# A Mitochondrial Uncoupler, BAM15, Inhibits Liver Tumor Promotion in the Context of a High Fat Diet Enriched in Saturated Fat

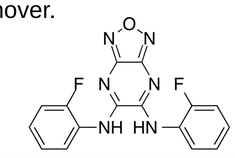

**Theresa Nguyen[1], Kim Pedersen[1], Tomislav Jelesijevic[3], Christopher Axelrod[2], Elizabeth Zunica[2], John Kirwan[2], Martin Ronis[1]**

LSU Health NEW ORLEANS School of Medicine

**LSU Health Sciences Center, Department of Pharmacology[1]; Pennington Biomedical Research Center, Baton Rouge[2]; Department of Comparative Biomedical Studies, LSU School of Veterinary Medicine, Baton Rouge[3]**

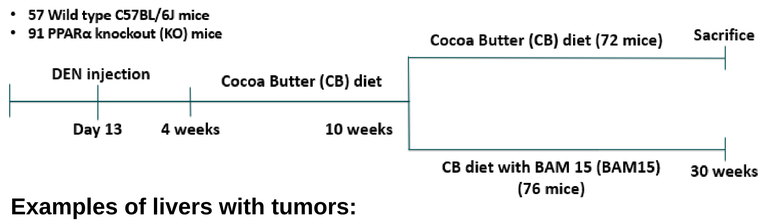

## Introduction

Liver cancer ranks as the third deadliest cancer globally and is on the rise due to the obesity epidemic. The incidence is higher in males than females. BAM15, a mitochondrial uncoupler, has demonstrated protective effects against weight gain in obesity models in mice. Our objective was to assess the effect of BAM15 on tumor promotion caused by a high saturated fat diet in mice. We also aimed to determine the role of PPARα, which is a transcription factor stimulating the expression of rate-limiting enzymes of fatty acid oxidation. Since bone marrow is enriched with adipocytes in adulthood, we finally assessed the role of BAM15 on bone turnover.

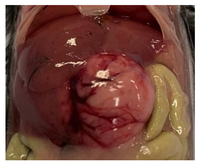



**BAM 15**

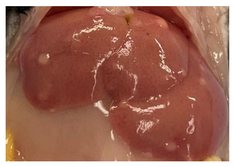
## Methods




**Examples of livers with tumors:**



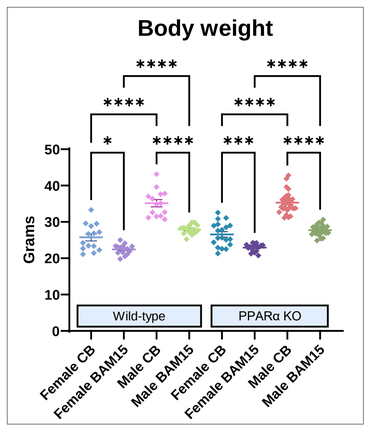
**A large hepatocellular carcinoma**



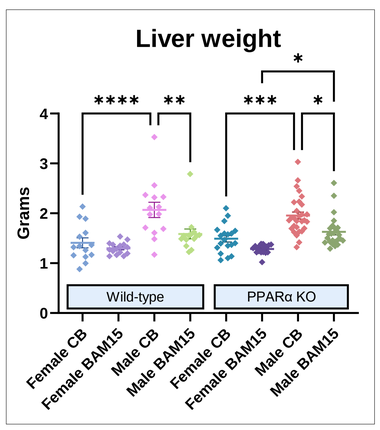
**Multiple small liver nodules**

- Wild type (wt) C57BL/6J and PPARα knockout (KO) mice were injected intraperitoneally with 20 mg/kg diethyl nitrosamine (DEN) on postnatal day 13.
- From weeks 4-10, mice were fed a high saturated fat diet with cocoa butter as a saturated fat (CB diet).
- At 10 weeks of age, mice were either continued on the CB diet or were fed a CB diet supplemented with a 0.1% (w/w) BAM15.
- The mice were sacrificed at 30 weeks of age with recording of visible liver tumors and collection of serum and tissues.
- From the serum, severity of liver tumorigenesis was determined by ELISA of the tumor stem cell marker alpha-fetoprotein (AFP) and liver injury by a kinetic enzymatic assay of alanine transaminase (ALT).
- Serum markers for bone synthesis (Procollagen 1A1) and bone resorption (collagen crosslinks CTX-1) were assessed by ELISA.
- RNA was isolated from randomly selected subsets of mice and RNA quality was validated by TapeStation analysis.
- Gene expression was determined by qRT-PCR assays.

