Cafestol and Quinic Acid as Novel Agents for the Attenuation of Type-2 Diabetes

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Abstract

In developed nations, Type-2 diabetes (T2D) is a public health concern that affects over 462 million individuals worldwide and is becoming increasingly more prevalent (*1*). With nearly 90 thousand deaths being attributed to T2D in 2019, it currently stands as the seventh leading cause of mortality in the United States (*2*). Furthermore, it has been reported that diabetes is the most expensive chronic condition in the United States, with $1 for every $4 spent on healthcare being used to care for diabetics (*3,4*). Thus, the pursuit of novel, low-cost agents capable of attenuating T2D and its sequelae as well as an understanding of the molecular mechanisms by which they promote insulin sensitivity and glucose disposal are of great value with respect to the development of more effective and affordable treatments for T2D. A review of the current literature has revealed that moderate habitual coffee consumption is inversely associated with the risk of T2D development (*6, 7, 8*). Cafestol (CF) and quinic acid (QA) are two polyphenols found naturally in coffee that have been shown to increase glucose-stimulated insulin secretion (*9, 10*). More specifically, CF is a diterpene that promotes glucose disposal in skeletal muscle, and QA is a phenolic acid that has been found to promote mitochondrial function in pancreatic β-cells and safeguard against oxidative and STZ-induced damage to the liver, kidney, and pancreas (*10, 11*). However, there remains a gap in knowledge as to the ability of CF and QA to exert these effects in adipose tissue, along with their mechanisms of action.

Research Question

The primary goal of this research was to determine whether CF and QA stimulate glucose disposal in adipocytes.

Methods

3T3-L1 fibroblasts were differentiated into adipocytes (Figure 1) according to supplier instructions (ATCC, Manassas, VA). A glucose uptake assay was conducted with the differentiated adipocytes according to manufacturer protocol (Cayman, Ann Arbor, MI). Briefly, the cells were exposed to a fluorescent derivative of glucose (2-NBDG), PBS, and either cafestol or quinic acid in a concentration-dependent manner (Figure 1). Insulin (10 nM) was used as a positive control. The negative control contained glucose, PBS, and the vehicle (diH2O for quinic acid and ethanol for cafestol). The cells were incubated for 30 minutes with each compound and analyzed for fluorescence relative to the controls: excitation/emission = 485/535 nm. The optimal concentration to promote glucose uptake was determined for both cafestol and quinic acid based on these results.

Acknowledgements

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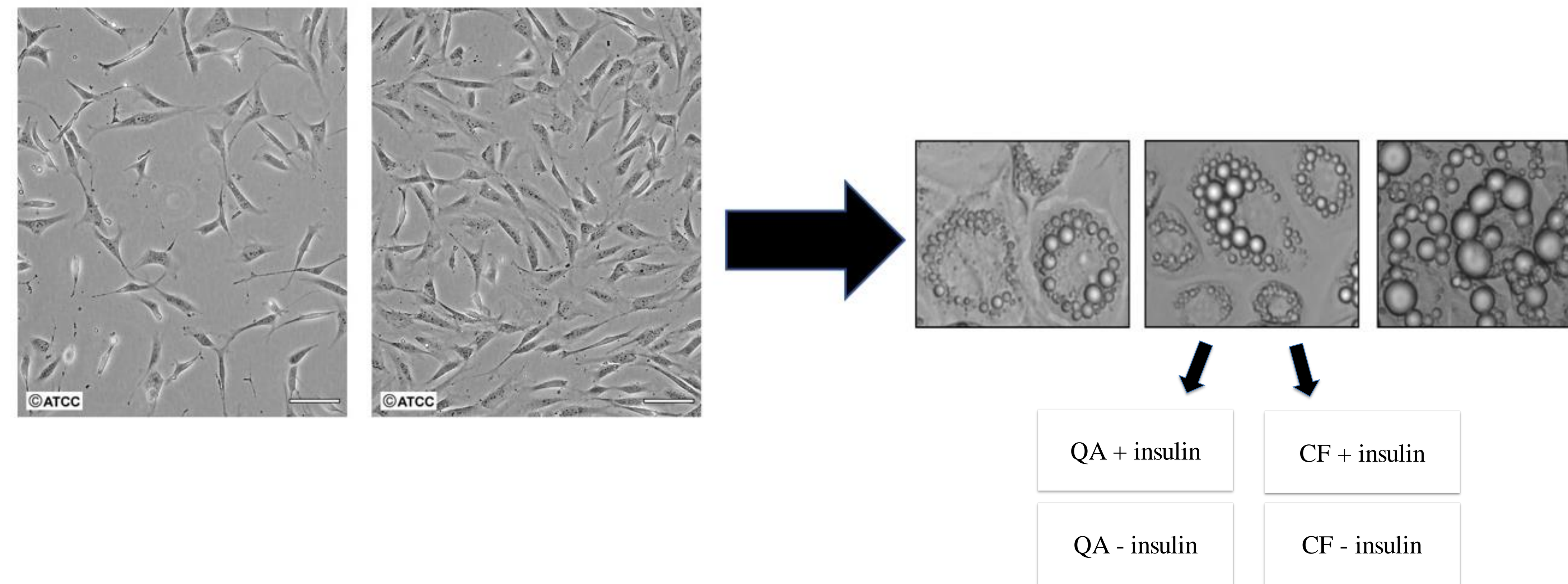

