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Polyphenol combinations improve quercetin absorption and inhibit carbohydrate digestion while reducing glucose transport

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High saccharide intake contributes to the development of obesity and type 2 diabetes. Polyphenols such as quercetin, kaempferol, and isoquercetin have been shown to reduce carbohydrate digestion and monosaccharide uptake. This study examined their individual and combined effects on α-amylase and α-glucosidase activity using spectrophotometry-based assays as well as on glucose and fructose transport and polyphenol absorption in differentiated Caco-2 cells. Quercetin (200 μM) significantly inhibited α-glucosidase activity by ~94%, while ~10% inhibition of α-amylase activity was observed. A three-polyphenol mixture (equimolar 1 : 1 : 1 quercetin : kaempferol : isoquercetin) significantly inhibited α-amylase by ~30%. Quercetin and kaempferol (100 μM) significiantly reduced glucose absorption by ~50% and ~25%, respectively. A mixture of all polyphenols (100 μM) significantly reduced glucose absorption by ~57%. None of the tested polyphenols altered fructose absorption compared to the control. The mixture doubled quercetin absorption compared to quercetin alone at equal doses, which could be explained by kaempferol's inhibition of efflux transporters. These findings suggest that multi-component polyphenol formulations can enhance α-amylase inhibition and quercetin bioavailability while maintaining strong α-glucosidase inhibition.

1. Introduction

Consumption of high-glycemic-index foods leads to rapid glucose absorption and pronounced postprandial glucose spikes in the blood. This pattern contributes to the development of metabolic health impairments, such as insulin resistance and pancreatic β-cell dysfunction, thereby increasing the risk of developing type 2 diabetes mellitus (T2D).[1] Dietary components such as polyphenols have been identified as modulators of postprandial glucose responses. These mechanisms include the inhibition of carbohydrate-digesting enzyme activity and intestinal saccharide absorption, improvement of insulin sensitivity, stimulation of insulin secretion, and reduction of oxidative stress and inflammation associated with metabolic disorders.[2]

Polyphenols can be broadly divided into flavonoids and non-flavonoids.[3] Flavonoids are especially abundant in fruits and vegetables, tea, wine, and spices. Common dietary flavonoids include quercetin, kaempferol, and myricetin.[4] Polyphenol concentrations vary considerably among crops and between cultivars. By selecting cultivars naturally rich in these compounds, it may be possible to enhance their health-promoting effects, including glucose homeostasis.

Polyphenols can improve glycemic control *via* several mechanisms. After ingestion, polyphenols that reach the small intestine can inhibit the activity of digestive enzymes that are involved in saccharide breakdown. Starch is broken down into maltose, maltotriose, and related α-1,6-oligomers by salivary and pancreatic α-amylases. In the small intestine, brush-border α-glucosidases release glucose from maltose, maltotriose, and related α-1,6-oligomers. The glucose can subsequently be absorbed into enterocytes.[5] Quercetin is a potent inhibitor of α-glucosidase and a moderate inhibitor of α-amylase. It has been shown to be as potent as or even more effective than the drug acarbose at inhibiting α-glucosidase.[6] Kaempferol has also been shown to inhibit both enzymes, although it is less potent than quercetin.[6] Enzyme inhibition can occur *via* competitive binding at the catalytic residues or non-competitive binding at allosteric sites.[7,8] Because quercetin, kaempferol, and isoquercetin inhibit α-amylase and α-glucosidase through different binding mechanisms, their combined presence in a single food matrix may yield additive or synergistic effects on carbohydrate digestion.

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Following enzymatic breakdown, glucose is transported into enterocytes by sodium–glucose transporter 1 (SGLT1) and solute carrier family 2 (GLUT2), while fructose is absorbed *via* facilitated glucose transporter member 5 (GLUT5).[9] When luminal glucose concentrations in the small intestine exceed 25 mM, GLUT2 transporters translocate to the apical membrane in enterocytes, thereby enhancing glucose absorption.[10] Polyphenols can inhibit transporters involved in the absorption of monosaccharides from the intestinal lumen into the enterocytes by direct inhibition or by reducing their expression.[11,12] Quercetin has been shown to be a potent inhibitor of α-glucosidase and can inhibit GLUT2 *via* direct binding,[13] and isoquercetin can directly inhibit SGLT1.[14] Combining multiple polyphenols that inhibit SGLT1 and/or GLUT2 may more effectively reduce saccharide absorption than targeting only one transporter, such as SGLT1, alone.

Only a fraction of dietary polyphenols reaches systemic circulation, while their chemical structure strongly influences absorption. The bioavailability of polyphenols is also influenced by digestive stability and their release from food matrices.[15,16] The majority reach the colon unabsorbed and are either degraded by microbiota or excreted in feces.[17] Intestinal efflux transporters such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP) further limit systemic availability by pumping absorbed polyphenols back into the intestinal lumen.[18] Quercetin can inhibit these efflux transporters, including BCRP, suggesting that combinations with other polyphenols may enhance overall absorption.[19,20] Once absorbed, polyphenols can modulate glucose metabolism in peripheral tissues by promoting insulin signaling or inhibiting gluconeogenesis.[21–23]

Polyphenols may act complementarily to reduce saccharide absorption by targeting both digestive enzymes (α-amylase and α-glucosidases) and intestinal glucose transporters (SGLT1 and GLUT2). To explore these potential combined effects, quercetin, kaempferol, and isoquercetin were selected based on their dietary abundance and complementary mechanisms of action. We hypothesized that the equimolar combination of quercetin, kaempferol and isoquercetin in a 1 : 1 : 1 ratio will inhibit α-amylase, α-glucosidase, and saccharide absorption more effectively than individual polyphenols. Furthermore, we hypothesized that the mixture enhances the cellular uptake of these polyphenols compared with individual treatments, potentially through the inhibition of efflux transporters, thereby increasing intracellular retention. These hypotheses were tested using α-amylase and α-glucosidase inhibition assays and differentiated Caco-2 monolayers to assess transporter-mediated glucose uptake and polyphenol absorption.

2. Methods

2.1 Reagents and materials

High-glucose Dulbecco's modified Eagle's medium (DMEM), heat-inactivated fetal bovine serum (hiFBS), Dulbecco's phosphate-buffered saline (DPBS) with calcium and magnesium, minimum essential medium (MEM) non-essential amino acids (NEAA), penicillin–streptomycin (P/S), and D-glucose were purchased from Invitrogen (Breda, the Netherlands). Bovine serum albumin (BSA), phloretin, horseradish peroxidase (HRP), glucose oxidase from *Aspergillus niger* (GO), porcine pancreatic α-amylase, α-glucosidase from *Saccharomyces cerevisiae*, acarbose, quercetin, dimethyl sulfoxide (DMSO, 100%), 4-nitrophenyl α-D-glucopyranoside (pNPG), sodium phosphate, sodium chloride, monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), and 10-acetyl-3,7-dihydroxyphenoxazine (Ampliflu™ Red) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). 1-Deoxy-1-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-fructose (NBD-F) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Isoquercetin and kaempferol were purchased from Tebu-bio B.V. (Heerhugowaard, the Netherlands). Twelve-well plates containing 0.4 μm pore size polycarbonate Transwell® inserts with a cell growth area of 1.12 cm^2 and 96-well black flat-bottom microplates were obtained from Corning Incorporated (Corning, NY, USA). For determination of Caco-2 barrier integrity, a EVOM2 epithelial voltohmmeter (World Precision Instruments Europe, Friedberg, Germany) was used. A BioTek Synergy HTX multi-mode plate reader (BioTek Instruments, Winooski, VT, USA) was used for spectrometric and fluorometric measurements. Polyphenol HPLC analyses were performed on a Shimadzu Prominence system equipped with a UV/vis detector (SPD-20A; Shimadzu Corporation, Kyoto, Japan). For chromatographic separation, a Discovery® C18 column (Supelco®, Sigma-Aldrich, St Louis, MO, USA; 5 μm, 12.5 cm × 2.1 mm) maintained at 40 °C was used. HPLC samples were processed using an ultrasonic homogenizer (Biosafer 150-96; Biosafer, Nanjing, China).

