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Absorption of Black Currant Anthocyanins by Monolayers of Human Intestinal Epithelial Caco-2 Cells Mounted in Ussing Type Chambers

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Anthocyanins (ACNs) have been reported to have multiple biological properties imparting benefits to human health. Their role in human nutrition, however, needs to be related to biokinetic data, such as bioavailability. The purpose of the present study was to focus on the potential absorption of black currant (*Ribes nigrum L.*) ACNs. Caco-2 monolayers were used as an in vitro model of the absorptive intestinal epithelium. For absorption studies, Caco-2 cells grown on permeable filters were mounted into Ussing type chambers. The monolayer integrity was monitored by measuring the transepithelial electrical resistance (TEER). Luminal to serosal transport of ACNs was examined by comparing ACN disappearance from the luminal solution of Ussing chambers not containing any inserts (control chambers) with that of Ussing chambers containing inserts. ACNs ($C_{total\ ACN} \sim 180\ \mu M$) were not detected in any serosal solution. However, it was shown that ACNs disappeared from the luminal side, not due to ACN degradation processes but rather—at least in part—due to physiological actions of the cells. The luminal net disappearance of ACNs was calculated ($max_{t\ 20\ min} \sim 11\%$ for total ACNs) and labeled as "absorption efficiency". This apical transport might occur to a much larger extent than the further translocation across the basolateral membrane. Thus, cell metabolism and translocation across the basolateral membrane may be the key determinants of ACN absorption and bioavailability.

KEYWORDS: Anthocyanins; absorption; Caco-2 monolayer; Ussing type chambers

INTRODUCTION

Anthocyanins (ACNs) are part of a very large and widespread group of water-soluble plant constituents known collectively as flavonoids (*1*). With the main sources being berries as well as other fruits and vegetables, the mean dietary intake of ACNs in the United States has been estimated to be 12.5 mg/day/person (*2*). This intake is in the same range as the consumption of numerous other flavonoids including quercetin, kaempferol, myricetin, apigenin, and luteolin in the Dutch diet (23 mg/day) (*3*). ACNs have been reported to have multiple biological properties imparting benefits to human health, such as anti-inflammatory actions (*4*), anticarcinogenic effects (*5*), and antioxidant activities (*6*). In addition, flavonoids have been suggested to reduce the risk of cardiovascular diseases by preventing the oxidation of plasma low-density lipoprotein (LDL). The oxidation of circulating LDL is recognized to be an important step in the formation of atherosclerotic plaques and subsequent cardiovascular disease (*7*). The so-called "French paradox" is characterized by the fact that people in France suffer a relatively low incidence of coronary heart disease (CHD) despite their consumption of saturated fats at high levels, which are positively correlated with increased risks of CHD (*8*). It is suggested that France's high consumption of red wine (containing considerable amounts of polyphenolics including ACNs) is one reason for this paradox (*9, 10*). However, ACN functions and their roles in human nutrition can only be discussed on the basis of biokinetic data, such as ACN absorption, distribution, and metabolism. ACNs that are present in plants as glycosides have long been considered to be nonabsorbable by the human body unless they are first hydrolyzed by the intestinal bacterial microflora (*11*). Today, several studies have shown that ACNs can be rapidly absorbed as intact glycosides in rats and humans (*12–15*). Nevertheless, information on the absorption and bioavailability is still very limited. The purpose of the present study was to evaluate the potential absorption of the ACNs delphinidin 3-*O*-β-glucopyranoside (del-3-gluc), delphinidin 3-*O*-β-rutinopyranoside (del-3-rut), cyanidin 3-*O*-β-glucopyranoside (cya-3-gluc), and cyanidin 3-*O*-β-rutinopyranoside (cya-3-rut) derived from black currant (*Ribes nigrum* L.) extract. As an in vitro model of the principal barrier of intestinal absorption, Caco-2 cells were mounted in Ussing type chambers.

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Caco-2 cells are well-differentiated human intestinal cells, which have been extensively characterized (*16, 17*).