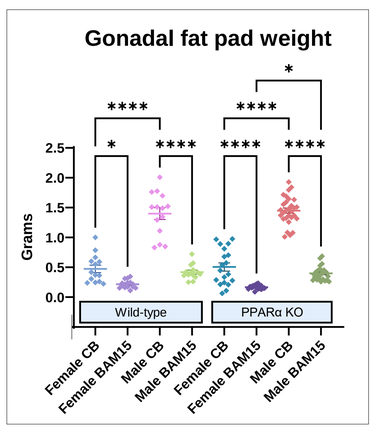
## Results



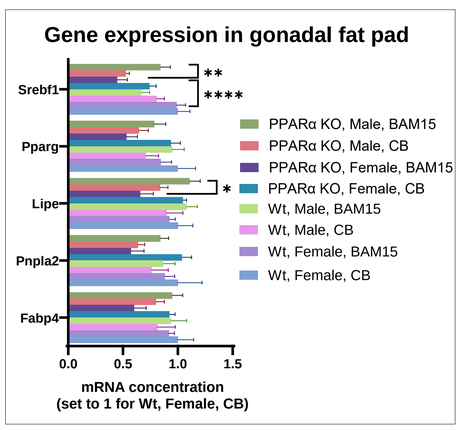

Body weight of mice fed a cocoa butter diet (CB), or a CB diet supplemented with BAM15 (BAM15) at sacrifice[1].



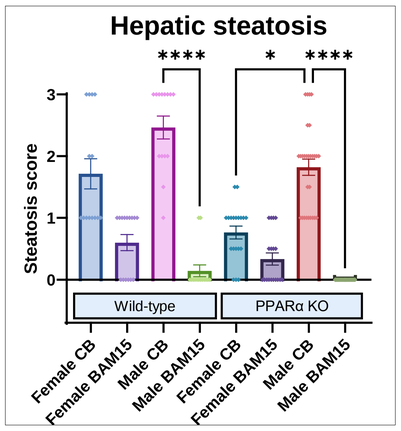

Liver weight of mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) at sacrifice[1].



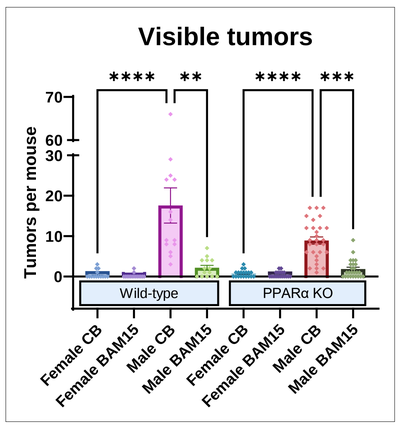

Gonadal fad pad weight of mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) at sacrifice[1].



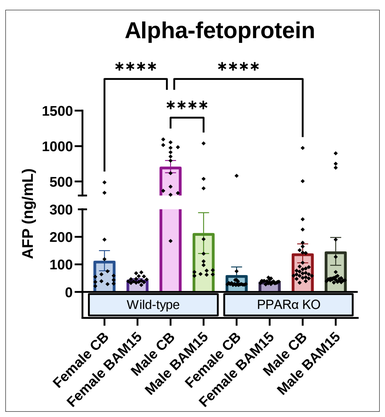

mRNA expression in gonadal fat tissue from mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) was determined by qRT-PCR relative to the expression of 18S rRNA. N = 6 per group. ANOVA of $\Delta C_T$ values was performed. There were no significant effects of the BAM15 exposure[1].



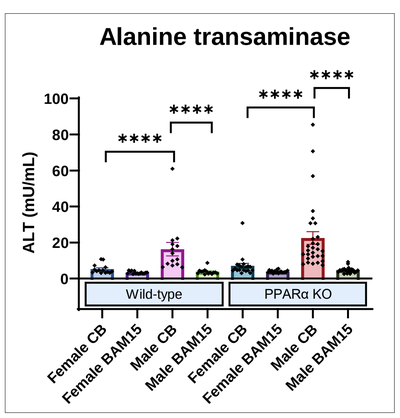

Steatosis scores of H&E-stained liver sections from mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) were recorded[2].



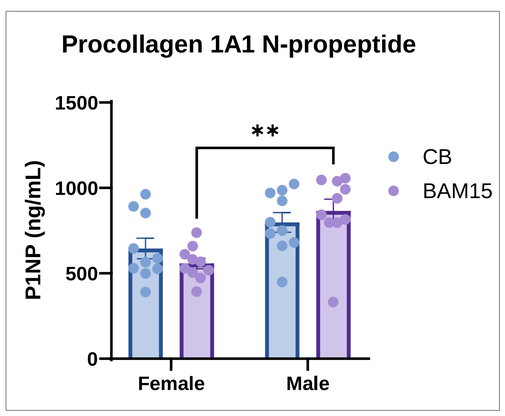

Visible liver tumors and nodules were counted at sacrifice for mice fed CB diet, or a CB diet supplemented with BAM15 (BAM15)[2].