2.2 α-Glucosidase inhibition assay

α-Glucosidase inhibitory activity was determined by a spectrophotometric method using pNPG, based on a previously described method with slight modifications, including using kinetic measurements instead of an endpoint assay.[24] Quercetin, isoquercetin and kaempferol stock solutions were prepared in DMSO (0–10 mM) and were further diluted in phosphate buffer (0.1 M, pH 6.8) to final concentrations of 0–200 μM and pre-incubated with α-glucosidase (0.0125 U mL^{-1}) at 37 °C for 10 minutes. The final concentration of DMSO in the reaction mixture was 2%. A 2% DMSO vehicle control confirmed that DMSO alone did not affect α-glucosidase activity relative to the solvent-free control. The reaction was initiated by adding pNPG (5 mM) and incubated for 30 minutes at 37 °C. Acarbose, dissolved in distilled water, served as a positive control (125–1000 μM). Product formation (*p*-nitrophenol liberation) was measured kinetically during the 30 minutes of incubation at λ = 405 nm using a microplate reader. The percentage inhibition was calculated as follows: inhibition (%) = $[(S_0 - S_1)/S_0] \times 100$, where S_0 and S_1 are the reaction slopes calculated from the first 10 minutes of the 30-minute incubation with pNPG without and with the test

compound, respectively. To correct for intrinsic compound absorbance at 405 nm, wells containing the buffer instead of pNPG were included for each compound concentration, and the corresponding slopes were subtracted prior to inhibition calculation.

2.3 α-Amylase inhibition assay

α-Amylase inhibition activity was determined using the DNS colorimetric assay, following previously reported protocols with minor modifications to the α-amylase concentration (2 U mL^{-1}) and pre-incubation time (20 minutes).[25] Porcine pancreatic α-amylase was used due to its high sequence identity with the human enzyme (87.1%)[26,27] and conserved active site architecture.[28] Polyphenolic compounds were initially dissolved in DMSO and subsequently diluted in sodium phosphate buffer (20 mM sodium phosphate containing 6.7 mM sodium chloride, pH 6.9) to achieve a final DMSO concentration of 2% (v/v) in the reaction mixture. A 2% DMSO vehicle control demonstrated that the solvent did not significantly interfere with α-amylase activity when compared to the solvent-free control. Acarbose, dissolved in distilled water, served as the positive control (125–1000 μM). Polyphenolic compounds (0–200 μM) were pre-incubated with α-amylase (2 U mL^{-1}) in sodium phosphate buffer for 20 minutes at 37 °C. Subsequently, 1% (w/v) starch solution was added as the substrate, and the mixture was incubated for an additional 30 minutes at 37 °C. The enzymatic reaction was terminated by adding the DNS reagent, followed by boiling in a heating block at 100 °C for 10 minutes to develop color. After cooling to room temperature, 100 μL of the samples was transferred to a flat-bottom 96-well microplate, and the absorbance was measured at 540 nm using a microplate reader. The percentage of α-amylase inhibition was calculated using the following equation: inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 represents the absorbance of the control (without the test compound) and A_1 represents the absorbance in the presence of the test compound, both measured after DNS color development.

2.4 Cell culture

All experimental procedures utilized Caco-2 cells (DSMZ, German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) within passage numbers 4–8 to ensure consistency. Routine cell maintenance was conducted in complete growth medium formulated with high-glucose DMEM containing 20% (v/v) hiFBS, 100 μM MEM NEAA, and 1% (v/v) P/S (100 U mL^{-1}). Cultures were maintained in T175 flasks under standard incubation conditions (37 °C, 5% CO_2, humidified atmosphere). The medium was replaced every 2–3 days, with subcultivation performed as needed to prevent overconfluency (maintained below 80%). For experimental applications, cells were harvested at approximately 50% confluency to preserve their differentiation capacity.[29] The cell seeding density was set at 7.2×10^4 cells per insert using 12-well Transwell® systems, targeting a final confluency of 95–100% to promote optimal cellular attachment and subsequent differentiation. The differentiation protocol involved daily changes of growth medium during the initial 72-hour period, transitioning to medium renewal every other day for the remaining 18 days of the 21-day differentiation protocol.

2.5 Monolayer validation and compound preparation

The barrier integrity of differentiated Caco-2 cells was assessed by measuring transepithelial electrical resistance (TEER) prior to acute polyphenol treatment. Individual polyphenolic compounds (quercetin, isoquercetin and kaempferol) were applied at 100 μM concentrations. These concentrations were chosen based on previous literature showing no toxic effects of the compounds at the concentrations and incubation time used.[30–33] Additionally, there was no significant difference in LDH release between the control conditions and the tested polyphenols (SI Fig. S1). LDH was measured *via* the optical density of NADH at λ = 340 nm as LDH catalyzes the reversible reaction of pyruvate and NADH to lactate and NAD+. The polyphenol combination consisted of equal proportions (1 : 1 : 1) of these three compounds, maintaining a total concentration of 100 μM (33.3 μM each).

Exposure to 1 mM phloretin for 1 hour was used as a positive control for inhibiting glucose uptake as it is a known GLUT2 inhibitor.[10,11,34–36] All test compounds were initially solubilized in DMSO, filter-sterilized through a 0.22 μm membrane, and subsequently diluted in DPBS to achieve working concentrations while maintaining DMSO levels below 1% (v/v) in the final treatment medium. The addition of 1% of DMSO did not induce cytotoxicity (data not shown).

2.6 Exposure and transport assay

Following TEER validation, cell monolayers were gently rinsed twice with pre-warmed DPBS to remove residual culture medium without compromising monolayer integrity. Transport experiments were initiated by adding the exposure medium containing test compounds in DPBS supplemented with 2.5 mM glucose and 0.5 mM NBD-fructose to the apical compartment, while fresh DPBS was added to the basolateral chamber. Upon completion of the transport assay after 60 minutes, basolateral samples were divided into duplicate aliquots: one set designated for glucose/fructose quantification and the second acidified with 0.2% formic acid to optimize the pH conditions for polyphenol stability during HPLC analysis. HPLC-designated samples were stored in amber plastic Eppendorf tubes to reduce light exposure and snap frozen in liquid nitrogen after collection. The schematic overview of the experimental setup is given in Fig. 1.

2.7 Measurement of absorbed glucose and NBD-fructose

Glucose concentrations were determined using the Ampliflu™ Red enzymatic fluorometric assay, while NBD-fructose levels were quantified through direct fluorescence measurements. The Ampliflu™ Red working solution was freshly prepared containing 100 μM Ampliflu™ Red reagent, 0.2 U mL^{-1} horseradish peroxidase (HRP), and 2 U mL^{-1} glucose oxidase. To reduce the influence of the polyphenols on the Ampliflu™ Red assay, the collected basolateral samples were diluted 10 times.

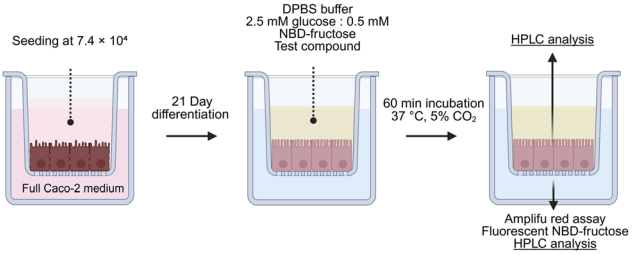

Fig. 1 Schematic overview of the experimental setup. Cells were differentiated for 21 days after validation of monolayer integrity *via* TEER; the polyphenols and glucose/NBD-fructose were added apically. Basolateral samples were collected for the determination of glucose and fructose concentrations, and polyphenols were measured in apical and basolateral samples *via* HPLC. The figure was created using BioRender.com. DPBS, Dulbecco's phosphate-buffered saline; HPLC; high-performance liquid chromatography.

