Miller DB et al., Am J Respir Crit Care Med, 193 (12): 1382–1391, 2016

Ozone Exposure Increases Circulating Stress Hormones and Lipid Metabolites in Humans

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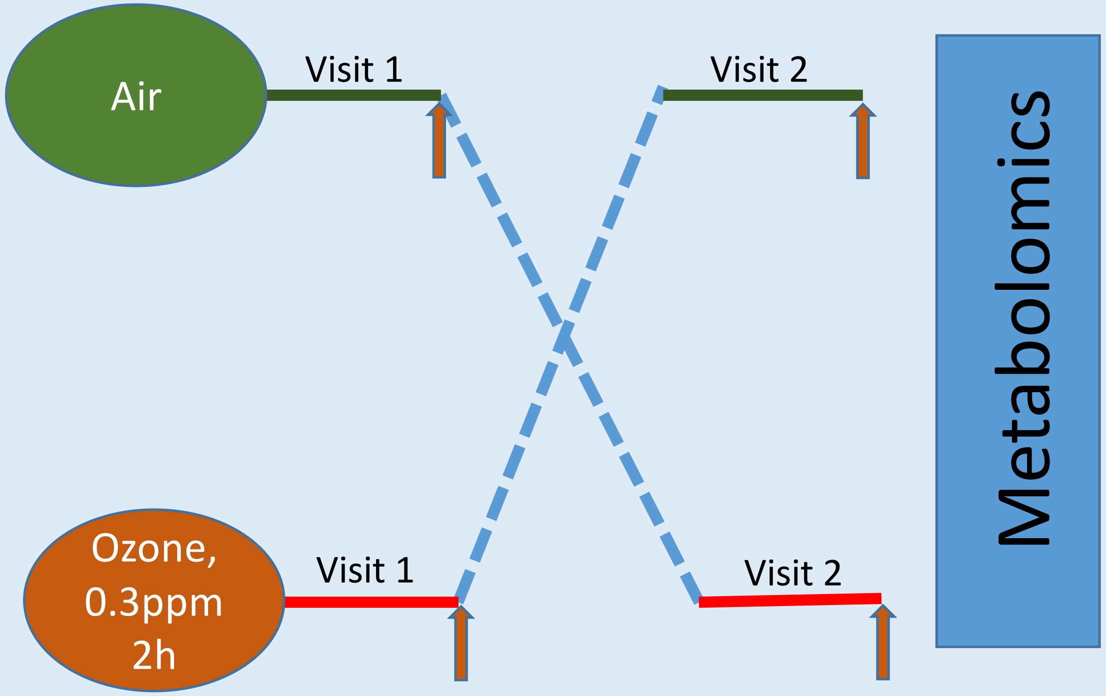
- Air pollution and association with a wide array of non-pulmonary diseases
- Interest in identifying circulating mediators responsible for peripheral effects
- Inconsistent data on increases in circulating cytokines but significant elevations in acute phase proteins and circulating stress hormones
- In the rat, ozone increases in circulating stress hormones and hundreds of metabolites, typical of a fight-or-flight response



Study participants.

Study Subjects	Ratio
Gender	
Male	20/24
Female	4/24
Race	
White	22/24
Hispanic	2/24
Smoking	
Never Smoker	21/24
Former Smoker*	3/24
Medication †	3/24
Exercise‡	
Mild	2/24
Moderate	9/24
Heavy	13/24

†Smoking before the year 2006.
‡Current medication (Multivitamins, and/or Lexapro).

Exercise level

Mild	90 to 180 min/wk
Moderate	180 to 360 min/wk
Heavy	over 360 min/wk

	Mean
Age (yr)	**25.6±3.8**
Height (cm)	**177.9±9.3**
Weight (kg)	**78.5±13.9**
BMI	**24.7±3.0**
Systolic	**121.0±8.4**
Diastolic	**73.9±6.8**
Heart Rate (bpm)	**73.4±14.4**

