Eur J Nutr (2007) 46:329–336
DOI 10.1007/s00394-007-0670-y

ORIGINAL CONTRIBUTION

Clara Lemos
Godefridus J. Peters
Gerrit Jansen
Fátima Martel
Conceição Calhau

Modulation of folate uptake in cultured human colon adenocarcinoma Caco-2 cells by dietary compounds

Received: 20 December 2006
Accepted: 28 June 2007
Published online: 21 August 2007

Abbreviations: AfBeer: Alcohol-free beer, AfRW: Alcohol-free red wine, AfWW: Alcohol-free white wine, BT: Black tea, EGCG: Epigallocatechin 3-gallate, ^{3}H-FA: ^{3}H-folic acid, GT: Green tea, Lbeer: Lager beer, OJ: Orange juice, ^{3}H-MTX: ^{3}H-methotrexate, RFC: Reduced folate carrier, RW: Red wine, Sbeer: Stout beer, WW: White wine

C. Lemos (✉) · F. Martel · C. Calhau
Dept. of Biochemistry (U38-FCT)
Faculty of Medicine
University of Porto
4200-319 Porto, Portugal
Tel.: +351-22/551-3624
Fax: +351-22/551-3624
E-Mail: clara006@med.up.pt

C. Lemos · G.J. Peters
Dept. of Medical Oncology
VU University Medical Center
Amsterdam, The Netherlands

G. Jansen
Dept. of Rheumatology
VU University Medical Center
Amsterdam, The Netherlands

Abstract Folate is a water-soluble B vitamin with a crucial role in the synthesis and methylation of DNA and in the metabolism of several amino acids. In the present study we investigated whether beverages like wine, beer and tea, or some of their specific constituents, affect the intestinal uptake of ^{3}H-folic acid or ^{3}H-methotrexate (an antifolate). All tested beverages significantly inhibited the uptake of ^{3}H-folic acid by Caco-2 cells. Most of these beverages, with the exception of wines (not tested), also inhibited ^{3}H-methotrexate uptake in these cells. Additionally, ethanol, when tested separately, inhibited the uptake of both compounds. Some of the tested phenolic compounds, namely myricetin, epigallocatechin gallate (EGCG) and isoxanthohumol, markedly inhibited ^{3}H-folic acid uptake. Myricetin and EGCG also had a concentration-dependent inhibitory effect upon the uptake of ^{3}H-methotrexate by Caco-2 cells. Resveratrol, quercetin and kaempferol were able to inhibit the transport of both compounds, but only in the concentration of 100 μM. In conclusion, dietary constituents may impact on intestinal folate uptake, as here shown for phenolic compounds.

Key words beverages – Caco-2 cells – flavonoids – folate – intestinal transport

Introduction

Folate (a generic name for a family of compounds which includes folic acid, an oxidized form, and reduced folates) is a water-soluble B vitamin essential for normal cellular functions, growth and development. It acts as a coenzyme in the synthesis of precursors of DNA and RNA and in the metabolism of several amino acids [1]. An adequate supply of this vitamin is therefore necessary for normal human health. Folate deficiency is a highly prevalent vitamin deficiency throughout the world and occurs due to a variety of reasons, including impairment in intestinal absorption of this vitamin [2]. There is a variety of clinical pathologies associated with folate deficiency,

EJN 670

including megaloblastic anemia [3], neural tube defects [4], occlusive vascular disease [5], cancer [6], Down's syndrome [7] and Alzheimer's disease [8]. As human cells cannot synthesize folate and thus must obtain the vitamin from dietary sources, through intestinal absorption the intestine plays a crucial role in regulating folate body homeostasis.

Methotrexate (MTX) is a well known antifolate used as a chemotherapeutic agent since the early 1950s. This compound is commonly used to treat leukaemia [9], osteosarcoma, breast cancer, and head and neck cancer [10]. It is also used in a variety of other pathogenic conditions, including rheumatoid arthritis [11]. MTX is a dihydrofolate reductase (DHFR) inhibitor, resulting in inhibition of synthesis of dTMP and purine precursors for DNA synthesis [10, 12]. Although MTX is usually given as an infusion to cancer patients, in other diseases such as rheumatoid arthritis and in childhood leukaemia it is given as oral maintenance therapy. MTX was found to share the transport system for natural folates, the reduced folate carrier (RFC), and also to be metabolized to poly(γ-glutamates) in mammalian cells, similarly to natural folates.