Figure 1. The differentiation of 3T3-L1 fibroblasts. 3T3-L1 fibroblasts were differentiated into adipocytes and treated with either QA or CF in a concentration-dependent manner in the presence and absence of insulin.

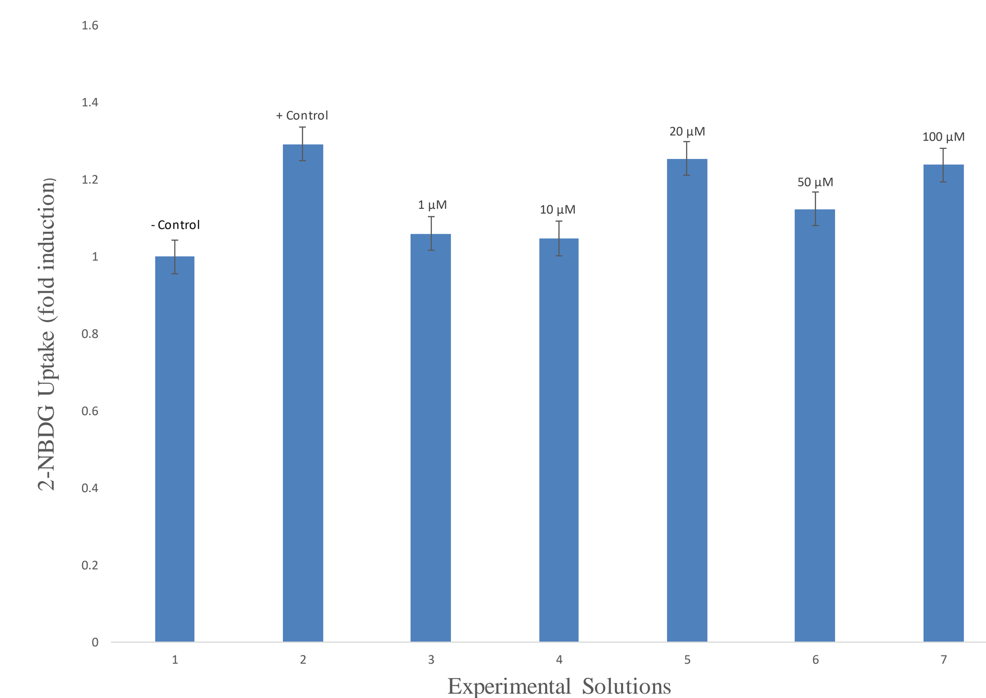

Figure 2. Quinic acid was assayed in a dose-dependent manner for its ability to stimulate glucose uptake in 3T3-L1 adipocytes. Quinic acid (1, 10, 20, 50, and 100 µM) were tested. A negative control containing glucose, PBS, and the vehicle (diH2O) was used. A positive control containing insulin (10 nM), glucose, and PBS was used. Concentrations of 20 and 100 µM significantly increased glucose uptake in the adipocytes to an extent that is no different from the positive control ($p < 0.05$).

