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Skeletal Muscle Mitochondrial Respiration Is Elevated in Female Cynomolgus Macaques Fed a Western Compared with a Mediterranean Diet

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ABSTRACT

Background: Western diets are associated with increased incidences of obesity, hypertension, diabetes, and hypercholesterolemia, whereas Mediterranean diets, richer in polyphenols, monounsaturated fats, fruits, vegetables, poultry, and fish, appear to have cardiometabolic health benefits. Previous work has included population-based studies with limited evidence for causation or animal studies focused on single macro- or micronutrients; therefore, primate animal models provide an opportunity to determine potential mechanisms underlying the effects of dietary patterns on health and disease.

Objective: The aim of this study was to determine the effects of whole dietary patterns, either a Western or Mediterranean diet, on skeletal muscle mitochondrial bioenergetics in cynomolgus macaques.

Methods: In this study, 22 adult female cynomolgus macaques (~11–14 y by dentition) were fed either a Western or Mediterranean diet for 30 mo. The Western diet was designed to mimic the diet of a middle-aged American woman and the Mediterranean diet included key aspects of Mediterranean diets studied in humans, such as plant-based proteins and fat, complex carbohydrates, and fiber. Diets were matched on macronutrient composition (16% protein, 54% carbohydrate, and 31% fat) and cholesterol content. Skeletal muscle was collected for high-resolution respirometry, citrate synthase activity, and western blot measurements. Pearson correlation analysis between respirometry measures and measures of carbohydrate metabolism was also performed.

Results: We found that consumption of a Western diet resulted in significantly higher mitochondrial respiration with fatty acid oxidation (FAO) (53%), FAO + complex I (52%), complex I + II (31%), max electron transport system (ETS) (31%), and ETS rotenone sensitive (31%) than did consumption of a Mediterranean diet. In addition, measures of respiration in response to fatty acids were significantly and positively correlated with both insulin resistance and plasma insulin concentrations.

Conclusions: This study highlights the importance of dietary composition in mitochondrial bioenergetics and that diet can influence skeletal muscle mitochondrial respiration independently of other factors such as macronutrient composition. *J Nutr* 2019;149:1493–1502.

Keywords: Western, Mediterranean, diet, skeletal muscle, mitochondria, bioenergetics, nonhuman primates

Introduction

Typical Western diets contain high amounts of saturated fats, sucrose and fructose, proteins from red meats, and sodium, and low amounts of monounsaturated and polyunsaturated fats. Consumption of a Western diet has been associated with increased incidences of obesity, all-cause mortality, cancer, kidney disease, osteoporosis, hypertension, type 2 diabetes, and hypercholesterolemia (1–3). In contrast, Mediterranean diets are composed of high amounts of monounsaturated fats from mainly plant sources such as olive oil, fruits, vegetables, and proteins from poultry and fish sources. Consumption of a Mediterranean diet has been associated with reduced risk of developing diabetes, cancer, cardiovascular disease, and Alzheimer disease (4–7). These associations with dietary patterns have been elucidated primarily by population-based epidemiological studies; therefore, there is

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limited evidence for causation and a need for mechanistic insights.

Animal studies have reported that a high-fat diet increases fatty acid– but not pyruvate-, malate-, or glutamate-mediated respiration of mitochondria isolated from skeletal muscle (8–10). Increased expressions of oxidative phosphorylation proteins and uncoupling protein 3 were also observed. When mice were fed a high-fat and high-sucrose diet, increased mitochondrial respiration in permeabilized skeletal muscle fibers, increased citrate synthase (CS) and carnitine palmitoyl transferase (CPT) activity, and increased expression of uncoupling protein 3, CS, and complex I were observed (11). Rodents in these studies were obese and insulin resistant, but not overtly diabetic. In humans, previous studies demonstrate decreased mitochondrial enzyme activity in subjects with type 2 diabetes and obesity. Obese subjects had lower succinate dehydrogenase (complex II) activity (12), lower CPT activity (13), and lower cytochrome c oxidase activity (complex IV) (14). When mitochondrial function was examined by respirometry, no differences in respiration between obese and lean subjects were observed (15). Diabetic subjects had lower NADH:ubiquinone oxidoreductase (complex I) activity and CS activity (16). Diabetics also exhibited lower skeletal muscle mitochondrial respiration than healthy controls (17). These subjects also had decreased levels of mitochondrial DNA and CS activity, suggesting that there was lower mitochondrial density in their skeletal muscle, a potential cause for their decreased mitochondrial respiration.

In this study, we have examined the effects of whole dietary patterns, either a Mediterranean or a Western diet, on skeletal muscle mitochondrial bioenergetics. It is unlikely that the negative effects of a Western diet or the positive effects of a Mediterranean diet are due to a single dietary component. To our knowledge, the study presented here is the first to report on the effects of whole dietary patterns on skeletal muscle mitochondrial bioenergetics. We utilized cynomolgus macaques to examine the effects of whole diets administered for 30 mo, equivalent to ~8 human years. We performed bioenergetic profiling of skeletal muscle comprised of high-resolution respirometry of permeabilized muscle fibers with and without fatty acid substrates, tissue CS activity, and western blot analyses.

