Epigenetic BET Reader Inhibitor Apabetalone (RVX-208) Counters Proinflammatory Aortic Gene Expression In a Diet-Induced Obesity Mouse Model

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BACKGROUND

Obesity is associated with insulin resistance, dyslipidemia, high blood pressure, chronic inflammation and it increases the risk of type 2 diabetes (DM2) and cardiovascular disease (CVD) in patients. Bromodomain and extraterminal (BET) proteins enhance the expression of proinflammatory genes in DM2 and CVD models by recruiting the transcription regulation factors. Apabetalone is a small molecule that inhibits BET interactions with chromatin, leading to anti-inflammatory and anti-atherogenic effects.

OBJECTIVE: Here we evaluate the effects of apabetalone on vascular inflammation in a mouse model of diet induced obesity.

METHODS

From 8 to 30 weeks of age, C57 BL/6J mice were fed a low-cholesterol high-fat (HFD) or low-fat diet (LFD). Serum glucose, GTT and ITT were measured every 6 weeks. Mice received apabetalone at 150 mg/kg b.i.d between 14 and 30 weeks. Gene expression was analyzed post necropsy in aortic tissue by PCR and nCounter® Inflammation Panel.

RESULTS

Metabolic dysregulation in the DIO model: As previously published, HFD induced weight gain, visceral obesity, high fasting blood glucose, glucose intolerance and insulin resistance compared to LFD controls (not shown), confirming metabolic dysregulation. Apabetalone treatment did not impact metabolic parameters measured.

Gene expression profiling in the aorta: Analysis showed an upregulation of 27 inflammatory genes in HFD-fed mice as compared to LFD, including transcription factors *Rela* (22%), *Hif1a* (25%) and *Tcf4* (44%) ($p<0.05$) which are associated with DM2 risk. Differentially expressed genes map to cytokine signaling, cytoskeletal remodeling, coagulation and complement pathways.

Effects of apabetalone treatment: Apabetalone reduced the expression of proinflammatory transcription factors *Rela* and *Nfkb* as well as the expression of potent chemokines *Ccl2* and *Ccl8*, and the chemokine receptor *Ccr2*. Genes associated with vascular inflammation were also reduced by apabetalone, including leukocyte receptors *Itgam* (Cd11b*) and Ccr2,* as well as endothelial receptors *Sele* and *Icam1*.

CONCLUSION

HFD induces vascular inflammation in mice. Apabetalone treatment diminishes this proinflammatory phenotype, providing mechanistic insight into how apabetalone reduces CVD risk in DM2 patients in clinical trials.

FIG 1: THE DIET INDUCED OBESITY (DIO) MOUSE MODELS HUMAN CHARACTERISTICS OF OBESITY, PRE-DIABETES AND DYSLIPIDEMIA

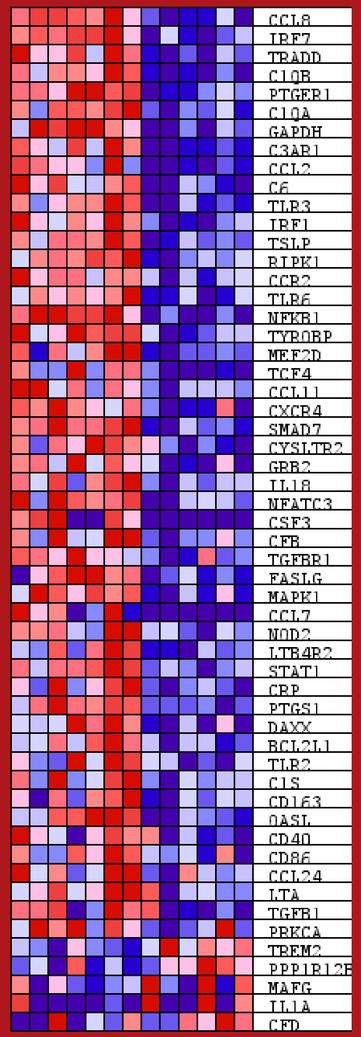
Pharmacological intervention with APABETALONE (clinical-stage BET inhibitor)

Age:	8 weeks	14 weeks	20-21 weeks	30 weeks
	Low-fat (LFD) or High-fat (HFD) Diet	Vehicle or **APABETALONE 150 mg/kg b.i.d.**	Tests: Fasting glucose Glucose tolerance Insulin resistance	Aorta dissection Gene expression with Nanostring Inflammation Panel (254 genes)

DIO Characteristics: Increased adiposity Glucose intolerance Insulin resistance

FIG 2: HFD INDUCES PROINFLAMMATORY AORTIC GENE EXPRESSION

Gene expression (mouse aorta)

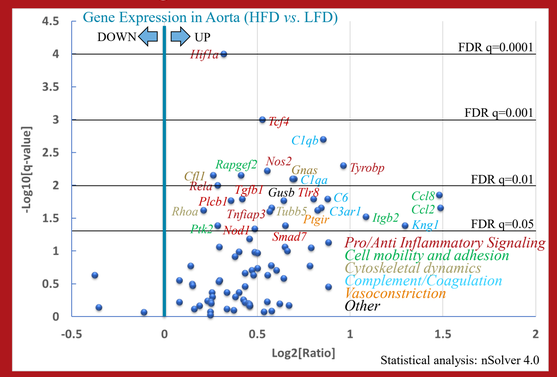


Gene Set Enrichment Analysis (GSEA)
Red: Upregulated
Blue: Downregulated

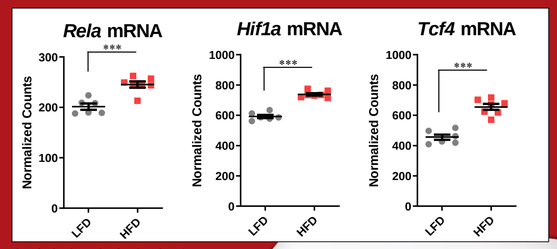
HFD enhances the expression of pro-inflammatory gene sets in the mouse aorta

