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Bioavailability of quercetin in humans and the influence of food matrix comparing quercetin capsules and different apple sources

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ABSTRACT

The objective of the four-way cross-over pilot study was the investigation of quercetin bioavailability after ingestion of apple quercetin incorporated in different matrices and quercetin dihydrate capsule. Six healthy volunteers were given a standard diet supplemented with 71 μmol quercetin equivalents from vacuum impregnated apple chips, apple peel extract capsules and apple peel. Quercetin dihydrate capsules were used as control. The vacuum impregnated apple chips were enriched with a quercetin rich apple peel extract. The ingestion of vacuum impregnated apple chips, apple peel extract capsules and apple peel resulted in similar plasma quercetin and plasma total flavonol concentrations compared to the quercetin dihydrate capsule. With regard to the bioavailabilities of all quercetin treatments, a reduced release from vacuum impregnated apple chips can be excluded. The apple peel matrix which is rich in indigestible dietary fibers impacted the profile of the plasma flavonol time curve similar to poor soluble quercetin dihydrate. Finally, the quercetin bioavailability of vacuum impregnated apple chips ($AUC_{0-1440\ min}$: 104 ± 24 μmol·min·L^{-1}) as functional food was similar to the supplementation with apple peel extract capsules ($AUC_{0-1440\ min}$: 87 ± 27 μmol·min·L^{-1}).

1. Introduction

Consuming fruits and vegetables which are rich in polyphenols may reduce the risk of chronic diseases including cardiovascular diseases (Liu, 2003; Pallauf, Giller, Huebbe, & Rimbach, 2013). The daily dietary quercetin intake varies widely. 73–76% of the flavonoid intake (20–22 mg/d) in US is contributed by quercetin (Sampson, Rimm, Hollman, de Vries, & Katan, 2002) whereas in a Finnish cohort, on average about 95% of the flavonoid intake was due to quercetin (Knekt, Jarvinen, Reunanen, & Maatela, 1996). In Japan a quercetin intake of 8.28 mg/d was reported (Kimira, Arai, Shimoi, & Watanabe, 1998) and in Germany 10.3 mg/d quercetin (Linseisen, Radtke, & Wolfram, 1997). Apples and apple pomace are a good source for quercetin and contain quercetin monoglycosides, which are glycosylated in the 3-position with sugars like glucose, galactose, rhamnose, xylose and arabinose (Lu & Foo, 1997). Apple quercetin derivatives are predominately located in apple peel which probably can be incorporated as apple peel extract or powder for healthy functional food products (Wolfe & Liu, 2003).

In humans, the absorption of the different quercetin glycosides is particularly dependent on the glycosidic moiety (Arts, Sesink, Faassen-Peters, & Hollman, 2004). Wolffram, Blöck, and Ader (2002) indicated that, quercetin-3-*O*-glucoside can pass the enterocyte by the sodium dependent SGLUT1 transporter in the small intestine. Further, quercetin-3-*O*-glucoside is a substrate for lactase phloridzin hydrolase (LPH), a luminal brush border enzyme that catalyzes the deglycosylation and enables the aglycone to pass the enterocyte via passive diffusion (Day, Gee, DuPont, Johnson, & Williamson, 2003). Any other apple quercetin glycosides may not be metabolized in the small intestine but fermented and degraded by heterocyclic ring cleavage due to the colonic microbiota (Kahle et al., 2006). After absorption, quercetin undergoes glucuronidation, methylation, and/or sulfation in the small intestine, liver, colon and kidney (Mullen, Edwards, & Crozier, 2006).

The aim of this pilot study was to investigate the influence of the carrier or matrix on the bioavailability of quercetin and quercetin glycosides after consumption: freeze dried apple peel and enriched, vacuum impregnated apple chips were compared with quercetin dihydrate capsule functioned as reference treatment and apple peel extract

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capsules. In recent studies, the influence of the quercetin sugar moiety has been discussed to be stronger compared to the effect of the food matrix (Hollman et al., 1997; Graefe et al., 2001), however systematic studies are currently lacking. Our previous study demonstrated a higher bioavailability for quercetin aglycone when incorporated as an extract into a cereal bar compared to quercetin dihydrate in a capsule (Egert et al., 2011). Thus our objective was to further investigate the food matrix effects compared to capsule treatment in healthy woman on the systemic availability using a single dosage of 71 μmol quercetin equivalents either from quercetin dihydrate or the natural occurring quercetin glycosides from apples.