Methods

Experimental model and study design

The ancillary study described here examined 22 randomly selected female cynomolgus macaques (*Macaca fascicularis*) ranging in age from ~11 to 14 y that were available from a larger parent study. The parent study included 42 female cynomolgus macaques and was designed to test the effects of Mediterranean compared with Western diet on a broad range of health outcomes (18).

Animals were housed in small social groups of 3–4 in pens measuring 3.3 m × 3.3 m × 3.3 m, on a 12 h/12 h light/dark schedule. Animals were fed either a Western or Mediterranean diet designed and produced by the Primate Nutrition and Diet Laboratory at Wake Forest School of Medicine for 30 mo. For the parent study, animals were randomly assigned to either diet treatment using stratified randomization, balanced on pretreatment characteristics that reflect overall health, including body weight, BMI, basal cortisol, and plasma lipid concentrations. Diet composition is shown in **Supplemental Table 1** and macronutrient composition is shown in **Supplemental Table 2**. These semipurified diets were matched on protein, fat, carbohydrate, and cholesterol content. The Western diet was designed to be similar to consumption of middle-aged American women, with protein and fat derived from mainly animal sources and high in saturated fats and sodium (19). The Mediterranean diet was designed to mimic key aspects of the Mediterranean diet, including protein and fats from mainly plant sources, higher amounts of monounsaturated fats, high in complex carbohydrates and fiber, and lower amounts of sodium (20, 21). In particular, the Mediterranean diet included English walnut powder and extra virgin olive oil, which were key ingredients included in the PREDIMED study (22). Water was available ad libitum. Skeletal muscle tissue (*Vastus lateralis*) for bioenergetics analyses was collected at the time of necropsy. Animals were first sedated with intramuscular ketamine hydrochloride (15 mg/kg body weight), then intravenous sodium pentobarbital (~13 mg/kg body weight) was administered to achieve surgical anesthesia, and animals were exsanguinated in accordance with guidelines established by the Panel on Euthanasia of the American Veterinary Medical Association. All procedures were conducted in compliance with state and federal laws, standards of the US Department of Health and Human Services, and the Animal Care and Use Committee of Wake Forest School of Medicine. All procedures and protocols were reviewed and approved by the Wake Forest School of Medicine Animal Care and Use Committee.

Body mass measurements

Body length (suprasternal notch to pubic symphysis) was measured during month 27 of the treatment phase and body weight was measured on the day of necropsy. BMI (in kg/m^2) was calculated as described previously (23).

Insulin, glucose, and insulin sensitivity measurements

Intravenous glucose tolerance tests with insulin responses were done during treatment phase month 26 as previously described (24). Briefly, animals were sedated with intramuscular ketamine hydrochloride (15 mg/kg body weight) after being deprived of food for 18 h, then administered 500 mg dextrose/kg body weight. Blood samples were taken at 0, 5, 10, 20, 30, 40, and 60 min. Glucose AUC and k were calculated as previously described (23). Insulin AUC was calculated using insulin responses between 10 and 40 min. Glucose concentrations were determined by colorimetric assay using reagents and instrumentation (ACE Alera autoanalyzer) from Alfa Wasserman Diagnostic Technologies (25). Insulin was determined by ELISA (Mercodia). HOMA-IR was calculated as described previously (26).

Energy intake measurements

Energy intake was measured in the parent study (18). Briefly, each monkey was offered 120 kcal diet per kilogram of body weight per day for the duration of the study (30 mo) and experimental diets were weighed before and after the meal to calculate calories consumed. In addition, all monkeys were provided enrichment several times per week, including flavored noncaloric ice cubes 2 times/wk, rice krispies 1 time/wk, and low-calorie vegetables such as celery 1 time/wk.

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Supplemental Tables 1–5 and Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: CPT, carnitine palmitoyl transferase; CS, citrate synthase; ETS, electron transport system; FAO, fatty acid oxidation; FCCP, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone; Rot, rotenone; SUIT, substrate-uncoupler-inhibitor titration.

Preparation of permeabilized skeletal muscle fibers

Immediately after killing, skeletal muscle tissue (*Vastus lateralis*) was removed. Approximately 10–15 mg tissue was selected for mechanical separation as described previously (27). The tissue was placed in ice-cold BIOPS (10 mM Ca-EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM potassium morpholineethanesulfonic acid, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1). The tissue was cut into smaller bundles of ~5 mg and separated mechanically with sharp angular forceps under magnification, permeabilized with saponin (30 mg/mL) for 30 min on ice, and washed with MiR05 (110 mM sucrose, 60 mM K^+-lactobionate, 0.5 mM EGTA, 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 1 mg/mL BSA, pH 7.1) or buffer Z (105 mM K-MES, 30 mM KCl, 1 mM EGTA, 10 mM K_2HPO_4, 5 mM $MgCl_2$·6H2O, 0.5 mg/mL BSA, pH 7.4) for 15 min on ice before analysis.

High-resolution respirometry of permeabilized skeletal muscle fibers

Two substrate-uncoupler-inhibitor titration (SUIT) protocols were used to examine mitochondrial bioenergetics with and without fatty acid oxidation (FAO). Approximately 2.5 mg of tissue was added to each chamber and steady-state rate of respiration measurements were obtained after every substrate addition and expressed as picomoles per second per milligram.