For the assay, 50 μL of working solution was combined with an equal volume of sample (1 : 1 ratio) in black flat-bottom 96-well microplates. Glucose quantification was performed through kinetic fluorescence monitoring of resorufin formation over a 35-minute period. Measurements were conducted at 37 °C using a microplate reader with excitation and emission wavelengths of λ = 571 nm and λ = 585 nm, respectively. Following completion of the glucose assay, NBD-fructose fluorescence was measured using the same samples, with excitation at λ = 472 nm and emission at λ = 538 nm. Quantitative analysis was performed using standard calibration curves prepared for both analytes spanning concentrations from 0 to 50 μM. At the used concentrations, polyphenols did not interfere with the NBD-fructose read-out.

2.8 Polyphenol absorption

Prior to HPLC analysis, samples were thawed at 4 °C and centrifuged at 12 800 rpm for 10 seconds, and DPBS was exchanged for ethanol. The sample was sonicated at 8 °C for 5 minutes with a pulse cycle of 4 seconds on and 2 seconds off. PBS was then added at a 1 : 1 ratio with the original DPBS and sonicated again to homogenize the mixture, then filtered (0.45 μm PES) prior to injection. Chromatographic separation used acetonitrile and 0.5% acetic acid under a gradient from 18% to 18.5% acetonitrile over 7 min, held for 5.5 min, at 0.6 mL min^{-1} with a 5 μL injection volume. Detection wavelengths were as follows: λ = 356 nm and λ = 368 nm. Peaks were identified by retention time and UV spectra relative to authenticated standards and quantified using external calibration curves for quercetin, isoquercetin, and kaempferol. Additionally, absorption was calculated as the percentage of the initial apical dose that crossed the monolayer: absorption (%) = (amount transported to basolateral side/total polyphenols added) × 100.

2.9 Statistics

All experiments were conducted with a minimum of three independent biological replicates, each with technical replicates. Data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism. Differences in inhibition across polyphenols and concentrations were analyzed using one-way ANOVA followed by

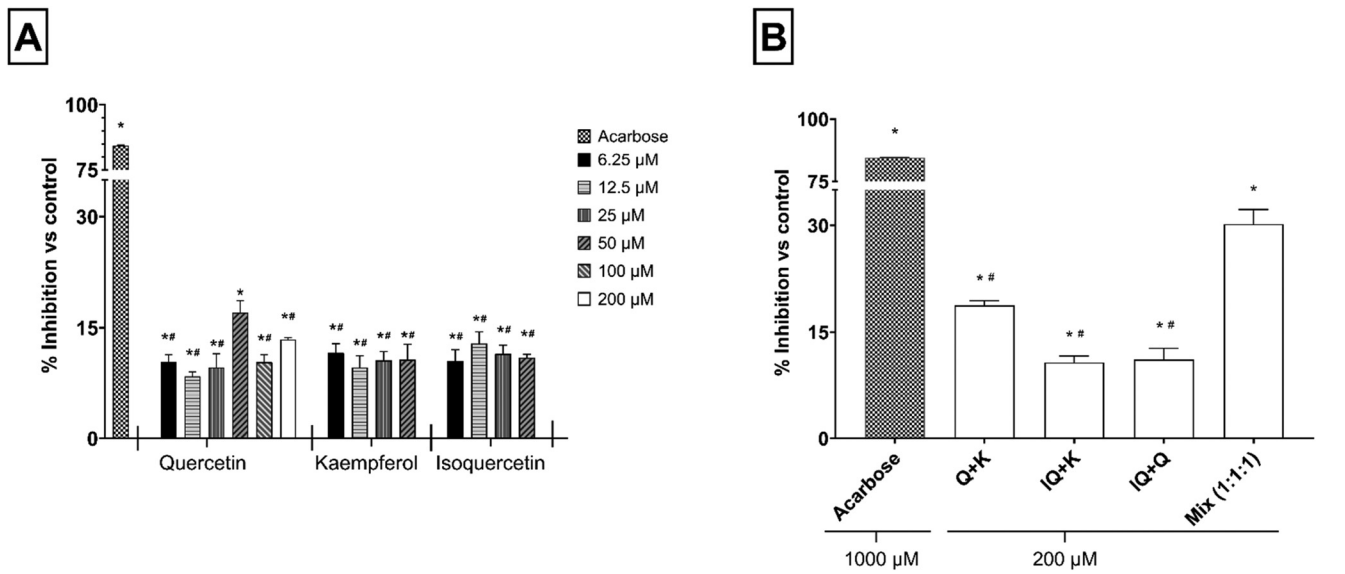

Fig. 2 α-Amylase inhibition by (A) individual polyphenols (quercetin, kaempferol, and isoquercetin), (B) combinations of quercetin and kaempferol (Q + K), isoquercetin and kaempferol (IQ + K), and isoquercetin and quercetin (IQ + Q), and an equimolar mixture of quercetin, kaempferol and isoquercetin (Q + K + IQ), each tested at 200 μM. Acarbose (1000 μM) served as positive control. Bars represent mean ± SEM (N = 3). * Indicates a statistically significant difference compared to the control (p < 0.05), as determined by one-way ANOVA followed by Dunnett's multiple comparisons test. # indicates a statistically significant difference compared to the mixture at the same concentration (p < 0.05), as determined by one-way ANOVA followed by Tukey's multiple comparisons test.

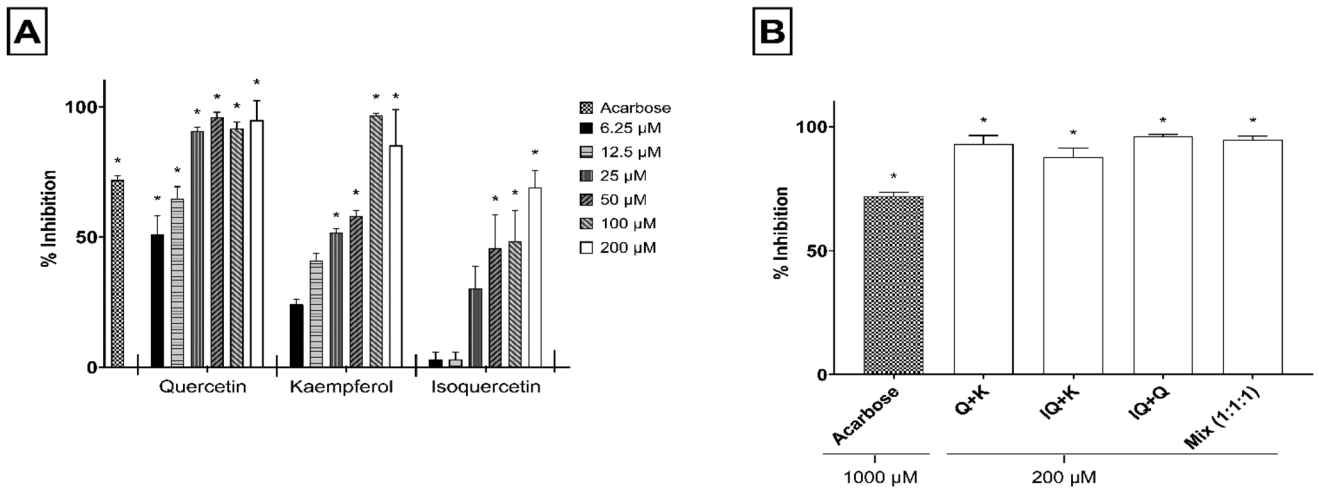

Fig. 3 α-Glucosidase inhibition by (A) individual polyphenols (quercetin, kaempferol, and isoquercetin), (B) combinations of quercetin and kaempferol (Q + K), isoquercetin and kaempferol (IQ + K), and isoquercetin and quercetin (IQ + Q), and a mixture of quercetin, kaempferol and isoquercetin (Q + K + IQ), each tested at 200 μM. Acarbose (1000 μM) served as positive control. Bars represent mean ± SEM (N = 3). * Indicates a statistically significant difference compared to the control ($p < 0.05$), as determined by one-way ANOVA followed by Dunnett's multiple comparisons test.

Tukey's or Dunnett's *post hoc* tests. Polyphenol absorption was analyzed using Mann Whitney or unpaired *t*-tests to compare absorption across quercetin, isoquercetin, kaempferol, and their combination. All data were tested for normality using the Shapiro–Wilk test, with transformations applied as needed. A significance threshold of $p < 0.05$ was used.