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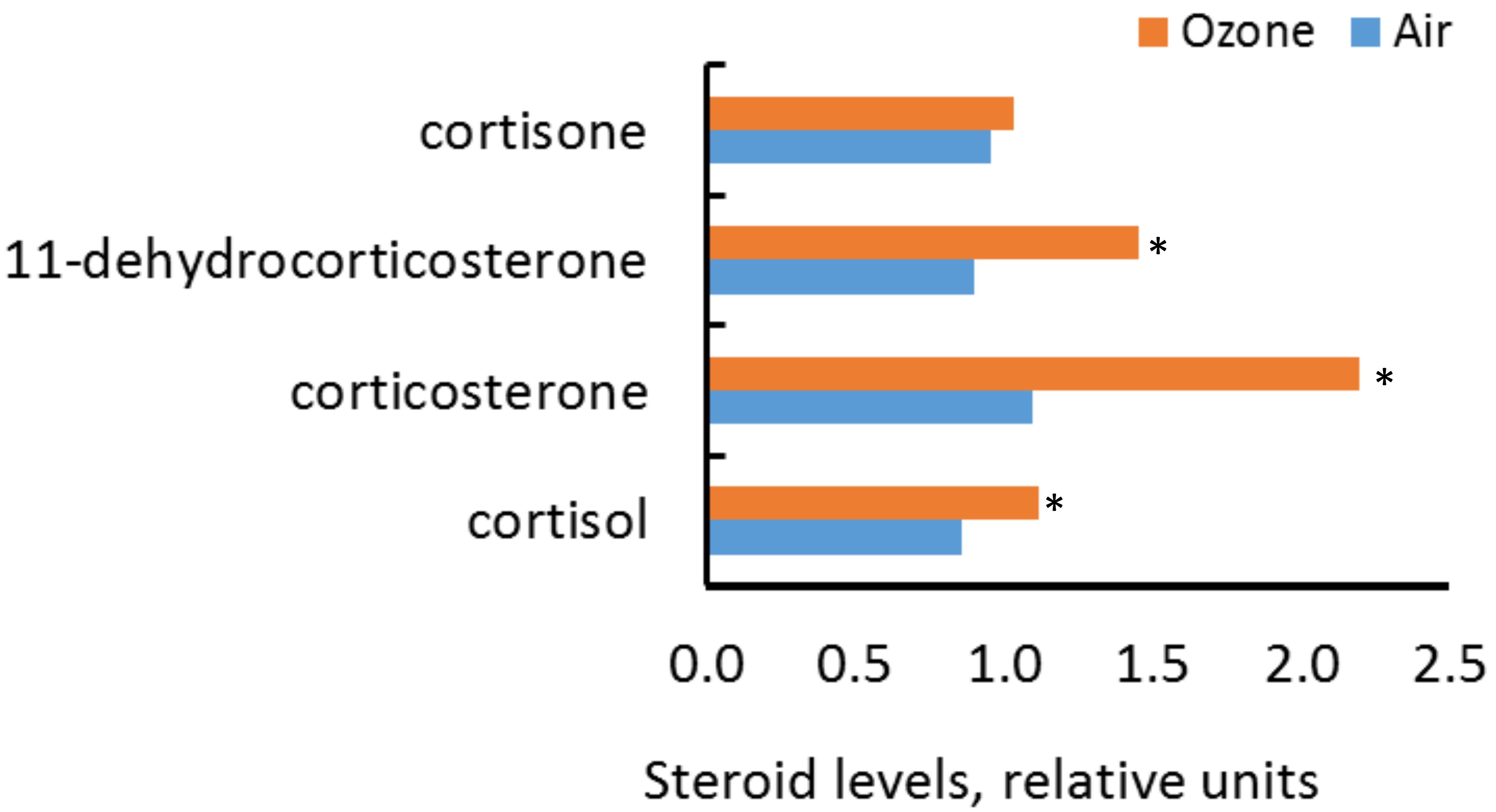
Ozone-induced changes in levels of steroid –stress hormones



