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An in-vitro Examination of the Effects of Enterodiol on Glucose Disposal in Human Adipocytes

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Abstract

Recent research has shown a relationship between moderate coffee consumption and a decreased risk of developing Type 2 Diabetes (T2D). Furthermore, it has been shown that the attenuated risk is not conferred by caffeine since decaffeinated coffee has the same effect. Through a systematic review of the literature, we identified six compounds, either found in coffee or that are metabolites of compounds founds in coffee, that have been shown to have anti-diabetic effects. In this study, we explore the effects of enterodiol (EDL), which is a direct gut microbial metabolite of secoisolariciresinol, a lignan found in coffee. While EDL has not been directly assayed for its ability to promote glucose disposal, research has shown that EDL inhibits 3T3-L1 adipogenesis and triacylglycerol (TAG) uptake in HEPA 1-6 cells. Inhibition of fat uptake and storage supports the rationale that EDL may foster an augmented reliance on carbohydrates to maintain energy balance. These data thusly suggest that EDL may contribute to attenuated T2D pathogenesis by promoting glucose disposal. In this study, we show that EDL promotes basal glucose uptake in human adipocytes. To test this, 3T3-L1 fibroblasts were differentiated into adipocytes and treated with EDL in a concentration-dependent manner. A fluorescent analog of glucose was used to measure glucose uptake in response to EDL. Future studies will further investigate the effects of EDL on insulin-stimulated glucose uptake and the mechanisms by which its effects are exerted.

Introduction

Type-2 Diabetes (T2D) is an increasingly common public health concern, with confirmed cases exceeding 462 million globally and 35 million in the United States alone.[1] Furthermore, T2D is the most expensive chronic health condition in the United States, with over $200 billion being spent on medical care for diabetics annually.[1,2] T2D is characterized by chronically elevated blood glucose levels due to acquired insulin resistance.[3] Insulin is primarily responsible for glucose uptake in specific cell types; however, in T2D pathogenesis, these cells become desensitized to insulin leading to insulin resistance and diminished glucose uptake. This diminishing leads to chronic hyperglycemia, contributing to the pathogenesis of the disease and damage to other vital organs, such as the kidneys and the heart.[4] Due to the increased prevalence and cost of this disease, the pursuit of novel low-cost agents that can help treat and prevent T2D is crucial.

Previous research has shown that the moderate consumption of coffee has an inverse association with T2D risk.[5] Moreover, caffeine is unlikely to be associated with this correlation due to research suggesting that consumption of decaffeinated coffee elicits the same effects.[6] Through an extensive literature review, six compounds found in coffee or that are direct metabolites of those compounds have been shown to have anti-diabetic effects. In this study, we examine enterodiol (EDL) in this context.

EDL is a gut metabolite of secoisolariciresinol, which is classified as a lignan and is found in coffee. EDL has been shown to inhibit triacylglycerol (TAG) synthesis uptake in HEPA 1-6 cells and adipogenesis in 3T3-L1 adipocytes.[6] This inhibition would suggest that the adipocytes meet energy demand via metabolizing alternative substrates. EDL has not been directly assayed for glucose disposal in human adipocytes, which is considered a primary insulin-responsive tissue in the pathogenesis of diabetes. Thus, we hypothesized that EDL could promote glucose disposal in human adipocytes, which may contribute to attenuated T2D pathogenesis.

Methods

3T3-L1 Differentiation

3T3-L1 fibroblasts were seeded in black, clear bottom, 96-well plates and grown to ~80% confluency in complete growth medium comprised of DMEM supplemented with 10% bovine calf serum. The fibroblasts were then differentiated for seven days in complete growth medium supplemented with 0.5 mM IBMX, 1.0 µM dexamethasone, and 10 µg/mL human insulin. On day three of the differentiation protocol, differentiation medium was replaced with post-differentiation medium which consisted of complete growth medium and 10 µg/mL insulin.

