Assessing Alaskan Blueberry Bioavailability in vitro
Savanah Owen, Aline Collin, and Thomas Kuhn

Abstract
There is a strong correlation between type II diabetes (T2D) and obesity determined by body mass index (1). T2D is characterized by a lack of insulin-sensitive regulation of blood glucose despite all components of the regulatory system being properly expressed. Notably, rural Alaskan communities exhibit a strange phenomenon where the prevalence of T2D is considerably lower than that of obesity, which may be attributed to the berry rich diets as a part of their subsistence lifestyle. Rescue or restoration of insulin sensitivity has been shown in cell cultures assays but it remains to be shown whether this potency is actually transported from the digestive system (intestine) to the blood. Hence we assessed in vitro the bioavailability of blueberry botanicals relying on the widely used Caco-2 cell model. These cells form a diffusion barrier in vitro comprising the quantitative transport of pharmacological and/or botanical compounds across the intestine. Our study showed that blueberry botanicals presented unique problems for the caco-2 system, including cytotoxicity and interference with insert filters. Resolution of the issues presented in this paper will allow us to use the Caco-2 bioavailability assay to collect Alaskan blueberry metabolites in vitro for further analysis using LC-MS.

Methods
Caco-2 cell monolayers, that can mimic transport across the intestinal lining were grown in DMEM+20% FBS until adequate concentrations (~2.4 x 10^3) were obtained. Cells were then transferred onto transwell membrane inserts with 0.4µm pore filters. Once caco-2 cells became confluent on inserts, they were chronically exposed to different concentrations of botanical extract: 0 µg/ml, 5 µg/ml, 10 µg/ml. After complete maturation, 21-29 days post-seeding, monolayers were put through control transport testing that evaluated monolayer intactness.

Background
Berries contain high levels of vitamins, minerals, fibers and polyphenols with the latter likely responsible for most of the health benefits of berries (2). There is mounting evidence, obtained in in vitro studies, that polyphenols from Alaskan blueberries potently enhance sensitivity to insulin (1,3). The principal obstacle remains biotransformation. Bioavailability addresses the crucial step of absorption of botanicals across the intestinal barrier while chronic exposure to botanicals has been shown to influence their absorption (4).

Figure 1. Trial 1, cumulative percent Dextran transported for experimental groups: 0, 5, and 10 µg/ml (n=9, for each). Standard error is expressed by gray bars.

Results
Trial 1 showed monolayers were intact prior to berry transport (Fig. 1. & Fig. 2.), but were compromised after berry transport (Fig 3. & Fig 4.), leading us to suspect berry cytotoxicity. MTT tests showed that acute concentrations from trial 1 i.e. 100 µg/ml killed cells (64.2 - 69.2% viability) and compromised monolayers, while chronic concentrations i.e. 5, and 10 µg/ml actually increased cell proliferation (105.5-108.6% & 107-114.6% viability respectively). Trial 2 testing, using a reduced acute concentration, showed controls inconsistent with trial 1. Controls appeared to improve upon the caco-2 bioavailability assay monolayer integrity must be maintained if results are to be meaningful. During this study, we found that use of ECM MaxGel® with high seeding count improved cell proliferation speeds.

Conclusions
Of the two trials performed monolayers were not sufficiently intact. To improve upon the caco-2 bioavailability assay monolayer integrity must be improved. Further investigation into berry caco-2 cytotoxicity could indicate the maximum non-toxic berry concentration, thus eliminating the destructive effect of berry solutions on monolayers. Investigation into the impact of berry solutions on monolayer transport is needed to ensure that berry particles do not impact monolayer transport. Caco-2 cells appear to be successful, however being able to grow sufficient amounts of cells in a timely manner would make this assay more useful. During this study, we found that use of ECM MaxGel® with high seeding count improved cell proliferation speeds.

References

UAF is an equal opportunity employer and auxiliary aids and services, services to individuals with disabilities are available upon request. Research reported in this publication was supported by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Numbers U01GM119911, T32GM18952, or R8S199849. The content is solely the responsibility of the authors and not necessarily represent the official views of the National Institutes of Health.