The Effects of Coffee Extract on Adipogenesis

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Introduction

Obesity rates in the United States have increased from 30.5% in 2000 to 42.4% in 2018 (Center for Disease and Control, 2020). Obesity is characterized as an increased accumulation of adipocytes within the body. An increased accumulation of adipocytes can lead to other health conditions such as heart disease, type 2 diabetes, and premature death (Center for Disease and Control, 2020).

Adipogenesis is the development of an adipocyte from a preadipocyte. 3T3-L1 preadipocytes are embryonic fibroblasts that are derived from mice and used as a model system to study adipogenesis. Adipocytes are mature fat cells that are differentiated from 3T3-L1 preadipocytes. In order to form an adipocyte, a preadipocyte must go through a regulated differentiation process. Preadipocytes are grown to confluency in a growth supplement, Calf serum media. Two days post confluency, the preadipocytes are stimulated with media containing IBMX, dexamethasone, and insulin until day 8, when full differentiation is achieved.

A number of reports have investigated the effects of a variety of compounds on the process of adipogenesis in 3T3-L1 cells. Coffee extract has been reported to inhibit adipogenesis in 3T3-L1 preadipocytes by downregulating IRS1 (Maki et al., 2017). The downregulation of IRS1 causes a cascade of downregulation in the adipogenesis pathway. Target proteins required for adipogenesis, such as PPARγ and Cdc451, were no longer expressed. Mitotic clonal expansion was consequently down regulated. Mitotic clonal expansion is essential for adipogenesis because it allows the cells to divide and replicate so they can differentiate into mature adipocytes. Because mitotic clonal expansion was downregulated, the preadipocytes were not able to divide and replicate. Overall, this led to a decrease in adipocytes. The proposed effects of coffee extract on the process of adipogenesis are shown in Figure 1.

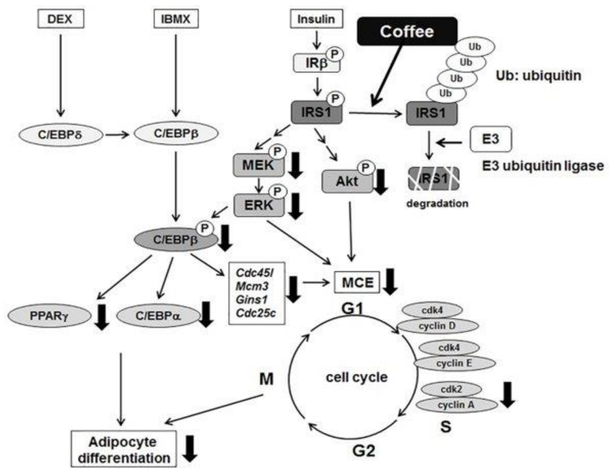

Figure 1: The proposed pathway for downregulation of the adipogenesis pathway when 3T3-L1 cells are treated with coffee extract (Kim et al., 2016). Coffee directly affects IRS1 which leads to the downregulation of key transcription factors in the pathway.

Coffee extract is made from coffee beans and contains over a thousand compounds. The role of caffeine in the inhibition of adipogenesis has been studied, and it is thought to be the compound responsible for this cascade of events. In a study done in 2016, caffeine suppressed 3T3-L1 adipocyte differentiation by inhibiting expression of CCAAT/enhancer binding proteins and PPARγ (Kim et al., 2016). Since these proteins were no longer expressed, the adipogenesis pathway was inhibited. However, another study showed that caffeine failed to inhibit PPARγ expression, and therefore wasn't the compound responsible for inhibiting adipogenesis (Maki et al., 2017).

To study whether caffeine is the compound responsible for inhibiting the adipogenesis pathway, I tested the effects of caffeinated and decaffeinated coffee extracts on 3T3-L1 preadipocytes during days 0, 2, 4 and 6 of differentiation.

Methods and Materials

Preparation of Coffee Extract
Ground Pike Place roast coffee and Pike Place roast decaffeinated coffee was obtained from Starbucks Coffee. Each coffee extract was prepared through a drip style method, in which 4.47 g of ground coffee was poured with 78 mL of hot water. The extract was filtered through a paper filter, filter sterilized, and divided into smaller aliquots. The extract was stored in the freezer and thawed when needed.
Adipocyte Differentiation
3T3-L1 preadipocytes were grown to confluency in Calf Serum Media on 6-well plates. Two days after confluency, day 0, the cells were stimulated with MDI Induction Media. MDI Induction media consisted of 10% FBS/DMEM with 1:1000 IBMX, 1:1000 dexamethasone, and 1:1000 insulin. The cells in the decaffeinated wells were stimulated with 5% decaffeinated coffee extract, and the cells in the caffeinated wells were stimulated with 5% caffeinated coffee extract. Two days later, day 2, the cells were stimulated with Insulin Media. The cells on the coffee plates were stimulated with 5% of the corresponding coffee extract. On days 4 and 6 the media was changed to 10% FBS/DMEM and the cells on the coffee plates were stimulated with 5% of the corresponding coffee extract again. On day 8 full differentiation was achieved.

