LIBERTY UNIVERSITY

Evaluation of enterolactone for the treatment and prevention of type-2 diabetes

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Background

Type-2 diabetes (T2D) is an increasingly common public health concern that affects over 35 million Americans[1] and over 462 million people worldwide[2]. T2D is characterized by elevated blood glucose levels caused by insulin resistance[3]. Despite its role in maintaining blood glucose levels, adipose tissue is often overlooked as a potential therapeutic target for T2D. Previous literature has indicated that moderate habitual coffee consumption, both caffeinated and decaffeinated, is inversely associated with the risk of developing T2D[4]. Therefore, there is a current interest in this relationship, particularly the effects of small bioactive compounds found in coffee and their metabolites, and T2D. Several metabolites of compounds found in coffee have potential as anti-diabetic agents. One such compound is enterolactone (ENL), a gut microbial metabolite of matairesinol, which has also shown promise as an anti-diabetic agent[5]. Previous literature has shown that ENL promotes basal glucose uptake in L6 myotubules, however its effect on adipose tissue is still unknown[5]. Here, we show that low concentrations of ENL promotes basal glucose disposal in differentiated 3T3-L1 adipocytes.

Research Question

How does enterolactone effect glucose uptake in 3T3-L1 adipocytes?

Methods

3T3-L1 fibroblasts were grown in complete growth media (DMEM + 10% Bovine Calf Serum). Once grown to confluency, a timeline of experiments was followed.

Day 0: Ten thousand cells were seeded into each well of a 96-well plate.
Day 1: The complete growth media was aspirated and 100 µL of differentiation media (complete growth media + 0.5 mM IBMX + 1 µM dexamethasone + 10 µg/mL insulin) was added to each well.
Day 4: The differentiation media was aspirated and 100 µL of post-differentiation media (complete growth media + 10 µg/mL insulin) was added to each well.
Day 7: The post-differentiation media was aspirated and 100 µL of complete growth media was added to each well, supplemented with increasing concentrations of ENL (1 µM, 10 µM, 20 µM, 50 µM, 100 µM) and 200 µg/mL of 2-NBDG, a fluorescently labeled glucose molecule.

After treatment, the plate was incubated at 37 °C for 45 minutes. The plate followed a centrifugation and washing procedure. Then the fluorescence intensity was read, the data was graphed (Fig. 1,3), and analyzed by a student t-test (Table 1,3).

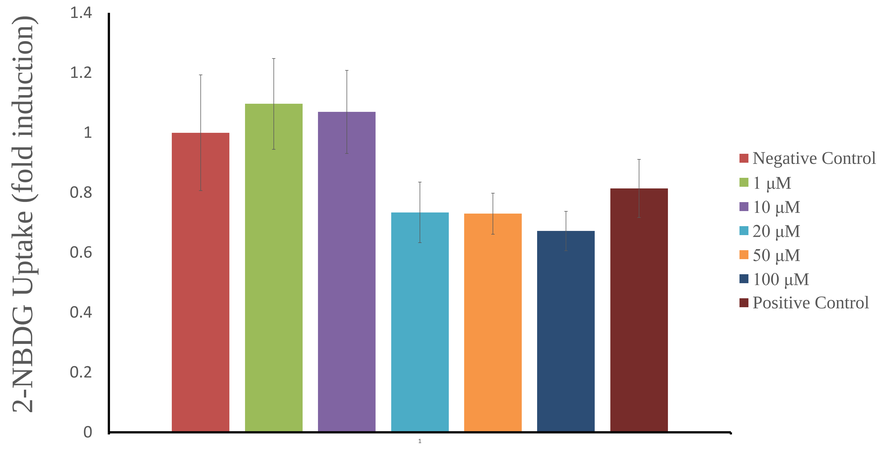

Figure 1. Normalized glucose uptake vs the different treatments given to 3T3-L1 adipocytes. Two trials of the assay were run, with this graph showing the composite data of both trials. The negative control was a vehicle control of 100 µL ethanol. The positive control was 10 nm of insulin. The ENL concentrations were 1 µM, 10 µM, 20 µM, 50 µM, and 100 µM.