Fluorescence Glucose Uptake Assay

On day eight of the protocol the adipocytes were treated for 30 minutes with differing concentrations of EDL (1, 10, 20, 50, and 100 µM) or vehicle (methanol), or insulin (10 nM). The range of EDL concentrations used in this study were based on those used in other studies that assay the various metabolic effects of EDL. A fluorescent glucose analog, 2-NBDG, was also added to each treatment in a concentration of 100 µg/mL. After the incubation time, plates were washed with 1X phosphate buffered saline (in mM: 137 NaCl, 2.7 KCl, 8 Na_2HPO_4, and 2 KH_2PO_4, pH 7.4) according to manufacturer protocol (Cayman, Ann Arbor, MI). Once complete the resultant fluorescence was analyzed at excitation/emission 485/535 nm via a Tecan Infinite 200 Pro plate reader (Grödig, Austria).

Statistical Analysis

Data were analyzed by one-way ANOVA. Duncan's multiple range test was performed for pairwise comparison of observed significant differences ($p < 0.05$). Values are expressed as mean ± the standard error of the mean.

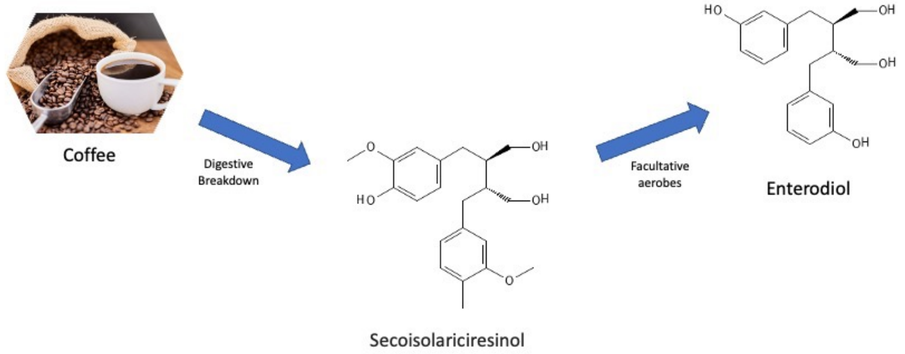

Figure 1. Secoisolarciresinol is broken down in the gastrointestinal tract via facultative aerobes to produce the primary metabolite enterodiol. Secoisolariciresinol is a common organic compound in the class of lignans that is found in coffee. Secoisolariciresinol is then broken down in the gastrointestinal tract via facultative aerobes and is converted into a direct metabolite, enterodiol.[7]

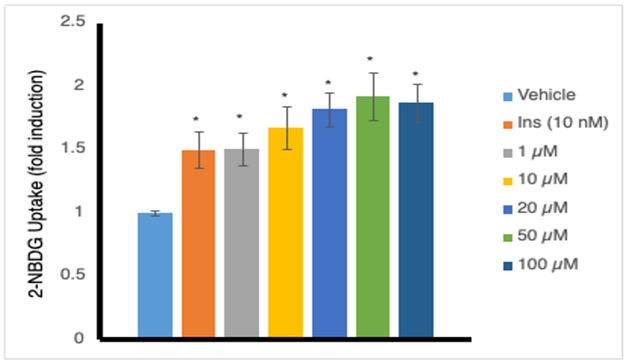

Figure 2. Enterodiol stimulates glucose uptake to the same extent as insulin in human adipocytes. Three glucose uptake assays were performed, and the data collected was normalized to the negative control. Insulin in the concentration of 10 nM was used as the positive control. Each concentration of EDL exhibited a similar effect to the positive control on glucose disposal in human adipocytes. This could suggest that EDL mimics insulin in the upregulation of GLUT4 translocation. Furthermore, the EDL concentration of 50 µM exhibited the highest effect before showing a slight decrease in the 100 µM concentration. This shows that the optimal concentration of EDL on glucose disposal may be 50 µM.

