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Identification of select low-molecular weight compounds, found in coffee, that promote glucose homeostasis in 3T3-L1 adipocytes

Sierra Hall, Brooke Chapple, Alexis Ramerth, Josiah Chung, Spencer Powers, Abigail McLaughlin, William Moore Ph.D.

Purpose of Project

The purpose of this study was to determine whether the following compounds affect biomarkers of type-2 diabetes (T2D) pathogenesis in 3T3-L1 adipocytes: cafestol (CF), secoisolariciresinol (SL), and enterodiol (EDL). To do this, three specific objectives were been proposed:

1. Determine whether these compounds promote glucose uptake in 3T3-L1 adipocytes.
2. Determine whether CF, EDL, and SL promote glucose uptake via a Glut1-, or Glut4-dependent mechanism in 3T3-L1 adipocytes.
3. Characterize the effect of CF, EDL, and SL on biomarkers associated with pertinent Glut activity.

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 fasting blood glucose levels due to insulin resistance.[3] Insulin is primarily responsible for glucose uptake in specific cell types; however, in T2D pathogenesis, these cells become desensitized leading to insulin resistance and diminished glucose uptake. This 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. Multiple studies collectively demonstrate that habitual coffee consumption and T2D risk are inversely related.[5]

Via a systematic literature review, six compounds that are either naturally found in coffee or are metabolites of compounds found in coffee were determined to have the potential to behave as anti-diabetic agents. Three of these compounds were studied in this project. One of these compounds, cafestol (CF), has previously been shown to promote insulin secretion[6,7] and glucose disposal in SkM.[7] However, its ability to promote glucose uptake in adipocytes has not yet been assessed. The second compound, secoisolariciresinol (SL), has exhibited an ability to delay the onset of STZ-induced T2D, which suggests that it may convey a protective effect on β-cells.[8] Finally, enterodiol (EDL) has been shown to inhibit triacylglycerol uptake in HEPA1-6 cells, as well as adipogenesis in 3T3-L1 fat cells.[9] These data evidence the rationale that these compounds may attenuate T2D pathogenesis by preventing β-cell damage and promoting glucose uptake in SkM and adipose tissues.

In summation, the available literature indicates that these compounds may promote β-cell function and survival, induce glucose uptake in SkM and adipocytes, and/or promote anti-diabetic effects in vivo. A gap in knowledge remains as to the ability of these compounds to affect multiple tissue types, as well as the mechanisms by which they promote these effects. Thus, the aim of this work was to determine their effects on differentiated 3T3-L1 adipocytes.

Methods

Cell Culture
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 (day 0) 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. Cells were maintained in post-differentiation medium until day six at which point the experiments were conducted.

Fluorescence Glucose Uptake Assay
On day six of the protocol the adipocytes were treated for 30 minutes with either compound (in a concentration-dependent manner), vehicle (the negative control) or 10 nM insulin (the positive control). The following were used in initial assays to determine the optimal concentration(s) of each compound:

EDL: 0,1, 10, 20, 50, 100 µM
SL: 0.01, 0.1, 1, 10, 15, 20, 100 µM
CF: 0.01, 0.1, 1, 10, 20 µM

A fluorescent glucose analog, 2-NBDG (100 µg/mL), was also added to each treatment. Following the incubation, plates were washed with 1X PBS according to manufacturer protocol (Cayman, Ann Arbor, MI). Fluorescence was analyzed at excitation/emission 485/535 nm via a Tecan Infinite 200 Pro plate reader (Grödig, Austria).

BAY-876-Treated Glucose Uptake Assay
On day six of the protocol the adipocytes were treated for 30 minutes with either compound (concentrations were determined based on the two optimal concentrations in the previous glucose uptake assay), vehicle (the negative control), 10 nM insulin (the positive control), compound plus either 2 or 290 nM BAY-876, or 10 nM insulin plus 2 nM BAY-876 (ensures effectiveness of BAY-876).

2-NBDG (100 µg/mL), was added to each treatment as described in the original glucose uptake assay. Immediately following a 30-minute incubation period, plates were washed with 1X PBS according to manufacturer protocol (Cayman, Ann Arbor, MI). Fluorescence was then measured 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.

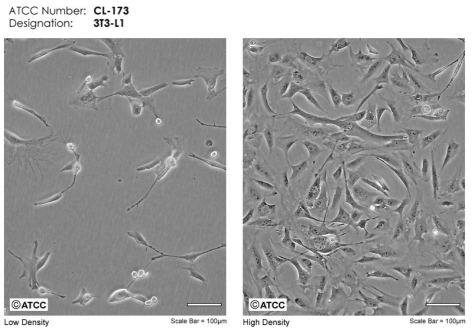

Figure 1. 3T3-L1 fibroblasts at low vs high confluency rates.

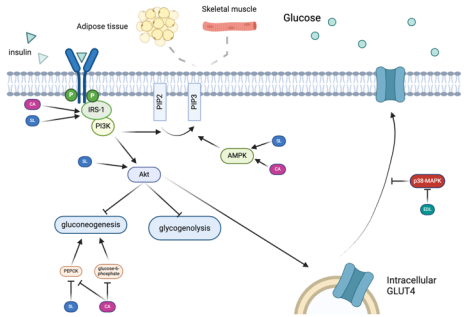

Figure 2. Potential mechanisms of stimulation of glucose uptake by CF, EDL, and SL.

