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Uptake of Quercetin and Quercetin 3-Glucoside from Whole Onion and Apple Peel Extracts by Caco-2 Cell Monolayers

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Evidence suggests that regular consumption of fruits and vegetables may reduce the risk of chronic diseases, and phytochemicals from fruits and vegetables may be responsible for this health benefit. However, there is limited knowledge on the bioavailability of specific phytochemicals from whole fruits and vegetables. This study used Caco-2 cells to examine uptake of quercetin aglycon and quercetin 3-glucoside as purified compounds and from whole onion and apple peel extracts. Pure quercetin aglycon was absorbed by the Caco-2 cells in higher concentrations than quercetin 3-glucoside ($p < 0.05$). Caco-2 cells treated with quercetin 3-glucoside accumulated both quercetin 3-glucoside and quercetin. Caco-2 cells absorbed more onion quercetin aglycon than onion quercetin 3-glucoside ($p < 0.05$), and the percentage of onion quercetin absorbed was greater than that of pure quercetin, most likely due to enzymatic hydrolysis of quercetin 3-glucoside and other quercetin glucosides found in the onion by the Caco-2 cells. Caco-2 cells absorbed low levels of quercetin 3-glucoside from apple peel extracts, but quercetin aglycon absorption was not detected. Caco-2 cell homogenates demonstrated both lactase and glucosidase activities when incubated with lactose and quercetin 3-glucoside, respectively. This use of the Caco2 cell model appears to be a simple and useful system for studying bioavailability of whole food phytochemicals and may be used to assess differences in bioavailability between foods.

KEYWORDS: Quercetin; phenolics; flavonoids; cell culture; apple; onion

INTRODUCTION

Epidemiological studies have shown that the ingestion of diets high in fruits and vegetables may decrease the risk of cancer and cardiovascular disease (*1*, *2*). Much of the protective effects of fruits and vegetables has been attributed to phytochemicals, possibly due to their ability to protect against oxidative stress (*3*). A major class of phytochemicals found commonly in fruits and vegetables are the flavonoids, and the most common flavonoids found in fruits and vegetables are quercetin and its conjugates (*4*). Flavonoid intake, particularly quercetin, has been inversely associated with incidences of heart disease, cerebrovascular disease, and several types of cancer (*5*). Other studies have also shown an inverse relationship between coronary heart disease and flavonoid intake, particularly when adults consumed apples, onions, and tea, foods high in quercetin conjugates (*6*−*8*). Although some groups have found links between flavonoids and cancer (*9*, *10*), others have found no relationship (*11*). This discrepancy appears to be more common when researchers seek to identify correlations between a specific, isolated compound or class of compounds and reduced cancer risk (*1*). However, there is strong evidence supporting a lower cancer risk associated with diets high in fruits and vegetables, and this correlation may be due to a complex combination of phytochemicals rather than the action of a single compound (*12*).

To this date, little literature exists that addresses the bioavailability of phytochemicals from whole foods. The use of human and animal clinical trials for studying bioavailability is complex, time-consuming, and expensive. Alternatively, in vitro models are attractive options due to their potential overall simplicity and lower cost. We selected a well-established in vitro model that uses the Caco-2 cell line. The Caco-2 cells are derived from human adenocarcinoma and will differentiate into polarized enterocyte-like monolayers, acting similarly to intestinal epithelial cells. The model has been developed for pharmaceutical assay and more recently used for micronutrient assay (*13*). It has been used to evaluate cell transport and/or accumulation of pure phytochemicals such as quercetin, quercetin glucosides, chrysin, flavone, epicatechin, and proanthocyanidin (*14*−*20*), as well as to estimate carotenoid bioavailability from fresh stir-fried vegetables and from spinach puree (*21*, *22*).

Understanding the bioavailability of pure compounds is a necessary start to understanding the bioavailability of these same compounds when present as a complex mixture of phytochemicals within whole fruits and vegetables. The latter is more complicated because many factors, such as concentrations and forms of individual phytochemicals, interactions with other chemicals in the food, and the properties of the food matrix

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and chyme, affect the bioavailability of phytochemicals from foods. Previous work from our laboratory has suggested that it may be the important synergistic and additive effects of compounds in whole fruit and vegetable extracts, not single compounds, which provide health benefits (*12*, *23*, *24*). It is possible that the bioavailability of the same compounds may differ among foods or may differ from the bioavailability of pure compounds. Using the Caco-2 cell model, it has been found that red grape juice and prune juice inhibit iron uptake, but other juices such as pear, apple, and orange juices increased iron bioavailability (*25*). The inhibitory or promotional effect on iron is most likely due to phytochemicals in the fruit juices, demonstrating that different mixtures of phytochemicals between fruits can have significant effects on bioavailability.