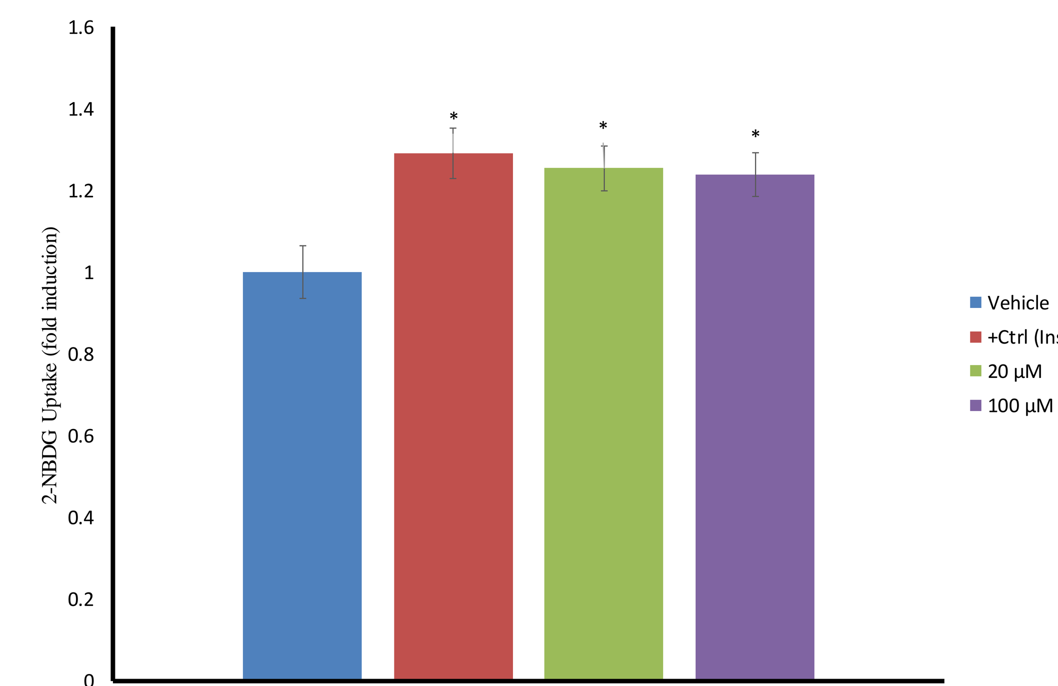

Figure 3. Optimal concentrations of quinic acid were determined. Based on the original bar graph of concentrations, the optimal concentrations of quinic acid for glucose uptake appeared to be 20 µM and 100 µM with fluorescent readings of 1.25 and 1.24. Both concentrations statistically varied from the negative control and promoted levels of glucose disposal that are not statistically distinguishable from the positive control of insulin.