MATERIALS AND METHODS

ACNs. Black currant extract was purchased from Rudolf Wild GmbH & Co. KG Eppelheim (Germany). The high-performance liquid chromatography (HPLC) profile showed the four characteristic black currant ACN peaks with retention times and proportion as follows: del-3-gluc, 6.94 min (10−11%); del-3-rut, 7.83 min (61−62%); cya-3-gluc, 10.14 min (2−3%); and cya-3-rut, 12.12 min (24−25%). ACN standard compounds were obtained from the Institute of Enology and Beverage Research Geisenheim (Geisenheim, Germany; del-3-rut), Polyphenols Laboratories AS (Hanaveien, Norway; del-3-gluc), and Carl Roth GmbH (Karlsruhe, Germany; cya-3-rut and cya-3-gluc). For each experiment, a stock solution containing the black currant extract dissolved in Hank's balanced salt solution (HBSS) (0.13 g/L $CaCl_2$, 0.10 g/L $MgCl_2 \times 6\ H_2O$, 0.04 g/L $MgSO_4$, 0.40 g/L KCl, 0.06 g/L KH_2PO_4, 0.35 g/L $NaHCO_3$, 8.00 g/L NaCl, and 0.06 g/L $Na_2HPO_4 \times 2\ H_2O$, pH 7.2) was prepared. Because preliminary tests showed that the strongly acidic extract seriously harmed the monolayer's integrity, the stock solution was neutralized with NaOH to pH 7.2 immediately before each experiment.

Cell Culture. The human colon adenocarcinoma cell line Caco-2 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The cells were routinely grown in 25 cm^2 culture flasks using Dulbecco's modified Eagle's medium (DMEM) at pH 7.2. The medium was supplemented with 4.5 g/L D-glucose, 1% nonessential amino acids (NEAA), and 10% fetal calf serum (FCS) as well as 50 μg/mL gentamycin. Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C. Stock cells were subcultured every 7 days before reaching confluence by harvesting them with trypsin/EDTA (0.05/0.02%).

Experimental Setup of Absorption Studies. For absorption studies, Caco-2 cells (passages 25−30) were seeded according to Hidalgo et al. (*16*) at a density of 63000 cells/cm^2 onto Snapwell inserts with a polycarbonate membrane (12 mm diameter, 0.4 μm pore size, CostarCorning Inc., United States). Inserts were placed in six well plates, and cells were allowed to grow and differentiate to confluent monolayers for 19−21 days. The culture medium was changed every 3 days at the luminal side (500 μL) and every 7 days at the serosal side (5 mL). To ensure that the monolayer exhibits the properties of a tight biological barrier, transepithelial electrical resistance (TEER) was monitored using a Millicell-ERS voltohmmeter (Millipore, United States). Because TEER values exceeding 200 Ω cm^2 are generally considered as acceptable (*18*), monolayers with TEER above 200 Ω cm^2 were used for absorption experiments exclusively. For experiments, the medium was decanted, and cells were carefully washed with prewarmed (37 °C) HBSS. The inserts were mounted in individual Ussing type chambers, originally established by Ussing and Zerahn (*19*). Donor compartments (luminal side) and receiver compartments (serosal side) were each filled with 3.7 mL of prewarmed (37 °C) HBSS (transport buffer). To sustain tissue viability, HBSS containing 4.5 g/L glucose was used for the serosal side. To give an osmotic load, HBSS containing 4.5 g/L mannitol was used for the luminal side. The chambers were kept at 37 °C, and HBSS was aerated with carbogen (95% O_2/5% CO_2). Absorption studies were conducted in an unclamped state as this more closely represents the in vivo situation. Chambers were allowed to equilibrate for 15 min, so that the Caco-2 cells could adapt to the changed conditions in the diffusion chambers. The ACN stock solution (0.8 mL) was added to the donor compartment, leading to an average initial concentration of $C_{\text{total ACN}} = 180.3 \pm 19.3$ μmol/L. The concentration of total ACNs (tACNs) referred to the sum of individual ACNs (cya-3-gluc = 6.0 ± 0.7 μmol/L, cya-3-rut = 45.4 ± 4.4 μmol/L, del-3-gluc = 18.4 ± 2.2 μmol/L, and del-3-rut = 110.5 ± 12.2 μmol/L). Simultaneously, ACN-free HBSS was added to the receiver compartment. ACN transport across the monolayer was followed in the luminal to serosal direction as a function of time. At regular time intervals (0, 20, 40, 60, and 80 min), samples (0.2 mL) were taken from each side, and the sampled volume was replaced with fresh HBSS. Electrophysiological measurements were continually made on the mounted monolayers to observe monolayer viability and integrity. TEER was recorded by voltage/current clamp electronics (Scientific Instruments K. Mussler, Aachen, Germany). Control chambers without filter and monolayer were run as a control to measure ACN degradation due to experimental conditions. Chambers with a blank filter in the absence of cells were measured to ensure that ACN absorption was not interfered by the filter membrane.