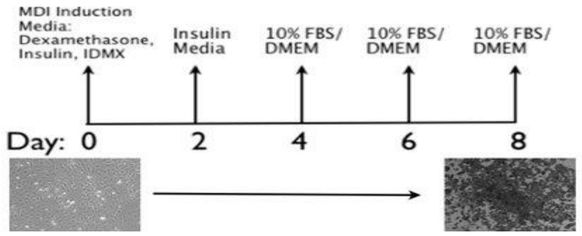

Figure 2: Regulated differentiation process from a 3T3-L1 preadipocyte to an adipocyte.
Oil-Red O staining and quantification
The differentiation media was removed from the cells and washed with phosphate-buffered saline (PBS). The cells were fixed with 4% formaldehyde solution. The formaldehyde solution was removed, and 1.25 mL of Oil red O solution was added to each well. After incubation, the staining solution was removed, and each well was washed five times with 1 mL of distilled water. Next, 2.5 mL of 2-propanol was added to the wells and set on an orbital shaker. After ten minutes, 1 mL of the extracted dye was put into a cuvette and the absorbance was read in a spectrophotometer at 510 nm.

Results

I investigated the effects of caffeinated and decaffeinated coffee extracts on adipocyte differentiation. In Figure 3, the impact coffee extracts had on adipogenesis overall is shown. Significant Oil Red O staining can be seen in photos A and D. Staining was decreased by the coffee extract treatments, as not as many adipocytes were formed.

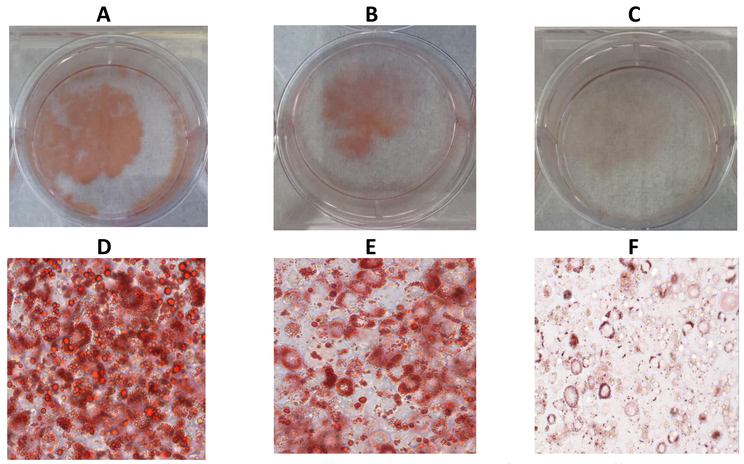

Figure 3: Inhibition of adipogenesis by coffee extract. On day 8 of adipocyte differentiation, Oil-Red-O staining was used to visually see the formation of adipocytes in each treatment. Photos A, B, and C are one well on a six well plate. A is the control, B was treated with 5% caffeinated extract, and C was treated with 5% decaffeinated extract. Photos D, E, and F are micrographs of adipocytes at 400 X magnification. Photo D is the control, E was treated with 5% caffeinated extract, and F was treated with 5% decaffeinated extract. Decreased adipocyte accumulation was observed in the wells treated with 5% coffee extract.

Results Continued

Moderate staining was observed in the wells that were treated with 5% caffeinated coffee extract. This decrease in adipocyte formation supports the hypothesis that coffee extract decreases adipogenesis, presumably through directly downregulating IRS1. In the wells treated with 5% decaffeinated coffee extract, we observed little staining of adipocytes. Adipogenesis was highly suppressed in this treatment, disputing previous research that showed that caffeine was the compound responsible for decreased adipogenesis.

To quantify relative adipogenesis in each treatment, samples of each well were measured in a spectrophotometer at 510 nm. These absorbances can be viewed in Figure 4.

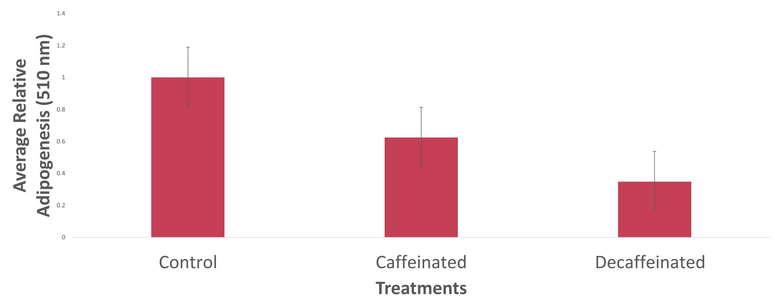

Figure 4: Inhibition of adipogenesis with decaffeinated and caffeinated coffee extracts. Relative adipogenesis was determined by Oil Red O staining, measured at 510 nm. Overall, inhibition of adipogenesis occurred in both coffee groups. Adipogenesis was decreased the most when treated with 5% decaffeinated coffee extract.

In Figure 4, a significant difference in absorbances can be observed between the groups. The cells treated with either coffee extract showed an overall reduction of adipogenesis. Adipogenesis was reduced even more when treated with decaffeinated coffee extract.

The results of this study refute the hypothesis that caffeine is the compound in coffee extracts responsible for downregulating the adipogenesis pathway. Cells treated with decaffeinated coffee extract showed lower levels of relative adipogenesis than those treated with caffeinated coffee extract.

Conclusions and Future Work

Conclusions
- Inhibition of adipogenesis was seen with both decaffeinated and caffeinated coffee extracts
- Inhibition of adipogenesis was greater with decaffeinated coffee extract
- These results support that hypothesis that caffeine is not the compound in coffee responsible for the inhibition of adipogenesis

Future Work
- Study different concentrations of coffee extract to determine the minimum amount necessary to decrease adipogenesis
- Look at other compounds in coffee, such as antioxidants or diterpenes, to determine if they are the compound inhibiting adipogenesis

References

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