For the SUIT protocol with fatty acids, high-resolution oxygen flux measurements were conducted in 2 mL MiR06Cr (MiR05 containing 20 mM creatine and 280 U/mL catalase) using the Oroboros Oxygraph-2k (O2k; Oroboros Instruments). This protocol was adapted from Pesta and Gnaiger (27) and completed as follows: 7.5 mM ADP, 0.5 mM octanoylcarnitine, 2 mM malate, 10 μM cytochrome c to test for mitochondrial membrane integrity, 5 mM pyruvate, 10 mM glutamate, 50 mM succinate, 2 additions of 0.25 μM carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) followed by a titration of 0.5 μM FCCP to obtain maximal electron transport system (ETS) capacity, 10 mM glycerol-3-phosphate, 0.5 μM rotenone (Rot), and 5 μM antimycin-A.

For the SUIT protocol without fatty acids, high-resolution oxygen flux measurements were conducted in 2 mL buffer Z containing 20 mM creatine and 25 μM blebbistatin to inhibit contraction (28). This protocol was completed as follows: 2 mM malate, 4 mM ADP, 20 mM pyruvate, 10 mM glutamate, 10 mM succinate, 10 μM cytochrome c to test for mitochondrial membrane integrity, 2 additions of 0.25 μM FCCP followed by a titration of 0.5 μM FCCP to obtain maximal ETS capacity, 0.5 μM Rot, and 5 μM antimycin-A.

Respiration parameters measured in these SUIT protocols are summarized in **Supplemental Table 3**.

To further examine the effects of diet on mitochondrial bioenergetics we also calculated flux control ratios. Flux control ratios were calculated by dividing each respiration parameter by maximum respiration (27).

CS activity assay

CS activity was determined according to the manufacturer's instructions (Citrate synthase assay kit, Sigma CS0720). Briefly, skeletal muscle (*Vastus lateralis*) was homogenized in cold CelLytic MT (Sigma C3228) at pH 7.4 and a protease inhibitor cocktail (Sigma P8340). The homogenized sample was centrifuged at 12,000 × *g* for 10 min at 4°C and the supernatant containing the protein was collected. Protein concentration was determined by bicinchoninic acid protein assay (Pierce). CS activity was measured by continuous spectrophotometric rate determination at 412 nm. Each sample was run in triplicate.

Western blotting

Skeletal muscle (*Vastus lateralis*) homogenate was loaded at a concentration of 30 μg total protein per well for separation by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked at room temperature for 1 h in 5% nonfat dry milk in tris-buffered saline containing 0.1% Tween-20. Blots were probed overnight at 4°C with primary antibodies (VDAC/Porin, Abcam, ab15895; GAPDH, Abcam, ab9484; CPT1b, Invitrogen, PA5-12218) and incubated with the appropriate HRP-conjugated anti-IgG antibody. Antibody-bound protein was detected by enhanced chemiluminescence and quantified by densitometry with ImageJ (National Institutes of Health).

Statistical analysis

Distributions of all variables were examined before any further analysis. Normality was assessed by Shapiro–Wilk tests and any variables that were not normally distributed were log transformed to achieve a normal distribution (FAO + complex I, FAO max ETS capacity, FAO ETS Rot sensitive, body weight, fasting glucose, fasting insulin, glucose AUC, and insulin AUC). Statistical significance between groups was evaluated by unpaired 2-tailed Student's *t* tests using Microsoft Excel software. Significance between groups was defined as $P \leq 0.05$. Pearson correlation coefficients were assessed between all variables and partial correlations were adjusted for age and weight. Analysis was performed using SAS Enterprise Guide version 7.12 (SAS Institute Inc.).

Results

Demographic and bioenergetic characteristics of nonhuman primate subjects

Age, weight, BMI, HOMA-IR, fasting blood glucose, fasting blood insulin, glucose AUC, and insulin AUC for the subcohort of animals utilized for this ancillary study are summarized in **Table 1**. Cross-sectionally, there were no statistically significant differences between groups for any of these characteristics. However, this ancillary study was not adequately powered for between-group differences in these characteristics. In the larger parent study, body weight was significantly increased in the Western diet group at all time points after 6 mo on the diet; these changes were not observed in the Mediterranean diet group (18). In addition, the parent study found that Western diet resulted in increased body fat, activity, energy expenditure, TG concentrations, insulin resistance, and hepatosteatosis when compared with the Mediterranean diet (18).

Overall energy intake for animals in this ancillary study was not significantly different between the Western or Mediterranean diet groups, both in mean calories consumed ($P = 0.30$) and in mean calories consumed per kilogram of body weight ($P = 0.83$) (**Supplemental Figure 1**).

Representative bioenergetic profiles from a single animal are shown in **Figure 1**. All bioenergetic measurements of permeabilized muscle fiber respiration and CS activity for both diet groups are summarized in **Supplemental Table 4**.

Diet induces bioenergetic changes in permeabilized fibers

Western diet–fed primates exhibited higher oxygen flux across all respirometry measurements in permeabilized fibers than did primates fed a Mediterranean diet (**Figure 2**A). There was significantly higher respiration with FAO, FAO + complex I, complex I + II, max ETS, and ETS Rot sensitive. There were also trends for higher FAO + complex I + II, complex I, and ETS Rot insensitive.

