The ACLY inhibitor SB204990 does not alter lysine histone acetylation in mouse liver.

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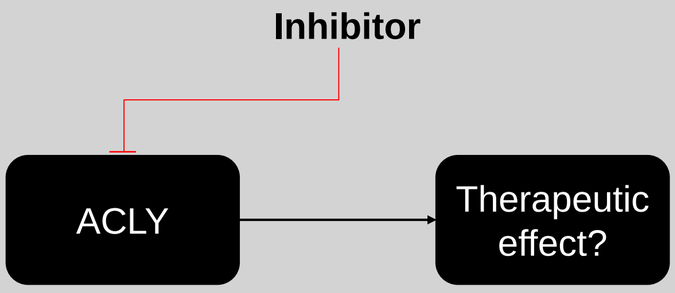
· Introduction ·

ATP-citrate lyase (ACLY) is an enzyme that cleavage citrate into acetyl-coenzyme A (acetyl-CoA) and oxaloacetate in the nucleus and cytoplasm, where acetyl-CoA is used for important cellular functions such as histone acetylation or fatty acid synthesis (1). ACLY has been shown to play a role in a glucose-to-acetate metabolic switch (2).

Inhibition of ACLY, which is reasonably well tolerated in adult animals, impairs tumor growth and produces blood lipid-lowering effects (2). Thus, ACLY inhibitors as SB could represent a therapeutic opportunity for the treatment of cancer and metabolic diseases.



· Background ·

Previously, mice were exposed for 16 weeks to 4 experimental conditions: standard diet (STD), STD + SB (250 mg/Kg of food), high fat diet (HFD) and HFD + SB (250 mg/Kg of food). Beneficial metabolic effects were observed in HFD + SB when compared to HFD.

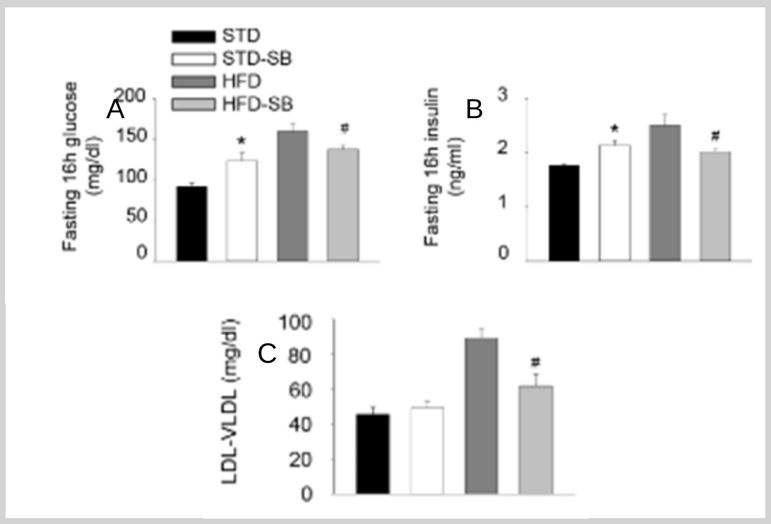


Figure 1. Metabolic parameters levels in mice treated with SB with healthy and obesogenic diets. A) Glucose in blood after 16 hours fasting. B) Insulinemia after 16 hours fasting. C) circulating LDL-VLDL levels. Two-way ANOVA.