The use of the Caco-2 cell line may thus be a useful system to estimate bioavailability of specific phytochemicals present as a component of a complex whole food. Quercetin and its glycosides are commonly found in foods; therefore, we analyzed these compounds as markers of bioavailability from food extracts. The objectives of these experiments were (1) to use the Caco-2 cell culture model to evaluate and compare the bioavailability of quercetin aglycon and quercetin 3-glucoside from whole onion and apple peel extracts and (2) to determine whether the bioavailability of pure quercetin aglycon and quercetin 3-glucoside differs from the bioavailability of the same compounds from onion and apple peel extracts. As a result, we hope to establish the Caco-2 cell culture model as a viable option for in vitro analysis of phytochemical bioavailability from whole foods. Onion and apple peels were chosen because they are commonly consumed foods that contain significant amounts of quercetin (*26*). The apple peel was used instead of the whole apple because the apple peel is higher in quercetin glycosides, antioxidant activity, and antiproliferative activity (*27*).

MATERIALS AND METHODS

Phytochemical Extract Preparation. Apple peel extracts and shallot extracts were prepared using a method similar to that reported previously by our laboratory (*27*, *28*). Shallots were obtained from Oswego County (NY) Cornell Cooperative Extension, and Rome Beauty apples were obtained from Red Jacket Orchards (Geneva, NY). Preliminary research from our laboratory as well as other laboratories (*29*) has shown that the shallot is significantly higher in phenolic compounds when compared to other onion varieties. Four gram aliquots of shallots or apple peel were homogenized for 3 min with 32 g of chilled 80% acetone using a Virtis 45 homogenizer and filtered through no. 1 Whatman paper using a Büchner funnel under vacuum, and the solids were rehomogenized in 25 mL of 80% acetone before refiltering. The total acetone filtrate was evaporated using a rotary evaporator and recovered with water to a final volume of 8 mL. These reconstituted extracts were extracted four times with equal volumes of ethyl acetate, evaporated to dryness, and reconstituted in methanol to a final volume of 4 g/mL. These stock solutions were further diluted to final test concentrations of 25, 50, 75, 100, and 150 mg/mL in Hank's balanced salt solution (HBSS).

HPLC analysis showed that the apple peel extract (200 mg/mL) in methanol contained 5.4 nmol/mL quercetin 3-glucoside and no detectable amounts of quercetin aglycon. The shallot extract (200 mg/mL) in methanol contained more quercetin 3-glucoside (8.1 nmol/mL) than the apple peel extract, and it contained some free quercetin (2.8 nmol/mL).

Cell Culture. Caco-2 cells, obtained from the American Type Culture Collection (Rockville, MD), were seeded at a density of 5×10^5 cells per well in a six-well flat-bottom plate. The cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco Life Technologies, Grand Island, NY) supplemented with 5% fetal bovine serum (Gibco Life Technologies), 10 mM HEPES, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin and were maintained at 37 °C in 5% CO_2. Caco-2 cells were used between passages 10 and 25, and the cells reached confluency ~5 days postseeding. Culture medium was changed three times a week. On day 15 postseeding, the DMEM was removed and the cells were rinsed three times with phosphate-buffered saline (PBS).

Kinetics and Dose−Response Experimental Design. Stock solutions of quercetin and quercetin 3-glucoside (Indofine Chemical Co., Hillsborough, NJ) in methanol were diluted to concentrations within a range from 10 to 100 μM (final concentration of methanol as 1%) in HBSS. For kinetics experiments, 1 mL of 100 μM quercetin 3-glucoside in HBSS and 1 mL of 50 μM quercetin were added to PBS-rinsed cells and incubated for 20, 40, 60, and 90 min at 37 °C. The apical solution was immediately removed, and the cells were rinsed three times with 20% methanol in PBS before cell extraction. For dose−response experiments, 1 mL aliquots of the full concentration range from 10 to 100 μM of pure quercetin and quercetin 3-glucoside were each placed in individual wells of Caco-2 cells and incubated for 40 min. One milliliter doses of apple peel extract and shallot extract (0, 25, 50, 75, 100, 150, and 200 mg/mL) were placed in individual wells of cells and incubated for 40 min. After incubation, the apical solution was removed and the cells were rinsed three times with 20% methanol in PBS. For each dose−response and kinetics experiment, at least three replications of each sample were collected.

After each kinetics or dose−response experiment, the rinsed cells were scraped in methanol (Fisher Scientific, Pittsburgh, PA) and each well was washed three times with methanol. The cells and the wash solvent were collected and immediately extracted using methanol. The cells were sonicated for 15 min at room temperature and centrifuged at 1600*g* for 5 min. The methanol supernatant was collected, and the cells were rinsed with 1 mL of methanol, vortexed for 1 min, and centrifuged at 1600*g*. The methanol supernatant was collected, and the cells were rinsed two more times with methanol, evaporated to dryness at 35 °C under nitrogen, and reconstituted in 400 μL of methanol.

