

Identification of Small Bioactive Molecules for the Treatment and Prevention of Type-2 Diabetes

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Abstract

Prior research has indicated that matairesinol (ML) and secoisolariciresinol (SL) may have potential as anti-diabetic agents. ML has been shown to inhibit body weight gain and increased fat mass¹, while SL has displayed an ability to delay the onset of STZ-induced type-2 diabetes (T2D) in rats². The present study aims to determine the ability of these compounds to promote glucose disposal in adipose tissue, as well as determine the mechanism by which these compounds exert their anti-diabetic effects. To accomplish this, 3T3-L1 fibroblasts were differentiated into adipocytes and exposed to each of the compounds in a concentration-dependent manner. Glucose uptake was measured by treating the cells with a fluorescent derivative of glucose (2-NBDG) and measuring fluorescence relative to the controls. Here, we report that various concentrations of these two compounds significantly increased glucose uptake in adipocytes relative to the controls. The optimal concentrations determined from these studies will be used in future studies to characterize the mechanism(s) by which glucose uptake is promoted. Briefly, the role of Glut-4, insulin signaling, and AMPK activation will be determined. Because of the previously reported effects on adiposity, we further intend to test their effects on the secretion of adiponectin, leptin, visfatin, and omentin, which are hormones that can lead to attenuated insulin sensitivity, reduced white fat cell depots, increased glucose utilization, and increased Akt phosphorylation, respectively.

Research Question

In this study, we aim to determine how matairesinol (ML) and secoisolariciresinol (SL) affect biomarkers of T2D pathogenesis in 3T3-L1 adipocytes. To do this, two specific objectives have been proposed.

1. Determine whether these compounds promote glucose disposal in differentiated 3T3-L1 adipocytes.
2. Characterize the molecular mechanism(s) by which these compounds exert their anti-diabetic effect(s).

Results

A glucose uptake assay was performed in which 3T3-L1 fibroblasts were differentiated into adipocytes and treated with either matairesinol (ML) or secoisolariciresinol (SL) in a concentration-dependent manner. Glucose uptake was then measured by assessing the relative fluorescence of the cells treated with 2-NBDG. We found that ML (0.1, 10, 15, 50, and 100 μ M) significantly increased glucose uptake compared to the negative control ($p < 0.05$). Interestingly, we found that the extent to which 100 μ M ML increased glucose uptake was not statistically different from the positive control (insulin), which might suggest that ML works as an insulin mimic. SL (20 and 100 μ M) significantly increased glucose disposal relative to the negative control ($p < 0.05$). Moreover, 100 μ M SL increased glucose uptake to a significantly greater extent than insulin ($p < 0.05$). Given that SL has been previously reported to delay T2D onset in STZ-induced rats, it is thus possible that it may promote glucose tolerance by more than one mechanism.

Methods

Cell Culture

3T3-L1 fibroblasts were seeded into 96 well plates at 2×10^5 cells/mL in complete growth medium consisting of DMEM supplemented with 10% fetal calf serum. On day 0, the complete growth medium was replaced with differentiation medium (complete growth medium + 0.5 mM IBMX + 1 μ M dexamethasone + 10 μ g/mL insulin) to promote differentiation into adipocytes. On day three, the differentiation medium was replaced with post-differentiation medium (complete growth medium + 10 μ g/mL insulin). On day five, the post-differentiation medium was replaced with complete growth medium. On day six, the glucose uptake assay was performed.

Glucose Uptake Assay

The complete growth medium was discarded, and the cells were treated with either ML (0.01, 0.1, 10, 15, 50, or 100 μ M) plus 100 μ g/mL 2-NBDG for 20 minutes or SL (0.01, 0.1, 1, 10, 15, 20, or 100 μ M) plus 200 μ g/mL 2-NBDG for 30 minutes. DMSO, which is the vehicle for these compounds was the negative control while insulin (10 nM for ML and 100 nM for SL) served as the positive control. All treatments were prepared in 1X phosphate buffered saline (PBS). Following incubation, both plates were centrifuged at 400 x g for 5 minutes at room temperature. The supernatant was discarded and 200 μ L 1X PBS was added to each well. The plates were centrifuged at 400 x g for 5 minutes at room temperature. The supernatant was discarded and 100 μ L 1X PBS was added to each well. A plate reader was then used to immediately analyze the relative fluorescence (excitation/emission = 485/538 nm).

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 \pm the standard error of the mean.

Future Work

Future studies will be conducted to further elucidate the ability of ML and SL to promote glucose disposal by testing their effect on insulin-stimulated glucose uptake. We further plan to characterize the mechanism(s) by which these compounds exert their anti-diabetic effects. We anticipate that the concentrations deemed optimal by these initial glucose uptake assays will be substantiated by their effects on Akt phosphorylation, AMPK activity, and/or Glut-4 activity and translocation. Lastly, we anticipate that these compounds will promote production of the hormones adiponectin, leptin, visfatin, and omentin, all of which can contribute to improved insulin sensitivity, reduced white fat cell depots, increased glucose utilization, and increased Akt phosphorylation, respectively.