2. Experimental section

2.1. Participants

Six healthy female volunteers (mean age 26.8 ± 2.6 years) were initially recruited. Participants were in good health, were non-smokers with a BMI range 21.0–23.9 kg/m^2. Exclusion criteria were BMI < 20 kg/m^2, BMI > 25 kg/m^2, smoking, pregnancy, lactation, metabolic and endocrine diseases, use of dietary supplements and use of any form of medication except oral contraceptives. The descriptive participant characteristics are presented in Table 1. In principle all collected data were within the normal range.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the ethical committee of the Medical Faculty of the Christian-Albrechts-University of Kiel (Germany) and the participants gave their written informed consent after explanation of the study details before investigation. Volunteers were given the order to maintain their physical exercises and usual lifestyle during the 8 study weeks.

2.2. Study design

2.2.1. Run-in phase

Participants were instructed to follow a diet low in quercetin for 1 week prior the start of the experimental period (washout phase). For this, participants were given a list of 'foods to avoid', which included several quercetin sources. The compliance of the quercetin low diet was controlled with three-day food records. After a one day (24 h) treatment, the participants were allowed to return to their normal eating habits for one week. Before the next quercetin treatment a washout phase for 1 week was again performed. This cycle was repeated until the participants got all 4 treatments in randomized crossover design.

2.2.2. Treatment period

The bioavailability of quercetin in women was investigated with a randomized, diet controlled, cross-over pilot study with 4 treatments. The dosage in every quercetin treatment was 71 μmol quercetin equivalents. The participants were randomly assigned to the treatments (a, b, c, d). The treatments were a) vacuum impregnated apple chips containing apple peel extract; b) quercetin and quercetin glycoside free apple chips and apple peel extract capsules; c) quercetin and quercetin glycoside free apple chips with one quercetin dihydrate capsule and d) quercetin and quercetin glycoside free apple chips combined with freeze dried apple peel. The treatment was given together with 200 mL mineral water.

In the morning of the intervention study a fasting blood sample as reference for the quercetin baseline was taken after insertion of an indwelling cannula. Accordingly the volunteers got the quercetin rich test meal in form of different apple chip types, apple peel or quercetin equivalent containing gelatin capsules. Afterwards, blood samples were collected 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h and 24 h after supplementation. On the study days, the participants got a standardized quercetin-free diet according to their energy requirement which was as follows. The morning and evening meal consisted of cheese filled rolls and additional yoghurt in the evening, the lunch was pasta with cheese sauce, the afternoon snack was a piece of cake. The breakfast on the study day was given 70 min, the lunch 200 min and the evening meal 10 h after the test meal. Within 10 h after supplementation the volunteers drank 1.5 L mineral water. The day after supplementation, fasting venous blood samples were taken.

Table 1
Baseline characteristics of participants (n = 6 women).

Parameter	Mean ± SEM
Age, y	26.8 ± 1.1
BMI, kg/m^2	22.4 ± 0.5
Body height, cm	169.4 ± 2.4
Body weight, kg	64.4 ± 1.5
Fasting plasma glucose, mmol/L	4.6 ± 0.1
Fasting serum total cholesterol, mmol/L	5.4 ± 0.5
Fasting serum HDL cholesterol, mmol/L	2.1 ± 0.2
Fasting serum LDL cholesterol, mmol/L	2.7 ± 0.3
Fasting serum triglycerides, mmol/L	1.3 ± 0.3
Fasting systolic blood pressure, mm Hg	124.2 ± 3.7
Fasting diastolic blood pressure, mm Hg	73.3 ± 4.9

2.3. Study products

2.3.1. Quercetin dihydrate capsules

The hard gelatin capsules contained quercetin dihydrate (Voigt Global Distribution Inc., Lawrence, KS, USA), mannitol and silicium dioxide. One capsule filled with quercetin dihydrate powder containing 22 mg quercetin (71 μmol) was defined as the control treatment.

2.3.2. Vacuum impregnated apple chips

Apples (*cv*. Elstar) of a high maturity level from the same growing region in Germany were used for vacuum impregnation (VI). The plant material was stored at 4 °C until the apple chips production was prepared in pilot plant scale. Prior to VI the apples were washed, peeled. After removal of the core the apples were sliced (thickness was 6 mm). The impregnation solution was a mixture of commercial apple juice (11.1 ± 0.1°Brix) and 0.45% apple peel extract. The apple peel extract (pomactive hfv) contained large amounts of flavonoids and was purchased from Val de Vire Bioactives (Condé sur Vire, France). The apple peel extract is approved for food use and the composition is described by Auclair et al. (2008). The VI was performed in a vacuum dryer (Type VDL 53, Binder, Tuttlingen, Germany). The vacuum phase (100 mbar, 5 min) was followed by a 10 min atmospheric pressure phase at 20 °C (Schulze, Peth, Hubbermann, & Schwarz, 2012). After enrichment, the apple chips were immediately frozen and freeze dried (Christ Gamma 1–20, Osterode am Harz, Germany). The apple products were stored until start of the study at 20 °C in the absence of air and protected against humidity. The portion size for a supplementation of 71 μmol total quercetin was 47.5 g vacuum impregnated apple chips.