HPLC Analysis. Quercetin and quercetin 3-glucoside concentrations of cellular extracts were determined using an RP-HPLC procedure with a Supelcosil LC-18-DB column (150 mm × 4.6 mm, and 3 μm pore size). A Waters 515 HPLC pump (Waters Corp., Milford, MA) and a Waters 2487 dual-wavelength absorbance detector set at 370 nm was used for all HPLC analyses. Quercetin and quercetin 3-glucoside were used as standards. For quercetin, quercetin 3-glucoside, apple peel extract, and shallot extract analyses, the solvent system used was (A) acidified water (pH 2; triflouroacetic acid) and (B) acetonitrile. The gradient method for quercetin was the following: flow rate = 1.2; 0.0 min, (A) 80% and (B) 20%; 7 min, (A) 67% and (B) 33%; 13 min, (A) 67% and (B) 33%; 14 min, (A) 80% and (B) 20%. The gradient program used for quercetin 3-glucoside was the following: flow rate = 1.0; 0.0 min, (A) 83% and (B) 17%; 8 min, (A) 70% and (B) 30%; 15 min, (A) 70% and (B) 30%; 16 min, (A) 83% and (B) 17%. The gradient used for the apple peel extracts and the shallot extracts was the following: 0.0 min, flow rate = 1.4, (A) 90% and (B) 10%; 53 min, flow rate = 1.5, (A) 80% and (B) 20%; 58 min, flow rate = 1.7, (A) 65% and (B) 35%; 64 min, flow rate = 1.4, (A) 90% and (B) 10%. Fifty microliter injections were made for each sample. Quercetin and quercetin 3-glucoside concentrations in the apple peel extracts, shallot extracts, and cellular extracts were extrapolated from the pure quercetin and quercetin 3-glucoside standard curves. Quercetin 3-glucoside recovery was 92 ± 5.0% and quercetin recovery was 91 ± 5.5% from the incubation media.

Lactase Activity. Lactase activity of Caco-2 cells was measured using methods adapted from Dahlqvist (*30*). Briefly, Caco-2 cells were cultured for 14 days. Cells were trypsinized, collected, centrifuged, and resuspended in homogenization buffer (50 mM sodium phosphate; 1 mM EDTA; 10 mM DTT; protease inhibitor cocktail, Sigma Chemical Co., St. Louis, MO). Cells were homogenized 5 times for 30 s with 1 min of cooling between bursts using a benchtop homogenizer. Cells were diluted 1:2 in a sodium maleate buffer (0.1 M, pH 6.0). Dilute homogenates were treated with an equal amount of lactose (56 mM) and incubated at 37 °C for 40 min. Glucose oxidase, peroxidase, and *o*-dianisidine were applied to the cell homogenates, and the final colored products were measured at 420 nm using a spectrophotometer (*30*). The results were compared to a glucose standard curve to determine

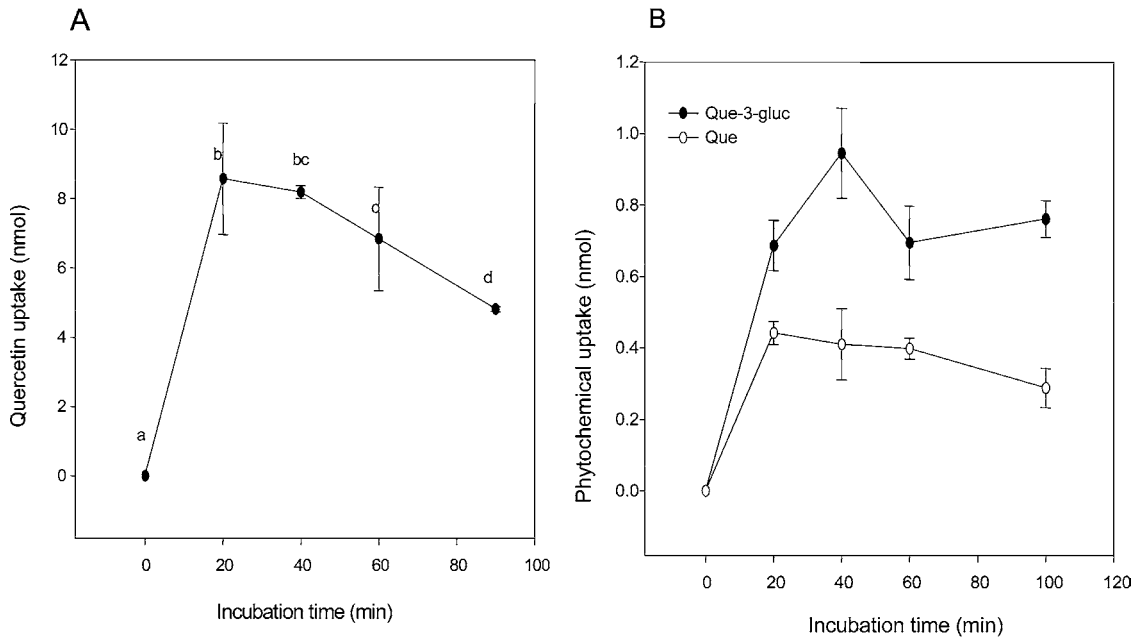

Figure 1. Effect of incubation time on quercetin and quercetin 3-glucoside uptake by a Caco-2 cell monolayer. Caco-2 cells were incubated with 1 mL of 50 μM quercetin (**A**) or 1 mL of 100 μM quercetin 3-glucoside (**B**) in HBSS for the indicated times. Each point represents the mean ± standard deviation (SD) of triplicate observations within the same experiment. Different letters indicate significantly different observations ($p < 0.05$).

the amount of glucose released by lactase in the Caco-2 cell monolayer. Protein was determined from crude cell homogenates colorimetrically using the Biuret reagent and Folin−Ciocalteau phenol reagent with comparisons to bovine serum albumin (BSA) as standard. Results are expressed as milliunits per milligram of protein, and 1 unit is defined as the lactase activity that hydrolyzes 1 μmol of lactose per minute at 37 °C.

