Digestive stability and transport of equol by Caco-2 cells.

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ABSTRACT

Equol has been reported to have greater estrogenic activity and antioxidant capacity than its soy isoflavonoid precursor daidzein. However, only 30 - 50% of humans have intestinal microflora capable of converting daidzein to equol. This suggests that dietary supplementation of equol may represent a useful strategy to provide all individuals with the health-promoting benefits of this metabolite. Here we characterized the digestive stability and bioaccessibility of equol, as well as its uptake and efflux by differentiated Caco-2 cells. Bovine milk (3.3% fat) supplemented with equol (160 μg) was subjected to simulated gastric and small intestinal digestion and the aqueous (bioaccessible) fraction was isolated from digesta by centrifugation and filtration (0.2 μm). Recovery of equol following simulated digestion was 106% ± 6%. Equol efficiently (≥ 93%) partitioned into the aqueous fraction of chyme regardless of the amount of bile extract present. Monolayers of Caco-2 cells (12 – 15 days post confluent) attached to culture dishes were incubated with 2.5 to 20 μM equol to characterize uptake. After 4 h, cellular content of equol was proportional to the medium concentration (r^2 = 0.99) and represented 5 to 7% of the starting amount of equol. Intracellular equol was present as the un-conjugated form. Maximum accumulation was observed within 1 h of exposure and cellular equol content continually declined after 4 h as phase II conjugates of equol were excreted into the medium. In conclusion, equol is stable during simulated digestion, partitions into the bioaccessible fraction of chyme, and is rapidly taken up by Caco-2 cells and metabolized to phase II conjugates that are excreted.

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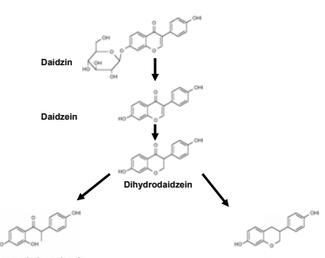
INTRODUCTION

- Equol is a microbial metabolite of the isoflavonoid daidzein (Figure 1).
- Equol has greater estrogenic activity than daidzein.
- Studies suggest that only 30 - 50% of humans have intestinal microflora capable of producing equol.
- Equol supplementation may represent a useful strategy to provide all individuals with its proposed health-promoting benefits.

Figure 1. Metabolism of Daidzin to Equol

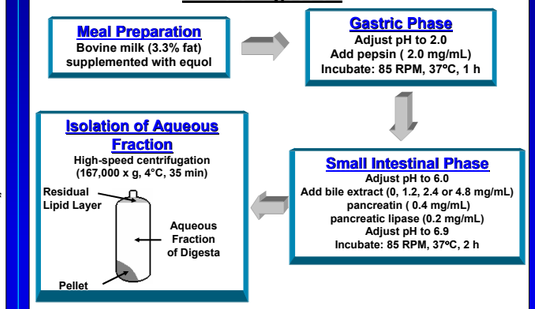


SPECIFIC AIMS

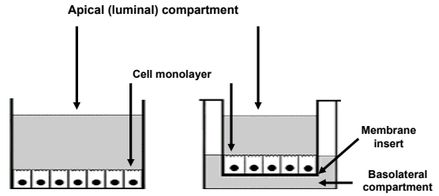
1. Characterize the digestive stability and bioaccessibility of equol during simulated gastric and small intestinal digestion.
2. Characterize equol and daidzein uptake, transport and metabolism by differentiated Caco-2 human intestinal cells.

MATERIALS & METHODS

***In Vitro* Digestion**



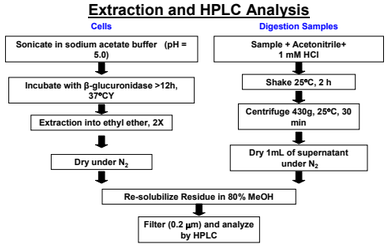
Culture of Caco-2 Cells



Uptake Experiments
Dose: 2.5 to 20 μmol/L for 4h
Time: 0.5 to 8 h (10 μmol/L)

Transepithelial Transport Experiments
Time: 0.5 to 8 h (10 μmol/L)

Extraction and HPLC Analysis



HPLC SYSTEM

Column	3.9 x 150 mm Nova-Pak C18
Separation	Waters 2695 Separations Module
Detector	Waters 2996 Photodiode Array (PDA)
Software	Empower

RESULTS

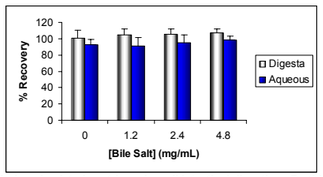


Figure 2
Equol is stable during simulated gastric and small intestinal digestion. Partitioning of equol into the aqueous (bioaccessible) fraction of the digesta was > 90% regardless of the bile salt concentration.