2.3.3. Apple peel extract capsules

The apple peel extract pomactive hfv was mixed with mannitol and the flow-regulating excipient silicium dioxide prior to filling of the hard gelatin capsules. For a total quercetin intake of 71 μmol 0.33 g apple peel extract were required and divided to 10 gelatin capsules for a single treatment.

2.3.4. Apple peel

Apples of a high maturity grade (*cv*. Jonagored) were thoroughly washed. Afterwards the apple peel was removed and immediately shock frozen in liquid nitrogen. The peels were freeze dried for 72 h (Christ Gamma 1–20, Osterode am Harz, Germany), roughly crushed and well mixed for the supplementation. A portion size of 8.1 g dried apple peels corresponded to 71 μmol total quercetin.

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2.4. Flavonoid analysis of study products

The quercetin glycoside concentrations of the vacuum impregnated apple chips, the apple peels, quercetin free chips and the two capsule types were determined by HPLC analysis (Schulze, Hubbermann, & Schwarz, 2014). For identification of quercetin glycosides data of HPLC-DAD with coupled ESI-MS^n (Agilent 1100 Series LC/MSD Trap) were used. The apple material samples were extracted with of 70% acetone and 30% aqueous 0.1% (*w*/w) citric acid, which were purchased from Roth (Karlsruhe, Germany). Approximately 2 g freeze dried and ground apple material samples were extracted in 4 extraction steps. For cell disruption the samples were treated four times with an ultrasound horn (Sonopuls HD 2070, MS 73, Bandelin, Berlin, Germany) for 1 min. After the first application of ultrasound the samples soaked in the solvent for 5 h. After centrifugation and removal of the concentrated supernantant the sediment was extracted directly again for three times (step 2, 3, 4). The combined supernantants was filled up to a defined volume. Prior the HPLC analysis the extracts were filtered through MN 615 ¼ no. 1 filter paper (Machery & Nagel, Düren, Germany). Prior the HPLC analysis the extracts were filtered through MN 615 ¼ no. 1 filter paper (Machery & Nagel, Düren, Germany). For the determination of the capsules, the content was dissolved in the solvent and analyzed. Quercetin, quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside were quantified after calibration with quercetin dihydrate, quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside, respectively. Quercetin-3-*O*-xyloside was used as reference compound for the quantification of quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinoside and quercetin-3-*O*-rhamnoside. All reference compounds were purchased from Roth (Karlsruhe, Germany). Quantitative HPLC-DAD analyses of quercetin derivatives were performed on an Hewlett-Packard 1100 HPLC (Agilent Technologies, Waldbronn, Germany) using a tempered Nucleodur Sphinx RP-C18 column (125 × 4 mm; 5 μm, Machery & Nagel, Düren, Germany) with the corresponding Sphinxs precolumn at 30 °C. The injection volume was 10 μL. The mobile phase consisted of a binary gradient with 0.5% (*v*/v) formic acid in water (eluent A) and 100% acetonitrile (eluent B) with a flow rate of 1 mL/min. The gradient program started at 5% solvent B in 20 min to 20%, from 20% to 35% in 8 min, from 35% to 80% in 2 min, from 35% to 80% in 5 min, from 80% to 5% in 5 min and additional 5 min stabilization with 5% of solvent B. Formic acid and acetonitrile were of HPLC-grade and purchased from Roth (Karlsruhe, Germany). Analytes were detected at DAD wavelengths of 280 nm and 365 nm. Analyses were carried out in triplicates.

2.5. Blood analysis

The venous blood samples were collected in lithium heparin vacuum tubes (S-Monovette®; Sarstedt, Nümbrecht, Germany) and centrifuged for plasma separation (2000 x *g*, 15 min, 4 °C). All measurements were performed without knowledge of treatment assignment. Until analysis, the plasma samples were stored at −80 °C. Prior HPLC analysis all plasma samples were incubated with with β-glucuronidase/sulfatase type H-2 (crude enzyme extract from *Helix pomatia*; Sigma-Aldrich AG, Taufkirchen, Germany). The measurement of plasma flavonols were performed by HPLC with fluorescence detection after a postcolumn chelation with aluminium as described previously (Cermak, Landgraf, & Wolffram, 2003). For quantitative measurement of quercetin and the metabolites isorhamnetin, tamarixetin and kaempferol authentic flavonols as external standards were purchased from Roth (Karlsruhe, Germany). Total flavonols were characterized as the sum of quercetin, isorhamnetin, tamarixetin and kaempferol.