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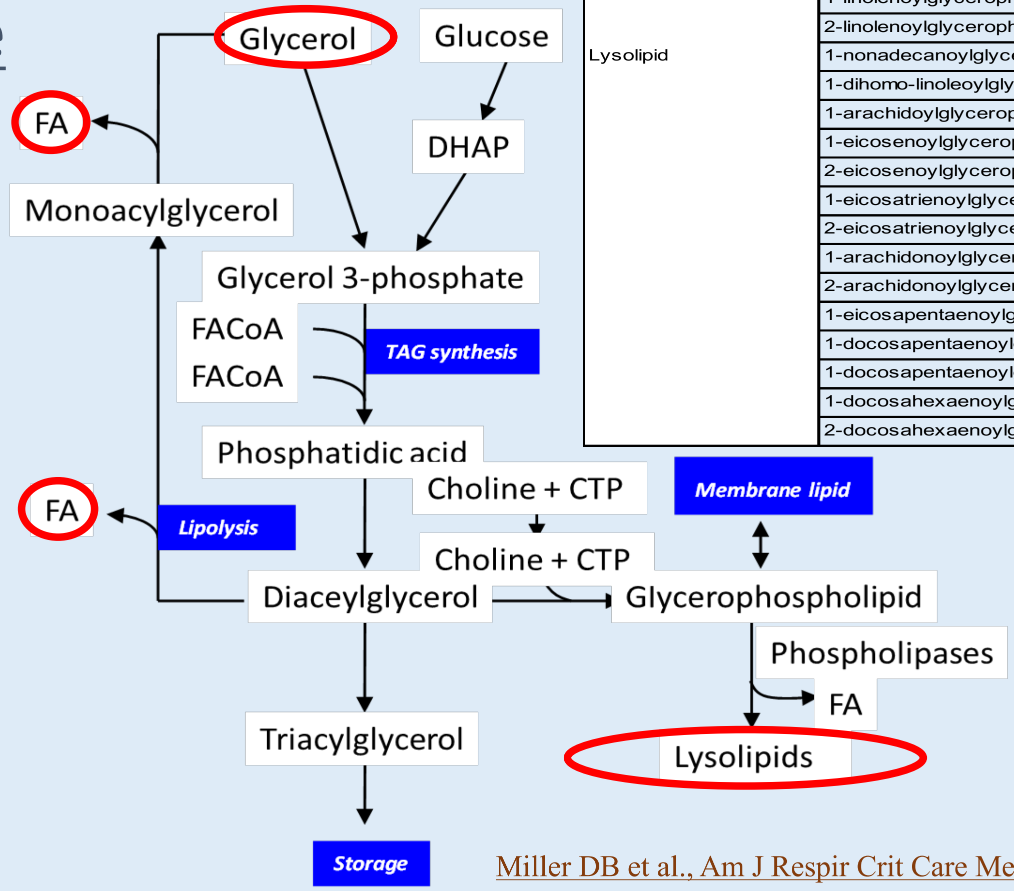
Metabolites changed after ozone exposure

Statistical Summary	
ANOVA contrasts	Ozone / Air
Total number of biochemicals with p<0.05	121
Biochemicals () p<0.05	85 | 36

Fatty Acid Lipolysis and Ozone Exposure

Sub Pathway	Biochemical Name	OZONE / AIR
Medium Chain Fatty Acid	caproate (6:0)	1.17
	laurate (12:0)	1.17
	5-dodecenoate (12:1n7)	1.57
Long Chain Fatty Acid	myristate (14:0)	1.42
	myristoleate (14:1n5)	1.80
	palmitoleate (16:1n7)	1.72
	margarate (17:0)	1.30
	10-heptadecenoate (17:1n7)	1.48
	oleate (18:1n9)	1.38
	cis-vaccenate (18:1n7)	1.20
	10-nonadecenoate (19:1n9)	1.54
	eicosenoate (20:1n9 or 11)	1.44
Glycerolipid Metabolism	glycerol	1.34
Monoacylglycerol	1-stearoylglycerol (1-monostearin)	2.32
	2-stearoylglycerol (2-monostearin)	1.89
	1-oleoylglycerol (1-monoolein)	1.56
	2-oleoylglycerol (2-monoolein)	1.85



Sub Pathway	Biochemical Name	OZONE / AIR
Lysolipid	1-pentadecanoylglycerophosphocholine (15:0)*	1.10
	1-palmitoylglycerophosphocholine (16:0)	1.08
	2-palmitoylglycerophosphocholine*	1.06
	1-palmitoleoylglycerophosphocholine (16:1)*	1.11
	1-margaroylglycerophosphocholine (17:0)	1.24
	1-stearoylglycerophosphocholine (18:0)	1.16
	2-stearoylglycerophosphocholine*	1.19
	1-oleoylglycerophosphocholine (18:1)	1.18
	2-oleoylglycerophosphocholine*	1.11
	1-linoleoylglycerophosphocholine (18:2n6)	1.08
	2-linoleoylglycerophosphocholine*	1.14
	1-linolenoylglycerophosphocholine (18:3n3)*	0.82
	2-linolenoylglycerophosphocholine(18:3n3)*	0.85
	1-nonadecanoylglycerophosphocholine(19:0)	1.29
	1-dihomo-linoleoylglycerophosphocholine (20:2n6)*	1.22
	1-arachidoylglycerophosphocholine (20:0)	1.44
	1-eicosenoylglycerophosphocholine (20:1n9)*	1.33
	2-eicosenoylglycerophosphocholine(20:1n9)*	1.48
	1-eicosatrienoylglycerophosphocholine (20:3)*	1.22
	2-eicosatrienoylglycerophosphocholine*	1.20
	1-arachidonoylglycerophosphocholine (20:4n6)*	1.15
	2-arachidonoylglycerophosphocholine*	1.16
	1-eicosapentaenoylglycerophosphocholine (20:5n3)*	1.17
	1-docosapentaenoylglycerophosphocholine (22:5n3)*	1.11
	1-docosapentaenoylglycerophosphocholine (22:5n6)*	1.25
	1-docosahexaenoylglycerophosphocholine (22:6n3)*	1.25
	2-docosahexaenoylglycerophosphocholine*	1.21