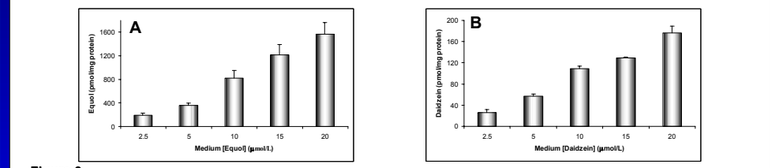


Figure 3
Uptake of equol (Panel A) and daidzein (Panel B) by Caco-2 cells cultured on plastic is directly proportional ($r^2 \geq 0.99$) to the medium concentration. Uptake of equol is approximately 10-fold greater than that of daidzein for each treatment concentration.

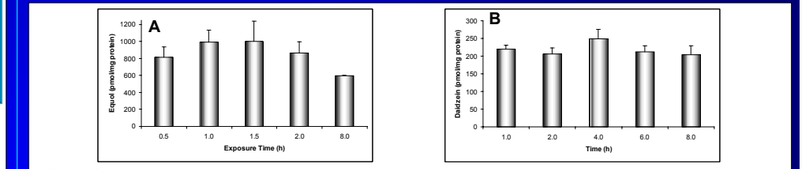


Figure 4
Uptake of equol (Panel A) and daidzein (Panel B) by Caco-2 cells cultured on plastic is rapid with maximum intracellular levels attained within 1 h of incubation. Intracellular equol begins to decline by 2 h suggesting efflux is occurring. Intracellular levels of daidzein remained consistent for each incubation period.

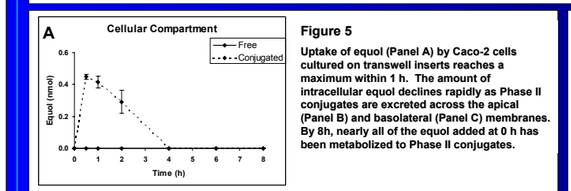


Figure 5
Uptake of equol (Panel A) by Caco-2 cells cultured on transwell inserts reaches a maximum within 1 h. The amount of intracellular equol declines rapidly as Phase II conjugates are excreted across the apical (Panel B) and basolateral (Panel C) membranes. By 8h, nearly all of the equol added at 0 h has been metabolized to Phase II conjugates.

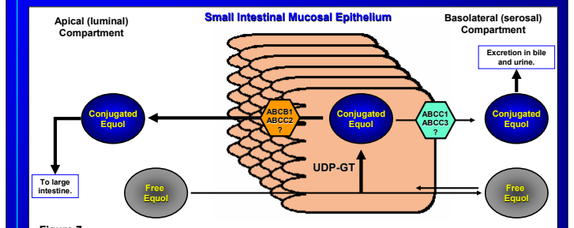


Figure 7
Schematic representation of transepithelial transport and metabolism of equol. The participation of multidrug resistance proteins in efflux of phase II metabolites of equol is currently being investigated. (ABCB1 = PgP, MDR1; ABCC1 = MRP1; ABCC2 = MRP2; ABCC3 = MRP3)

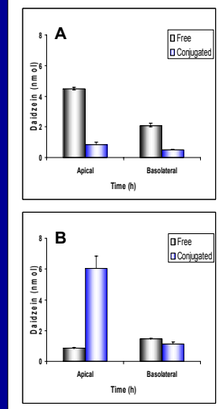


Figure 6
Daidzein in the apical and basolateral compartments is predominantly free at 2 h (Panel A). Phase II metabolites are the primary species in the apical compartment by 8 h (Panel B), whereas similar amounts of free and conjugated daidzein are present in the basolateral compartment. Neither free nor conjugated daidzein was detected in cells grown on inserts.

SUMMARY & CONCLUSIONS

Equol is stable during simulated digestion and partitions into the aqueous (bioaccessible) fraction of digesta.

Uptake of equol and daidzein by Caco-2 cells rapidly reaches its maximum by 1 h.

Equol and daidzein are extensively metabolized to Phase II conjugates that are excreted primarily across the apical membrane of small intestinal mucosa epithelial cells. This suggests that the bioavailability of equol is limited and may contribute to classification of ≤ 50% of individuals as "non-producers."

Despite limited bioavailability of equol, supplementation may provide individuals with its health-promoting benefits.