

Supplementary Data

Supplementary Information Regarding Mitochondrial Reactive Oxygen Species Measurements

It is important to note that the dichlorodihydrofluorescein diacetate assay has limitations and lacks some of the specificities of other assays such as electron paramagnetic resonance spectroscopy- or high-performance liquid chromatography-based methods (1). With this said, however, the nature of the reactive species most likely being formed and detected in our isolated mitochondria-based assay is hydrogen peroxide formed from the enzymatic and/or nonenzymatic dismutation of superoxide that is generated as a byproduct of electron transport. Approximately, 2%–5% of electrons “leak” from the respiratory chain complexes (I and III) to molecular O₂ to form superoxide and subsequently hydrogen peroxide. Note that studies were performed using isolated mitochondria in the presence of the oxidizable substrate succinate. Mitochondria were extensively washed (four wash steps); therefore, contaminating nitric oxide (NO) synthase would be minimal. Therefore, we do not anticipate NO and ensuing peroxynitrite formation. Also, assays were done in the absence of NO

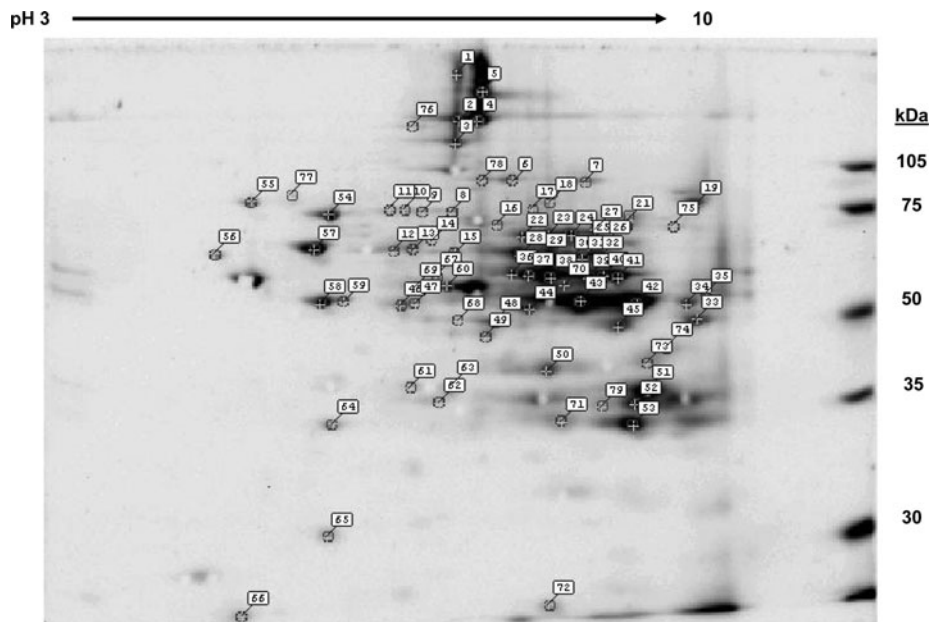
synthase cofactors and substrates (*e.g.*, no L-arginine); thus, we would not expect NO generation. Together, this supports hydrogen peroxide as the reactive oxygen species (ROS) agent. This is further supported as ROS measurements were membrane potential sensitive (*i.e.*, ROS decreased by treatment with the uncoupler FCCP).

Reference

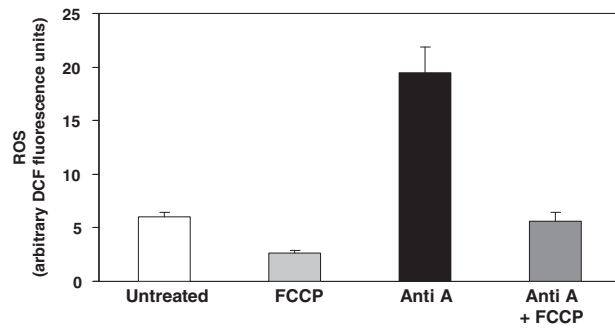
1. Tarpey MM, Wink DA, and Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. *Am J Physiol Regul Integr Comp Physiol* 286: R431–R444, 2004.

Abbreviations Used

FCCP = carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazone
NO = nitric oxide
ROS = reactive oxygen species



SUPPLEMENTARY FIG. S1. Master map gel of proteins identified by mass spectrometry. See Supplementary Table S1 for protein IDs. Note that this gel and the table do not contain proteins that were altered as a consequence of high-fat diet. Information regarding the proteins that were altered as a consequence of a high-fat diet is included within the main body of the manuscript in Figure 4 and Tables 2 and 3.