Blood biochemical parameters were also determined after overnight fasting, in addition to the 24 h plasma sample, including serum triglycerides, serum total cholesterol, serum LDL-cholesterol, serum HDL-cholesterol, fluoride plasma glucose concentrations, liver and kidney function markers in serum on each treatment day. The fasting blood concentrations were evaluated in routine methods in the clinical chemistry laboratory. Fluoride plasma glucose, serum lipids as well as liver and kidney function markers were determined on a VITROS 950 Chemistry System autoanalyzer (Ortho-Clinical Diagnostics, Neckargemünd, Germany) with the manufacturer's assay kits, quality controls, and reagents.

The calculations of pharmacokinetic parameters were performed with Origin 8G software program (OriginLab Corporation, Northampton, MA, USA). The bioavailability was determined by calculating the area under the plasma concentration time curve ($AUC_{0-1440\ min}$) using the trapezoidal rule. The plasma flavonol concentrations obtained at different time points (T30–T1440) were corrected for the individual baseline flavonol concentrations (T0). The maximum plasma concentration (c_{max}) and the time when the maximum plasma concentration was reached (t_{max}) were determined by observation the plasma concentration curves of each volunteer and treatment.

2.6. Statistical evaluation

Descriptive statistics are given as mean value and standard error. For treatment comparisons analysis of variance (ANOVA) with Tukey post hoc test were used for statistical analyses. *P*-values < 0.05 were considered significant. All statistical analyses were performed with the software program SPSS® (v.19.0, SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Apple products

The composition of the quercetin derivatives of apple products and capsules administered to the participants are listed in Table 2. As expected, the composition of the apple extract capsules and the vacuum impregnated apple chips were similar. Apple peel of the Jonagored cultivar was characterized by a lower proportion of quercetin aglycone and quercetin-3-*O*-glucoside as well as a higher proportion of quercetin-3-*O*-rhamnoside compared to the apple peel extract and vacuum impregnated apple chips.

3.2. Plasma variables

The pharmacokinetic parameters for plasma quercetin and plasma total flavonols of the participants are given in Table 3. The plasma concentration time profiles after the 4 different quercetin treatments are shown in Fig. 1 for the plasma quercetin concentrations and in Fig. 2 for the plasma total flavonol concentrations. Only minor amounts of kaempferol, isorhamnetin and tamarixetin were detected in plasma. The comparison between the four quercetin carriers (quercetin dihydrate capsule as the reference treatment, vacuum impregnated apple chips, the apple peel extract capsules and the apple peel demonstrated no significant differences in terms of the areas under the plasma concentration-time curves ($AUC_{0-1440\ min}$) or maximum plasma peak concentrations (c_{max}). However, the plasma concentration time profiles differed markedly between the quercetin dihydrate capsule (control) and the apple peel treatment versus treatments with apple peel extract capsules or vacuum impregnated apple chips. The time between oral administration and the maximal plasma peak (t_{max}) was the slowest after quercetin capsule treatment for the quercetin plasma concentration ($p = 0{,}017$) compared to apple peel extract capsules. Regarding maximum plasma peak concentration of the total flavonols after quercetin capsule administration, t_{max} of the apple peel extract capsules ($p = 0{,}013$) and vacuum impregnated apple chips ($p = 0{,}043$) treatments were reached earlier.

There was a high inter-individual variability among the 6 participants in quercetin and total flavonols plasma concentrations at T0 (baseline) as well as after the quercetin source applications. The baseline concentrations for all study participants differed between

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Table 2
Quercetin compositions of quercetin dihydrate capsule, apple peel extract capsules, enriched apple chips and apple peel (cv. Jonagored).

	Ratio of quercetin glycosides and quercetin, % of the total quercetin content			
	Quercetin dihydrate capsule	Apple peel extract capsules	Vacuum impregnated apple chips	Apple peel
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Quercetin-3-*O*-galactoside	–	35.6 ± 0.1	35.2 ± 0.1	32.1 ± 0.4
Quercetin-3-*O*-glucoside	–	9.2 ± 0.1	9.6 ± 0.1	5.6 ± 0
Quercetin-3-*O*-xyloside	–	10.0 ± 0.1	10.2 ± 0.2	11.3 ± 0.1
Quercetin-3-*O*-arabinoside	–	24.2 ± 0.1	25.7 ± 0.1	29.6 ± 0.2
Quercetin-3-*O*-rhamnoside	–	11.2 ± 0	10.6 ± 0.2	21.2 ± 0.4
Quercetin	100 ± 0	9.9 ± 0.2	8.6 ± 0.1	0.1 ± 0.2
Sum	100 ± 0	100 ± 0	100 ± 0	100 ± 0

Values are means with standard deviation (SD), $n = 3$.

