Effects of Cigarette Smoke Exposure on the Insulin Resistance-Related Metabolic Changes in the High Fat Diet-fed ApoE Knock-Out Mice

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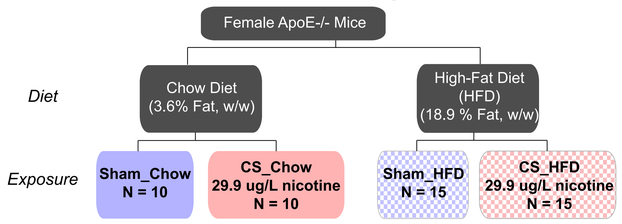
Introduction

Insulin resistance (IR) has been linked to cigarette smoking and is known to contribute to diabetes and lipid disorders. Cigarette smoke (CS) induces inflammation and alters the normal immune responses, both of which can exacerbate diabetes and contribute to atherosclerosis development. We use Apolipoprotein-E (ApoE)$^{-/-}$ mice to examine if CS exposure impacted the IR-related metabolic changes. The ApoE$^{-/-}$ mice is a mouse model of dyslipidemia and atherosclerosis, which upon high fat diet (HFD) feeding has been reported to exhibit diabetes-related pathological alterations. The impact of CS on the IR-related changes were evaluated by measuring the fasting glucose and insulin levels, aortic plaque area, serum levels of lipids and metabolic analytes, as well as transcriptomes and lipidomes of the major cardiometabolic organs.

Materials & Methods

Experimental Design



Animals -- Female B6.apoE-/- mice (6-8 weeks old, bred under specific pathogen free conditions, Taconic Farms USA). All procedures were carried out in accordance with current guidelines of National Advisory Committee on Laboratory Animal Research and approved by Phillip Morris International Institutional Animal Care and Use Committee.

Exposure -- Mainstream cigarette smoke (CS) from 3R4F University of Kentucky reference cigarettes, or fresh air (sham) for 11 weeks (3 hours daily, 5 days/week, whole body exposure). Food was removed during the exposure.

Diet -- Chow groups were fed T2914C pellet diet and HFD groups were fed TD.88137 high fat rodent diet, Harland, U.K.

Fasting (6 h) Blood Glucose -- Measured in whole blood using a handheld glucometer (Accu Check® Performa, Roche).

Fasting Plasma Insulin -- Measured in plasma using Mouse Insulin ELISA Kit (Cat # 90080, Crystal Chem, Inc.).

Blood Cholesterol and Triglycerides – Measured using Beckman Unicell® DXC 600 clinical auto analyzer.

Serum Analytes -- Measured using multiplexed immunoassays.

Aortic Plaque -- Plaque size was identified as oil red O-positive material in the aortic arch and morphometric evaluation of total plaque area (using Visopharm software).

Transcriptomics Analysis -- Generated from the liver, skeletal muscle (mix of gastrocnemius and soleus muscle), and fat (mesenteric fat, white adipose tissue). A threshold free approach using GSEA (Gene Set Enrichment Analysis) was conducted to evaluate the biological impact of HFD and CS.

Lipidomics Analysis -- Generated from the plasma, liver, and mesenteric fat. Shotgun and triacylglycerol lipidomics were analyzed using NanoMate (Advion Biosciences) and QTRAP 5500 MS (Applied Biosystems). Ceramide lipidomics were analyzed using UHPLC autosampler, Rheos Allegro pump and QTRAP 500 MS. Unpaired t-test was performed on log-transformed concentrations to calculate the p-values (* $p < 0.05$). Group differences are reported as relative percentage differences of average concentrations of the groups.

References

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Reference for the 3R4F cigarettes can be found in www.ca.uky.edu/refcig

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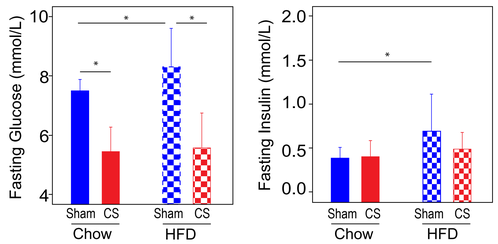
Results