To examine potential mechanisms of action, we measured CS activity, a marker of mitochondrial content (29, 30). Mean CS activities were similar for the Western and Mediterranean diet groups (Figure 2B). This finding was confirmed by performing western blots for VDAC/Porin, a mitochondrial structural protein. Mean VDAC/Porin protein expression relative to

TABLE 1 Characteristics at necropsy of female cynomolgus macaques fed either a Western diet or Mediterranean diet[1]

	Western diet	Mediterranean diet	*P* value
Age, y	12.3 ± 0.2 (11.5–13.2)	12.2 ± 0.2 (11.1–13.7)	0.75
Weight, kg	3.65 ± 0.40 (2.59–7.10)	3.19 ± 0.33 (2.42–5.24)	0.32
BMI, kg/m^2	46.7 ± 3.4 (36.3–73.9)	41.3 ± 2.5 (31.7–56.4)	0.18
HOMA-IR	13.4 ± 3.2 (1.0–34.2)	11.4 ± 2.4 (2.9–26.2)	0.62
Fasting blood glucose, mg/dL	78 ± 3 (61–96)	91 ± 7 (64–133)	0.12
Fasting blood insulin, mIU/L	66.4 ± 16.7 (6.4–175)	48.4 ± 9.5 (16.9–117)	0.80
Glucose AUC, mg · dL^{-1} · min	2950 ± 430 (1720–6070)	2830 ± 159 (1960–3680)	0.84
Insulin AUC, mg · dL^{-1} · min	6820 ± 2450 (1480–28,900)	4520 ± 1540 (1060–18,700)	0.43

[1]Values are mean ± SEM (range), $n = 11$ per group. Differences between groups were evaluated by unpaired 2-tailed Student's *t*tests. Glucose and insulin measurements were obtained from plasma samples.

GAPDH was similar for the Western and Mediterranean diet groups (**Supplemental Figure 2**). To examine differences in the relative contributions of substrates to mitochondrial respiration, we calculated flux control ratios for key respiratory parameters. We found trends for higher FAO flux control ratio ($P = 0.08$) and FAO + complex I flux control ratio ($P = 0.10$) in the Western diet group than in the Mediterranean diet group. To further examine these differences in FAO capacity, we performed western blots for CPT1, the rate-limiting step in the transport of fatty acids into mitochondria and β-oxidation (Supplemental Figure 1). Although there was no significant difference between the 2 groups, we found a trend for increased expression of CPT1 in the Mediterranean diet group ($P = 0.09$).