Quercetin Glucosidase Activity in Cell-Free Caco-2 Homogenates. Six plates of Caco-2 cells, seeded at 5×10^5, were cultured for 14 days, trypsinized, and collected in PBS. The cells were centrifuged, rinsed, and then suspended in 2 mL of homogenization buffer. Cells were homogenized 5 times for 30 s using a benchtop homogenizer with 1 min of cooling between bursts. Small aliquots (50 μL) of homogenate were placed in Eppendorf tubes, and 5 μL of 1 mM quercetin 3-glucoside was added to each tube. The homogenates plus quercetin 3-glucoside (measured in triplicate) were incubated for 1, 2, 4, 8, 12, or 24 h. Following incubation, methanol (195 μL) was added, and the tubes were vortexed prior to sampling by HPLC, using the procedures described above. A dose−response was measured by adding 0.25, 0.5, 1, or 5 mM solutions of quercetin 3-glucoside to the homogenates.

Statistical Analysis. All data were reported as mean ± standard deviation (SD) for three replicates of each treatment. An analysis of variance (ANOVA) was used to compare results between treatment groups, and pairwise multiple comparisons were performed using Fisher's LSD with an individual error rate of 0.05. The statistical analysis was completed using Minitab release 12 software (State College, PA).

RESULTS AND DISCUSSION

Pure Compound Uptake by Caco-2 Cells. Figure 1 shows quercetin 3-glucoside and quercetin uptake by the Caco-2 cell monolayers after different incubation time periods. In cells treated with 50 nmol of quercetin (**Figure 1A**), quercetin reached its peak concentration in the Caco-2 cells after only 20 min (8.6 ± 0.8 nmol), stabilized through 40 min, and decreased after 60 min ($p < 0.05$). In cells treated with 100 nmol of quercetin 3-glucoside (**Figure 1B**), peak accumulation of quercetin 3-glucoside occurred in the Caco-2 cell extracts at 40 min (0.95 ± 0.12 nmol), but decreased after 60 min ($p < 0.05$). The decrease in quercetin and quercetin 3-glucoside concentrations may be attributed to either decomposition of the compounds during incubation or metabolism of the compounds by the Caco-2 cell layer. Following HPLC analysis of the cellular extracts treated with quercetin, only very minor, unidentified peaks were seen in the HPLC chromatograms, which were most likely products of degradation or metabolism. Work by Boulton et al. (*31*) showed that quercetin was degraded over time when incubated with Caco-2 cells, and quercetin was both degraded and metabolized through ortho-methylation when incubated with Hep G2 cells.

Murota et al. (*17*) found substantial quercetin conjugates in Caco-2 cellular extracts following incubations with quercetin. Interestingly, we did not find significant conjugation of quercetin in our intracellular Caco-2 extracts during any of the incubation time periods. Not only have quercetin conjugates been seen in previous Caco-2 cell culture work, but quercetin metabolites have also been found in both animal and human bioavailability studies. A 30-min perfusion of a rat intestine with quercetin resulted in a majority of quercetin being glucuronidated and to a lesser extent sulfated and methoxylated (*32*). Ingestion of quercetin and quercetin 3-glucoside by pigs also resulted in circulating quercetin conjugates, but no circulating quercetin (*33*). In humans, onion ingestion led to an increase in quercetin conjugates in the plasma, but no quercetin aglycon (*34*). Furthermore, when humans consumed quercetin 3-glucoside and quercetin 4-glucoside, no quercetin glucosides were found in the plasma. Trace amounts of quercetin were seen, but the major plasma components were quercetin glucuronides (*35*).

It is possible that we did not see glucuronidation in the intracellular Caco-2 extracts because of the short incubation times. However, Moon et al. (*34*) saw conjugation after a 30-min incubation in Caco-2 cells. Therefore, the lack of glucuronidation in our Caco-2 cells may be a unique characteristic of this Caco-2 strain, or the glucuronide conjugates were at levels below the detection limit. Our Caco-2 model has the potential to be a useful predictor of quercetin intestinal uptake, but not of further metabolism.