CS exposure did not affect the body weight of HFD-fed mice

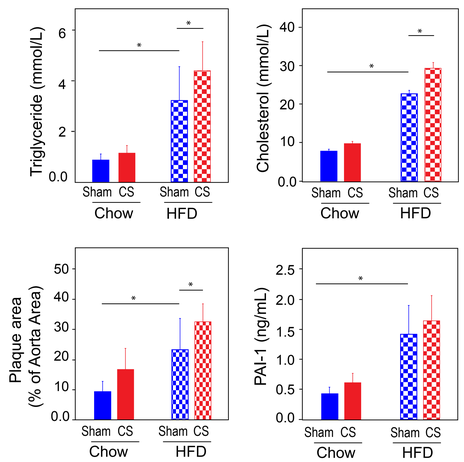
Group	Mean Body Weight (g ± SD)	
	Week 0	Week 11
Sham_Chow	20.93 ± 1.19	23.86 ± 1.39
CS_Chow	20.36 ± 1.01	22.67 ± 1.98
Sham_HFD	20.11 ± 1.19	26.02 ± 2.45
CS_HFD	20.11 ± 0.86	25.82 ± 2.13

* $p < 0.05$

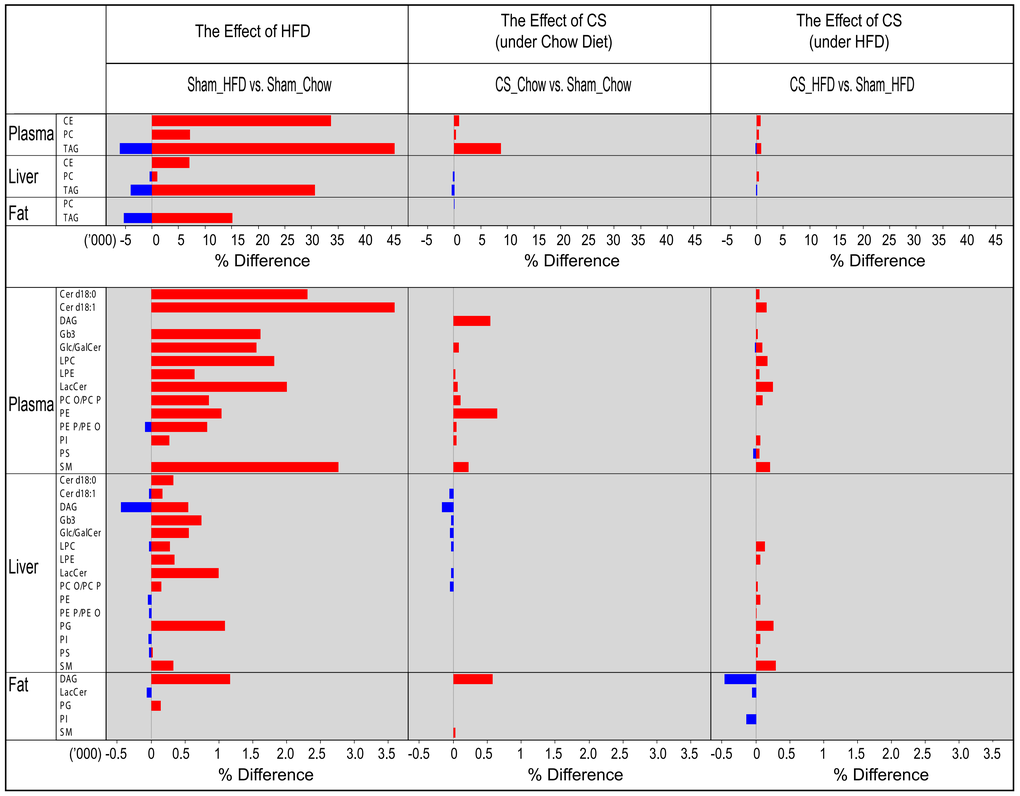
CS exposure was associated with reduced levels of fasting blood glucose but not insulin (mean ± SD, * $p < 0.05$)



CS exposure was linked to elevated levels of circulating atherogenic lipids and analytes in the HFD-fed mice (mean ± SD, * $p < 0.05$)

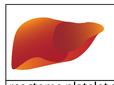


Lipidomics analysis indicated increased levels of various lipids in the chow and HFD-fed mice that were exposed to CS



Shown are the % difference at *$p < 0.05$. Abbreviations: CE, cholesterol esters; Cer, ceramides; DAG, diacylglycerols; Gal/GlcCer, galactosyl and glucosylceramides; Gb3Cer, globotriosylceramides; LacCer, lactosylceramides; LPC, lysophosphatidylcholines; LPL, lysophospholipids, PA, phosphatidic acid; PC, phosphatidylcholines, PC O, ether-linked phosphatidylcholines; PE, phosphatidylethanolamines; PG, phosphatidylglycerols; PI, phosphatidylinositols; PL O, other ether-linked phospholipids; PS, phosphatidylserines; SM, sphingomylines; TAG, triacylglycerols.