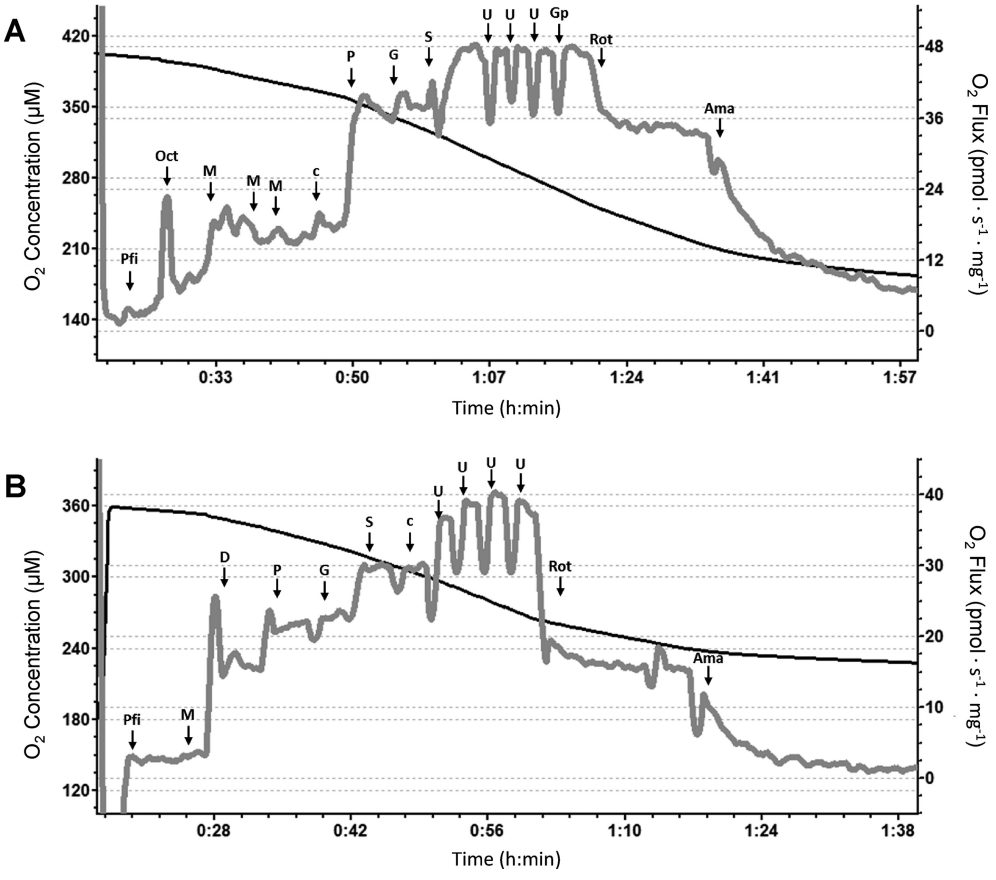

FIGURE 1 Representative traces of high-resolution respirometry of permeabilized skeletal muscle fibers from a single female cynomolgus macaque. (A) High-resolution respirometry of permeabilized fibers with the SUIT protocol with fatty acids. (B) High-resolution respirometry of permeabilized fibers with the SUIT protocol without the addition of fatty acids. Ama, antimycin-A; c, cytochrome c; D, ADP; G, glutamate; Gp, glycerol-3-phosphate; M, malate; Oct, octanoylcarnitine; P, pyruvate; Pfi, permeabilized fibers; Rot, rotenone; S, succinate; SUIT, substrate-uncoupler-inhibitor titration; U, FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone).

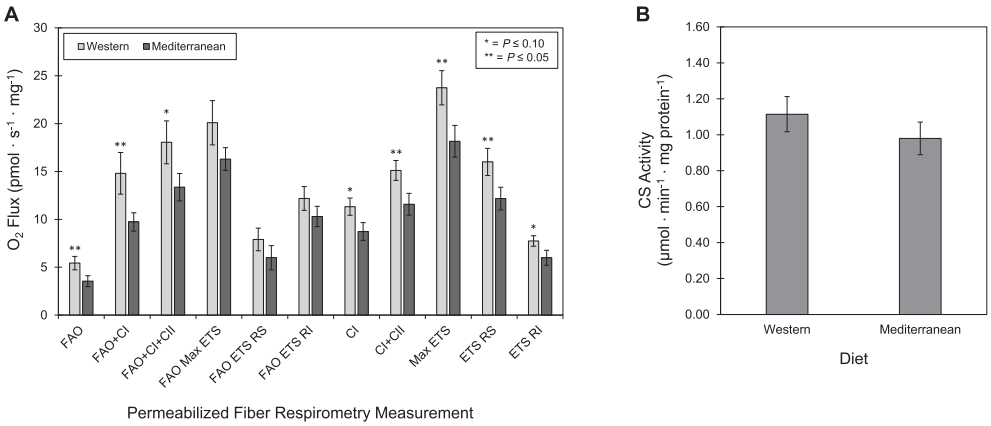

FIGURE 2 Effects of Western or Mediterranean diet on skeletal muscle bioenergetics of female cynomolgus macaques. (A) High-resolution respirometry of permeabilized muscle fibers. (B) CS activity. *,**Different from Mediterranean group: $^{*}P \leq 0.10$, $^{**}P \leq 0.05$. Data are presented as mean ± SEM, $n = 11$. C, complex; CS, citrate synthase; ETS, electron transport system; FAO, fatty acid oxidation; RI, rotenone insensitive; RS, rotenone sensitive.

Correlations between permeabilized muscle fiber bioenergetics and insulin sensitivity, glucose, and insulin measurements

Pearson correlation coefficients were used to assess relationships between bioenergetic parameters and insulin sensitivity, glucose, and insulin measurements by diet group and are shown in **Table 2** (Western diet) and **Table 3** (Mediterranean diet); relationships with both groups combined are shown in **Supplemental Table 5**. For correlation analysis, our primary metabolic outcome was insulin sensitivity calculated by HOMA-IR.

Regression plots illustrating the statistically significant relationships with HOMA-IR are shown in **Figure 3**A–E for both groups combined. HOMA-IR was significantly positively correlated with FAO (Figure 3A), FAO + complex I (Figure 3B), FAO + complex I + II (Figure 3C), FAO max ETS (Figure 3D), and FAO ETS Rot insensitive (Figure 3E). These relationships between measures of FAO and HOMA-IR were strongest in the Western diet group and are shown in Figure 3F–K. HOMA-IR was positively correlated with FAO (Figure 3F), FAO + complex I (Figure 3G), FAO + complex I + II (Figure 3H), FAO max ETS (Figure 3I), FAO ETS Rot sensitive (Figure 3J), and FAO ETS Rot insensitive (Figure 3K).

In addition, with both groups combined, fasting blood insulin was positively correlated with FAO + complex I + II (**Supplemental Figure 3**A) and FAO ETS Rot sensitive (Supplemental Figure 3B). Insulin AUC was positively correlated with FAO + complex I + II (Supplemental Figure 3C). In the Western diet group, fasting blood insulin was positively correlated with FAO + complex I (Supplemental Figure 3D) and FAO + complex I + II (Supplemental Figure 3E). Insulin AUC was significantly positively correlated with FAO (Supplemental Figure 3F), FAO + complex I (Supplemental Figure 3G), FAO + complex I + II (Supplemental Figure 3H), FAO max ETS (Supplemental Figure 3I), and FAO ETS Rot sensitive (Supplemental Figure 3J).