3. Results

3.1 The quercetin–kaempferol–isoquercetin mixture exhibits greater α-amylase inhibitory activity than individual polyphenol components

All three polyphenols moderately inhibited α-amylase (Fig. 2A). Quercetin reduced activity at all concentrations (max 17.0 ± 1.61% at 50 μM, $p < 0.05$), whereas kaempferol and isoquercetin were effective mainly at lower concentrations (kaempferol 11.6 ± 1.20% at 6.25 μM; isoquercetin 12.9 ± 1.58% at 12.5 μM, $p < 0.05$). Isoquercetin followed a similar pattern, with a maximum of 12.9 ± 1.58% at 12.5 μM. At higher concentrations, their effects were not significant. The positive control, acarbose, inhibited α-amylase at all tested concentrations, reaching 84.5 ± 0.12% at 1000 μM ($p < 0.05$) (Fig. 2 and SI Fig. S4). To assess potential combined effects, we evaluated combinations of the polyphenols (Fig. 2B and SI Fig. S2, S3). Quercetin–kaempferol reached 18.9 ± 0.70% at 200 μM, exceeding individual kaempferol or isoquercetin ($p < 0.05$). The inhibition of α-amylase by isoquercetin–kaempferol peaked at 200 μM showing 10.5 ± 0.938% inhibition and was significantly more effective than the individual compounds at 200, 100, and 6.25 μM ($p < 0.05$). The inhibition of α-amylase by the quercetin–isoquercetin combination was 11.1 ± 1.56% at 200 μM ($p < 0.05$). The mixture of all three polyphenols showed the strongest inhibition, 30.5 ± 1.33% at 200 μM ($p < 0.05$) compared to the control. This effect was significantly greater than the inhibition by quercetin (30.5 ± 1.33% *vs.* 13.4 ± 0.241%), kaempferol (30.5 ± 1.33% *vs.* 5.91 ± 1.08) and isoquercetin (30.5 ± 1.33% *vs.* 7.03 ± 1.09%) alone at a concentration of 200 μM ($p < 0.05$) (Fig. 2B). Across all concentrations tested, the mixture outperformed quercetin individually, with the exception of 50 μM where the difference was minimal (17.0% *vs.* 15.8%) and did not reach statistical significance. The mixture also inhibited α-amylase significantly more than the combinations of isoquercetin and kaempferol across all concentrations ($p < 0.05$). Compared with quercetin–kaempferol, the mixture was only significantly more effective at 200 μM, while consistently outperforming the other two double combinations across all tested concentrations ($p < 0.05$).

3.2 α-Glucosidase is strongly inhibited by quercetin and by the quercetin–kaempferol–isoquercetin mixture at concentrations >25 μM

Quercetin strongly inhibited α-glucosidase at all concentrations tested ($p < 0.05$), with maximal inhibitions of 95.9 ± 2.04% at 50 μM and 94.8 ± 7.52% at 200 μM. Even at the lowest concentration of quercetin (6.25 μM), α-glucosidase inhibition was substantial (50.9 ± 7.29%). Kaempferol also reduced α-glucosidase activity but only above 25 μM, reaching 96.8 ± 0.588% inhibition at 100 μM ($p < 0.05$). Isoquercetin inhibition was comparatively weak compared with quercetin and kaempferol: α-glucosidase activity was significantly inhibited only at 50, 100 and 200 μM, with a maximum inhibition of 69.0 ± 6.46% at 200 μM and 48.3 ± 12.0% at 100 μM ($p < 0.05$) (Fig. 3A). By contrast, the positive control acarbose inhibited α-glucosidase at all concentrations, peaking at 71.9 ± 1.65% at 1000 μM ($p < 0.05$) (SI Fig. S7).

The combination of quercetin and kaempferol inhibited α-glucosidase at all concentrations, with the highest inhibition at 50 μM (97.3 ± 0.545%) ($p < 0.05$) (SI Fig. S5). The combi-

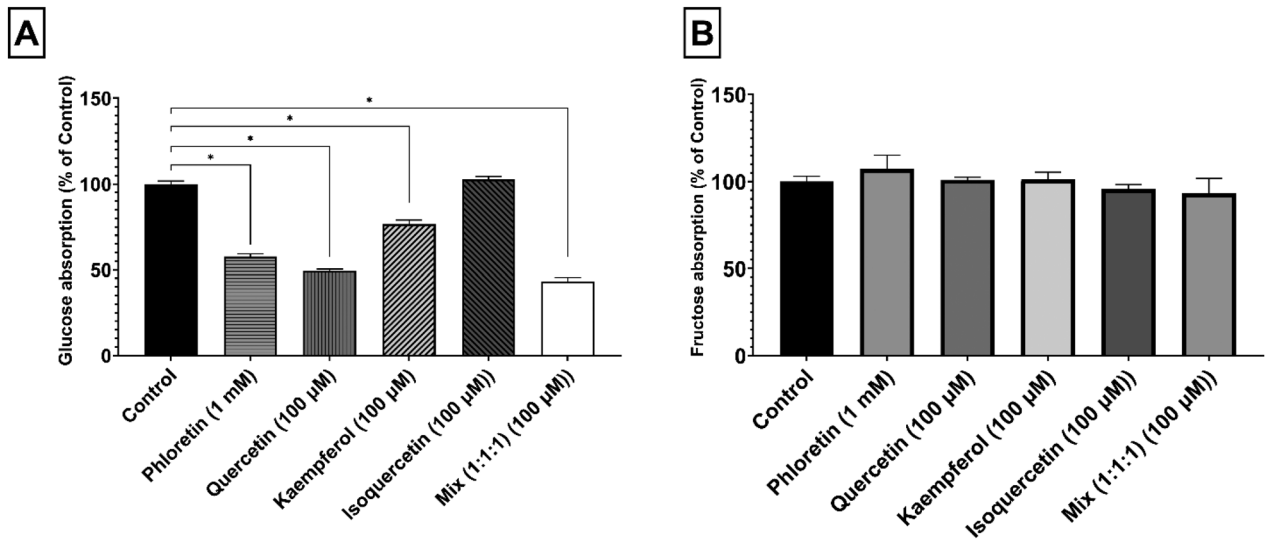

Fig. 4 Basolateral glucose (A) and NBD-fructose (B) concentration (μM) following a 1-hour exposure of differentiated Caco-2 cells to the test compound and 2.5 mM glucose and 0.5 mM NBD-fructose. Glucose concentrations were reduced after exposure to phloretin, kaempferol, quercetin and a mixture of quercetin, kaempferol and isoquercetin compared to the control. Polyphenols did not affect fructose absorption. $N = 3$, $n \geq 1$ and data are presented as mean ± SEM; * $p \leq 0.05$, significantly different from the control (Kruskal–Wallis test with Dunn's multiple comparisons).

nation of isoquercetin and kaempferol similarly reduced α-glucosidase activity at 200 μM but did not exceed the inhibition achieved by quercetin or kaempferol alone. Similarly, the combination of isoquercetin and quercetin did not show significantly different activity from isoquercetin or quercetin alone at 200 μM. The mixture of quercetin, kaempferol and isoquercetin significantly inhibited α-glucosidase compared to the control ($p < 0.05$), with a maximum inhibition of 96.3 ± 0.712% at 100 μM (SI Fig. S6), followed by 94.0 ± 1.33% at 200 μM (Fig. 3B).

3.3 Quercetin, kaempferol and the quercetin–kaempferol–isoquercetin mixture reduce basolateral glucose absorption without affecting fructose transport in differentiated Caco-2 cells

Cells treated with phloretin reduced basolateral glucose concentrations compared to the control by 42.3 ± 1.53% ($p < 0.001$; Fig. 4A). Quercetin (100 μM) also significantly decreased basolateral glucose concentrations by 50.7 ± 1.07% ($p < 0.001$). Kaempferol (100 μM) significantly reduced basolateral glucose by 23.4 ± 2.40% ($p < 0.001$). Isoquercetin (100 μM) did not significantly affect basolateral glucose concentrations relative to the control. The equimolar mixture of quercetin, kaempferol, and isoquercetin (1 : 1 : 1 ratio) reduced glucose absorption by 56.8 ± 2.44% ($p < 0.01$), comparable to quercetin alone. For all conditions tested, basolateral fructose did not differ significantly from the control condition (Fig. 4B).