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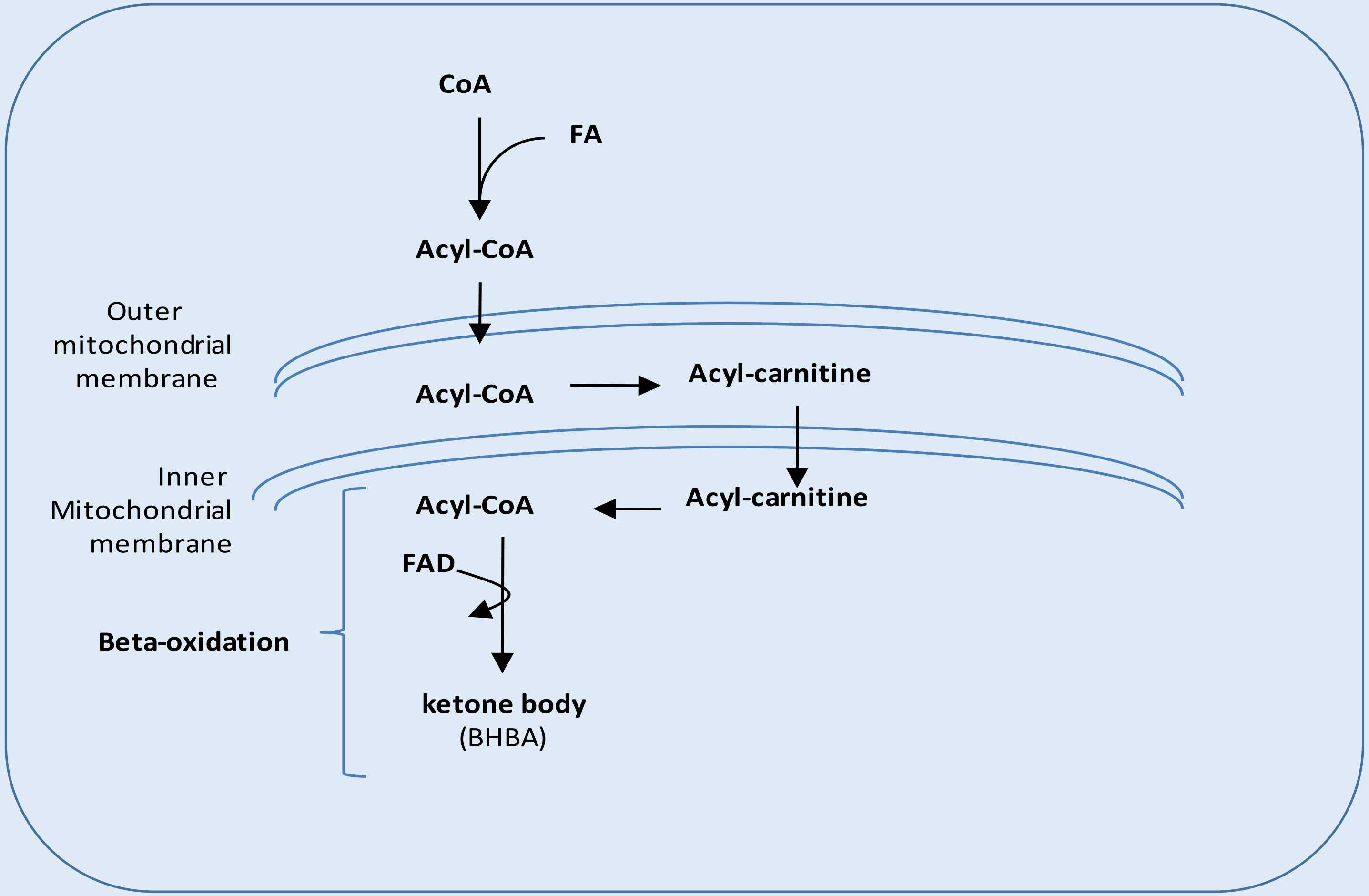
Indications of Altered Fatty Acid β-oxidation