Discussion

We observed significantly higher respiration both with and without FAO in permeabilized skeletal muscle fibers from animals fed a Western compared with a Mediterranean diet. These changes were independent of CS activity, a marker of mitochondrial volume or content (29, 30), and expression of VDAC/Porin. These data suggest that the increase in respiration with a Western diet is likely not due to differences in mitochondrial content and could be the result of alterations in other factors such as mitochondrial morphology and composition, mitochondrial quality control, or other intracellular interactions. For example, differences in mitochondrial morphology have been linked to differences in respiration: more fragmented mitochondria are associated with nutrient-rich environments and inefficient energy production, whereas elongated mitochondria are associated with periods of starvation and more efficient production of ATP (31).

We also observed a trend for increased FAO and FAO + complex I flux control ratios in the Western diet group. To examine a potential mechanism for this observation, we looked at the expression of CPT1 by western blot. Interestingly, we found a trend for increased CPT1 expression in the Mediterranean diet group. For the measurement of FAO, we used a combination of octanoylcarnitine and malate to induce respiration from FAO. This combination bypasses the rate-limiting step of conversion of acyl-CoAs to acylcarnitines by CPT1, because octanoylcarnitine can freely cross the mitochondrial membrane (32). If we had used octanoyl-CoA and carnitine separately as substrates to induce FAO, we may have observed different results. It is important to consider that this study was not able to examine the stimulation of FAO by longer fatty acids such as palmitate, MUFAs such as oleate, or PUFAs such as linoleic acid. These limitations may account for the lower FAO observed in the Mediterranean diet group despite marginally higher CPT1 expression in skeletal muscle. Indeed, muscle has different capacities for the oxidation of various types of fatty acids (33). Notably, other studies have reported that oleate increases both gene and protein expression of CPT1 (34, 35), which is consistent with our observation that a Mediterranean diet rich in monounsaturated fats increased expression of CPT1 in skeletal muscle.

Previous studies of mitochondrial bioenergetics have reported that high-fat diets in rodents are associated with increased mitochondrial respiration in response to both fatty acid and carbohydrate substrates, increased mitochondrial biogenesis, and increased mitochondrial content, despite

TABLE 2 Correlations between carbohydrate metabolism phenotypes and respirometry of permeabilized muscle fibers of female cynomolgus macaques fed a Western diet[1]

Western diet	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	0.77**	0.86***	0.84**	0.77**	0.74*	0.75**	0.30	0.19	0.32	0.41	−0.01
Fasting blood glucose	0.20	0.18	0.11	0.02	0.03	0.05	0.46	0.38	0.53	0.50	0.42
Fasting blood insulin	0.57	0.63*	0.63*	0.58	0.58	0.60	0.30	0.15	0.37	0.47	0.02
Glucose AUC	0.25	0.18	0.11	0.09	0.11	0.03	−0.36	−0.15	0.00	0.03	−0.09
Insulin AUC	0.64*	0.68*	0.65*	0.66*	0.66*	0.59	−0.17	−0.03	−0.10	−0.05	−0.17
Partial for age + weight											
HOMA-IR	0.81**	0.90**	0.86**	0.81*	0.73*	0.82**	0.30	0.18	0.47	0.62	−0.01
Fasting blood glucose	0.09	0.11	0.00	−0.05	−0.11	0.03	0.48	0.36	0.70	0.71	0.46
Fasting blood insulin	0.66	0.68	0.66	0.62	0.56	0.65	0.27	0.15	0.53	0.69	0.01
Glucose AUC	0.03	−0.05	−0.05	0.00	0.16	−0.08	0.02	−0.02	0.34	0.42	0.07
Insulin AUC	0.86**	0.83**	0.85**	0.88**	0.92***	0.78**	0.51	0.60	0.71	0.26	−0.12

[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. *,**,***Significant correlation: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. ETS, electron transport system; FAO, fatty acid oxidation; Rot, rotenone.

TABLE 3 Correlations between carbohydrate metabolism phenotypes and respirometry of permeabilized muscle fibers of female cynomolgus macaques fed a Mediterranean diet[1]

Mediterranean diet	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	−0.04	0.13	0.06	0.15	−0.25	0.34	−0.33	−0.32	−0.21	0.02	−0.48
Fasting blood glucose	0.44	−0.10	−0.07	0.47	0.41	0.00	−0.11	−0.13	0.03	0.14	−0.14
Fasting blood insulin	−0.26	0.04	−0.03	−0.05	−0.30	0.21	−0.43	−0.36	−0.30	−0.07	−0.54
Glucose AUC	0.14	−0.09	0.20	0.24	0.04	0.19	−0.14	−0.27	0.01	0.24	−0.36
Insulin AUC	−0.52	−0.14	−0.12	−0.54	−0.32	−0.14	−0.24	−0.09	−0.38	−0.39	−0.22
Partial for age + weight											
HOMA-IR	−0.10	0.07	0.08	0.12	−0.27	0.37	−0.48	−0.43	−0.40	−0.14	−0.65
Fasting blood glucose	0.38	−0.39	−0.09	0.45	0.54	−0.12	−0.36	−0.33	−0.24	−0.11	−0.36
Fasting blood insulin	−0.26	0.09	0.01	−0.05	−0.36	0.29	−0.43	−0.33	−0.39	−0.17	−0.57
Glucose AUC	0.18	−0.07	0.17	0.31	0.09	0.19	0.03	−0.16	0.22	0.46	−0.29
Insulin AUC	−0.73*	0.19	−0.30	−0.75*	−0.62	−0.16	0.17	0.43	0.05	−0.01	0.13

[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. *Significant correlation, $P \leq 0.05$. ETS, electron transport system; FAO, fatty acid oxidation; Rot, rotenone.

