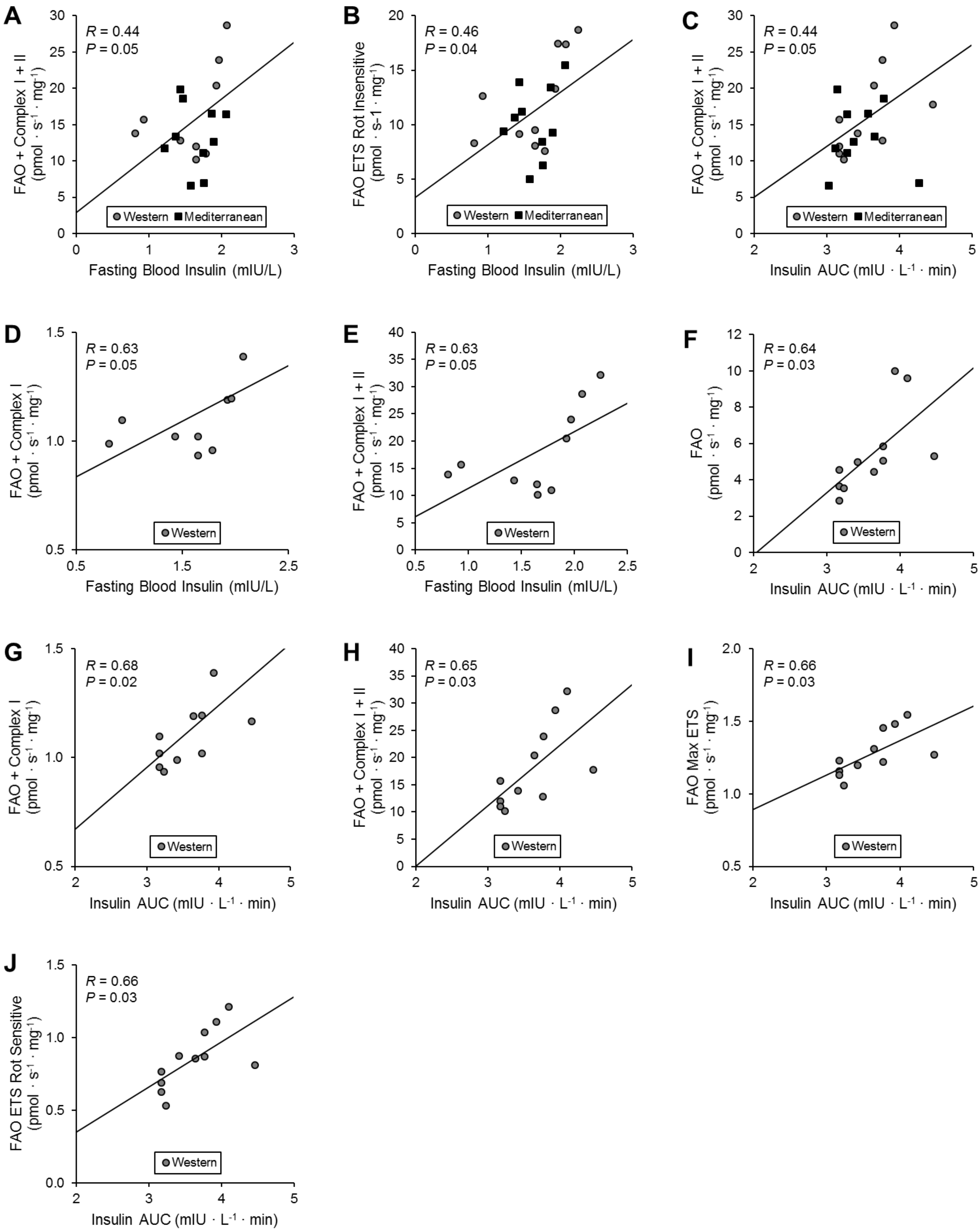


(A) Mean calories consumed per day by diet group. Data are presented as means ± SEM, *n* = 11.

(B) Mean calories consumed per kilogram of body weight per day by diet group. Data are presented as means ± SEM, *n* = 11.

Supplementary Data

Supplemental Figure 2. Related to Figure 3. Correlations between female cynomolgus macaque permeabilized muscle fiber bioenergetics and fasting blood insulin and insulin area under the curve



Supplementary Data

(A-B) Plots of fatty acid oxidation measures versus fasting blood insulin for all animals in the study. Pearson correlation (*R*) and *P*-values are shown on each plot.