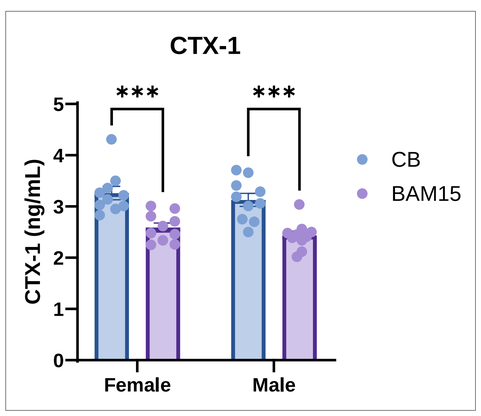

Serum alpha-fetoprotein (AFP) content in mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) was determined by ELISA. Logarithm-transformed data were analyzed[1].



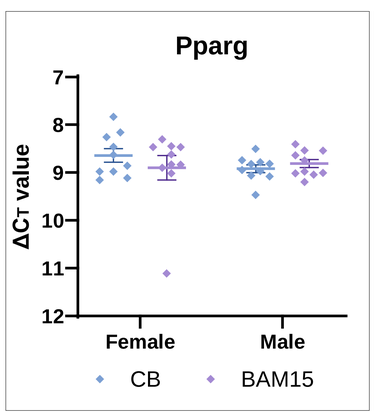

Serum alanine transaminase (ALT) activity in mice fed a cocoa butter (CB) diet, or a cocoa butter diet supplemented with BAM15 (BAM15) was determined by an enzymatic assay. Logarithm-transformed data were analyzed[1]. The main effect of the genotype was significant (P = 0.003).

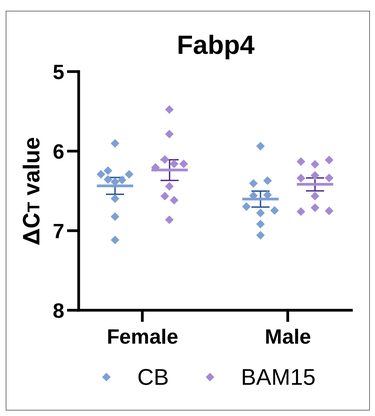



The bone formation marker, serum procollagen 1A1 N-propeptide (P1NP), in wild-type mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) was determined by ELISA[1].




The bone resorption marker, serum CTX-1, in wild-type mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) was determined by ELISA[1].




Expression of Fabp4 and PPARg mRNA (marker genes for adipocytes) in bone marrow from wild-type mice fed a cocoa butter (CB) diet, or a CB diet supplemented with BAM15 (BAM15) was determined by qRT-PCR relative to the expression of beta-Actin mRNA as $\Delta C_T$ values. ANOVA indicated no significant differences.

## Summary

- BAM15 led to significantly (P<0.05) lower body weight and weight of gonadal fat pads.
- Weight loss of gonadal fat pads were not explained by changes in gene expression of *Fabp4, Pnpla2, Lipe, Pparg,* or *Srebf1.*
- In males, BAM15 significantly decreased liver weight and hepatic steatosis.
- The number of tumors per mouse was significantly higher in male compared to female mice fed the CB diet.
- In male mice, BAM15 led to significantly fewer tumors and significant decreases in serum AFP and serum ALT activity.
- Knockout of PPARα did not stimulate hepatic steatosis, but led to higher ALT levels and significantly lower AFP levels in males fed the CB diet.
- In wt mice, the BAM diet had no effect on Procollagen 1A1 abundance but caused a significant decrease in serum CTX-1 content in both sexes.
- Expression of two adipocyte marker genes (*Fabp4* and *Pparg*) in femoral bone marrow was unaffected by BAM15.

[1]Data were analyzed by ANOVA followed by comparisons with Tukey's adjustment. *, **, ***, ****: P < 0.05, 0.01, 0.001, 0.0001.

[2]Data were analyzed by Kruskall-Wallis test and Dunn's multiple comparisons test. *, **, ***, ****: P < 0.05, 0.01, 0.001, 0.0001.

## Conclusion

- In addition to protection from obesity, BAM15 inhibits liver tumor promotion caused by a high-saturated fat diet, particularly in males.
- PPARα has a dual effect, with knockout of the gene promoting liver injury, but reducing the tumor severity.
- BAM15 may inhibit bone resorption without a decrease in bone marrow adiposity.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