3.4 Kaempferol and isoquercetin co-administration enhances quercetin absorption in differentiated Caco-2 cells

After 60 minutes of exposure, apical concentrations of quercetin were 51.4 ± 4.46 μM following single-compound treatment and 16.1 ± 0.873 μM in the polyphenol mixture. In single-compound exposure, quercetin reached a basolateral concentration of 10.1 ± 0.663 μM, corresponding to a transport efficiency of 17.11 ± 1.32%. In the mixture, quercetin transport efficiency significantly increased to 29.30 ± 1.30% (6.53 ± 0.190 μM basolateral concentration; $p < 0.001$ *vs.* single exposure). Isoquercetin showed the highest apical concentration among the single-compound treatments (81.63 ± 2.13 μM) and a concentration of 32.72 ± 1.70 μM in the mixture. However, isoquercetin was not detected in the basolateral compartment under any condition, indicating negligible transport across the differentiated Caco-2 monolayer. Kaempferol reached apical concentrations of 41.75 ± 2.90 μM in single-compound exposure and 35.2 ± 1.32 μM in the mixture. Basolateral concentrations were 6.24 ± 0.375 μM (13.49 ± 1.58% transport efficiency) in single-compound exposure and 6.67 ± 0.281 μM (16.00 ± 0.744% efficiency) in the mixture. The difference in transport efficiency between single-compound exposure and exposure to the kaempferol mixture was not statistically significant ($p > 0.05$).

4. Discussion

This study examined the inhibitory effects of quercetin, kaempferol, and isoquercetin, tested individually, in pairwise combinations, and as a mixture of all three, on key enzymes and transporters involved in saccharide digestion and monosaccharide absorption. Among the individual polyphenols, quercetin exhibited the greatest inhibitory activity across both the α-amylase and α-glucosidase assays. Polyphenol combinations significantly enhanced the inhibitory potency against α-amylase and monosaccharide transporters compared with

individual compounds. The mixture of quercetin, kaempferol and isoquercetin at 200 μM demonstrated the strongest α-amylase inhibition, achieving 30% inhibition.

Quercetin, kaempferol, isoquercetin, and their combinations inhibited α-glucosidase in a concentration-dependent manner. Among the combinations, quercetin–kaempferol exhibited the greatest inhibitory effect; however, quercetin alone was the most potent overall, surpassing kaempferol, isoquercetin, and all mixtures at equivalent concentrations. For instance, at 200 μM, the mixture achieved 94% inhibition, which was not significantly different from that of quercetin alone at the same concentration. These findings indicate that at higher concentrations, quercetin alone may be as effective as more complex mixtures at inhibiting α-glucosidase. Quercetin reduced glucose absorption by approximately 50% and the mixture reduced it by 57%, whereas fructose absorption remained unaffected for all the polyphenols tested. Furthermore, cells exposed to the mixture demonstrated a two-fold increase in quercetin uptake compared to those treated with quercetin alone. Although the catalytic domain of *S. cerevisiae* α-glucosidase is conserved compared with that of human intestinal α-glucosidase, validation using human-relevant systems (*e.g.* Caco-2 cell lysates) would strengthen physiological relevance.[37,38]

Quercetin, kaempferol, and isoquercetin strongly inhibited α-glucosidase but weakly inhibited α-amylase, which is consistent with prior findings.[6] Although the α-amylase inhibition observed here is modest, partial inhibition is often therapeutically desirable. The clinical agent acarbose illustrates this point, as strong α-amylase inhibition is effective but frequently causes gastrointestinal side effects such as flatulence, abdominal pain and diarrhoea.[39] In contrast, the milder inhibition exhibited by the polyphenols in this study may be sufficient to reduce postprandial glucose spikes while avoiding these adverse effects.[39] In a previous study, the dietary polyphenol EGCG inhibited pancreatic α-amylase by only 34% at 20 μM, yet this was sufficient to reduce peak postprandial blood glucose by 50% and the glycemic area under the curve by 20% in an *in vivo* starch challenge model. This suggests that even partial α-amylase inhibition can meaningfully blunt postprandial glucose spikes.[40]

The mixture consistently outperformed the individual polyphenols in inhibiting α-amylase across the tested concentration range. The sole exception was observed at 50 μM, where the difference between quercetin and the mixture did not reach statistical significance. The mechanisms by which polyphenols inhibit α-amylase are compound-specific. Quercetin has been reported to act as a mixed-type inhibitor of α-amylase, indicating that it can interact with both the free enzyme and the enzyme–substrate complex.[41,42] Although mixed-type inhibition often includes a non-competitive component, some studies have also described competitive inhibition by quercetin, suggesting that its mode of action may depend on the experimental conditions or the enzyme source.[43] Kaempferol, in contrast, has consistently been reported to inhibit α-amylase *via* a non-competitive mechanism.[44,45] The inhibitory mechanism of isoquercetin is less well defined; however, available evidence suggests a predominantly competitive mode of inhibition, potentially involving stable binding to the enzyme active site.[41] The enhanced α-amylase inhibition observed with the mixture may reflect additive inhibitory mechanisms, whereby quercetin and kaempferol interact with non-competitive sites, while isoquercetin exerts competitive inhibition at the active site, collectively leading to greater suppression of enzymatic activity.

At lower concentrations, polyphenols may compete for the same primary binding site, limiting the inhibitory effect of the mixture. At higher concentrations, secondary or allosteric binding sites become occupied, enabling simultaneous binding and thereby producing greater inhibition.[46]

The strong α-glucosidase inhibition by quercetin is consistent with previous studies, and similar inhibitory effects have been described for kaempferol and isoquercetin.[6] Quercetin inhibits yeast α-glucosidase *via* a mixed mechanism,[47] and kaempferol displays a similar mixed-type inhibition.[48] The inhibitory mechanism of isoquercetin remains incompletely characterized. The absence of combined effects among the polyphenol combinations suggests that quercetin is the primary contributor to the observed α-glucosidase inhibition. Quercetin alone showed strong inhibition across all tested concentrations, and the addition of other polyphenols did not further enhance this inhibition.

Quercetin significantly reduced glucose absorption in differentiated Caco-2 cells, consistent with its known non-competitive inhibition of GLUT2 at a non-sugar binding site.[13,14,49] Isoquercetin showed no inhibitory effect, whereas previous research showed that isoquercetin inhibits glucose uptake in SGLT1-transfected oocytes.[50] Kwon *et al.* (2007) found no effect on SGLT1 but observed GLUT2 inhibition at 100 μM.[51] It is possible that isoquercetin inhibition of GLUT2 in our study is limited due to the lack of absorption of the compound across the monolayer. GLUT2 is situated basolaterally and might require uptake and/or absorption for inhibition. Torres-Villarreal *et al.* showed a 56% decrease in methylglucoside uptake in glucose starved differentiated Caco-2 when incubated with 60 μM of isoquercetin for 15 minutes.[52] However, in that study, cellular uptake was assessed rather than transepithelial absorption (cells were not grown on Transwells), which limits direct comparison. These findings suggest that isoquercetin does not effectively inhibit SGLT1 at 100 μM. This confirms that isoquercetin primarily exerts luminal effects rather than contributing directly to basolateral transporter inhibition. Kaempferol caused a relatively smaller reduction in glucose absorption, consistent with reports of weak intestinal transporter inhibition.[53,54] The mixture of polyphenols reduced glucose absorption similarly to quercetin alone. This could be attributed to the increased uptake and/or absorption of quercetin,[13] potentially increasing basolateral GLUT2 inhibition. However, further validation is required to determine if lower concentrations of quercetin have similar effects. Although the Ampliflu™ Red assay is commonly employed for measuring glucose levels in various sample types, polyphenols

have been reported to interfere with this reaction due to their capacity to inhibit HRP.[55] In the present study, sample dilution effectively mitigated this interference, as the resulting polyphenol concentrations fell below 0.5 μM. Nevertheless, since basolateral phloretin concentrations were not determined, the possibility that residual interference contributed to an overestimation of inhibition under phloretin treatment cannot be fully excluded.