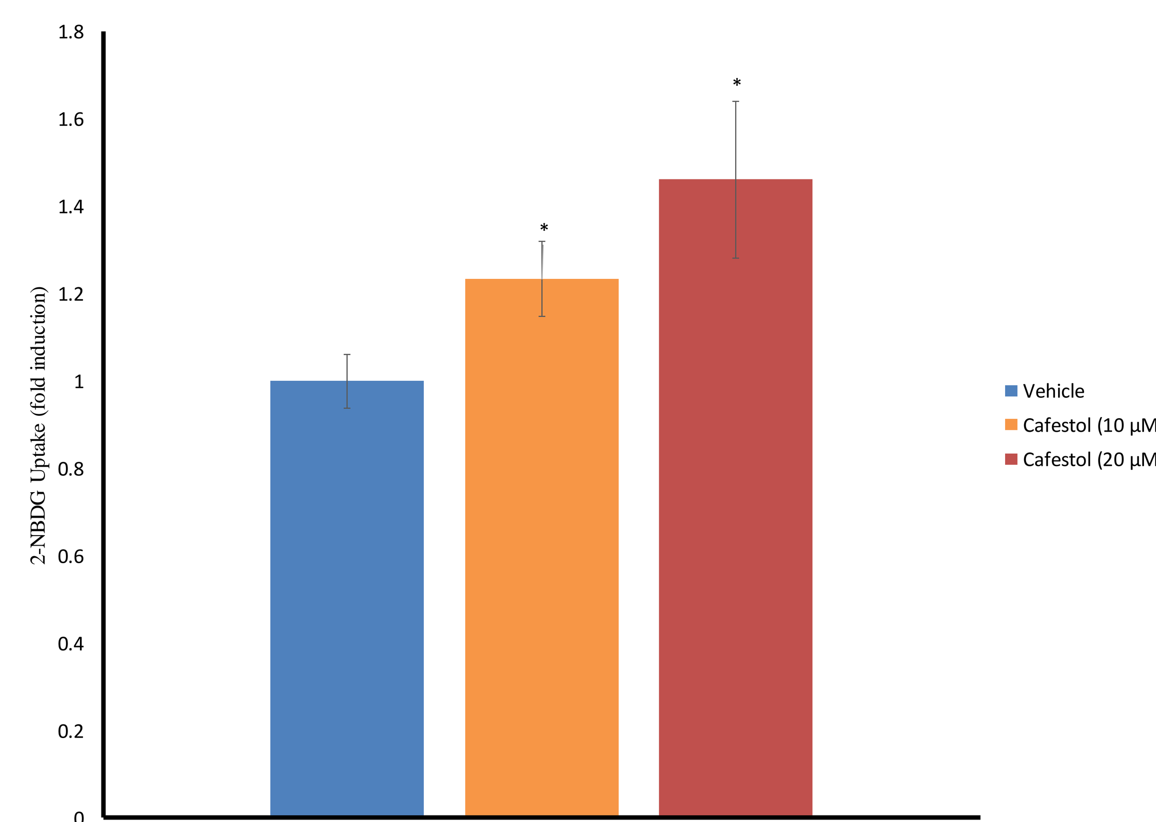

Figure 4. Cafestol was assayed for its ability to stimulate glucose uptake in human adipocytes in a dose-dependent manner. Cells were treated with either cafestol (0.01, 0.1, 1, 10, and 20 µM), 10 nM insulin (positive control), or the vehicle (ethanol). The 10 and 20 µM concentrations of CF significantly increased glucose uptake relative to the negative control.

Results and Conclusions

To determine whether QA increases glucose disposal in adipocytes, we incubated differentiated 3T3-L1 adipocytes with QA (1, 10, 20, 50, and 100 µM) and 2-NBDG (Figure 2). We found that 20 and 100 µM QA significantly increased glucose disposal in adipocytes (Figure 3) compared to the control ($p < 0.05$). Interestingly, we found that the extent to which these two concentrations increase glucose uptake was not statistically different from the positive control (insulin), which may indicate that QA acts as a mimetic of insulin (Figure 3). To determine the impact of CF on glucose uptake in adipose tissue, 3T3-L1 adipocytes were incubated in CF (0.01, 0.1, 1, 10, and 20 µM) and 2-NBDG (Figure 4). All concentrations except the 0.01 µM solution resulted in increased glucose uptake compared to the negative control. Furthermore, the adipocytes treated with the 10 and 20 µM solutions stimulated significantly greater glucose disposal than the negative control and the other tested concentrations (Figure 5).

Studies have shown that QA increases glucose-stimulated insulin secretion in INS-1E cells (*6*). QA has also been found to promote mitochondrial function in pancreatic β-cells and safeguard against oxidative and STZ-induced damage to the liver, kidney, and pancreas in a STZ-induced diabetic rat model (*7*). However, its ability to promote glucose disposal in adipocytes has remained enigmatic. Here, we show that QA stimulates glucose uptake in adipocytes. While we have not yet began to investigate the underlying mode of action, the fact that QA stimulates glucose uptake to the same extent as insulin may indicate that its mechanism is similar to insulin, which is to promote glucose uptake by stimulating the translocation of Glut4-containing vesicles to the plasma membrane. Similarly, CF has previously been shown to promote insulin secretion and stimulate glucose disposal in skeletal muscle, but the mechanism by which it exerts these anti-diabetic effects and their impact in adipose tissue have not yet been assessed. Here, we show that CF stimulates glucose disposal in 3T3-L1 adipocytes, with 10 and 20 µM concentrations demonstrating the greatest increase in glucose uptake compared to the negative control. There are several biochemical mediators of Akt phosphorylation that lead to Glut4 translocation. These will be the focus of our future research in which we will aim to address the QA and CF-stimulated glucose uptake mechanisms.

Future Work

Using the optimal concentrations of these compounds, future research should aim to further characterize the molecular mechanism(s) by which they exert their effects. Because of previous studies showing that another chemically related compound in coffee, enterolactone, uses a Glut4- and AMPK-dependent mechanism in skeletal muscle, these regulators will be assessed in response to CF and QA (*12*). In addition, given its pivotal role as a regulator of Glut 4 translocation, compound-induced Akt phosphorylation will be measured. The ability of these compounds to stimulate the anti-diabetic endocrine effects associated with adipocytes will also be assayed.

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