Sample Preparation and HPLC Analysis. Samples (0.2 mL) from the luminal and serosal side were acidified with 50 μL of 5% formic acid/H_2O and subsequently stored at −80 °C until HPLC analysis. To determine the presence of minimal amounts of ACNs in the serosal compartment, 4 mL samples instead of 0.2 mL samples were collected in some experiments. The samples were then extracted with an octadecylsilane solid-phase extraction (SPE) cartridge (Sep-Pak C18 Plus; Waters Corp., Milford, MA) following the method of Felgines et al. (*20*) with minor modifications. The cartridge was washed with 10 mL of methanol and equilibrated with 10 mL of 12 mmol/L aqueous HCl before use. Four milliliters of the serosal samples diluted with 1 mL of 5% formic acid/H_2O was loaded onto the cartridge. The cartridge was then washed with 10 mL of 12 mmol/L aqueous HCl. ACNs were recovered with 10 mL of 10 mmol/L HCl in methanol into a glass tube. The extract of ACNs in the methanol phase was evaporated to dryness with a rotary vacuum evaporator (Heidolph Instruments, Germany) at ≤35 °C. The dried methanol extract was dissolved in 250 μL of 5% formic acid/H_2O, transferred into vials, and stored at −80 °C until HPLC analysis.

The analysis of ACNs was carried out by a reversed phase (RP)-HPLC. The RP-HPLC system consisted of a Merck-Hitachi intelligent autosampler AS-4000, a Merck-Hitachi high precision pump model L-6200, and a Merck-Hitachi L-4250 UV/vis detector (Merck-Hitachi, Darmstadt, Germany). The analytic column was a RP-C18 Prontosil Eurobond (250 mm × 4 mm, 5 μm particle size; Bischoff Chromatography, Leonberg, Germany), protected by a LiCrospher 100 RP-C18 guard column (4 mm × 4 mm, 5 μm particle size; Merck KGaA, Darmstadt, Germany). Chromatographic data were collected and recorded by a Merck-Hitachi D-2500 Chromato-Integrator. Single substances were separated by isocratic elution at a flow rate of 1.2 mL/min and detected at 520 nm. The mobile phase was water/formic acid/acetonitrile (81/10/9; v/v/v; pH 1.9). Del-3-gluc, del-3-rut, cya-3-gluc, and cya-3-rut were identified by comparing their retention times with those of commercially available standards. The quantitation was based on calibration curves achieved from standards with known concentrations. The injection volume was 50 μL for both luminal and serosal samples. Chromatography was performed at room temperature.

Data Treatment and Statistical Analysis. Statistical analysis was performed using the statistical software package SPSS for Windows V. 11.5 (SPSS Inc., Chicago, IL). ACN absorption efficiencies were calculated by comparing ACN disappearance in the luminal solution of transport chambers (chambers with mounted monolayer) with ACN disappearance in the luminal solution of control chambers (chambers without filter and monolayer). The equation used was

$$\text{ACN absorption efficiency (\%)} = (100 \times C_1)/C_3 - (100 \times C_2)/C_4 \quad (1)$$

where C_1 = ACN concentration at the luminal side of the transport chamber at $t = x_1$, C_2 = ACN concentration at the luminal side of the control chamber at $t = x_1$, C_3 = ACN concentration at the luminal side of the transport chamber at $t = 0$ min, and C_4 = ACN concentration at the luminal side of the control chamber at $t = 0$ min.