20 $nmol*L^{-1}$–130 $nmol*L^{-1}$. The observed $AUC_{(0–1440\ min)}$ range after supplementations were 38.1 $\mu mol*min*L^{-1}$–192.1 $\mu mol*min*L^{-1}$ for enriched apple chips, 12.8 $\mu mol*min*L^{-1}$–88.3 $\mu mol*min*L^{-1}$ for apple peel, 23.5 $\mu mol*min*L^{-1}$–196.5 $\mu mol*min*L^{-1}$ for apple peel extract capsules and 21.7 $\mu mol*min*L^{-1}$–86.7 $\mu mol*min*L^{-1}$ for the quercetin capsule control treatment. Fig. 3 depicts the high inter-individual variability and specified the $AUC_{(0–1440\ min)}$ range which varied between the participants considerably.

4. Discussion

To the best of our knowledge is the first study evaluating the bioavailability of quercetin derivatives at a non-pharmacological dose with regard to matrix effects due the administration of capsules, apple peels and apple chips. To determine the influence of the sugar moiety or matrix effects on the absorption of quercetin, 71 μmol quercetin equivalents were administered to 6 healthy women in a four-way cross-over study, which corresponds to 22 mg quercetin aglycone. Our data demonstrate that vacuum impregnated apple chips, apple peel extract capsules and apple peel single dose administrations showed the same quercetin bioavailability as the reference (quercetin dihydrate capsule). The maximum plasma concentrations were similar for the plasma quercetin and the plasma total flavonols. After enzymatic hydrolysis of the plasma metabolites mainly the quercetin aglycone was found. Isorhamnetin, kaempferol and tamarixetin accounted for a lower proportion of the plasma total flavonols. The natural occurrence of kaempferol in apples was responsible for the plasma kaempferol concentrations. In accordance to the daily total flavonoid intake (26 mg) in the Netherlands (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993a), the dosage of 22 mg quercetin equivalents corresponds to a non-pharmacological dose. To obtain the same quantity quercetin equivalents by the consumption of fresh apples, approximately 1 kg apples should be given, according to the apple variety (Hertog, Hollman, Katan, & Kromhout, 1993b). The quercetin dosage of this study corresponded to about the 2-fold daily quercetin intake in Germany (Linseisen et al., 1997) and the total quercetin intake in a Netherlands cohort (16 mg/d) (Hollman & Arts, 2000). There is a lack of human studies using apple quercetin glycosides in a moderate dose. Thus our findings are discussed in comparison either with different doses of quercetin supplementation (Egert et al., 2008) or with apple ingestion studies using higher dosage (Hollman et al., 1997).

4.1. Quercetin dihydrate capsule

Under consideration of the difference in dosage the c_{max} and $AUC_{(0–1440\ min)}$ after quercetin dihydrate capsule treatment are consistent with those reported by Egert et al. (2008). Administration of the double dose (50 mg quercetin dihydrate) resulted in c_{max} of 189 $nmol*L^{-1}$ and $AUC_{(0–1440\ min)}$ 76.1 $\mu mol*min*L^{-1}$. Quercetin aglycone showed a slow and irregular absorption from the gastrointestinal tract, probably due to the low water solubility (1.6×10^{-5}) (Piskula & Terao, 1998) and slow dissolution rate (Lauro et al., 2002). In contrast to our data Erlund et al. (2000) showed faster quercetin absorption after 20 mg quercetin capsule administration with a maximal concentration peak at 162 min. It is important to note that the quercetin dihydrate capsule administration was associated with the ingestion of quercetin free apple chips rich in dietary fiber. Dietary fiber appears to delay the flavonoid absorption (Perez-Jimenez et al., 2010). Investigations of the bioavailability of quercetin in rats showed that the addition of apple pectin in the diet prolonged the t_{max} of plasma metabolites (Nishijima, Iwai, Saito, Takida, & Matsue, 2009). After quercetin capsule administration in combination with a dietary fiber containing cereal bar Egert et al.

Table 3
Plasma pharmacokinetic parameters of quercetin and total flavonols after consumption of different apple quercetin glycoside and aglycone sources with equal intake of 71 μmol quercetin equivalents.