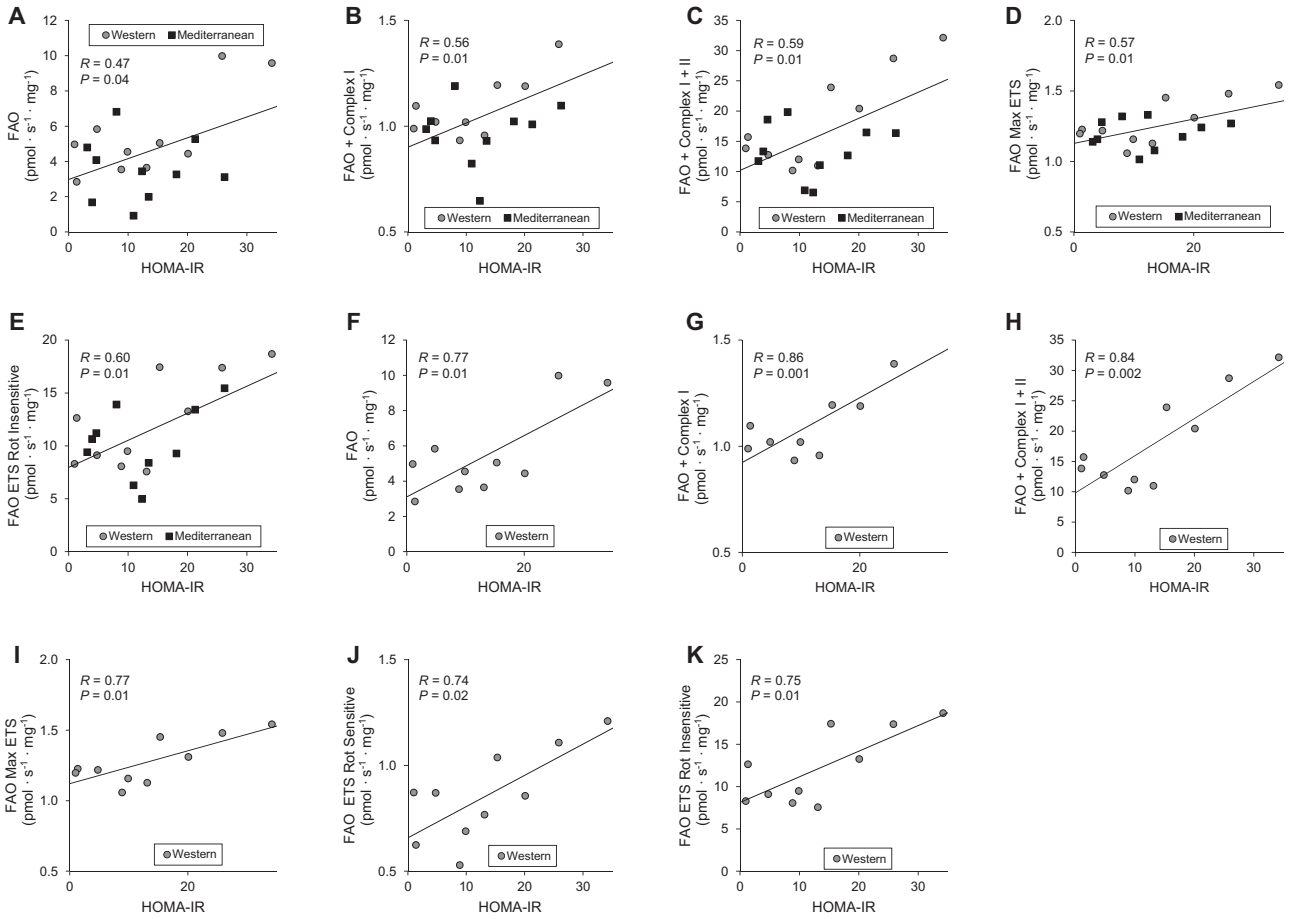

FIGURE 3 Correlations between permeabilized muscle fiber bioenergetics and insulin sensitivity of female cynomolgus macaques. (A–E) Plots of FAO measures against HOMA-IR for all animals in the study. (F–K) Plots of FAO measures against HOMA-IR for animals in the Western diet group. Pearson correlation coefficients (*R*) and *P* values are shown on each plot. ETS, electron transport system; FAO, fatty acid oxidation; Rot, rotenone.

increased insulin resistance (36–39). However, these studies included very-high-fat diets with 45–60% of total calories from fat that were compared with control diets that contained 8–12% of calories from fat. In addition, the composition of fats in these studies varies. Some studies included flax seed oil and olive oil as sources of fat, whereas others had lard as the major source. It should be noted that the study presented here does not compare high-fat and low-fat diets. Both the Western and Mediterranean experimental diets contain 31% of calories from fat. Indeed, we matched the experimental diets on protein, carbohydrate, and total fat content. The Western experimental diet has a larger amount of saturated fat, whereas the Mediterranean experimental diet has a larger percentage of monounsaturated and polyunsaturated fats. Our results suggest that manipulations of the types of fats consumed can alter mitochondrial bioenergetics independently of other factors such as macronutrient composition, energy intake, and obesity.