Absorption efficiencies of tACNs refer to the sum of individual ACNs. Accumulative absorption efficiencies were calculated by adding the single absolute absorption efficiencies. All data are presented as means ± standard deviation (SD). They were tested for normality using the Kolmogorov−Smirnov test. Significant differences between the means of ACN disappearance in transport chambers and the respective control chambers were assessed by one-way ANOVA (analysis of variance). The significance of differences for ACN degradation and accumulative/absolute absorption efficiencies was assessed using the t test. Differences were considered to be significant with $p < 0.05$. Tendencies were defined with $p < 0.07$.

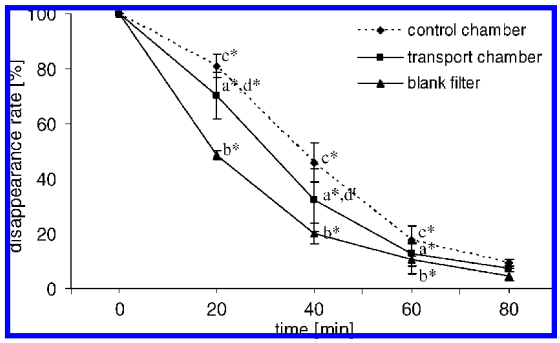

Figure 1. tACN disappearance rate (%) over time in luminal solution of control chambers, transport chambers, and blank filters. Values are the means ± SD ($n = 5$). *a* indicates differences over time in transport chambers, *b* indicates differences over time in blank filters, *c* indicates differences over time in control chambers, and *d* indicates differences between control chambers and transport chambers. $^{*}p < 0.05$ and $^{t}p < 0.07$.

RESULTS

Integrity/Viability of the Monolayers. Electrophysiological measurements (TEER) during absorption experiments showed that after 15 min of equilibration, TEER reached approximately 350 Ω cm^2. The addition of ACN resulted in a short increase in TEER. Following the experimental time course, TEER slowly decreased to 150 Ω cm^2 at 80 min.

Absorption Studies. After adding black currant ACNs (del-3-gluc, del-3-rut, cya-3-gluc, and cya-3-rut) to the donor compartment of the Ussing type chambers, ACN disappearance from the luminal side and ACN appearance at the serosal side were determined by HPLC. There was no detection of ACNs in any of the serosal solutions (0.2 mL samples; 4 mL samples, SPE procedure). To compare ACN disappearance in mucosal solutions of transport chambers with those of control chambers, measured ACN concentrations at the respective time points were related to the initial ACN concentrations at time point zero. ACN disappearance percentages were calculated.

A large decrease of ACNs over time could be seen in the control chambers as well as the transport chambers and the blank filters (**Figure 1**). Significant differences between transport chambers and the respective control chambers were defined as absorption efficiencies and were observed at 20 min, as well as tendential differences at 40 min. The very large decrease of ACNs in chambers with blank filters assured that there was no interference in ACN absorption by the filter membrane. ACNs rapidly diffused across the blank filter support from the luminal to the serosal compartment. Differences between control and transport chambers were used to calculate absolute and accumulative absorption efficiencies. When compared to sample time zero (no absorption), significant absolute absorption efficiencies were observed at 20 min (10.7 ± 7.2%) as well as tendential absolute absorption efficiencies at 40 min (13.4 ± 11.8%) (**Figure 2A**). A significant increase in accumulative absorption efficiencies was observed within 0−20 min as well as a tendential increase within 20−40 min (**Figure 2B**).