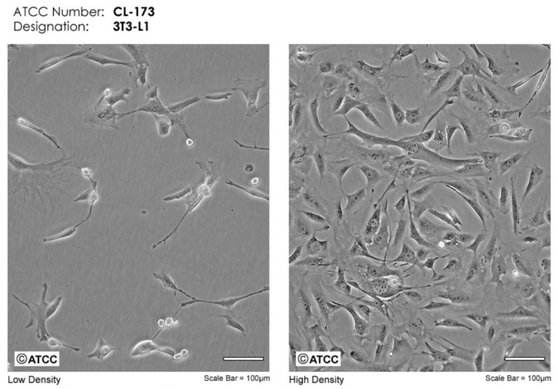

Figure 3. 3T3-L1 human fibroblasts at low vs high confluency rates

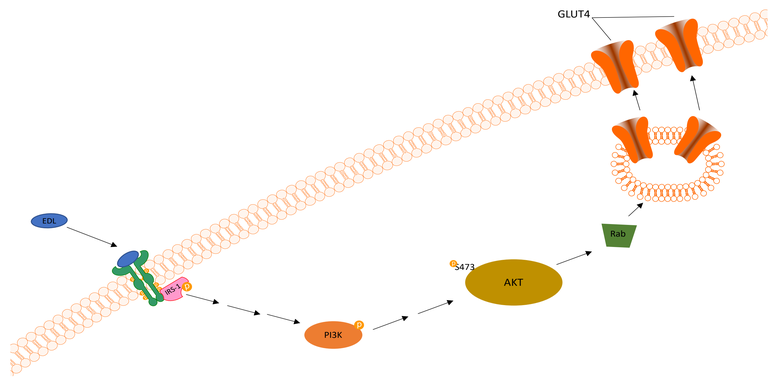

Figure 4. The hypothesized mechanism that enterodiol takes to upregulate GLUT4 translocation and glucose disposal in human adipocytes.[8] It has been hypothesized that EDL mimics insulin in the upregulation of GLUT4 translocators. EDL binds to an insulin receptor causing the autophosphorylation of tyrosine kinase receptors, this leads to the phosphorylation of insulin response element 1 (IRS1) leading to a cascade involving the phosphorylation of PI3K and AKT. This eventually leads to the exocytosis and incorporation of GLUT4 which are involved in glucose uptake.

Results and Conclusion

To determine whether EDL increases glucose disposal in adipocytes, we co-incubated differentiated 3T3-L1 adipocytes with EDL (concentrations) and 2-NBDG. EDL significantly increased glucose uptake as compared to the control ($p < 0.05$) (Fig. 2) with the 50 µM concentration increasing glucose uptake by over 50% ($p < 0.001$). Additionally, we found that all of the assayed concentrations increase glucose uptake to an extent that is not statistically different from the positive control (insulin). This suggests that EDL may act as an insulin mimetic.

Conclusions

Previous studies showed that EDL prevents TAG uptake and adipogenesis.[8] However, its effect on glucose uptake remained unclear. In this study, we demonstrate that EDL stimulates glucose uptake in adipocytes. While the mechanism that underlies this beneficial action remains unclear, the fact that EDL stimulates glucose uptake to the same extent as insulin suggests that it may behave similarly to insulin by facilitating GLUT4 translocation. GLUT4-mediated glucose transport in adipose tissue can be stimulated through at least two signaling cascades. It is well understood that insulin stimulates glucose uptake through PI3K/Akt-mediated GLUT4 trafficking (Fig. 4). It has also been shown that AMPK activation can stimulate the conversion of PIP2 to PIP3, which activates PI3K/Akt signaling.[9] This could suggest that EDL might function by activating PI3K/Akt signaling in one of several ways including binding directly to the insulin receptor thereby activating a tyrosine kinase to activate insulin receptor substrate 1 (IRS-1). Either mechanism would eventually lead to the incorporation of GLUT4 into the plasma membrane causing an increase in the uptake of glucose. In addition, due to research showing that EDL inhibits the uptake of TAGs and adipogenesis, it is also possible that glucose uptake could be an indirect effect of mitochondrial uncoupling. The inhibition of fatty acid synthesis is an indicator of negative energy balance. This supports the rationale that glucose uptake would be augmented to account for the resultant increase in cellular ATP demand.

Future Work

1. Confirming the role of GLUT4 in EDL-stimulated glucose uptake by inhibiting GLUT4 with the selective inhibitor, ritonavir.
2. Assaying the effect of EDL on insulin-stimulated glucose uptake
3. Determining the mechanism by which EDL stimulates glucose uptake by measuring its effect on IRS-1, Akt, and AMPK activation.

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All organic structures were drawn using PubChem Sketcher V2.4 Software.

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