Comparability of the enriched biological annotations associated with HFD and CS exposure derived from the transcriptomic data



Gene Set Enrichment Analysis (GSEA) Liver Transcriptomes	The Effect of HFD	The Effect of CS (under Chow Diet)	The Effect of CS (under HFD)
	Sham_HFD vs. Sham_Chow	CS_Chow vs. Sham_Chow	CS_HFD vs. Sham_HFD
reactome platelet activation signaling and aggregation			
pid TXA2 pathway			
kegg regulation of actin cytoskeleton			
reactome iron uptake and transport			
kegg chemokine signaling pathway			
reactome semaphorin interaction			
pid integrin1 pathway			
reactome axon guidance			
reactome muscle contraction			
kegg sphingolipid metabolism			
reactome IL-3, 5 and GM-CSF signaling			
kegg ECM receptor interaction			
reactome MHC class II antigen presentation			
reactome cell surface interactions at the vascular wall			
pid alphasynuclein pathway			
biocarta cdc42 rac pathway			
reactome ADP signaling through P2RY1			
pid cdc42 pathway			
reactome regulation of insulin secretion			
reactome the role of NEF in HIV1 replication and disease			
reactome apoptosis			
pid TCR pathway			
reactome G alpha-q signaling events			
kegg vasopressin regulated water reabsorption			
pid E-cadherin in the nascent adjerent junction			
reactome cytokine signaling in immune system			
reactome trans golgi network vesicle budding			
reactome extracellular matrix organization			
reactome inhibition of voltage gated Ca2 channels via g-beta			
reactome N-CAM signaling for neurite outgrowth			
reactome lipoprotein metabolism			
reactome GPCR ligand binding			
mips emerin complex 52			
reactome G protein activation			
kegg apoptosis			
kegg hypertrophic cardiomyopathy HCM			
biocarta BCR pathway			
kegg tight juntion			
reactome PI3K cascade			
reactome regulation of hypoxia inducible factor by oxygen			
kegg basal transcription factors			
reactome metabolism of vitamins and cofactors			
reactome fatty acyl Co-A biosynthesis			
mips spliceosome			
reactome cleavage of growing transcript in the termination region			
reactome cholesterol biosynthesis			

☐ * $p < 0.05$ ▫ Not significant — Enrichment Score -2.685 2.386

GSEA Muscle Transcriptomes	The Effect of HFD	The Effect of CS (under Chow Diet)	The Effect of CS (under HFD)
	Sham_HFD vs. Sham_Chow	CS_Chow vs. Sham_Chow	CS_HFD vs. Sham_HFD
reactome fatty acid triacylglycerol and ketone body metabolism			
kegg valine leucine and isoleucine degradation			
reactome lipoprotein metabolism			
reactome pyruvate metabolism and citric acid TCA cycle			
kegg oxidative phosphorylation			
kegg huntingtons disease			

☐ * $p < 0.05$ ▫ Not significant — Enrichment Score -2.510 2.994

GSEA Fat (Mesenteric) Transcriptomes	The Effect of HFD	The Effect of CS (under Chow Diet)	The Effect of CS (under HFD)
	Sham_HFD vs. Sham_Chow	CS_Chow vs. Sham_Chow	CS_HFD vs. Sham_HFD
kegg ribosome			
reactome translation			
reactome activation of the mRNA upon binding to the cap binding			
reactome metabolism of proteins			
reactome incretin synthesis, secretion and inactivation			
reactome interferon signaling			
reactome TRAF6-mediated NFKB activation			
reactome laggin strand synthesis			
pid p73 pathway			
mips 39s ribosomal subunit mitochondrial			
reactome fatty acid triacylglycerol and ketone body metabolism			
kegg oxidative phosphorylation			

☐ * $p < 0.05$ ▫ Not significant — Enrichment Score -2.527 4.078

Conclusion

Our study showed that HFD in ApoE$^{-/-}$mice increased fasting glucose and insulin levels. The impact of HFD was also associated with alterations in immune responses and lipid metabolism as demonstrated from the transcriptomics assessment of the liver, muscle, and fat. In contrast, CS exposure was unexpectedly linked to a reduction of fasting glucose. However, CS exposure was associated with increased accumulation of aortic plaque and elevated levels of lipids in the various cardiometabolic tissues despite a less robust impact on the transcriptomes. In addition, the results further indicated that the impact of HFD was more robust as compared with that of CS exposure in the ApoE$^{-/-}$mouse model.

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