No differences in degradation were observed between the individual delphinidin and cyanidin sugar conjugates (**Figure 3**). However, the delphinidins (del-3-gluc and del-3-rut) showed a significantly higher degradation than the cyanidins (cya-3-gluc and cya-3-rut) at 40, 60, and 80 min. Significant differences in the disappearance of delphinidins between control and transport chamber could be seen for del-3-gluc at 20 and 40 min. For del-3-rut, tendential differences were observed at 20 and 40 min (**Figure 4**). However, data show that the absorption

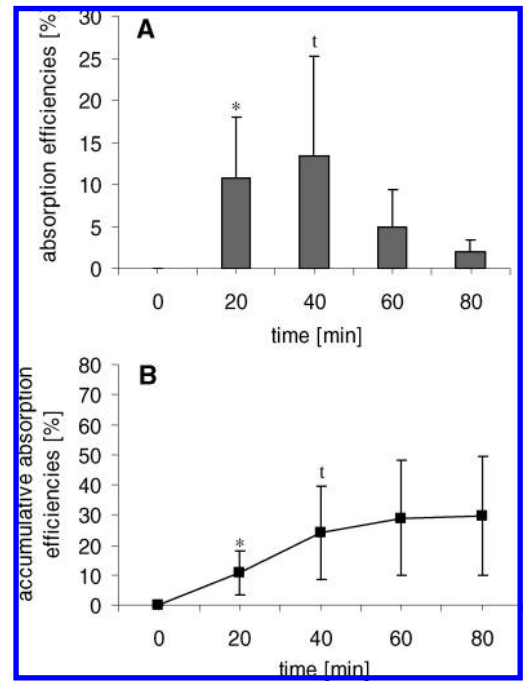

Figure 2. Absolute (**A**) and accumulative (**B**) absorption efficiencies of tACNs over time. Values are the means ± SD ($n = 5$). (**A**) Differences to time point 0 (no absorption). (**B**) Differences to prior sample time $^{*}p < 0.05$ and $^{t}p < 0.07$.

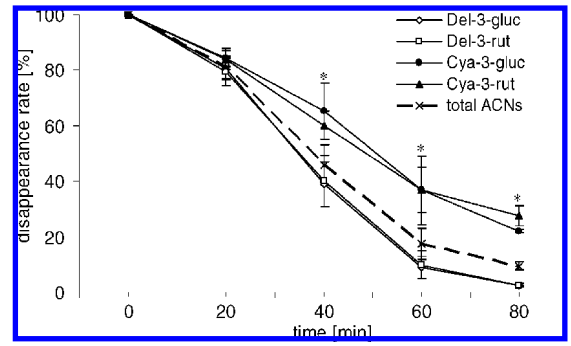

Figure 3. Disappearance rate (%) of del-3-gluc, del-3-rut, cya-3-gluc, and cya-3-rut together with tACN disappearance over time in luminal solutions of control chambers. Values are the means ± SD ($n = 5$). $^{*}p < 0.05$ indicates differences between delphinidins and cyanidins.

efficiencies were not markedly influenced by the different sugar moieties. For the cyanidins, statistical analysis did not show any differences between control and transport chambers for cya-3-gluc. For cya-3-rut, differences were obtained at 20 min (**Figure 5**). Absolute absorption efficiencies did not differ between individual delphinidins, whereas a significant difference was observed between individual cyanidins at 20 min (**Figure 6**). Although the cyanidins were more stable during transport studies and degradation was less, they showed minor absorption efficiencies when compared to the delphinidins. In particular, at 20 min, del-3-gluc showed a significantly higher absorption efficiency than cya-3-gluc and cya-3-rut. At 40 min, tendential absorption differences ($p < 0.07$) were observed between del-3-gluc (16.2 ± 12.0) and cya-3-rut (7.9 ± 5.5%). From 60 min on, delphinidin absorption efficiencies noticeably decreased.

DISCUSSION

tACNs. It was demonstrated that, although black currant ACNs were not detected in any serosal solution, they disappeared from the luminal side. This ACN disappearance was not