Fructose absorption was unaffected by exposure to the polyphenols. The previous literature has shown a lack of GLUT5 inhibition by quercetin.[13] Extracts containing kaempferol showed NBD-fructose absorption inhibition in undifferentiated Caco-2 cells, but the role of kaempferol was only validated by molecular docking studies on interactions of kaempferol with GLUT5.[56] The appearance of GLUT2 at the apical membrane normally occurs when glucose concentrations exceed 25 mM.[57] The lack of effects in our current study could be due to the absence of apical GLUT2 at low glucose levels.

This study provides the first evidence that combining polyphenols increases quercetin absorption. By increasing intestinal quercetin absorption, higher systemic levels of quercetin could be reached, which might contribute to the modulation of glucose metabolism in peripheral tissues by further promoting insulin signaling or inhibiting gluconeogenesis.[58–60] Quercetin is taken up by Caco-2 cells through passive diffusion as well as a pH-dependent process mediated by the organic anion transporting polypeptide B (OATP-B).[56] Kaempferol is also absorbed mostly through passive diffusion.[61] Isoquercetin was not converted to quercetin in the Caco-2 model, likely due to limited lactase phlorizin hydrolase activity, consistent with reports that it is not actively transported across the intestinal epithelium.[62–64]

Enhanced quercetin uptake may result from inhibition of intestinal efflux transporters, which normally reduce absorption. Quercetin itself has been reported to inhibit P-glycoprotein (Pgp/ABCB1) and BCRP/ABCG2,[19] while kaempferol may further enhance absorption by modulating these transporters. Kaempferol absorption was not increased in the mixture, which could be due to quercetin and kaempferol having different transporters and/or different transporter substrate specificities and affinities.[65] Prior work indicates compound-specific interactions with P-gp and BCR transporters. In Madin–Darby canine kidney (MDCK) overexpressing ABCG2, co-exposure to quercetin and kaempferol substantially increased quercetin absorption,[66] suggesting that kaempferol may inhibit ABCG2-mediated efflux in the small intestine. These findings imply that kaempferol may function as an ABCG2 substrate or inhibitor, thereby enhancing quercetin absorption. Testing with selective ABCG2 inhibitors such as Ko143 or with genetic knockout models could clarify the mechanism.[67] Second, kaempferol or isoquercetin may inhibit intestinal phase I and II metabolism involved in the metabolism of quercetin. Polyphenols can suppress cytochrome p450 (CYP) activity either by direct inhibition at the enzyme active site or by altering CYP expression through nuclear receptor pathways, such as AhR-mediated modulation of CYP1 induction.[68]

Overall, the mixture offers significant advantages, combining enhanced α-glucosidase inhibition and α-amylase inhibition for broader regulation of starch digestion. Typical dietary quercetin intake is 20–35 mg per day,[69] which is unlikely to yield pharmacologically relevant luminal concentrations. Oral supplementation with 250–500 mg has been reported to achieve luminal concentrations of up to 100 μM.[70] However, achieving such concentrations through quercetin supplementation alone may be challenging due to its limited gastrointestinal solubility. A polyphenol mixture, by distributing the required dose across multiple compounds, therefore represents a more feasible approach to attaining effective luminal concentrations with a reduced risk of inducing adverse effects due to toxic high concentrations.

These findings require *in vivo* validation to assess the physiological relevance, including polyphenol stability, metabolism, and food matrix effects. A 1 : 1 : 1 equimolar mixture of quercetin, kaempferol, and isoquercetin (200 μM total) inhibited saccharide digestion and glucose absorption, outperforming other individual or pairwise treatments. The mixture targets α-glucosidase (94% inhibition), α-amylase (30% inhibition) and glucose absorption (57% inhibition) in differentiated Caco-2 cells, which could contribute to reducing postprandial glucose peaks. It also increased quercetin uptake, possibly through kaempferol-driven inhibition of efflux transporters such as ABCG2, though this mechanism and its generalizability to other combinations require further testing. Enhanced systemic quercetin exposure may additionally improve insulin sensitivity and hepatic glucose control.[71,72] These findings support multi-component polyphenol formulations as modulators of intestinal glucose handling and justify their evaluation in human models and using other polyphenol combinations.

Author contributions

C. G. M. Dohmen: conceptualization; methodology; data curation; formal analysis; investigation; writing – original draft; visualization; and writing – review & editing. M. M. J. P. E. Sthijns: conceptualization; methodology; writing – review & editing; supervision; and project administration. R. Slotboom: conceptualization; methodology; investigation; and formal analysis. F. J. Troost: conceptualization; writing – review & editing; supervision; and funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

Additional data supporting this article have been included as part of the supplementary information (SI). Supplementary

information is available. See DOI: https://doi.org/10.1039/d6fo00569a.

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References

1 E. E. Blaak, J. M. Antoine, D. Benton, I. Björck, L. Bozzetto, F. Brouns, M. Diamant, L. Dye, T. Hulshof, J. J. Holst, D. J. Lamport, M. Laville, C. L. Lawton, A. Meheust, A. Nilson, S. Normand, A. A. Rivellese, S. Theis, S. S. Torekov and S. Vinoy, Impact of postprandial glycaemia on health and prevention of disease, *Obes. Rev.*, 2012, **13**, 923–984.

2 D. de Paulo Farias, F. F. de Araújo, I. A. Neri-Numa and G. M. Pastore, Antidiabetic potential of dietary polyphenols: A mechanistic review, *Food Res. Int.*, 2021, **145**, 110383.

3 A. Durazzo, M. Lucarini, E. B. Souto, C. Cicala, E. Caiazzo, A. A. Izzo, E. Novellino and A. Santini, Polyphenols: A concise overview on the chemistry, occurrence, and human health, *Phytother. Res.*, 2019, **33**, 2221–2243.

4 A. N. Panche, A. D. Diwan and S. R. Chandra, Flavonoids: An overview, *J. Nutr. Sci.*, 2016, **5**, e47.

5 K. Hanhineva, R. Törrönen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen, H. Mykkänen and K. Poutanen, Impact of dietary polyphenols on carbohydrate metabolism, *Int. J. Mol. Sci.*, 2010, **11**, 1365–1402.

6 T. P. Lam, N. N. Tran, L. D. Pham, N. V. Lai, B. N. Dang, N. N. Truong, S. K. Nguyen-Vo, T. L. Hoang, T. T. Mai and T. D. Tran, Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: A systematic review of in vitro studies, *Nat. Prod. Bioprospect.*, 2024, **14**, 4.

7 L. Sun, F. J. Warren and M. J. Gidley, Natural products for glycaemic control: Polyphenols as inhibitors of alpha-amylase, *Trends Food Sci. Technol.*, 2019, **91**, 262–273.

8 Q. Peng, Y. Ma, Z. Wang and J. Wang, Inhibition mechanism of different structural polyphenols against α-amylase studied by solid-state NMR and molecular docking, *Int. J. Biol. Macromol.*, 2024, **275**, 133757.

9 I. S. Wood and P. Trayhurn, Glucose transporters (GLUT and SGLT): Expanded families of sugar transport proteins, *Br. J. Nutr.*, 2003, **89**, 3–9.

10 C. P. Kulkarni, J. M. Thevelein and W. Luyten, Characterization of SGLT1-mediated glucose transport in Caco-2 cell monolayers, and absence of its regulation by sugar or epinephrine, *Eur. J. Pharmacol.*, 2021, **897**, 173925.

11 C. G. M. Dohmen, F. J. Troost, A. Muijsenberg and M. M. J. P. E. Sthijns, Curcumin and bisdemethoxycurcumin inhibit glucose and enhance fructose absorption in differentiated Caco-2 cells, while dibenzoylmethane inhibits glucose absorption, *Food Res. Int.*, 2025, **221**, 117186.

12 D. Ni, Z. Ai, D. Munoz-Sandoval, R. Suresh, P. R. Ellis, C. Yuqiong, P. A. Sharp, P. J. Butterworth, Z. Yu and C. P. Corpe, Inhibition of the facilitative sugar transporters (GLUTs) by tea extracts and catechins, *FASEB J.*, 2020, **34**, 9995–10010.