Chronic alcohol addiction is a serious health problem worldwide and affects at least 5% of the US population [13]. Chronic alcohol intake is associated with deficiency of several nutrients, including the vitamin folate [13–15]. Approximately 60–70% of binge drinkers are folate deficient [15]. One of the possible causes of folate deficiency observed in chronic alcoholism is a reduction in the absorption of this vitamin at the intestinal level [15].

Recent studies suggest a health promoting effect of wine, beer and tea, concerning cardiovascular disease and certain types of cancer [16–19]. All these beverages are known to have a high content of phenolic compounds, which possess important beneficial properties for human health, namely antiinflammatory, antioxidant, antiallergic, antithrombotic and anticarcinogenic activities [20, 21]. Recent studies from our laboratory have shown that wine, beer and tea modulate intestinal thiamine uptake [22].

The intestinal uptake of folate and methotrexate has been characterized [23–25]; it has been reported that RFC is involved in the apical uptake of folate by Caco-2 cells and that the Multidrug Resistance Protein and/or Organic Anion Transporter may mediate apical efflux of folate [26]. Additionally, Subramanian et al. [27] have shown that folate deficiency up-regulates folate uptake as well as RFC protein and mRNA levels in these cells. Nonetheless, the nutritional modulation of folate and methotrexate is largely unknown. Therefore we determined whether the presence of some beverages largely consumed would affect the intestinal uptake of folate and methotrexate, possibly influencing their function as vitamin source or therapeutic effects. Uptake studies were performed in Caco-2 cells, an epithelial cell line derived from a human colon adenocarcinoma, which mimic the human intestinal absorptive epithelium [28].

Materials and methods

Materials

[3′,5′,7,9-^{3}H]Folic acid potassium salt (21.0 Ci/mmol) (Amersham Biosciences, Freiburg, Germany); [3′,5′,7-^{3}H(N)]Methotrexate disodium salt (33.5 Ci/mmol) (Moravek Biochemicals, Inc., Brea, CA, USA); (+)-catechin hydrate, chrysin (5,7-dihydroflavon), (−)-epicatechin, EGCG (epigallocatechin 3-gallate), genistein, HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), kaempferol, MES (2-[*N*-morpholino]ethanesulfonic acid hydrate), myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone), quercetin dihydrate (3,3′,4′,5,7-pentahydroxyflavone), resveratrol, rutin hydrate, Tris (tris-(hydroxymethyl)-aminomethane hydrochloride (Sigma, St. Louis, MO, USA); D-glucose, DMSO, Triton X-100 (Merck, Darmstadt, Germany); isoxanthohumol kindly supplied by Prof. Hans Becker (Pharmakognosie und Analytische Phytochemie, Universität des Saarlandes, Saarbrücken, Germany); xanthohumol, kindly provided by iBeSa (Instituto de Bebidas e Saúde, Portugal).

Lager type beer (Super Bock®), *stout* type beer (Super Bock Stout®) (both with an alcoholic content of 5.6% (v/v)) and alcohol-free beer (Cheers®) were Portuguese beers bought from the local market, as well as green tea (Lipton®), black tea (Rótulo Azul®) and orange juice (Fructis Natura®). Red and white wines (both with an alcoholic content of 12% (v/v)) were from Douro region (Portugal). Alcohol-free red and white wines were prepared by extracting ethanol from the intact wines (kindly prepared and supplied by Prof. Paula Guedes de Pinho, Escola Superior de Biotecnologia da Universidade Católica, Porto, Portugal).

Cell culture

The Caco-2 cell line (ATCC 37-HTB) was used between passages number 26–48. The cells were maintained as previously described [29, 30]. For uptake studies, Caco-2 cells were seeded on 24-well plastic cell culture clusters (1.91 cm^2; ø 16 mm; TPP®, Trasadingen, Switzerland) and the experiments were performed 8–10 days after the initial seeding. For 24 h before the experiment, the cell medium was free of fetal bovine serum. Each square centimeter contained about 130-170 μg cell protein.

Transport studies

Transport studies were performed in Krebs-Ringer buffer with the following composition (in mM): 123 NaCl, 4.93 KCl, 1.23 $MgSO_4$, 0.85 $CaCl_2$, 5 D(+)glucose, 5 glutamine, 10 HEPES and 10 MES, pH 5.5. Initially, the growth medium was aspirated and the cells were washed with Krebs-Ringer buffer at 37°C; then the cell monolayers were preincubated for 60 min in Krebs-Ringer buffer at 37°C. Uptake was initiated by the addition of 250 µl of buffer at 37°C containing 5 nM ^{3}H-folic acid (final specific activity 21.0 Ci/mmol) or ^{3}H-methotrexate (final specific activity 33.5 Ci/mmol). After 3 min, incubation was stopped by placing the cells on ice and rinsing the cells with 500 µl ice-cold Krebs-Ringer buffer. The cells were then solubilized with 300 µl 0.1% (v/v) Triton X-100 (in 5 mM Tris–HCl, pH 7.4), and placed at room temperature overnight. Radioactivity inside the cells was measured by liquid scintillation counting.