· Background ·

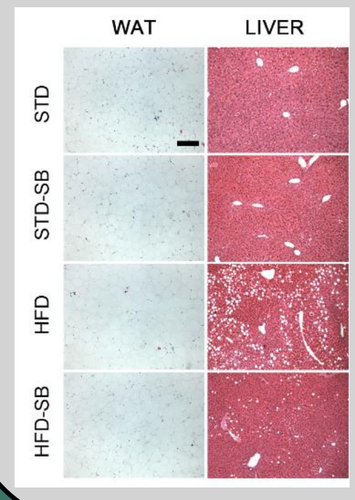


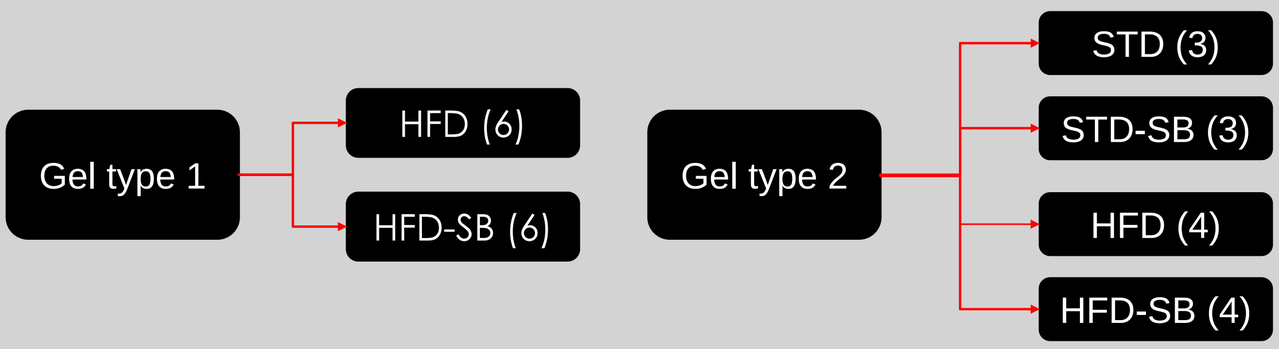
Figure 2. SB reduces lipid content in HFD-fed mice. Representative WAT and liver histology from STD, STD-SB, HFD and HFD-SB mice. HFD-SB treated mice exhibit smaller adipocytes and smaller lipid droplets when compared to HFD-fed mice. Scale bar: 200 µm.

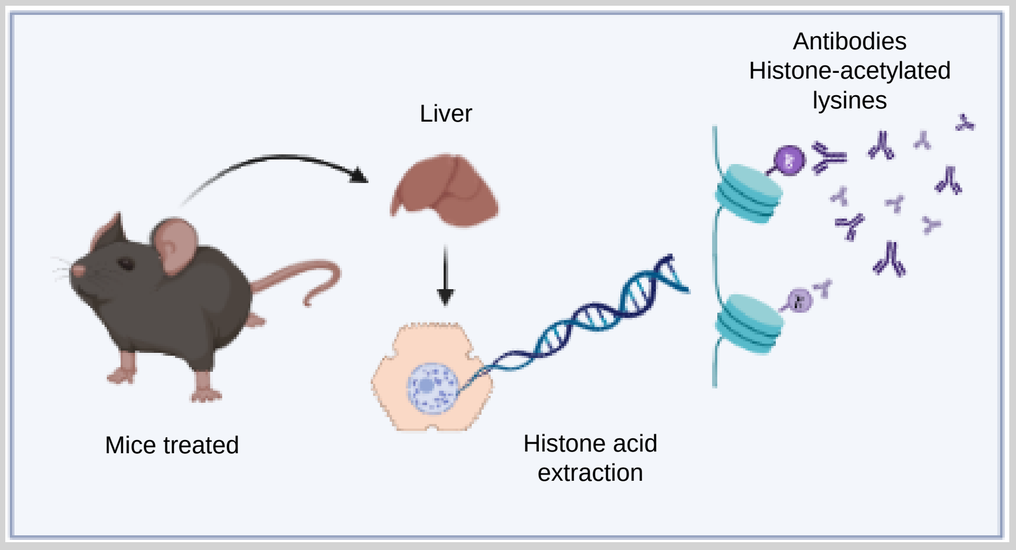
· Objetive ·

Due to the link between ACLY activity and histone acetylation determined in prior studies, we decided to evaluate whether beneficial metabolic effects observed are associated to modulations in histone acetylation in liver tissue lysates of mice treated with SB.