References

1. Biasiotto G, Zanella I, Predolini F, et al. 7-Hydroxymatairesinol improves body weight, fat and sugar metabolism in C57BL/6 mice on a high-fat diet. *Br J Nutr.* 2018;120(7):751-762. doi:10.1017/S0007114518001824
2. Prasad K, Mantha S, Muir A, Westcott A. Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism. *Mol Cell Biochem.* 2000;206:141-150.

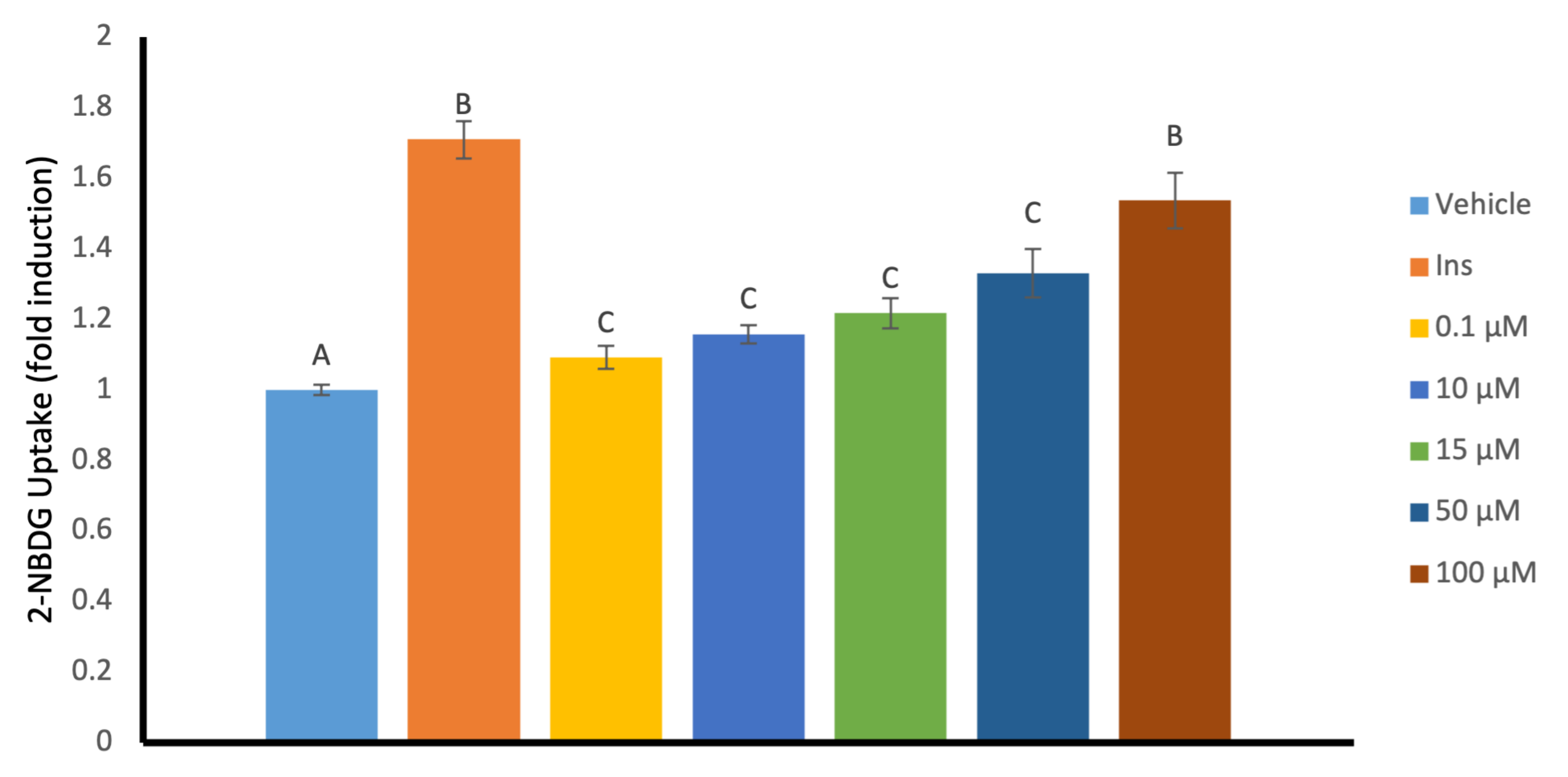


Figure 1. Matairesinol (ML) promotes glucose uptake in human adipocytes in a dose dependent manner. 3T3-L1 fibroblasts were differentiated into adipocytes and treated with the vehicle (DMSO), positive control (10 nM insulin), or ML (0.1, 10, 15, 50, or 100 μ M). Glucose uptake was measured by assessing the relative fluorescence of the cells treated with 2-NBDG, a fluorescent derivative of glucose. All assayed concentrations significantly increased glucose uptake relative to the negative control. Letters denote statistically significant differences ($p < 0.05$).