	Quercetin dihydrate capsule	Apple peel extract capsules	Vacuum impregnated apple chips	Apple peel
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Plasma quercetin				
$AUC_{0–1440\ min}$, $\mu mol \cdot min \cdot L^{-1}$	62 ± 12	87 ± 27	104 ± 24	52 ± 12
c_{max}, $nmol \cdot L^{-1}$	109 ± 24	200 ± 36	205 ± 26	111 ± 25
t_{max}, min	280^{b} ± 51	105^{a} ± 30	135^{ab} ± 34	140^{ab} ± 33
Plasma total flavonols				
$AUC_{0–1440\ min}$, $\mu mol \cdot min \cdot L^{-1}$	74 ± 13	112 ± 30	125 ± 27	62 ± 13
c_{max}, $nmol \cdot L^{-1}$	126 ± 23	267 ± 53	242 ± 33	124 ± 32
t_{max}, min	290^{b} ± 50	105^{a} ± 30	135^{a} ± 34	160^{ab} ± 36

Values are means ± SEM, $n = 6$ women;
$AUC_{0–1440\ min}$, area under the plasma concentration-time curve;
c_{max}, the maximum plasma concentration;
t_{max}, the time to reach the maximum plasma concentration;
Means in a row with superscripts without common letter differ, $P < 0.05$ (1-way ANOVA, Tukey).

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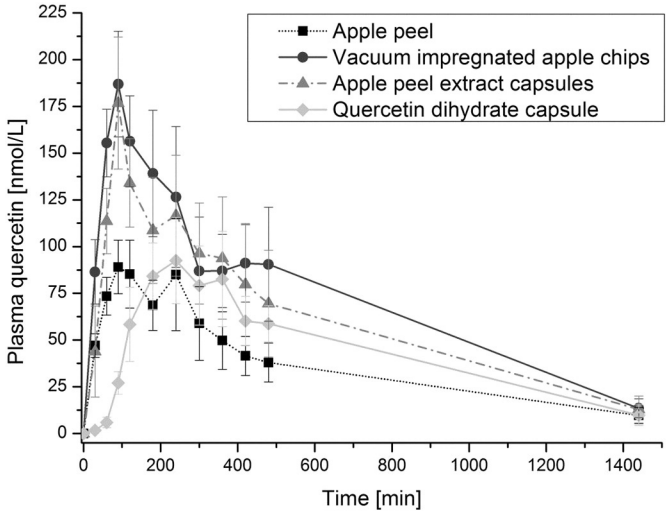

Fig. 1. Mean (±SEM) quercetin plasma concentration curve after consumption of apple peel (■), vacuum impregnated apple chips (●), apple peel extract capsules (▲) and the reference quercetin dihydrate capsule (♦).

(2011) reported similar t_{max} of plasma quercetin (310 min) to our study. Thus, the additional dietary fiber may have been partially responsible for the delayed t_{max} compared to quercetin dihydrate capsule treatment.

4.2. Vacuum impregnated apple chips vs. apple peel extract capsules

Hollman et al. (1997) detected after consumption of apple sauce with a 4.5-fold dosage (325 μmol quercetin equivalents) a quercetin plasma level of 0.3 μM, which corresponds to a 3-fold higher concentration than c_{max} for vacuum impregnated apple chips or the apple peel extract capsules in this study. There was no difference for the quercetin and total flavonol c_{max} values as well as the AUC values after ingestion of apple peel extract capsules compared with vacuum impregnated apple chips. It can thus be concluded that the inclusion of the apple peel extract into the apple tissue represented no significant barrier for the absorption process.

4.3. Apple peel extract capsules, vacuum impregnated apple chips vs. quercetin dihydrate capsule

The differences in $AUC_{(0–1440\ min)}$ were for apple peel extract containing products and the quercetin dihydrate administration not statistical significant. However, the differences of $AUC_{(0–1440\ min)}$ and c_{max} data and the curve shape (Fig. 1) between the control and supplementation groups were apparently large, it did not achieve significance, which could be due to the high inter-individual variation in the quercetin response, which is discussed below. The administration of a higher quercetin equivalents dose might give better indications of the bioavailability (Egert et al., 2008). The quercetin and total flavonol c_{max} values after ingestion of apple peel extract capsules compared to the quercetin dihydrate capsule were not significantly affected by the different profile of quercetin derivatives. Quercetin aglycone, present in apple peel extract and the quercetin dihydrate capsule is able to enter the enterocyte via passive diffusion (Day et al., 2003). Quercetin-3-*O*-glucoside can be transported via the sodium dependent SGLUT1 transporter (Wolffram et al., 2002) and it is substrate for lactase phloridzin hydrolase (LPH) the luminal brush border enzyme that catalyzes the deglycosylation to quercetin. Compared to quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside is a poor substrate for LPH (Arts et al., 2004). Morand, Manach, Crespy, and Rémésy (2000) reported that quercetin-3-*O*-glucoside possessed a stronger effect on the quercetin plasma concentration than quercetin aglycone itself. Except for quercetin-3-*O*-glucoside, the quercetin glycosides of the extract used in this study are considered to be resistant to intestinal hydrolysis (Arts et al., 2004) until reaching the colon, where fermentation processes by the colonic microbiota and degradation reactions by heterocyclic ring cleavage occurs (Kahle et al., 2006). Hydrolyzation of quercetin glycosides by bacteria metabolism resulted in quercetin, which can be absorbed effectively in Caco-2 cell monolayers (Murota, Sumie, Hitomi, Moon, & Terao, 2000). Despite of the low bioavailability of quercetin-3-*O*-galactoside, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinoside and quercetin-3-*O*-rhamnoside, the AUC and c_{max} of plasma quercetin and total flavonols seems to be higher for the apple peel extract treatments than for the quercetin dihydrate capsule. In fact, c_{max} and AUC can only be attributed to quercetin aglycone and quercetin-3-*O*-glucoside of the natural extract from apple peel. This difference indicated a trend and was not statistically significant, which