· Methods ·

An histone acid isolation was conducted using the livers of mice from the experimental conditions earlier described. Samples were processed and western blots using specific antibodies of several histone-acetylated-lysines were performed to evaluate potential modulation on histone acetylation levels. To compare the samples, two types of gels were prepared:





· Results (HFD/HFD-SB) ·

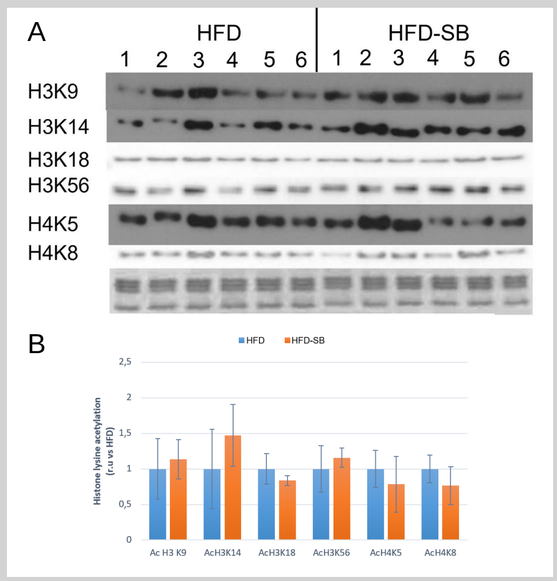


Figure 3. Histone acetylation levels of H3K9, H3K14, H3K18, H3K56, H4K5 y H4K8, and their corresponding ponceau. A) HFD (6) and HFD-SB (6). B) Acetylation levels, mean +/- SD. A t-test was applied. No significant differences has been observed between HFD and HFD-SB in acetylation levels of H3K9, H3K14, H3K18, H3K56, H4K5 and H4K8

· Results (all conditions) ·

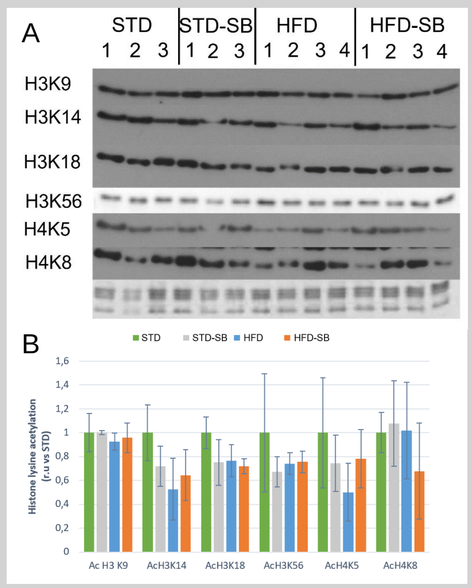


Figure 4. Histone acetylation levels of H3K9, H3K14, H3K18, H3K56, H4K5 y H4K8, and their corresponding ponceau. A) STD (3), STD-SB (3), HFD (4) and HFD-SB (4). B) Acetylation levels, mean +/- SD. A t-test was applied. Acetylation levels of H3K9, H3K14, H3K18, H3K56, H4K5 and H4K8 are not significantly altered in the different experimental conditions.

· Conclusion and future perspectives ·

These results indicate that beneficial effects produced by ACLY inhibition are not caused by changes in histone acetylation in the liver. Further analysis of others histone-lysines, such as H3K27 and H4K12, should be addressed in the nearly future. Total Histone 3 and histone 4 levels should be also performed.

Future perspectives will include the analysis of acetylation levels of proteins in other cellular compartments. In addition the determination of potential modulation in oxygen consumption rate will be performed in primary hepatocytes.

· References ·

- 1. Carrer, A., Parris, J. L., Trefely, S., Henry, R. A., Montgomery, D. C., Torres, A., ... & Wellen, K. E. (2017). Impact of a high-fat diet on tissue Acyl-CoA and histone acetylation levels. Journal of Biological Chemistry, 292(8), 3312-3322.
- 2. Zhao, S., Torres, A., Henry, R. A., Trefely, S., Wallace, M., Lee, J. V., ... & Wellen, K. E. (2016). ATP-citrate lyase controls a glucose-to-acetate metabolic switch. Cell reports, 17(4), 1037-1052.Lehmann,E.L. (1986) Testing Statistical Hypotheses. Springer Texts in Statistics. Vol. 1, 2nd edn. Springer-Verlag, New York.