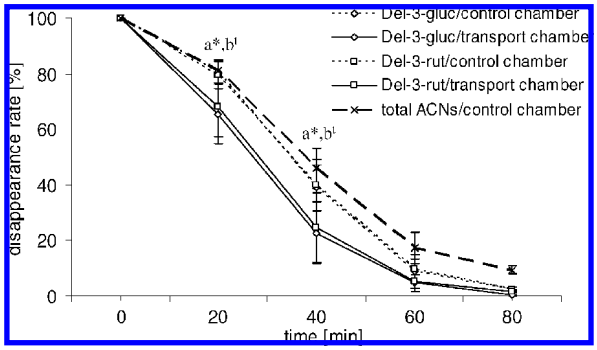

Figure 4. Disappearance rate (%) of del-3-gluc and del-3-rut over time in luminal solutions of control chambers and transport chambers, together with tACN disappearance. Values are the means ± SD ($n = 5$). *a* indicates differences of del-3-gluc between control chambers and transport chambers. *b* indicates differences of del-3-rut between control chambers and transport chambers. $^{*}p < 0.05$ and $^{!}p < 0.07$.

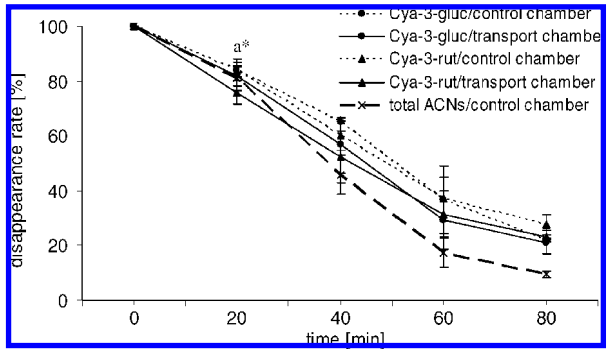

Figure 5. Disappearance rate (%) of cya-3-gluc and cya-3-rut over time in luminal solutions of control chambers and transport chambers, together with tACN disappearance. Values are the means ± SD ($n = 5$). *a* indicates differences of cya-3-rut between control chambers and transport chambers. $^{*}p < 0.05$.

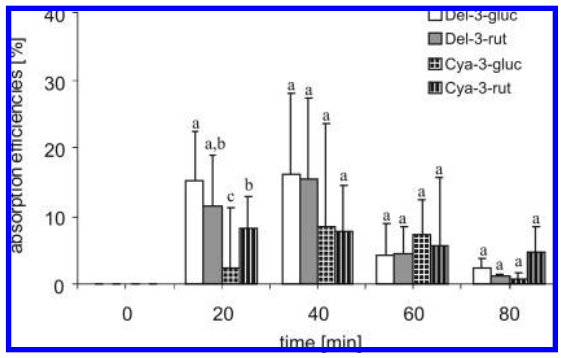

Figure 6. Absolute absorption efficiencies of del-3-gluc, del-3-rut, cya-3-gluc, cya-3-rut, and tACNs over time. Values are the means ± SD ($n = 5$). Significant differences between the individual ACNs are indicated by different indices within the respective sample times ($p < 0.05$).

due to the high degradation caused by experimental conditions because ACN degradation in control chambers (without filter and monolayer) was less than ACN disappearance in transport chambers (with mounted monolayer). Differences in ACN disappearance between transport and respective control chambers (net ACN decrease) could be rather due to physiological actions of the cells and were defined as "absorption efficiency". However, with a maximum absorption of about 11% at 20 min for tACNs, there are discrepancies with average data observed in several bioavailability studies. Miyazawa et al. (*14*) found that about 1% of ACNs was incorporated into rat plasma after oral supplementation. Numerous human intervention studies estimated the bioavailability of ACNs to range from 0.004 to 1.2% (*12, 14, 21, 22*). However, bioavailability studies cannot be fully compared with in vitro absorption studies as the term "bioavailability" not only displays the simple intestinal absorption: It rather reflects the dose available in systemic circulation, dependent on splanchnic metabolism, for example, first pass metabolism (*23*). Morazzoni et al. (*24*) investigated the pharmacokinetics of *Vaccinium myrtillus* ACNs in male rats and found an absolute bioavailability of about 1.2%, whereas the intestinal absorption was estimated to be 5%. An in vitro study by Matuschek et al. (*25*) showed a maximum ACN absorption of about 55% for jejunal mouse tissue mounted in Ussing chambers. In that study, ACNs were not detected in any serosal solution and ACN net disappearance (after correction for ACN degradation) was labeled as "ACN absorption". Because the data of Matuschek et al. (*25*) as well as the present data do not conform to data derived from in vivo studies, it is very likely that ACN net disappearance from the luminal side does not simply reflect bioavailability. Moreover, it is unknown to what extent luminal net disappearance is due to an adherence of ACNs to apical brush border microvilli rather than to cellular uptake.