Acute effect of drugs: drugs to be tested were present during both the preincubation and incubation periods. The pH of all beverages to be tested was adjusted to 5.5.

Chronic (48h) effect of drugs: drugs to be tested were dissolved in culture media (0.1%) in order to minimize the solvent effect. Medium was refreshed after 24 h of treatment. Drugs to be tested were not present during the preincubation and incubation periods.

Tea preparation

Teas were prepared by making an infusion of a tea bag (1.5 g—black tea; 1.3 g—green tea) in 250 ml of boiling water, for 2 min (black tea) or 5 min (green tea).

Protein determination

The protein content of cell monolayers was determined as described by Bradford [31], with bovine serum albumin as standard.

Total polyphenol content

The total polyphenol content of the beverages was determined following the Folin-Ciocalteu method adjusted to a microscale [32]. In an Eppendorf tube, 790 µL of distilled water, 10 µL of sample, and 50 µL of Folin-Ciocalteu reagent were mixed. After 1 min, 150 µL of aqueous 20% (w/v) Na_2CO_3 was added, and the mixture was mixed and allowed to stand at room temperature in the dark for 120 min. The absorbance was read at 750 nm, and the total polyphenol concentration was calculated from a calibration curve, using catechin as the standard. The results were expressed as mg/l catechin equivalents.

Calculations and statistics

Arithmetic means are given with SEM and geometric means are given with 95% confidence limits. Statistical significance of the difference between various groups was evaluated by one-way analysis of variance (ANOVA test) followed by the Bonferroni test. For comparison between two groups, Student's t-test was used. Differences were considered to be significant when $P < 0.05$.

Results

pH dependence

Both ^{3}H-folic acid and ^{3}H-methotrexate uptake in Caco-2 cells were highly pH dependent; the uptake of both compounds was higher at low pH and decreased as the pH increased (data not shown). Thus, and considering the existence of an acid microclimate in the luminal surface of the intestine [33], we used pH 5.5 in all the following experiments.

Effect of ethanol

Ethanol, the main alcohol present in wines and beers, had an acute inhibitory effect on both ^{3}H-folic acid and ^{3}H-methotrexate uptake (Fig. 1). This effect was concentration-dependent and was more potent in the case of ^{3}H-methotrexate (Fig. 1B). Ethanol, when tested chronically (0.01, 0.05 and 0.1%), had no effect on apical uptake of ^{3}H-folic acid (results not shown).

Effect of phenolic compounds

The acute effect of some of the phenolic compounds known to be present in wines, beers and/or teas was tested.

Myricetin, EGCG and isoxanthohumol had a concentration-dependent inhibitory effect on ^{3}H-folic acid uptake with IC_{50} values (95% confidence interval) of 12.5 (0.3–585.4) µM, 7.7 (2.8–20.7) µM and 35.7 (0.8–1693) µM, respectively. The effect of myricetin and EGCG was also tested on ^{3}H-methotrexate uptake and they also had a concentration-dependent inhibitory effect with IC_{50} values of 10.6 (1.3–88.7) µM and 10.1 (1.7–60.8) µM, respectively. Myricetin and EGCG together (50 µM) inhibited the uptake of ^{3}H-folic acid and ^{3}H-methotrexate to 55 ± 3% and 34 ± 1% of control, respectively.