In cell samples treated with quercetin 3-glucoside, one metabolic product seen in the Caco-2 cell extracts was quercetin (**Figure 1B**), as identified by using HPLC and GC-MS

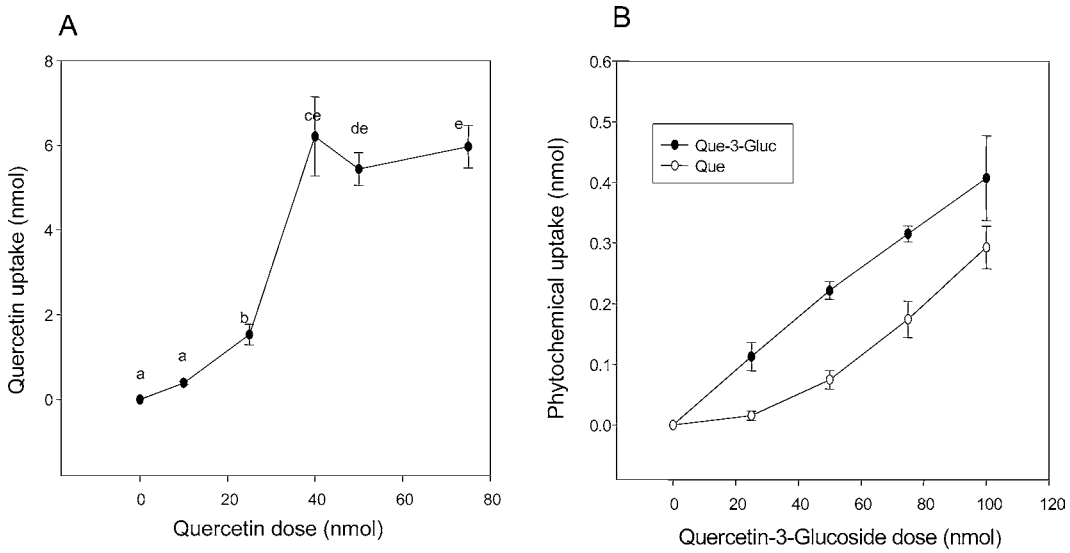

Figure 2. Caco-2 monolayer uptake of different concentrations of quercetin and quercetin 3-glucoside. Indicated amounts of quercetin (**A**) and quercetin 3-glucoside (**B**) in 1 mL of HBSS were applied to Caco-2 cell monolayers and incubated for 40 min. Each point represents the mean ± SD of triplicate observations within the same experiment. Different letters indicate significantly different observations ($p < 0.05$).

techniques. The cell extracts were derivatized and compared to derivatized quercetin standard using GC-MS (*36*). It has been hypothesized that an important step in quercetin glycoside intestinal absorption is an enzymatic hydrolysis. Two human small intestinal glucosidases, lactase phlorizen hydrolase and cytosolic β-glucosidases, have been isolated and have shown activity toward flavonoid glycosides (*37*). In our kinetic experiments, when cells were treated with quercetin 3-glucoside, peak quercetin accumulation was seen after only a 20-min incubation period (0.44 ± 0.03 nmol) and remained relatively stable after 40 and 60 min, but decreased by 100 min ($p < 0.05$; **Figure 1B**).

The amounts of pure quercetin and quercetin 3-glucoside taken up by the Caco-2 monolayers over a range of doses can be seen in **Figure 2**. Both quercetin and quercetin 3-glucoside uptake positively correlated with dosage up to a certain maximal dosage. Quercetin uptake increased through the 40 nmol doses and then remained similar for 50 and 75 nmol doses (**Figure 2A**). Quercetin 3-glucoside uptake continued to rise through the maximal dose of 100 nmol (**Figure 2B**). The percentage of pure quercetin absorbed by the cell was higher than that of quercetin 3-glucoside absorbed by the cell ($p < 0.05$), where only low amounts of quercetin 3-glucoside were detected. At the dose of highest cell uptake (40 nmol, quercetin; 100 nmol, quercetin 3-glucoside), quercetin uptake reached 15.9 ± 1.19% of the total quercetin applied to the monolayer, whereas quercetin 3-glucoside uptake reached only 0.45 ± 0.09%. This finding is consistent with past studies that have found that quercetin aglycon is absorbed by the Caco-2 monolayers or transported across the Caco-2 monolayers in much higher quantities when compared with quercetin glucosides (*17*, *20*).

When our cells were treated with quercetin 3-glucoside, quercetin absorption was also observed (**Figure 2B**). Quercetin absorption in Caco-2 cells treated with quercetin 3-glucoside also increased in a dose-dependent manner, reaching peak absorption (0.29 ± 0.03 nmol) when cells were treated with 100 nmol of quercetin 3-glucoside (**Figure 2B**). In an ileostomy study, Walle et al. measured different quercetin forms in the ileostomy fluid, and it was found that the fluid primarily contained the aglycon form. The group hypothesized that β-glucosidases hydrolyzed quercetin glucosides to quercetin, the form in which they were then passively transported (*38*). More recently, Day et al. (*39*) determined that quercetin glycosides are mainly deglycosylated by lactase phlorizen hydrolase before the aglycon then passes into the cell, but some intact glycoside transport by sodium-dependent glucose transporter 1 (SGLT1) occurs and the glucosides are deglycosylated within the cell by cytosolic β-glucosidase. Day et al. (*39*) also found that quercetin 3-glucoside appears to utilize only the lactase phlorizen hydrolase pathway, not the SGLT1 transporter, but that quercetin 4-glucoside uses both pathways. Walgren et al. (*40*) also found that quercetin 4-glucoside is transported by SGLT1, but its accumulation in the Caco-2 cell is limited by the apically expressed multidrug resistance-associated protein MRP2. This efflux pump transports intracellular quercetin 4-glucoside across the apical side of the Caco-2 cell monolayer. Efflux by MRP2 may be responsible for the low intracellular concentration of quercetin 3-glucoside in intracellular Caco-2 extracts.