13 J. S. Gauer, S. Tumova, J. D. Lippiat, A. Kerimi and G. Williamson, Differential patterns of inhibition of the sugar transporters GLUT2, GLUT5 and GLUT7 by flavonoids, *Biochem. Pharmacol.*, 2018, **152**, 11–20.

14 P. Ader, M. Blöck, S. Pietzsch and S. Wolffram, Interaction of quercetin glucosides with the intestinal sodium/glucose co-transporter (SGLT-1), *Cancer Lett.*, 2001, **162**, 175–180.

15 T. Bohn, Dietary factors affecting polyphenol bioavailability, *Nutr. Rev.*, 2014, **72**, 429–452.

16 M. D'Archivio, C. Filesi, R. Varì, B. Scazzocchio and R. Masella, Bioavailability of the polyphenols: status and controversies, *Int. J. Mol. Sci.*, 2010, **11**, 1321–1342.

17 A. Bertelli, M. Biagi, M. Corsini, G. Baini, G. Cappellucci and E. Miraldi, Polyphenols: From theory to practice, *Foods*, 2021, **10**, 2595.

18 A. Scheepens, K. Tan and J. W. Paxton, Improving the oral bioavailability of beneficial polyphenols through designed synergies, *Genes Nutr.*, 2010, **5**, 75–87.

19 Y. K. Song, J. H. Yoon, J. K. Woo, J. H. Kang, K. R. Lee, S. H. Oh, S. J. Chung and H. J. Maeng, Quercetin is a flavonoid breast cancer resistance protein inhibitor with an impact on the oral pharmacokinetics of sulfasalazine in rats, *Pharmaceutics*, 2020, **12**, 397.

20 M. A. Nguyen, P. Staubach, S. Wolffram and P. Langguth, Effect of single-dose and short-term administration of quercetin on the pharmacokinetics of talinolol in humans – Implications for the evaluation of transporter-mediated flavonoid-drug interactions, *Eur. J. Pharm. Sci.*, 2014, **61**, 54–60.

21 K. Hanhineva, R. Törrönen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen, H. Mykkänen and K. Poutanen, Impact of dietary polyphenols on carbohydrate metabolism, *Int. J. Mol. Sci.*, 2010, **11**, 1365–1402.

22 Z. Bahadoran, P. Mirmiran and F. Azizi, Dietary polyphenols as potential nutraceuticals in management of diabetes: a review, *J. Diabetes Metab. Disord.*, 2013, **12**, 43.

23 C. Sun, C. Zhao, E. C. Guven, P. Paoli, J. Simal-Gandara, K. M. Ramkumar, S. Wang, F. Buleu, A. Pah, V. Turi, G. Damian, S. Dragan, M. Tomas, W. Khan, M. Wang, D. Delmas, M. P. Portillo, P. Dar, L. Chen and J. Xiao, Dietary polyphenols as antidiabetic agents: Advances and opportunities, *Food Front.*, 2020, **1**, 18–44.

24 M. Daou, N. A. Elnaker, M. A. Ochsenkühn, S. A. Amin, A. F. Yousef and L. F. Yousef, In vitro α-glucosidase inhibi-

tory activity of *Tamarix nilotica* shoot extracts and fractions, *PLoS One*, 2022, **17**, e0264969.

25 M. N. Wickramaratne, J. C. Punchihewa and D. B. Wickramaratne, *In vitro* alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*, *BMC Complement. Altern. Med.*, 2016, **16**, 466.

26 S. Darnis, N. Juge, X.-J. Guo, G. Marchis-Mouren, A. Puigserver and J.-C. Chaix, Molecular cloning and primary structure analysis of porcine pancreatic α-amylase, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.*, 1999, **1430**, 281–289.

27 G. Ferey-Roux, J. Perrier, E. Forest, G. Marchis-Mouren, A. Puigserver and M. Santimone, The human pancreatic α-amylase isoforms: Isolation, structural studies and kinetics of inhibition by acarbose, *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.*, 1998, **1388**, 10–20.

28 S. Darnis, N. Juge, X. J. Guo, G. Marchis-Mouren, A. Puigserver and J. C. Chaix, Molecular cloning and primary structure analysis of porcine pancreatic alpha-amylase, *Biochim. Biophys. Acta*, 1999, **1430**, 281–289.

29 M. Natoli, B. D. Leoni, I. D'Agnano, M. D'Onofrio, R. Brandi, I. Arisi, F. Zucco and A. Felsani, Cell growing density affects the structural and functional properties of Caco-2 differentiated monolayer, *J. Cell Physiol.*, 2011, **226**, 1531–1543.

30 K. Min and S. E. Ebeler, Quercetin inhibits hydrogen peroxide-induced DNA damage and enhances DNA repair in Caco-2 cells, *Food Chem. Toxicol.*, 2009, **47**, 2716–2722.

31 T. Suzuki, S. Tanabe and H. Hara, Kaempferol enhances intestinal barrier function through the cytoskeletal association and expression of tight junction proteins in Caco-2 cells, *J. Nutr.*, 2011, **141**, 87–94.

32 H. Xu, T.-q. He, S.-y. Chen, R.-r. Shi, J. Xu, Y.-r. Xing, D. Shi, Y.-q. Liu, B.-s. He and J.-h. Gu, Isoquercitrin mitigates intestinal ischemia–reperfusion injury by regulating intestinal flora and inhibiting NLRP3 inflammasome activation, *Redox Biol.*, 2025, **86**, 103803.

33 Y. Fang, W. Cao, M. Xia, S. Pan and X. Xu, Study of structure and permeability relationship of flavonoids in Caco-2 cells, *Nutrients*, 2017, **9**, 1301.

34 E. J. Prpa, C. P. Corpe, B. Atkinson, B. Blackstone, E. S. Leftley, P. Parekh, M. Philo, P. A. Kroon and W. L. Hall, Apple polyphenol-rich drinks dose-dependently decrease early-phase postprandial glucose concentrations following a high-carbohydrate meal: A randomized controlled trial in healthy adults and in vitro studies, *J. Nutr. Biochem.*, 2020, **85**, 108466.

35 N. Andrade, J. R. Araújo, A. Correia-Branco, J. V. Carletti and F. Martel, Effect of dietary polyphenols on fructose uptake by human intestinal epithelial (Caco-2) cells, *J. Funct. Foods*, 2017, **36**, 429–439.

36 M. Wang, H. Mao, Z. Ke, J. Chen, L. Qi and J. Wang, Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) leaves proanthocyanidins inhibit intestinal glucose transport in human Caco-2 cells, *Front. Pharmacol.*, 2024, **15**, 1284268.

37 E. Barber, M. J. Houghton and G. Williamson, Flavonoids as human intestinal α-glucosidase inhibitors, *Foods*, 2021, **10**, 1939.

38 C. Proença, A. T. Rufino, J. M. P. Ferreira de Oliveira, M. Freitas, P. A. Fernandes, A. M. S. Silva and E. Fernandes, Inhibitory activity of flavonoids against human sucrase-isomaltase (α-glucosidase) activity in a Caco-2/TC7 cellular model, *Food Funct.*, 2022, **13**, 1108–1118.

39 A. Isman, A. Nyquist, M. Moel, X. Zhang and S. Zalzala, The efficacy and tolerability of intermittent prandial acarbose to reduce glucose spikes in healthy individuals, *Transl. Med. Aging*, 2023, **7**, 12–19.

40 S. C. Forester, Y. Gu and J. D. Lambert, Inhibition of starch digestion by the green tea polyphenol, (−)-epigallocatechin-3-gallate, *Mol. Nutr. Food Res.*, 2012, **56**, 1647–1654.

41 H. Shen, J. Wang, J. Ao, Y. Hou, M. Xi, Y. Cai, M. Li and A. Luo, Structure–activity relationships and the underlying mechanism of α-amylase inhibition by hyperoside and quercetin: Multi-spectroscopy and molecular docking analyses, *Spectrochim. Acta, Part A*, 2023, **285**, 121797.

42 M. Huang, Q. Xiao, Y. Li, M. Ahmad, J. Tang, Q. Liao and C. Tan, Inhibition of α-amylase activity by quercetin via multi-spectroscopic and molecular docking approaches, *Food Biosci.*, 2024, **61**, 104951.