However, quercetin glycosides (QGs) were shown to be absorbed in the small intestine by interacting with the intestinal glucose transporter (SGLT-1) (*26, 27*). The transporter is generally responsible for the "active" accumulation of hexoses into the cells and has been reported to rapidly increase in Caco-2 cells from day 12 up to day 20 postseeding (*28*). Milbury et al. (*29*) suggested that ACN absorption as unchanged glycosides may reflect the involvement of hexose pathway receptors. In addition, ACN aglycons and quercetin consist of a similar basic flavonoid structure. Urinary excretion of cya-3-gluc in humans after consumption of elderberry ACNs with and without simultaneous ingestion of sucrose was compared by Mulleder et al. (*30*). Because the ingestion of sucrose led to a reduced excretion of cya-3-gluc, he proposed that SGLT-1 may play a role in ACN absorption. However, very recently, Kottra and Daniel (*31*) showed for a variety of flavonoid glycosides that they are not transported by human SGLT-1 when expressed in *Xenupus laevis* oocytes.

Using the Caco-2 cell model, Walgren et al. (*32*) showed that although QGs were absorbed across the apical membrane via SGLT-1, they accumulated within the cell due to no further translocation across the basolateral membrane. This was suggested as being partly due to the action of MRP2 (multidrug resistance-associated protein-2) efflux pumps, which transported QGs out of the cell across the apical cell membrane (*33*). Those findings might explain at least in part the lack of ACN recovery in any serosal solution in the present study. Because it is suggested that ACNs could be a substrate for SGLT-1 due to their structural similarity to quercetin (*29*), it could also be assumed that ACNs are a substrate for MRP2 pumps. Thus, the efficiency of apical to basolateral translocation, which means ACN absorption could be inhibited by the efflux action of apical MRP2 pumps. Nevertheless, very recently, ACN absorption (apical to basolateral translocation) was shown using the Caco-2 cell model with an average absorption of about 3−4% (*34*). Further investigations are needed to establish the role of MRP2 pumps in ACN absorption.

The results of the present study correspond to several other in vitro Caco-2 studies investigating the transport of flavonoids. Although isoflavons (chrysin and genestein) and the flavanol epicatechin are absorbed in vivo, no apical to basolateral

absorption was detected across Caco-2 monolayers. Instead, the flavonoids and their metabolites were effectively effluxed by MRP2 pumps (*35–37*).

However, discussing ACN absorption, transport, and metabolism, another fact has to be considered. Miyazawa et al. (*14*) assumed that the flavylium cation structure may impart resistance to enzymatic conversions into conjugates. More recently, several authors demonstrated the presence of ACN metabolites, such as glucuroconjugates as well as sulfoconjugates (*38, 39*). This indicates that glycolytic processes may be involved in ACN absorption and their metabolic pathways. Talavera et al. (*40*) suggested that ACNs could partly be hydrolyzed by intestinal β-glucosidases within the cell. Subsequently, the aglycons could be degraded into phenolic acids due to their instability at physiological pH values. On the other hand, the liberated aglycons could be conjugated and then effluxed into the serosal fluid. This assumption would be in line with absorption studies of numerous flavonoids and their glycosides, which show extensive metabolism within the enterocyte during the transfer from the luminal to the serosal side (*41, 42*). Recent studies suggest that ACNs are absorbed and transported in human serum and urine primarily as metabolites (*39*). Because no ACN conjugates were measured in the present study, ACN absorption may be considerably underestimated, resulting in the lack of ACN detection at the serosal side.