Previous studies have linked Western diets to chronic low-grade inflammation with elevated concentrations of biomarkers such as C-reactive protein, IL-6 and IL-18, and fibrinogen (40). In the context of obesity and insulin resistance, increased concentrations of TNF-α, IL-6, and C-reactive protein have also been reported in both animal models and humans (41). Inflammation is associated with increased mitochondrial respiration in brown adipose tissue, adipocytes, and fibroblasts (42–44). Lark et al. (41) have suggested that high-fat diets that increase mitochondrial respiration and partially oxidized lipid metabolites also increase production of reactive oxygen species and H_2O_2 that could lead to the induction of proinflammatory cascades and prolonged inflammation. In the present study, the lower mitochondrial respiration we observed with the Mediterranean diet may be linked to reduced reactive oxygen species, with the potential to lower the systemic inflammatory burden. This diet is high in n–3 fatty acids and has a lower ratio of n–6:n–3 fatty acids; both of these have been associated with anti-inflammatory effects and could play a role in the differences in mitochondrial bioenergetics observed (45, 46). Future studies designed to investigate the effects of Western and Mediterranean dietary patterns on inflammation will need to be conducted in order to determine how these relate to differences in mitochondrial metabolism.

We observed strong positive correlations between FAO and HOMA-IR and fasting blood insulin. These results suggest that there is a potential link between increased FAO of octanoylcarnitine and the development of insulin resistance. Interestingly, we did not observe significant correlations between these parameters when respiration was examined in the absence of octanoylcarnitine. Within-group relationships were strongest in the Western diet group and generally nonsignificant in the Mediterranean diet group, perhaps reflecting greater heterogeneity in responses to diet in the Western diet group. However, when both diet groups are combined, similar relationships to

the Western diet group persist. Partial correlations controlling for body weight and age suggest that insulin AUC was positively associated with FAO and FAO max ETS capacity in the Western diet group and negatively associated in the Mediterranean diet group, suggesting a complex diet × body weight interaction. In light of these correlations, further studies are necessary to understand the link between FAO and insulin resistance. It should also be noted that we made multiple comparisons in this study, which increases the risk of type I errors and may lead to significant correlations that are false or incorrect. To mitigate these risks, we chose insulin sensitivity calculated by HOMA-IR as our primary metabolic outcome for correlation analysis. If we reduce the significance threshold to $P \leq 0.01$, many of these relationships are still significant including the relationships between measures of FAO and HOMA-IR. In addition, these results are consistent; not only do measures of FAO significantly and positively correlate with HOMA-IR, but so do other surrogate measurements of insulin resistance: fasting blood insulin and insulin AUC.

It should be noted that human studies of diabetics and obese individuals have reported reductions in skeletal muscle mitochondrial bioenergetics (12–17). In this study, metabolic perturbations associated with the Western diet may be related to a prediabetic state in humans. A significantly longer study would be required to determine the temporal relationship between bioenergetic changes associated with diet and the development of overt diabetes. Our results may reflect an early adaptation to insulin resistance which may be followed by bioenergetic decline upon disease progression.

Major strengths of this study include the use of high-resolution mitochondrial respirometric profiling with and without fatty acid substrate, the comparison of whole dietary patterns rather than manipulation of a single macronutrient, and the use of a well-established nonhuman primate model highly translatable to humans that allowed for tight experimental control that cannot otherwise be achieved. This study utilized similarly aged premenopausal female cynomolgus monkeys, so additional studies would be required to determine the effects of age, sex, and menopause. A major limitation of this study is the absence of a baseline measurement of mitochondrial respiration or a "control" diet. Muscle samples were obtained only after necropsy. Pre–post diet effects would require multiple biopsies and would, therefore, require an experimental design that is different from the parent study utilized here. Although baseline differences are less likely in animal studies than in human trials, the animals were monitored at baseline and randomly assigned to diet groups with stratified randomization that ensured the groups were balanced based on pretreatment characteristics (body weight, BMI, basal cortisol, and plasma lipid concentrations). The experimental diets were also carefully matched on macronutrient composition (16% protein, 54% carbohydrate, and 31% total fat) and cholesterol so that the only variables were fat composition and the sources of diet components: more animal sources for the Western diet and more plant sources for the Mediterranean diet.

We report that the consumption of a Western diet resulted in higher skeletal muscle mitochondrial respiration than consumption of a Mediterranean diet and that FAO significantly and positively correlated with insulin resistance, particularly in the Western diet group. Although future investigation is warranted, including analysis of mitochondrial function pre- and postdietary manipulation, these studies suggest a potential link between high skeletal muscle mitochondrial respiration, the consumption of a Western diet, and the development of insulin resistance. In addition, these results highlight the importance of dietary composition in the study of mitochondrial bioenergetics. We have shown that changes in the types of fat consumed can influence skeletal muscle mitochondrial respiration independently of other factors such as macronutrient composition.

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