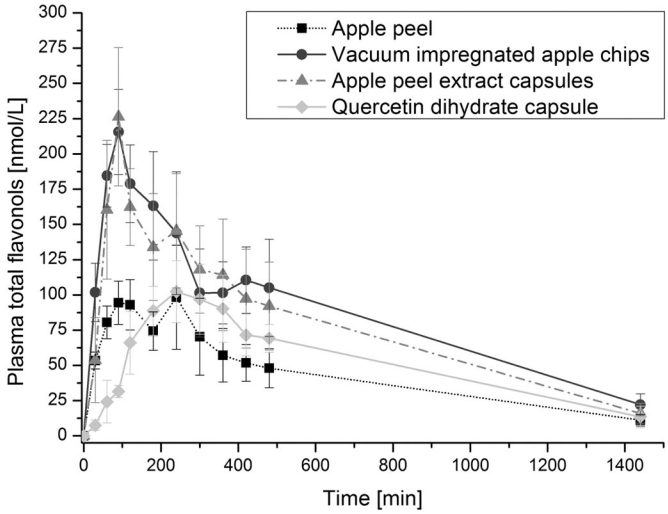

Fig. 2. Mean (±SEM) total flavonol plasma concentration curve after consumption of apple peel (■), vacuum impregnated apple chips (●), apple peel extract capsules (▲) and the control quercetin dihydrate capsule (♦).

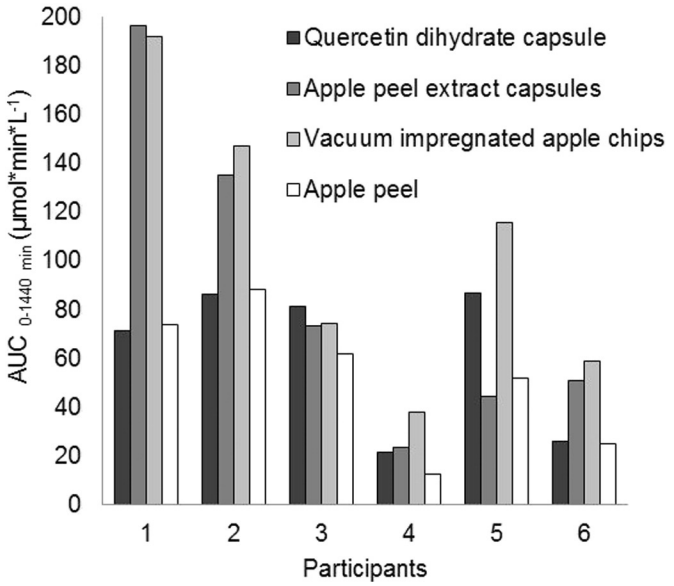

Fig. 3. Inter-individual plasma $AUC_{0–1440\ min}$ data of quercetin after consumption of different apple quercetin glycoside and aglycone sources with equal intake of 71 μmol quercetin equivalents.

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to some extend might be a result of the low quercetin dose of this study. In addition, t_{max} was significantly shifted for the quercetin dihydrate capsule compared to apple peel extract treatments.

4.4. Apple peel

For apple peel consumption matrix effects should be considered. The apple peel is characterized by high fiber content. For quercetin release a digestion of tissue prior to absorption is required. The plasma concentration time curve is not different to the other treatments. But it appears to consist of two peaks. Under this assumption the first peak (90 min) coincides with the time of the main peaks in the apple peel extract treatment. Thus, for the first elevating peak the quercetin glycosides content might be the main factor. But of the total 71 μmol quercetin equivalents the apple peel consists only 5% of the well absorbed quercetin-3-*O*-glucoside, i.e. ca. 1 mg quercetin equivalents. It seems to be questionable that quercetin-3-*O*-glucoside is exclusively responsible for the plasma increase of the first peak. Also deglycosylation of quercetin derivatives by the microbiota of the salvia as reported by Walle, Browning, Steed, Reed, and Walle (2005) must be taken into account. The second peak at 240 min — resulting in a broad plateau — was close to the c_{max} of the poor soluble quercetin dihydrate from capsules. In addition the concentration time curves of the quercetin dihydrate capsule and apple peel treatment were similar. This can be considered as an indication for the poor digestibility of the peel matrix, which resulted in a retardation of quercetin plasma concentration increase.