Phytochemical Uptake from Apple Peel and Shallot Extracts. As can be seen in **Figures 3** and **4**, the quercetin and quercetin 3-glucoside from the shallot and the quercetin 3-glucoside from the apple were taken up in a dose-dependent manner by the Caco-2 monolayers. Quercetin and quercetin 3-glucoside from shallot extracts were absorbed by cells in a manner similar to that exhibited by the pure compounds, in that uptake of quercetin was greater than that of the quercetin 3-glucoside ($p < 0.05$; **Figure 3**). At the highest absorption dose, the Caco-2 cells took up 45.8 ± 10.0% of the total quercetin (**Figure 3A**) and only 4.5 ± 0.4% of the total quercetin 3-glucoside (**Figure 3B**; $p < 0.05$,). Quercetin uptake from the shallot was greater than the uptake of pure quercetin ($p < 0.05$), most likely due to the hydrolysis of quercetin glycosides within the shallot by the Caco-2 cell glycosidases. Although onions contain some quercetin 3-glucoside, they are especially high in quercetin 4-glucoside as well as quercetin 3,4-diglucoside. Hydrolysis of these compounds could also increase the amount of free quercetin available for intestinal uptake. Day et al. (*39*) found that quercetin 4-glucoside was hydrolyzed 10 times more than quercetin 3-glucoside by enzymes from a rat intestine, giving more evidence to believe that the free quercetin accumulated from the shallot extracts may in part be from quercetin 4-glucoside hydrolysis. Quercetin 4-glucoside hydrolysis may also be higher due to its interactions with SGLT1 and the subsequent hydrolysis by cytosolic β-glucosidase.

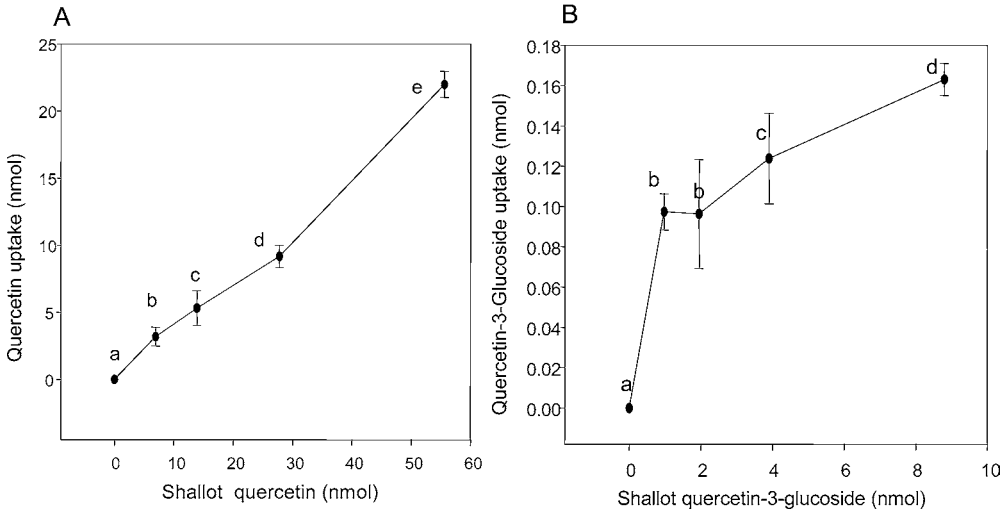

Figure 3. Caco-2 monolayer uptake of quercetin (**A**) and quercetin 3-glucoside (**B**) from shallot extract. Caco-2 cell cultures were incubated for 40 min with 1 mL of five different concentrations of shallot extract in HBSS (0, 25, 50, 100, and 150 mg/mL). Each point represents the mean ± SD of triplicate observations within the same experiment. Different letters indicate significantly different observations ($p < 0.05$).

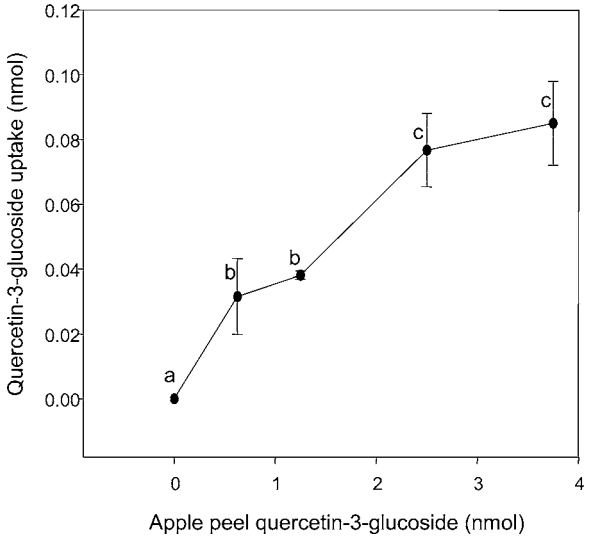

Figure 4. Quercetin 3-glucoside uptake from apple peel extract by Caco-2 cell monolayers. Caco-2 cell cultures were incubated for 40 min with 1 mL of five different concentrations of apple peel extract in HBSS (0, 25, 50, 100, and 150 mg/mL). Each point represents the mean ± SD of triplicate observations within the same experiment. Different letters indicate significantly different observations ($p < 0.05$).