Sub Pathway	Biochemical Name	OZONE / AIR
Long Chain Fatty Acid	myristate (14:0)	1.42
	myristoleate (14:1n5)	1.80
	palmitoleate (16:1n7)	1.72
	margarate (17:0)	1.30
	10-heptadecenoate (17:1n7)	1.48
	oleate (18:1n9)	1.38
	cis-vaccenate (18:1n7)	1.20
	10-nonadecenoate (19:1n9)	1.54
	eicosenoate (20:1n9 or 11)	1.44
Fatty Acid Metabolism(Acyl Carnitine)	acetylcarnitine	1.09
	hexanoylcarnitine	1.22
	octanoylcarnitine	1.32
	decanoylcarnitine	1.42
	cis-4-decenoyl carnitine	1.27
	laurylcarnitine	1.79
	myristoylcarnitine	1.57
	palmitoylcarnitine	1.25
	oleoylcarnitine	1.38
	linoleoylcarnitine*	1.31
	myristoleoylcarnitine*	1.81
Carnitine Metabolism	carnitine	0.92
Ketone Bodies	3-hydroxybutyrate (BHBA)	1.82



In addition to indications of increased fatty acid lipolysis following ozone exposure, there were also significant changes in biochemicals suggestive of increased fatty acid β-oxidation with ozone exposure.