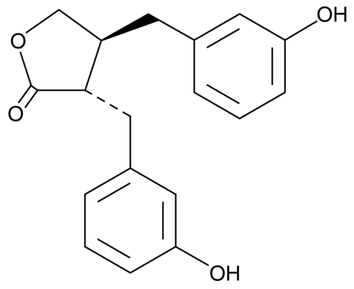

Figure 2. Chemical structure of enterolactone.

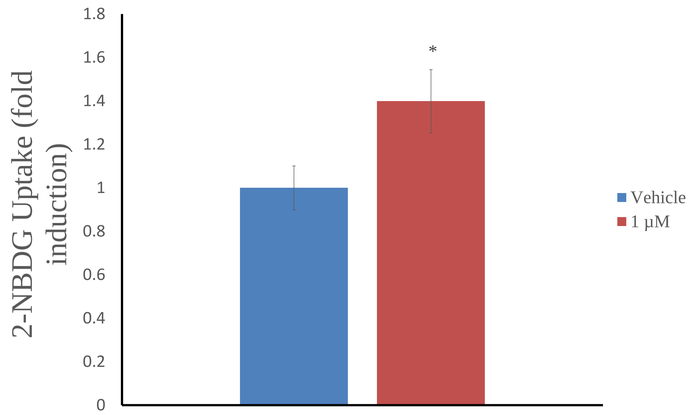

Figure 3. Normalized glucose uptake of the negative vehicle control and 1 µM ENL concentration. The negative vehicle control was 20 µL of ethanol.

Table 1. T-test results the negative control, positive control, and each ENL concentration from Figure 1.

Negative/Positive Control	0.2329

	Negative Control	**Positive Control**
1 µM	0.5843	0.0344
10 µM	0.6830	0.0411
20 µM	0.0948	0.4261
50 µM	0.0718	0.3227
100 µM	0.0302	0.0963

Table 2. T-test results the negative control and 1 µM ENL concentration from Figure 2.

Negative Control/ 1 µM ENL	0.03176

Results and Conclusion

Though analysis of the data indicated that higher concentrations of ENL have an inhibitory effect on glucose uptake, (Fig. 1), we also found that a lower concentration, 1 µM, significantly increased basal glucose uptake (Fig. 3). However, this data may only be described as a trend because of the negative and positive controls. Ideally, the negative control should show the least amount of glucose uptake and the positive control should be higher than the negative control. This discrepancy may be attributed to the cell's receiving too much insulin (100 nM).

The first set of t-test results indicate that there is no significant difference between the negative and positive controls (Table 1). The t-test results between the 20 µL negative vehicle control and 1 µM ENL concentration indicate that this relationship is statistically significant (Table 2).

Future Work

1. Repeat glucose uptake assays using 10 nM insulin rather than 100 nM insulin.
2. Extend the dose-dependent range below 1 µM.
3. Increase the time of ENL exposure up to 24 hours prior to measuring glucose uptake.
4. Determine the effect of ENL on insulin-stimulated glucose uptake.
5. Characterize the mechanism of ENL-stimulated glucose uptake.

References and Acknowledgments

1. Centers for Disease Control and Prevention. Diabetes. Published online 2021.
2. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Kaabi JA. Epidemiology of Type 2 Diabetes - Global
Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2020;10(1):107-111.
doi:10.2991/jegh.k.191028.001
3. Stoffers D. The development of beta-cell mass: recent progress and potential role of GLP-1. Horm Metab Res.
2004;36(11-12):811-821. doi:10.2337/diacare.21.9.1414
4. Kolb H, Martin S, Kempf K. Coffee and Lower Risk of Type 2 Diabetes: Arguments for a Causal Relationship.
Nutrients. 2021;13(4):1144. doi:10.3390/nu13041144
5. Zhou F, Furuhashi K, Jin Son M, Yoshizawa F, Miura Y, Yagasaki K. Antidiabetic effect of enterolactone in cultured
muscle cells and in type 2 diabetic model db/db mice. Cytotechnology. 2017;69(3):493-502. doi:10.1007/s10616-016-
9965-2

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