The percentage of quercetin 3-glucoside accumulated by the Caco-2 cells from the apple peel extracts (**Figure 4**) was similar to that from the shallot extracts. Apples contain phloridzin, an SGLT1 inhibitor, which, theoretically, may inhibit glycoside transport into the intestinal epithelial cell. We did not see evidence of this in our work, as quercetin 3-glucoside uptakes were similar between both the onion and the apple peel. The levels of quercetin 3-glucoside and phloridzin may both be too low to see an effect.

There was no quercetin aglycon absorbed by the Caco-2 cells after treatment with apple peel extract. Although apple peel extracts do contain quercetin 3-glucoside, the amount is very low, and if hydrolysis of quercetin 3-glucoside occurred, it may have been at levels too low to detect. It has been hypothesized that the small intestinal uptake of quercetin from apples may be low, because much of the quercetin in apples is not conjugated with glucosides (*37*). Instead, most apple quercetin is bound to rhamnosides and xylosides, neither of which is easily hydrolyzed by the intestinal glycosidases. However, quercetin in onion is predominantly bound to glucosides, which are readily hydrolyzed by intestinal glucosidases. This was reflected in the data obtained by our work with the Caco-2 cell monolayer. Quercetin bioavailability was greatest in shallot extracts when compared to pure compounds and the apple peel extracts. This may partially explain the findings of the Hollman et al. (*41*) study, in which human ileostomy patients exhibited a high degree of quercetin absorption from onions.

Lactase Activity in Caco-2 Cells. Following incubation with lactose, Caco-2 cell homogenates did contain glucose, indicating lactase activity. The crude Caco-2 cell homogenates demonstrated 3.1 ± 0.6 milliunits/mg of protein of lactase activity on lactose during a 40-min incubation at 37 °C. This amount of lactase activity is much greater than past estimates of lactase activity in Caco-2 cells. It has been estimated that the average Caco-2 cell has ~0.1 milliunit/mg of protein of lactase activity and that the PD-7 subclone has ~0.3 milliunit/mg of protein of lactase activity (*42*). Our Caco-2 cells had 10 times greater activity than the more active PD-7 Caco-2 subclone. However, on the basis of the glucosidase activity of the cells, it was expected that the lactase phlorizen hydrolase activity might be higher in these Caco-2 cells than the previously reported values. The lactase activity of the Caco-2 cells used in our experiments is still considerably lower than the lactase activity found in lactose-tolerant human small intestines (20−80 milliunits/mg of protein) (*43*). However, the lactase activity in our Caco-2 cells is more similar to the reported lactase activity of rat small intestines (10−20 milliunits/mg of protein) (*44*) and lactose-intolerant human small intestines (2−10 milliunits/mg of protein) (*43*).

Quercetin Glucosidase Activity of Cell-Free Caco-2 Homogenates. Cell-free Caco-2 homogenates did show glucosidase activity. In a 24 h period, the amount of quercetin 3-glucoside hydrolyzed in the Caco-2 homogenates continued to rise to a total of 69.7 ± 2.5% (**Figure 5A**). The hydrolysis of quercetin 3-glucoside resulted in the accumulation of free quercetin (**Figure 5B**). As the dose of quercetin 3-glucoside increased in the cell-free homogenates, the amounts of quercetin 3-glucoside and free quercetin recovered from the samples also increased