ACNs exist as a mixture of different molecular species, where the proportion of each structure depends on the pH. The chemistry of these transformations has been well-described (*43*). ACNs are stable under acidic conditions but rapidly broken down under neutral physiological pH values. Kang et al. (*44*) reported that ACNs as well as cyanidin aglycons spontaneously degrade at pH 7.0 and 37 °C. Therefore, ACN instability and degradation within the luminal solution may have been a limiting factor of ACN absorption in the present study. In addition, small amounts of ACNs might have been transported across the Caco-2 monolayers but were rapidly degraded at the serosal side.

Individual ACNs. It has been suggested that the aglycon structure as well as the structural features of the glycoside moiety of ACNs may play an important role in absorption and metabolism (*40, 45*). Therefore, black currant ACNs were considered individually. The loss of ACNs over time in the absence of cells (control chambers) showed no differences between the respective glucose and rutinose sugar moieties indicating that the glycosidic moiety did not influence ACN stability. However, delphinidins showed a significantly higher degradation than cyanidins. This is in line with data published by Yi et al. (*34*) and Talavera et al. (*40*), who observed a significantly higher loss of del-3-gluc as compared to other ACNs. Thus, the structural feature of the aglycon might be crucial for ACN stability, whereas the conjugated sugar appears to be of minor relevance.

ACN instability within the gastrointestinal tract has been suggested as a factor limiting their absorption (*46*). The results of the present study do generally conform to this assumption with some exceptions. Delphinidins were shown to be less stable than cyanidins, but they were absorbed more efficiently into the cell during the first 40 min of the experiment. After 40 min, delphinidin absorption efficiencies decreased, whereas the absorption of cyanidins remained nearly constant. On the basis of these findings, two factors might determine ACN absorption: Initially when equal doses of ACNs are present, the aglycon feature (but not ACN instability) may be the key determinant of ACN absorption. Talavera et al. (*47*) showed that ACNs are efficiently absorbed from the stomach in anesthetized rats with delphinidin glycosides being the most effectively absorbed. This demonstrates that when delphinidins are stable due to acidic pH, they are more efficiently absorbed than other ACNs. Later, following the time course of the experiments, ACN stability may become the main determinant of ACN absorption into the cell. Because of degradation processes, the concentration of delphinidins and therefore delphinidin absorption efficiencies clearly decreased. Cyanidins were more stable and therefore present at higher concentrations, resulting in more constant absorption efficiencies.

In addition, the proportion, which means the concentrations of individual ACNs present in the black currant extract, might have influenced absorption efficiencies. Miyazawa et al. (*14*) showed that cya-3-gluc plasma concentrations increased with increasing amounts of ingested ACNs, suggesting that ACN absorption may be dose-dependent. However, in the present study, del-3-rut, which was present at initial concentrations of about 110 μM, was slightly less absorbed (relative absorption) than del-3-gluc, present at initial concentrations of about 18 μM. This indicates that ACN absorption is not only dose-dependent and that chemical features may play a role in ACN absorption.

In conclusion, the present in vitro study showed that black currant ACNs disappeared from the luminal side of Caco-2 cell monolayers. This was demonstrably not only due to ACN degradation processes but could be due to physiological actions of the Caco-2 cells. In regard to other studies (*32–37*), it is likely that ACNs were transported via specific pathways across the apical side into the cell with no further translocation across the basolateral membrane. This apical transport/absorption might occur in a much larger extent than the further translocation across the basolateral membrane and does not reflect bioavailability. Cellular metabolism and translocation across the basolateral membrane may therefore be the key determinants of absorption and bioavailability of ACNs.

ABBREVIATIONS USED

ACNs, anthocyanins; ANOVA, analysis of variance; CHD, coronary heart disease; cya-3-gluc, cyanidin 3-*O*-β-glucopyranoside; cya-3-rut, cyanidin 3-*O*-β-rutinopyranoside; del-3-gluc, delphinidin 3-*O*-β-glucopyranoside; del-3-rut, delphinidin 3-*O*-β-rutinopyranoside; FCS, fetal calf serum; HBSS, Hank's balanced salt solution; MRP2, multidrug resistance-associated protein-2; NEAA, nonessential amino acids; tACNs, total ACNs; TEER, transepithelial electrical resistance; QGs, quercetin glycosides, SGLT-1, sodium-dependent glucose transporter.

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