SUPPLEMENTARY FIG. S2. Mitochondrial ROS production in the absence and presence of antimycinA and FCCP. Mitochondria were incubated using conditions described in the Methods section to detect ROS production using 2',7'-dichlorodihydrofluorescein diacetate. Succinate (0.2mM) was added as the oxidizable substrate to initiate respiration and establish membrane potential. AntimycinA (Anti A) was added at a final concentration of 15 μ M to stimulate ROS production at complex III and FCCP (1 μ M) was added to uncouple mitochondria and dissipate the mitochondrial membrane potential. After 60 min of incubation, dichlorofluorescein fluorescence was measured at excitation/emissions wavelengths of 488/525 nm, respectively. Data represent mean + standard error of the mean for $n = 3-6$ animals per treatment. Key *post hoc* statistical analyses: untreated *versus* antimycin, $p < 0.0001$; untreated *versus* FCCP, $p = 0.012$; antimycin *versus* Antimycin + FCCP, $p < 0.0001$; FCCP *versus* antimycin + FCCP, $p = 0.18$. ROS, reactive oxygen species.

(Supplementary Table follows →)

SUPPLEMENTARY TABLE S1. GLOBAL MOUSE LIVER MITOCHONDRIAL PROTEOME—LISTING OF PROTEINS UNAFFECTED BY A HIGH-FAT DIET

Spot no.	Protein	Mass (kDa)	MOWSE score	Accession no.
1	Pyruvate carboxylase, mitochondrial precursor (pyruvic carboxylase)	130	149	gi_94410786
2	Unnamed protein product	99.7	149	gi_74201196
3	Carbamoyl-phosphate synthetase 1 isoform 1	164.5	104	gi_82879179
4	Pyruvate carboxylase	129.6	105	gi_6679237
5	Carbamoyl-phosphate synthetase 1 isoform 1	164.5	161	gi_82879179
6	Carbamoyl-phosphate synthetase 1 isoform 1	164.5	134	gi_82879179
7	Dimethylglycine dehydrogenase precursor	97.2	111	gi_21311901
	Aconitase, mitochondrial (<i>Rattus norvegicus</i>)	85.4	101	gi_40538860
	Unnamed protein product	85.3	101	gi_74188189
	Aconitase, mitochondrial	85.4	100	gi_18079339
8	Propionyl-Coenzyme A carboxylase, alpha polypeptide	79.9	116	gi_21450241
9	Glycerol-3-phosphate dehydrogenase	80.9	119	gi_1339938
10	Trap1 protein	80.0	119	gi_13879408
11	Tumor necrosis factor type 1 receptor-associated protein (<i>R. norvegicus</i>)	80.4	93	gi_84781723
12	TNF receptor-associated protein 1	80.2	65	gi_13385998
	Protein disulfide-isomerase A3 precursor (disulfide isomerase ER-60)	56.6	100	gi_1352384
	(ERp60) (<i>R. norvegicus</i>)			
13	Unnamed protein product	56.7	100	gi_26353794
14	Protein disulfide isomerase associated 3	56.6	99	gi_112293264
15	Epoxide hydrolase (EC 3.3.2.3)	62.5	112	gi_477004
16	2-Hydroxyphytanoyl-CoA lyase	63.6	102	gi_31560355
17	Aldehyde dehydrogenase family 7, member A1	55.6	105	gi_20070418
18	Butyryl Coenzyme A synthetase 1	64.7	95	gi_16905127
19	Acyl-CoA synthetase long-chain family member 1	77.9	95	gi_31560705
20	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial precursor	79.3	97	gi_20138697
21	Peroxisomal bifunctional enzyme	78.2	100	gi_17366737
22	Unidentified	—	—	—
23	Programmed cell death 8	66.7	115	gi_6755004
24	Efdh protein	61.1	114	gi_34784638
25	Large subunit GTPase 1 homolog (<i>R. norvegicus</i>)	74.4	85	gi_61740615
26	Unnamed protein product	73.3	55	gi_74203215
27	Large subunit GTPase 1 homolog	73.1	54	gi_30017373
28	Sterol carrier protein 2, liver	59.1	61	gi_45476581
29	Sterol carrier protein 2, liver	59.1	107	gi_45476581
30	Sterol carrier protein 2, liver	59.1	101	gi_45476581
31	Acyl-Coenzyme A dehydrogenase, very long chain	70.1	125	gi_23956084
32	Aldehyde dehydrogenase family 6, subfamily A1	57.9	149	gi_19527258
33	Aldh6a1 protein	49.6	106	gi_21410418
34	Unnamed protein product	59.7	96	gi_74223714
35	Catalase	59.7	79	gi_115704

(continued)