Polyunsaturated Fatty Acids

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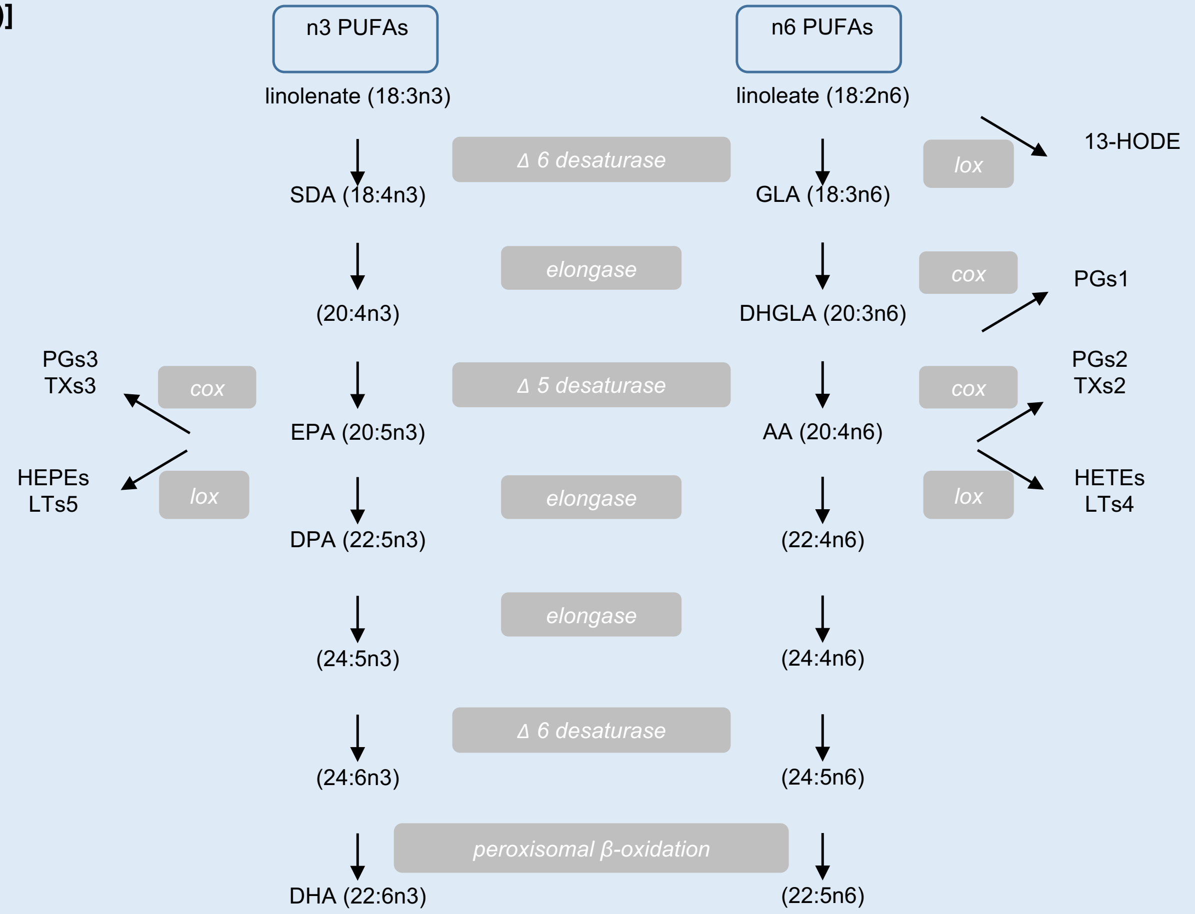
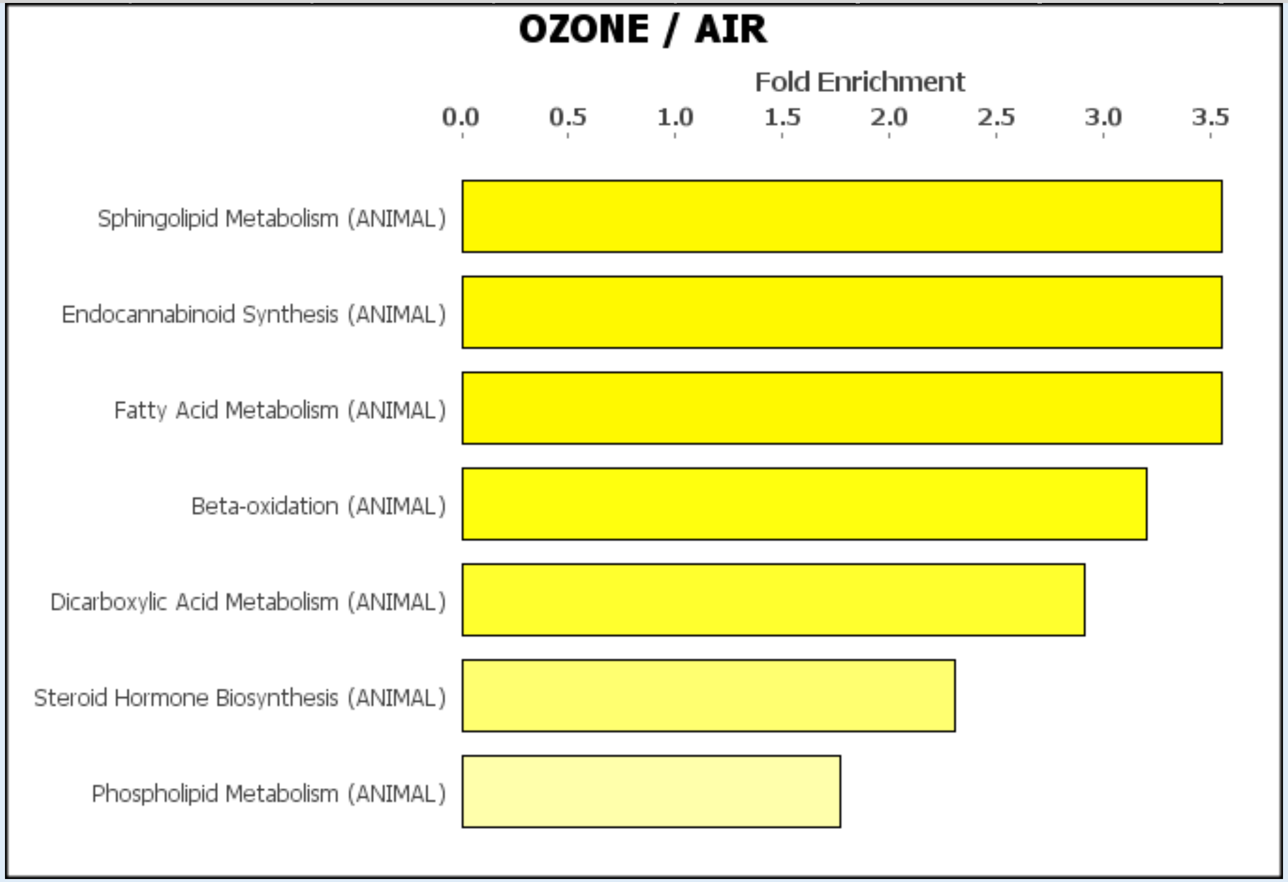
linolenate [alpha or gamma: (18:3n3 or n6)]

docosapentaenoate(n3 DPA: 22:5n3)