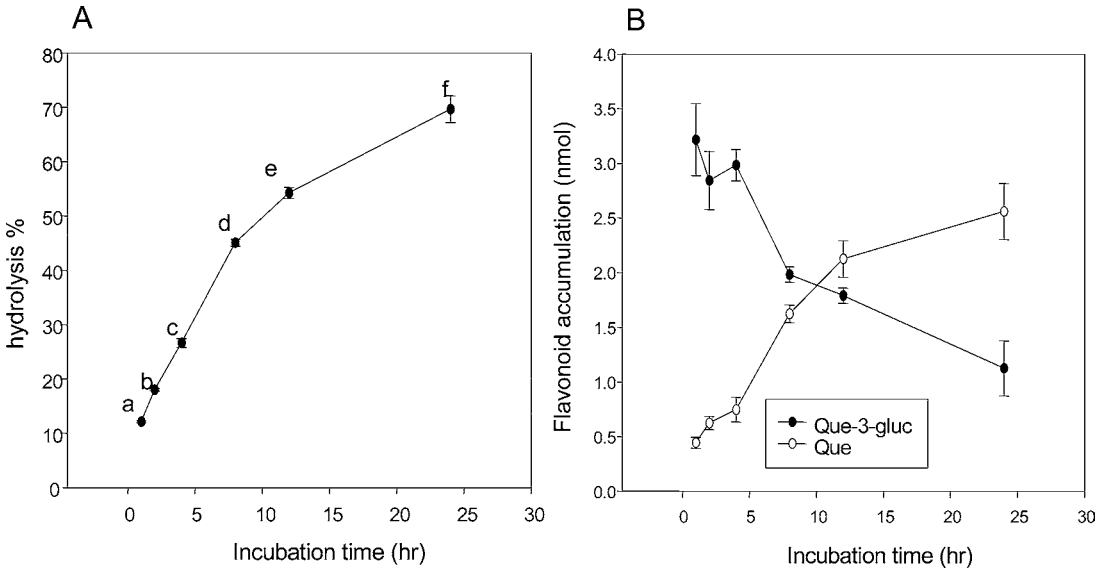

Figure 5. Hydrolysis (%) of quercetin 3-glucoside by Caco-2 cell homogenates (**A**) and accumulation of quercetin 3-glucoside and quercetin in Caco-2 cell homogenates (**B**). Caco-2 cell homogenates were incubated for 1, 2, 4, 8, 12, and 24 h with 5 nmol of quercetin 3-glucoside. Each point represents the mean ± SD of triplicate observations within the same experiment. Different letters indicate significantly different observations ($p < 0.05$).

linearly ($r^2 = 0.999$ for both quercetin and quercetin 3-glucoside accumulations). These data again demonstrate that the Caco-2 cells used in these experiments do possess glucosidase activity and are capable of hydrolyzing quercetin glycosides.

Caco-2 Model for Evaluating Phytochemicals from Foods. As the evidence builds to support the hypothesis that a diet high in fruits and vegetables may reduce the risk of chronic disease, it becomes more important to understand the bioavailability of compounds from whole foods. Single, isolated phytochemical compounds have not been conclusively linked to specific health benefits, although diets generally high in fruits and vegetables have been associated with decreased risks for cardiovascular disease and cancer. Therefore, it would be beneficial to understand the bioavailability of compounds within whole fruits and vegetables.

Results from our study demonstrate that the Caco-2 cell monolayer absorbs pure quercetin and quercetin 3-glucoside and quercetin and quercetin 3-glucoside from apple peel and shallot extracts, respectively, in a dose-dependent manner. Quercetin was absorbed much more efficiently than quercetin 3-glucoside from the shallot extracts, but much of the quercetin absorption may have been from hydrolysis of quercetin glycosides to quercetin aglycon. As most likely occurs in the human small intestines, the Caco-2 cells hydrolyzed some pure quercetin 3-glucoside to quercetin, and quercetin aglycon was detected in the Caco-2 cell. In the past, lactase activity has been found in the Caco-2 cells (*45*). However, more recently, Nemeth et al. (*37*) found no lactase activity in Caco-2 cells and some lactase activities in the TC-7 subclone of Caco-2 cells after long incubation periods. Our Caco-2 cells did demonstrate hydrolysis and uptake of quercetin 3-glucoside, and possibly other glucosides, from shallot extracts after short incubation periods, ranging from 20 to 90 min with a peak at 40 min. Using cell-free Caco-2 homogenates, it was found that the glucosidase activity increased through 24 h with some hydrolysis occurring at 1 h (12.1 ± 0.2%) and hydrolysis reaching 69.7 ± 2.5%. Using a lactase activity assay, it was found that our Caco-2 cells did have lactase activity, but that it was lower than that of lactose-tolerant human intestinal cells (*44*).

For an in vitro intestinal model to be considered a practical model of quercetin glucoside and other flavonoid glucoside absorption, it must contain glucosidase activity. The Caco-2 cells used in these experiments did show some lactase activity as demonstrated by the lactase enzyme activity assay. Although values were still lower than the approximate lactase activity level of normal human small intestines, the values were similar to the lactase activity of a rat small intestine, a model commonly used for studying flavonoid bioavailability.

Natural differences in total phytochemical profiles unique to apples and onions may thus affect the intestinal uptake of quercetin and quercetin conjugates. Our data suggest that quercetin from shallot extract is more readily absorbed by Caco-2 cells than that from apple peel extract and that the complex mixture of phytochemicals may influence intestinal uptake. It is likely that the intestinal uptake of other phytochemicals varies among foods, because of the complex and unique mixture of phytochemicals in various foods and because of differences in the food matrix. Other factors such as stage of harvest, storage conditions, processing, and digestion may also affect phytochemical uptake by Caco-2 cells. On the basis of our results, the Caco-2 cell model offers promise as a simple, rapid, and effective tool to evaluate phytochemical uptake from fruits and vegetables. In the future, the Caco-2 cell model may be used to examine the influence of other factors, such as digestion and processing, that may affect phytochemical uptake from foods.

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