4.5. Apple peel vs. vacuum impregnated apple chips and apple peel extract capsules

The equal quercetin and total flavonol concentration time curve after apple peel ingestion compared to apple peel extract capsules or vacuum impregnated apple chips administration can be attributed to the similar quercetin derivative profile. The comparison is limited by the quercetin glycoside profile: the apple extract contained approx. a 2-fold higher concentration of quercetin-3-*O*-glucoside than the apple peel and 10% of the extract consisted of the quercetin aglycone. But these slight differences should not markedly affect the bioavailability. Also there was no significant effect attributable to the matrix and the location of the quercetin derivatives in apple treatment products. The extract enrichment by VI is characterized by influx of quercetin containing solution into the intercellular spaces of apple tissue (Schulze et al., 2012). The quercetin glycosides were not incorporated into the apple cells, in contrast to the apple peel, where the quercetin glycosides are located in the cells. For a release of quercetin glycosides in freeze dried apple peel material, cell disintegration by digestion processes is necessary. The apple peel cell wall consists of apple fibers as pectin, hemicelluloses, cellulose and the cuticle wax (Voragen, Timmers, Linssen, Schols, & Pilnik, 1983). The location of quercetin derivatives as well as the poorly digestible fibers of the apple peel did not result in a signification difference for quercetin and total flavonol plasma concentration comparing vacuum impregnated apples chips and apple peel extract capsules. Possibly the grinding of the freeze dried peel resulted in a disintegration of the tissue having the same effect as chewing of chips on the release of flavonoids. Only a few studies have been performed to evaluate the bioavailability of apple quercetin derivatives. The maximal plasma concentration is in accordance with Hollman et al. (1997), who reported a peak level of 92 $ng*mL^{-1}$ after apple peel consisting apple sauce treatment with a dose of 325 μmol quercetin equivalents. The application of 100 mg quercetin equivalents by Lee and Mitchell (2012) resulted in a lower c_{max} (63.8 $ng*mL^{-1}$) calculated to 22 mg quercetin equivalents. The reason might be the different quercetin glycoside composition of the apple products between the studies.

4.6. Inter-individual variability

The four different quercetin treatments resulted in a high inter-individual variability of all plasma pharmacokinetic parameter. Fig. 3 demonstrates the inter-individual plasma $AUC_{0-1440\ min}$ data of the participants after equal intake of 71 μmol quercetin equivalents of the four apple product types. With the exception of the absorption level three probands showed similar $AUC_{0-1440\ min}$ profile. It is assumed that individual respond after quercetin ingestion varied between the participant that would enable to divide the participants into subgroups of high and low responders. Similar inter-individual variabilities are also reported in other studies (Graefe et al., 2001; Hollman & Arts, 2000; Lee & Mitchell, 2012). Reasons might be the impact of the complexity of food matrix, genetic polymorphism for intestinal enzymes influencing the absorption rate as well as for quercetin metabolizing phase II enzymes which are responsible for the glucuronidation and sulfation of quercetin and the varying digestive transit times (Arumugam et al., 2011). Further reasons for the inter-individual differences may be the individual microbiota of the intestinal tract (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). *Eubacterium ramulus* is an exemplary for inter-individual differences in gut microbiota and involved in metabolic cleavage of quercetin glycosides. Blaut et al. (2002) showed different individual concentrations of *E. ramulus* as well as different bacteria strains of the species. Because of the high inter-individual variability, the significance of prospective studies with low quercetin equivalent doses may benefit from a larger number of participants. The SEM range was a little higher for the present study compared to the study of Egert et al. (2011) although the quercetin dosage was more than five times higher in the latter one. Finally, to minimize the differences in the quercetin respond it may be useful to separate the participants into a high and low responding quercetin subgroup by preliminary investigations.

5. Conclusions

In conclusion, the present work indicated an equal increase in quercetin and total flavonol plasma concentrations, after administration of apple glycosides containing vacuum impregnated apple chip snacks, apple peel extract capsules or apple peel. Due to the large inter-individual variability of quercetin and quercetin glycoside responses, a study with a higher number of subjects may be necessary to demonstrate statistically significant differences. The inclusion of quercetin derivatives in the intercellular spaces of apple parenchyma as matrix modification by vacuum impregnation did not result in a retardation of their release or a lowered bioavailability. I.e. apple fruit flesh provides an adequate matrix for a functional food that is enriched with quercetin derivatives or other functional nutrient by vacuum impregnation.

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