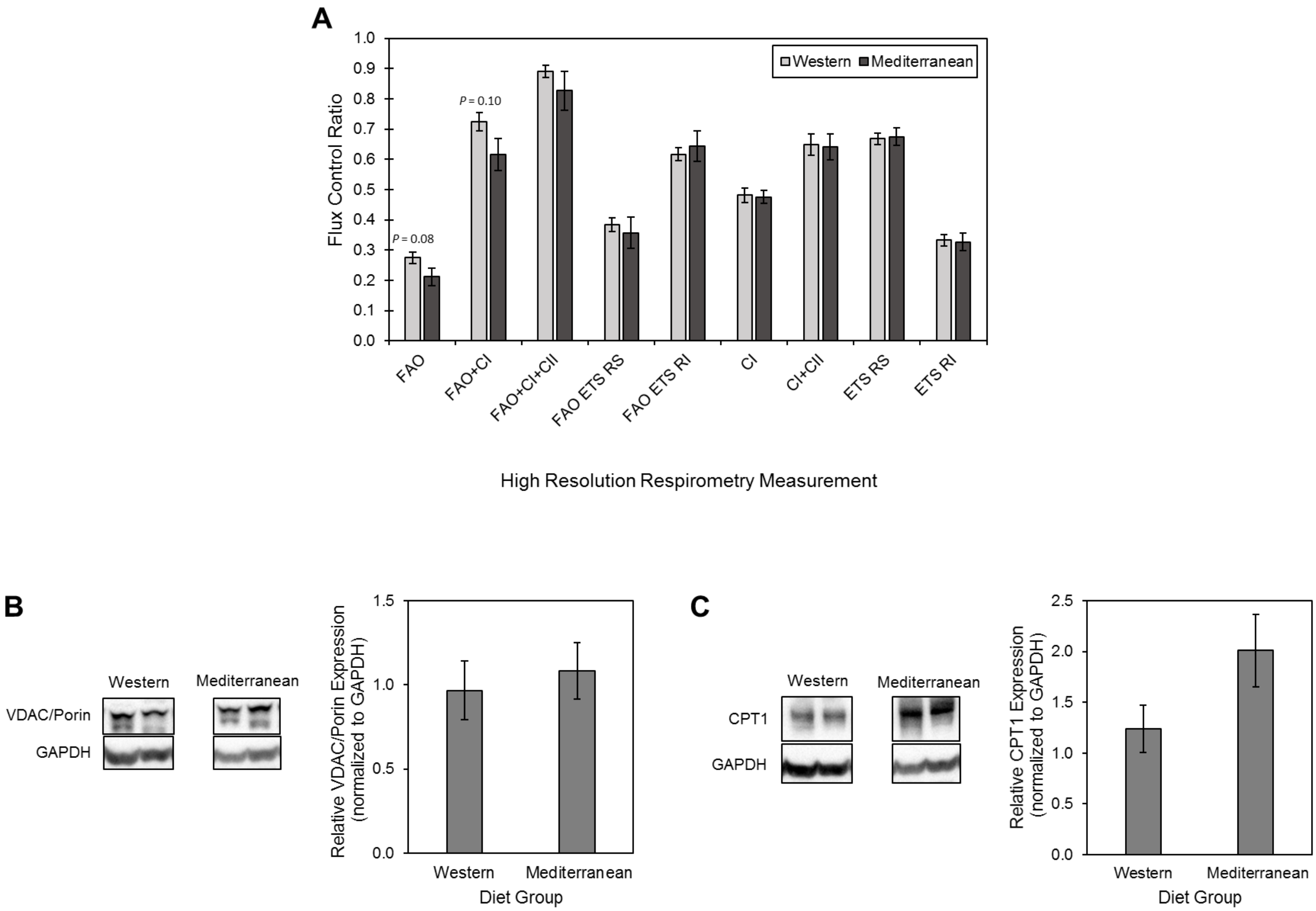
(C) Plot of FAO + Complex I + II respiration versus insulin area under the curve for all animals in the study. Pearson correlation (*R*) and *P*-value are shown on the plot.

(D-E) Plots of fatty acid oxidation measures versus fasting blood insulin for animals in the Western diet group. Pearson correlation (*R*) and *P*-values are shown on each plot.

(F-J) Plots of fatty acid oxidation measures versus insulin area under the curve for animals in the Western diet group. Pearson correlation (*R*) and *P*-values are shown on each plot.

Supplementary Data

Supplemental Figure 3. Flux control ratios and Western blot analysis of skeletal muscle from female cynomolgus macaques fed a Western or Mediterranean diet



(A) Flux control ratios (FCRs) of high-resolution respirometry of permeablized skeletal muscle fibers. FCRs were calculated by dividing each respiration parameter by maximum respiration to examine differences in relative contributions of substrates to mitochondrial respiration. Data are presented as means ± SEM, *n* = 11.

(B) Protein expression of VDAC/Porin in skeletal muscle tissue homogenate from cynomolgus macaques fed either a Western or Mediterranean diet. Data are presented as means ± SEM, *n* = 11.

(C) Protein expression of CPT1 in skeletal muscle tissue homogenate from cynomolgus macaques fed either a Western or Mediterranean diet. Data are presented as means ± SEM, *n* = 11.