GENE SETS (GSEA) Upregulated in HFD-fed mice	NES	Nominal p-value	FDR q-value
HALLMARK_INTERFERON_GAMMA_RESPONSE	1.43	0.019	0.364
HALLMARK_INTERFERON_ALPHA_RESPONSE	1.41	0.038	0.210
HALLMARK_COMPLEMENT	1.39	0.042	0.167
HALLMARK_ALLOGRAFT_REJECTION	1.38	0.033	0.135
HALLMARK_INFLAMMATORY_RESPONSE	1.34	0.117	0.170
HALLMARK_APOPTOSIS	1.34	0.089	0.142
HALLMARK_PI3K_AKT_MTOR_SIGNALING	1.28	0.200	0.182
HALLMARK_IL6_JAK_STAT3_SIGNALING	1.25	0.160	0.189

HFD enhances the expression of proinflammatory genes in the mouse aorta



HFD enhances the expression of key proinflammatory transcription factors



Statistical analysis: Student's *t*-test
***, $p<0.001$

ACC.21

APABETALONE INHIBITS EPIGENETIC ACTIVITY OF BET PROTEINS

Apabetalone is a clinical-stage small molecule that showed a trending reduction in CV events in high-risk CVD and DM2 patients. It binds competitively to bromodomains in BET histone acetylation "readers", causing their release from chromatin, and downregulation of BET-sensitive genes with proinflammatory and pro-atherosclerotic activities.

BET: bromodomain and extraterminal proteins; ac: acetylated lysine residue on DNA-associated proteins; BD: bromodomain; TF: transcription factor

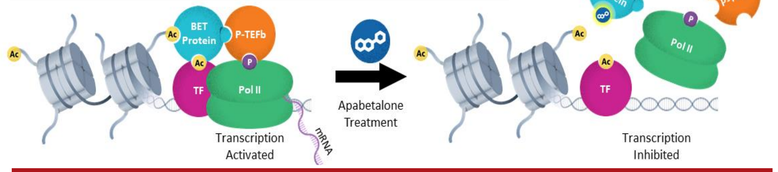


FIG 3: APABETALONE DOWNREGULATES NF-κB PATHWAY GENES ELEVATED IN DIO MOUSE AORTA

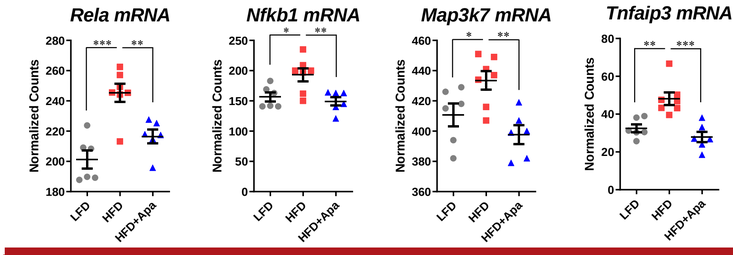


FIG 4: APABETALONE DOWNREGULATES KEY CHEMOKINES AND A CHEMOKINE RECEPTOR ELEVATED IN DIO MOUSE AORTA

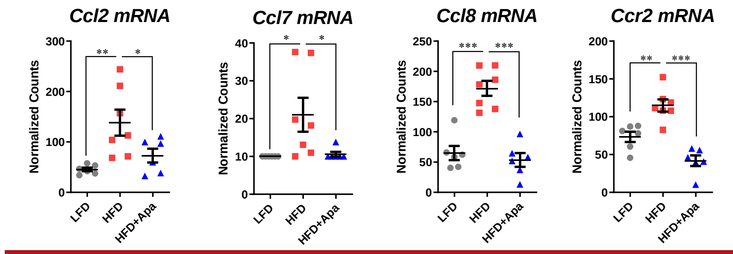
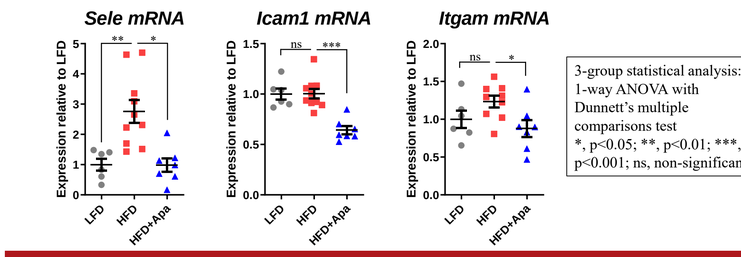


FIG 5: APABETALONE DOWNREGULATES VASCULAR INFLAMMATION MARKERS IN DIO MOUSE AORTA



3-group statistical analysis: 1-way ANOVA with Dunnett's multiple comparisons test
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, non-significant

DISCLOSURE INFORMATION

SW, ED, LMT, DG, CS, SCS, BDR, RJ, MS, JOJ, NCW and EK receive(d) a salary from Resverlogix and own stock and stock options. SA has no competing interests.