43 Y. Li, F. Gao, F. Gao, F. Shan, J. Bian and C. Zhao, Study on the Interaction between 3 flavonoid compounds and α-amylase by fluorescence spectroscopy and enzymatic kinetics, *J. Food Sci.*, 2009, **74**, C199–C203.

44 J. Sun, S. Dong, Y. Wu, H. Zhao, X. Li and W. Gao, Inhibitor discovery from pomegranate rind for targeting human salivary α-amylase, *Med. Chem. Res.*, 2018, **27**, 1559–1577.

45 A. A. Nada, A. M. Metwally, A. M. Asaad, I. Celik, R. S. Ibrahim and S. M. S. Eldin, Synergistic effect of potential alpha-amylase inhibitors from Egyptian propolis with acarbose using in silico and in vitro combination analysis, *BMC Complement. Med. Ther.*, 2024, **24**, 65.

46 E. Lo Piparo, H. Scheib, N. Frei, G. Williamson, M. Grigorov and C. J. Chou, Flavonoids for controlling starch digestion: structural requirements for inhibiting human alpha-amylase, *J. Med. Chem.*, 2008, **51**, 3555–3561.

47 X. Ren, X. Liu, B. z. Wang, M. Zhang and L. x. Wang, Inhibitory mechanism of apigenin, quercetin, and phloretin on α-glucosidase, *Food Biosci.*, 2024, **62**, 105398.

48 X. Peng, G. Zhang, Y. Liao and D. Gong, Inhibitory kinetics and mechanism of kaempferol on α-glucosidase, *Food Chem.*, 2016, **190**, 207–215.

49 J. Song, O. Kwon, S. Chen, R. Daruwala, P. Eck, J. B. Park and M. Levine, Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), Intestinal transporters for vitamin C and glucose, *J. Biol. Chem.*, 2002, **277**, 15252–15260.

50 G. Kottra and H. Daniel, Flavonoid glycosides are not transported by the human Na^+/glucose transporter when expressed in *Xenopus laevis* oocytes, but effectively inhibit

electrogenic glucose uptake, *J. Pharmacol. Exp. Ther.*, 2007, **322**, 829–835.

51 O. Kwon, P. Eck, S. Chen, C. P. Corpe, J.-H. Lee, M. Kruhlak and M. Levine, Inhibition of the intestinal glucose transporter GLUT2 by flavonoids, *FASEB J.*, 2007, **21**, 366–377.

52 D. Torres-Villarreal, A. Camacho, F. I. Milagro, R. Ortiz-Lopez and A. L. de la Garza, Quercetin-3-O-glucoside improves glucose tolerance in rats and decreases intestinal sugar uptake in caco-2 cells, *Nat. Prod. Commun.*, 2017, **12**, 1709–1712.

53 X. Xu, P. Wang, B. Wang, M. Wang, S. Wang, Z. Liu, Y. Zhang and W. Kang, Glucose absorption regulation and mechanism of the compounds in *Lilium lancifolium* Thunb on Caco-2 cells, *Food Chem. Toxicol.*, 2021, **149**, 112010.

54 Y. Yang, Z. Chen, X. Zhao, H. Xie, L. Du, H. Gao and C. Xie, Mechanisms of kaempferol in the treatment of diabetes: A comprehensive and latest review, *Front. Endocrinol.*, 2022, **13**, 990299.

55 B. Faerman, O. Chalifoux, M. Michalak, L. B. Agellon and R. J. Mailloux, The polyphenols phloretin and quercetin are potent horseradish peroxidase (HRP) inhibitors, *Biochim. Biophys. Acta. Gen. Subj.*, 2025, **1869**, 130833.

56 M. Zakłos-Szyda, N. Pietrzyk, A. Kowalska-Baron, A. Nowak, K. Chałaśkiewicz, M. Ratajewski, G. Budryn and M. Koziołkiewicz, Phenolics-rich extracts of dietary plants as regulators of fructose uptake in Caco-2 Cells via GLUT5 involvement, *Molecules*, 2021, **26**, 4745.

57 Y. Zheng, J. S. Scow, J. A. Duenes and M. G. Sarr, Mechanisms of glucose uptake in intestinal cell lines: Role of GLUT2, *Surgery*, 2012, **151**, 13–25.

58 R. Dhanya, Quercetin for managing type 2 diabetes and its complications, an insight into multitarget therapy, *Biomed. Pharmacother.*, 2022, **146**, 112560.

59 H. M. O'Neill, AMPK and exercise: Glucose uptake and insulin sensitivity, *Diabetes Metab. J.*, 2013, **37**, 1–21.

60 K. E. Hamilton, J. F. Rekman, L. K. Gunnink, B. M. Busscher, J. L. Scott, A. M. Tidball, N. R. Stehouwer, G. N. Johnecheck, B. D. Looyenga and L. L. Louters, Quercetin inhibits glucose transport by binding to an exofacial site on GLUT1, *Biochimie*, 2018, **151**, 107–114.

61 J. Gee and I. Johnson, Polyphenolic compounds: Interactions with the gut and implications for human health, *Curr. Med. Chem.*, 2001, **8**, 1245–1255.

62 S. Wolffram, M. Blöck and P. Ader, Quercetin-3-glucoside is transported by the glucose carrier SGLT1 across the brush border membrane of rat small intestine, *J. Nutr.*, 2002, **132**, 630–635.

63 A. J. Day, J. M. Gee, M. S. DuPont, I. T. Johnson and G. Williamson, Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter, *Biochem. Pharmacol.*, 2003, **65**, 1199–1206.

64 G. Kottra and H. Daniel, Flavonoid glycosides are not transported by the human Na^+/glucose transporter when expressed in *Xenopus laevis* oocytes, but effectively inhibit electrogenic glucose uptake, *J. Pharmacol. Exp. Ther.*, 2007, **322**, 829–835.

65 A. I. Alvarez, R. Real, M. Pérez, G. Mendoza, J. G. Prieto and G. Merino, Modulation of the activity of ABC transporters (P-glycoprotein, MRP2, BCRP) by flavonoids and drug response, *J. Pharm. Sci.*, 2010, **99**, 598–617.

66 G. An, J. Gallegos and M. E. Morris, The bioflavonoid kaempferol is an Abcg2 substrate and inhibits Abcg2-mediated quercetin efflux, *Drug Metab. Dispos.*, 2011, **39**, 426–432.

67 J. Zhu, F.-Y. Qin, S. Lei, R. Gu, Q. Qi, J. Lu, K. E. Anderson, P. Wipf and X. Ma, Inhibition of ABCG2 prevents phototoxicity in a mouse model of erythropoietic protoporphyria, *Nat. Commun.*, 2024, **15**, 10557.

68 A. Revel, H. Raanani, E. Younglai, J. Xu, I. Rogers, R. Han, J.-F. Savouret and R. F. Casper, Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects lung from DNA damage and apoptosis caused by benzo[a]pyrene, *J. Appl. Toxicol.*, 2003, **23**, 255–261.

69 C. Manach, G. Williamson, C. Morand, A. Scalbert and C. Rémésy, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies, *Am. J. Clin. Nutr.*, 2005, **81**, 230S–242S.

70 H. van der Woude, A. Gliszczyńska-Świgło, K. Struijs, A. Smeets, G. M. Alink and I. M. C. M. Rietjens, Biphasic modulation of cell proliferation by quercetin at concentrations physiologically relevant in humans, *Cancer Lett.*, 2003, **200**, 41–47.

71 N. Arias, M. T. Macarulla, L. Aguirre, M. G. Martínez-Castaño and M. P. Portillo, Quercetin can reduce insulin resistance without decreasing adipose tissue and skeletal muscle fat accumulation, *Genes Nutr.*, 2014, **9**, 361.

72 L. Chen, T. Shen, C. P. Zhang, B. L. Xu, Y. Y. Qiu, X. Y. Xie, Q. Wang and T. Lei, Quercetin and isoquercitrin inhibiting hepatic gluconeogenesis through LKB1-AMPKα pathway, *Acta Endocrinol.*, 2020, **16**, 9–14.