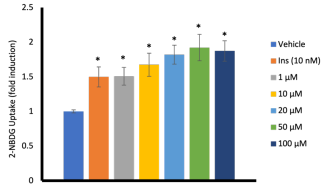

Figure 3. Enterodiol (EDL) significantly increased glucose uptake in differentiated 3T3-L1 adipocytes. Three glucose uptake assays were performed, and the data were normalized to the negative control (methanol). Insulin (10 nM) was used as the positive control. Each concentration of EDL significantly increased glucose uptake compared to the vehicle ($p < 0.05$) and in a similar manner as the positive control. The concentration of 50 µM elicited the highest effect.

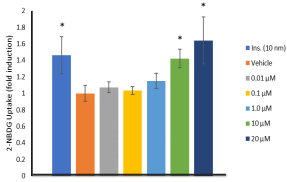

Figure 4. Cafestol (CF) significantly increased glucose uptake in differentiated 3T3-L1 adipocytes. Cells were treated with either CF (0.01, 0.1, 1, 10, or 20 µM), insulin (10 nM) or the vehicle (ethanol). Glucose uptake was measured in duplicate, and the data were normalized to the negative control (ethanol). The 10 and 20 µM concentrations of CF significantly increased glucose uptake relative to the negative control with 20 µM eliciting the greatest effect.

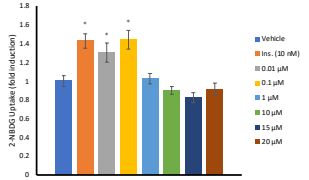

Figure 5. Secoisolariciresinol (SL) increased glucose uptake to an extent that is significantly greater than the negative control at concentrations of 0.01 µM and 0.1 µM. Three trials of the glucose uptake assay were conducted, and the data were normalized to the negative control (DMSO). Insulin (10 nM) was used as the positive control. SL (0.01, 0.1 µM) significantly increased glucose uptake compared to the vehicle ($p < 0.05$; $p < 0.01$).

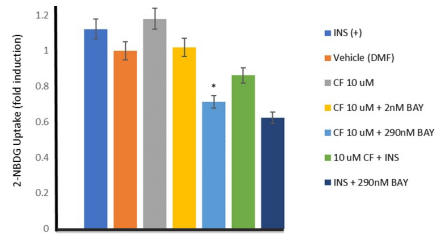

Figure 6. Inhibition of GLUT1 and GLUT4 significantly reduced CF-stimulated glucose uptake in 3T3-L1 adipocytes. Treatment with both 2 nM and 290 nM BAY-876 reduced glucose uptake in CF-treated adipocytes at the 10 µM concentration, with 290 nM BAY-876 resulting in the greatest inhibition of glucose uptake. Three biological replicates of this assay were performed, and the results were normalized to the negative control (DMF).

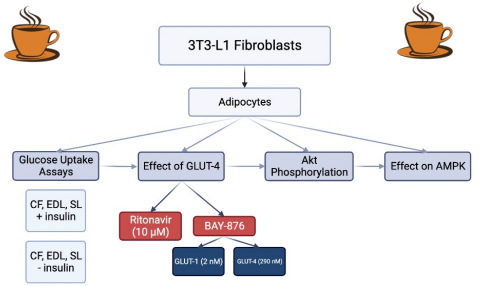

Figure 7. Experimental process flow chart (created in Biorender).

Results and Conclusions

Results
All compounds significantly increased glucose uptake in 3T3-L1 adipocytes relative to the negative control ($p < 0.05$).

Every tested concentration of EDL (1, 10, 20, 50, 100 µM) significantly increased glucose uptake in adipocytes compared to the control (Fig 3). Additionally, the extent to which the tested concentrations increased glucose uptake was not statistically distinct from the positive control (Fig 3).

CF (10, 20 mM) also caused a statistically significant increase in glucose uptake in 3T3-L1 adipocytes ($p < 0.05$) (Fig 4). The extent to which CF promoted glucose uptake promoted was not statistically distinguishable from the effect of insulin (Fig 4). Additionally, inhibition of GLUT1 with 2 nm BAY-876 did not significantly reduce glucose disposal, while inhibition of GLUT4 with 290 nm BAY-876 resulted in significantly reduced glucose disposal for adipocytes treated with 10 µM CF (Fig 6).

Two concentrations (0.01, 0.1 µM) of SL significantly increased glucose uptake relative to the control ($p < 0.05$; $p < 0.01$) (Fig 5). Both concentrations promoted glucose uptake to an extent that was statistically indistinguishable from that resulting from insulin (Fig 5).

Conclusions
Because all three compounds increased glucose uptake, this suggests these substances may promote an anti-diabetic effect in adipose tissue in vitro. The data show that select concentrations of these compounds stimulate glucose uptake to a similar extent as insulin. Future research will determine the effects of BAY-876 on EDL and SL and assays utilizing IRS-1 and Akt phosphorylation will be performed on all compounds depending on the results of initial testing.

Future Work

1. Determine whether CF, SL, and EDL stimulate glucose uptake via a GLUT4-dependent mechanism by using the selective inhibitor, BAY-876.
   a. BAY-876 can also be used to determine the potential effects on the GLUT1 pathway.
2. Perform an Akt phosphorylation assay on CF as the BAY-876 assay suggests a GLUT4-dependent mechanism.
3. Based on the data from the inhibitor assays, characterize the mechanisms by which each compound stimulates glucose uptake in 3T3-L1 adipocytes via various proteomic assays and arrays.
   a. Akt phosphorylation, AMPK, etc.

References and Acknowledgments

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