Polyunsaturated n3 and n6 fatty acids were also found to be higher within the ozone-exposed subjects and may support an increase in production of inflammatory mediators which would be consistent with ozone induced damage.

N= total number of detected metabolites in entire study		
n= total number of significant metabolites in selected contrast		
k= total number of significant compounds detected within the specific pathway		
m= total number of compounds detected within the specific pathway		
P= p value without any correction factor applied		



Sphingolipid Metabolism (ANIMAL)	3.6 P = 1.27E-5 k = 9 m = 14 n = 86 N = 475
Endocannabinoid Synthesis (ANIMAL)	3.6 P = 1.27E-5 k = 9 m = 14 n = 86 N = 475
Fatty Acid Metabolism (ANIMAL)	3.6 P = 1.27E-5 k = 9 m = 14 n = 86 N = 475
Dicarboxylic Acid Metabolism (ANIMAL)	2.9 P = 8.25E-5 k = 10 m = 19 n = 86 N = 475
Beta-oxidation (ANIMAL)	3.2 P = 1.00E-9 k = 22 m = 38 n = 86 N = 475
Steroid Hormone Biosynthesis (ANIMAL)	2.3 P = 0.01 k = 5 m = 12 n = 86 N = 475
Phospholipid Metabolism (ANIMAL)	1.8 P = 4.55E-5 k = 31 m = 97 n = 86 N = 475

Pathway enrichment analysis identified several biochemical pathways as having a high fold enrichment value which based on a number of parameters including the total number of detected.

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Interpretation

Ozone in exercising humans increased:

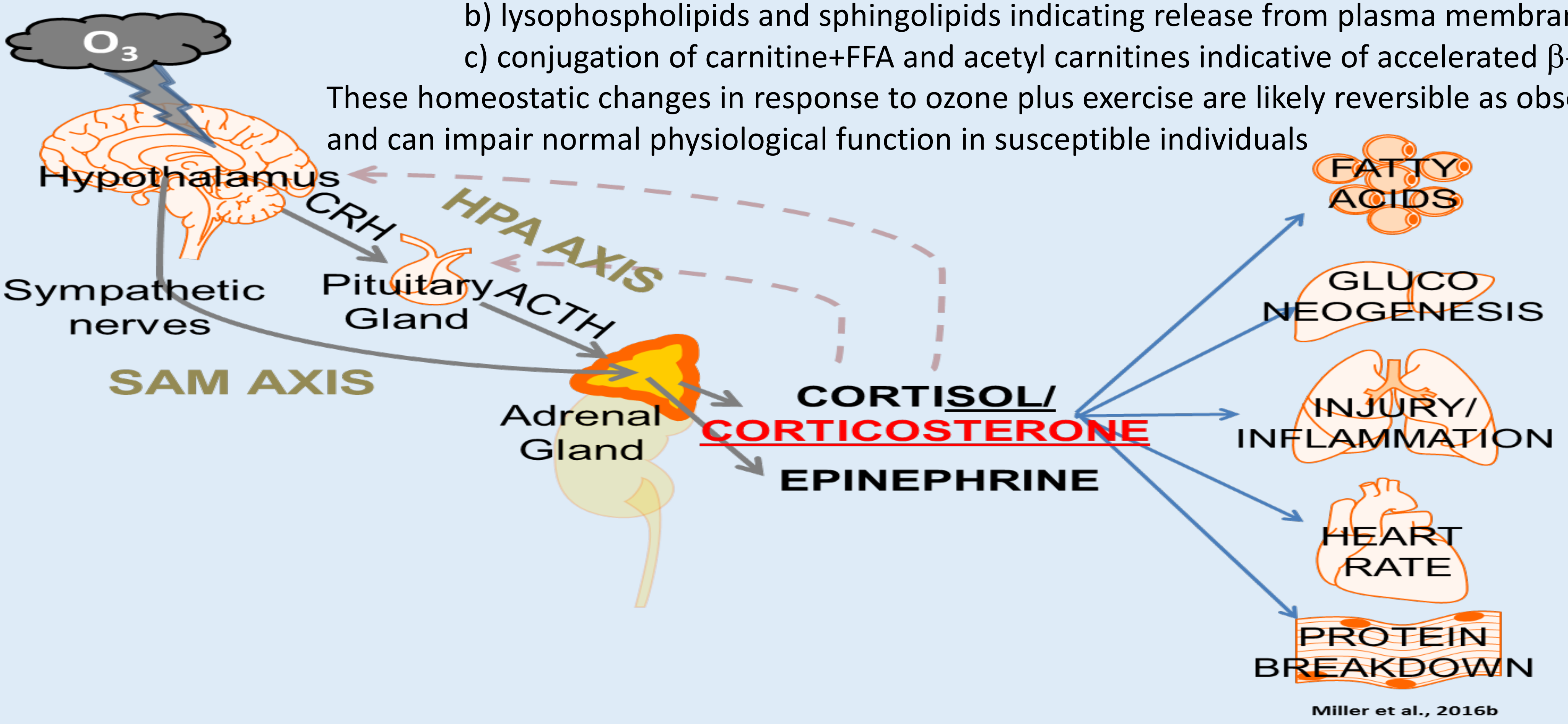
a) cortisol and 11-dehydrocorticosterone but not cortisone supporting the activation of hypothalamic–pituitary axis.