SUPPLEMENTARY TABLE S1. CONTINUED

Spot no.	Protein	Mass (kDa)	MOWSE score	Accession no.
31	Unnamed protein product	59.7	93	gi 74223714
	Catalase	59.7	74	gi 115704
32	Unnamed protein product	59.7	140	gi 74223714
	Catalase	59.7	115	gi 115704
33	Unnamed protein product	40.8	101	gi 74213886
	Aspartate aminotransferase, mitochondrial precursor (Transaminase A)	47.4	95	gi 94410786
34	Unnamed protein product	46.9	102	gi 12841359
	Ubiquinol cytochrome c reductase core protein 2	48.2	101	gi 22267442
35	Aspartate aminotransferase, mitochondrial precursor (Transaminase A)	47.4	89	gi 94410786
36	Glutamate dehydrogenase 1, mitochondrial precursor	54.2	123	gi 94396788
37	Glutamate dehydrogenase 1, mitochondrial precursor	54.2	113	gi 94396788
38	Glutamate dehydrogenase 1, mitochondrial precursor	54.2	132	gi 94396788
39	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	59.7	130	gi 6680748
40	Unnamed protein product	55.9	102	gi 74146998
	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	59.7	99	gi 6680748
41	Unnamed protein product	55.9	124	gi 74146998
	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	59.7	120	gi 6680748
42	Acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	41.8	128	gi 29126205
43	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 2	56.8	103	gi 31560689
44	Unnamed protein product	58.1	103	gi 74226121
	Sbno1 protein	55.5	80	gi 117938837
45	Acetyl-Coenzyme A acetyltransferase 1 precursor	44.8	86	gi 21450129
46	Ornithine aminotransferase	48.3	113	gi 8393866
47	Small GTPase homolog	44.1	87	gi 114145487
48	Chain A, crystal structure of rat short chain acyl-CoA dehydrogenase complexed with acetoacetyl-CoA (<i>R. norvegicus</i>)	42.1	104	gi 20150494
49	Acyl-Coenzyme A dehydrogenase, short chain	44.9	83	gi 31982522
	Unnamed protein product	46.2	123	gi 12836655
50	Isovaleryl coenzyme A dehydrogenase	46.3	122	gi 9789985
	Unnamed protein product	46.2	123	gi 12836655
51	Isovaleryl coenzyme A dehydrogenase	46.3	122	gi 9789985
	Uricase precursor (E.C. 1.7.3.3) (<i>R. norvegicus</i>)	33.3	86	gi 207619
52	Chondroitin sulfate GalNAcT-2	62.5	52	gi 31560032
	Unnamed protein product	32.6	113	gi 74182195
	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	59.7	97	gi 6680748
53	Unnamed protein product	27.3	91	gi 12832367
	Electron transferring flavoprotein, beta polypeptide	27.6	90	gi 38142460

54	Stress-70 protein (PBP74/CSA)	73.4	195	gi: 903309
55	Heat shock 70-kDa protein 5 (glucose-regulated protein)	72.4	143	gi: 31981722
56	Unnamed protein product	56.6	151	gi: 74203945
57	Prolyl 4-hydroxylase, beta polypeptide	57.0	150	gi: 42415475
	Unnamed protein product (<i>R. norvegicus</i>)	57.9	151	gi: 13344284
	Unnamed protein product	58.8	150	gi: 51452
	Hspd1 protein	59.4	150	gi: 76779273
58	Ubiquinol-cytochrome <i>c</i> reductase complex core protein I, mitochondrial precursor	52.7	98	gi: 14548301
59	Succinyl-CoA ligase (GDP-forming) beta-chain, mitochondrial precursor	46.8	144	gi: 85681897
60	Unidentified	—	—	—
61	Unidentified	—	—	—
62	Enoyl coenzyme A hydratase 1, peroxisomal	36.1	89	gi: 7949037
63	Unnamed protein product	29.8	120	gi: 74181431
	Prohibitin	29.8	119	gi: 6679299
64	Unidentified	—	—	—
65	Cytochrome <i>c</i> oxidase subunit 5A, mitochondrial precursor (cytochrome <i>c</i> oxidase polypeptide Va)	16.0	84	gi: 117099
66	Unidentified	—	—	—
67	Aldehyde dehydrogenase 2, mitochondrial	56.5	91	gi: 6753036
68	Unnamed protein product	46.3	101	gi: 12857011
	Isovaleryl coenzyme A dehydrogenase	46.3	101	gi: 9789985
69	Unidentified	—	—	—
70	Unnamed protein product	56.8	89	gi: 21758044
	3-Hydroxy-3-methylglutaryl-Coenzyme A synthase 2	56.8	88	gi: 31560689
71	Adenylate kinase 2 isoform a (<i>R. norvegicus</i>)	26.4	93	gi: 13591872
	Adenylate kinase 2 isoform b (<i>R. norvegicus</i>)	25.5	77	gi: 77020256
	Unnamed protein product	25.6	46	gi: 74207742
	Adenylate kinase 2 isoform b	25.6	45	gi: 34328230
72	Unidentified	—	—	—
73	Aldolase 2, B isoform	39.5	91	gi: 21450291
74	Unnamed protein product	42.0	85	gi: 74137706
	3-Ketoacyl-CoA thiolase B	44.0	84	gi: 22122797
75	Unidentified	—	—	—
76	Unidentified	—	—	—
77	Unidentified	—	—	—
78	Unidentified	—	—	—
79	Unidentified	—	—	—

Spot no. is the same as that used to identify proteins in the gel shown in Supplementary Figure S1. These numbers are not to be confused with the numbering system used within the main body of the manuscript. Mass is measured in kDa. The MOWSE score is an algorithmic calculation used to assign a statistical weight to each peptide match; therefore, a higher MOWSE score implies a higher statistical likelihood of the match being correct. The accession number is the unique NCBI label given to proteins.