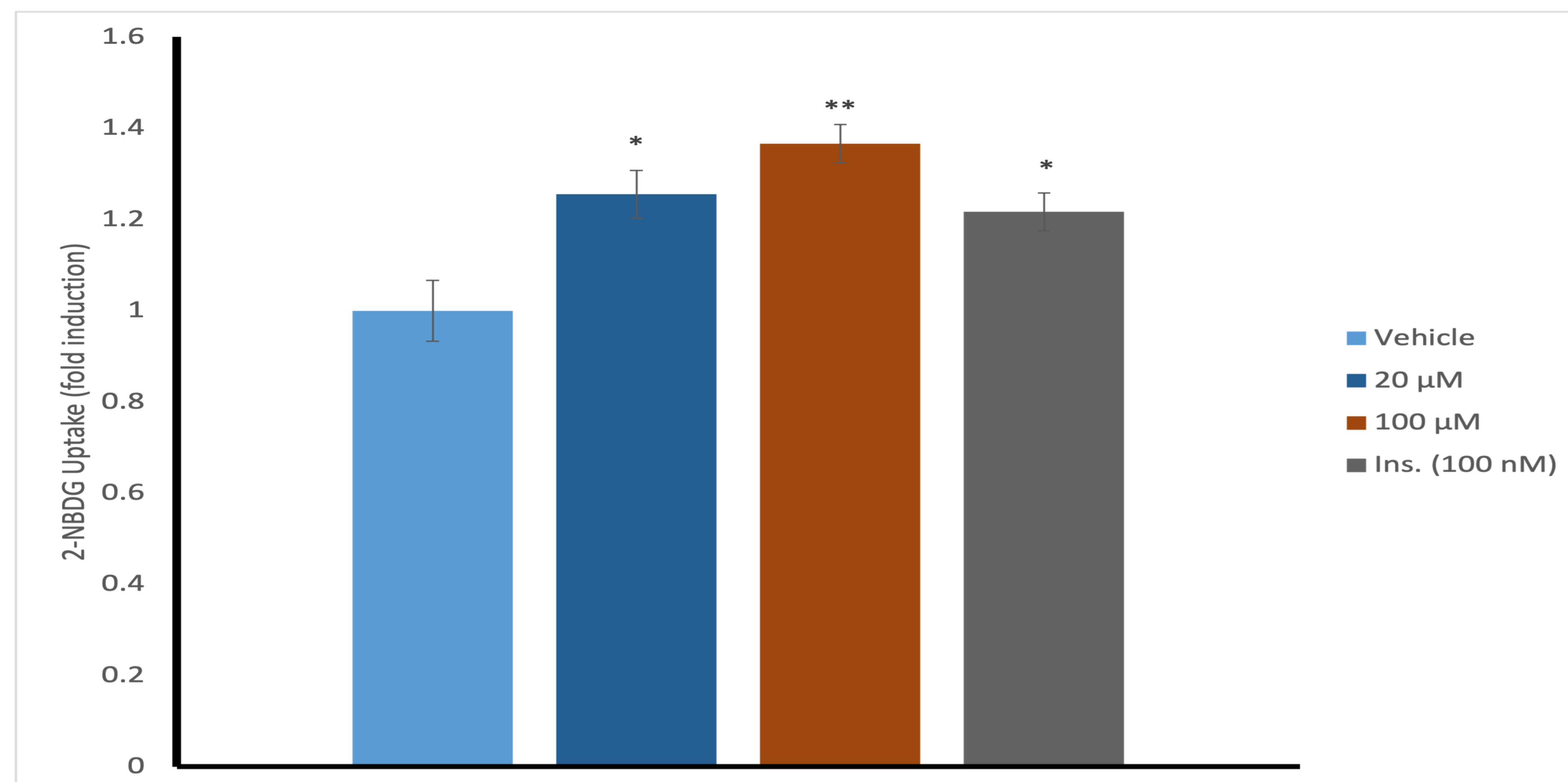


Figure 2. Secoisolariciresinol (SL) increases glucose uptake in human adipocytes. Differentiated 3T3-L1 fibroblasts were treated with either vehicle (DMSO), a positive control (100 nM insulin), or SL. Of the concentrations assayed, 20 and 100 μ M appears to promote glucose uptake to the greatest extent with 100 μ M stimulating glucose uptake to the same degree as insulin. *indicates significant difference from the negative control ($p < 0.05$); **indicates significant difference from the positive control ($p < 0.05$).