b) medium and long-chain FFA and plasma glycerol indicating enhanced lipolysis

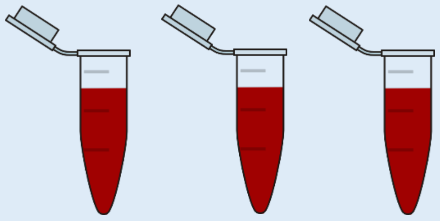
b) lysophospholipids and sphingolipids indicating release from plasma membranes

c) conjugation of carnitine+FFA and acetyl carnitines indicative of accelerated β-oxidation

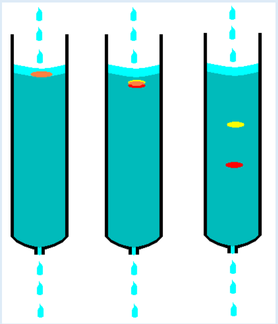
These homeostatic changes in response to ozone plus exercise are likely reversible as observed in rats, and can impair normal physiological function in susceptible individuals



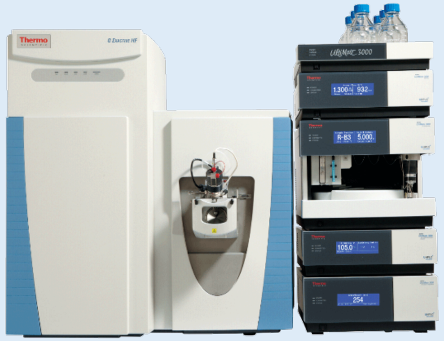
Untargeted Metabolomic Analysis Workflow



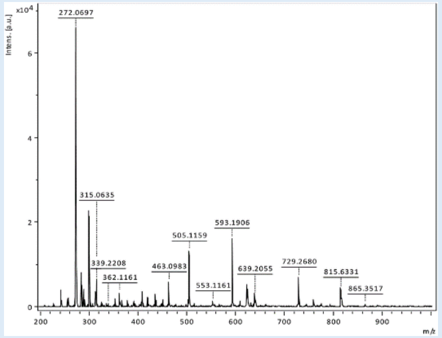
Serum



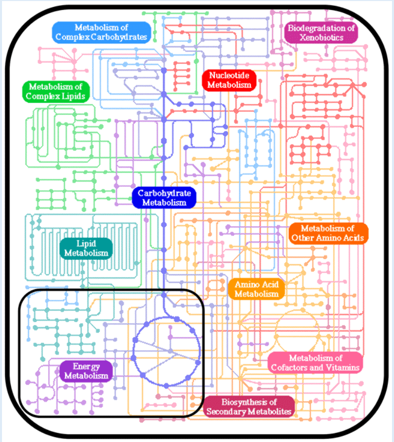
Metabolite separation



Mass spectrometry



Peak and feature identification, alignment and quantification



Statistical and pathway analysis