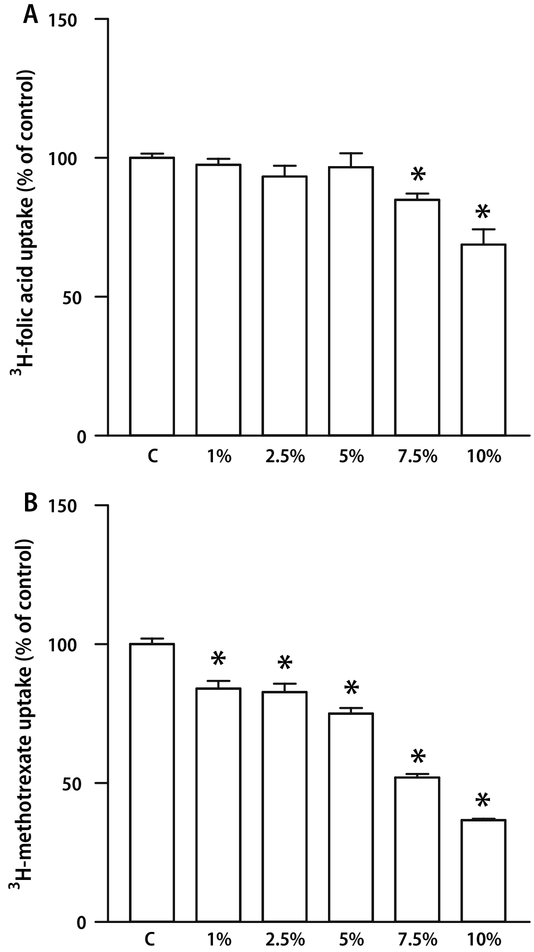

Fig. 1 Effect of ethanol (n = 4-14) on ^{3}H-folic acid (**A**) and ^{3}H-methotrexate (**B**) uptake by Caco-2 cells. Confluent monolayers were preincubated for 60 min and incubated at 37°C with 5 nM ^{3}H-folic acid or ^{3}H-methotrexate for 3 min, in the presence or absence (control; C) of this compound. Shown are arithmetic means ± SEM. $^*P < 0.05$ versus respective control.

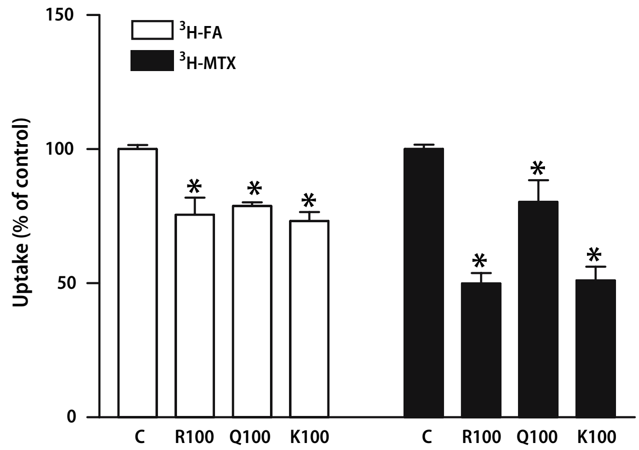

Fig. 2 Effect of resveratrol (100 μM, R100, n = 4), quercetin (100 μM, Q100, n = 4) and kaempferol (100 μM, K100, n = 4) on ^{3}H-folic acid and ^{3}H-methotrexate uptake by Caco-2 cells. Confluent monolayers were preincubated for 60 min and incubated at 37°C with 5 nM ^{3}H-folic acid or ^{3}H-methotrexate for 3 min, in the presence or absence (control; C) of these compounds. Shown are arithmetic means ± SEM. $^*P < 0.05$ versus respective control.

Resveratrol, quercetin and kaempferol moderately inhibited the uptake of both ^{3}H-folic acid and ^{3}H-methotrexate (Fig. 2). When these 3 compounds were tested together, a slightly increased inhibition of the uptake of both compounds was observed (data not shown).

Xanthohumol (100 μM) was able to inhibit ^{3}H-folic acid uptake in Caco-2 cells (to 82 ± 2% of control; n = 4). At the same concentration, crysin, rutin, genistein, epicatechin and catechin had no effect (data not shown).

We also tested the chronic effect of myricetin (25 μM), EGCG (25 μM) and isoxanthohumol (50 μM) on ^{3}H-folic acid uptake in Caco-2 cells. Myricetin and EGCG did not affect ^{3}H-folic acid uptake (data not shown) but isoxanthohumol had an inhibitory effect (70 ± 12% of control; n = 4), albeit less potent than the one shown in the acute studies. These results suggest that these phenolic compounds, when tested chronically, lose, at least in part, their ability to reduce the apical uptake of ^{3}H-folic acid in Caco-2 cells.

Effect of beverages

All beverages were tested in two different concentrations: 500 or 250 μl/ml of buffer solution.

Red and white wine, at both concentrations tested, significantly inhibited the apical uptake of ^{3}H-folic acid in Caco-2 cells. We compared these beverages with two different controls, a control of water (500 or 250 μl of water per ml of buffer solution) and another control with the same alcoholic content of the wines (500 or 250 μl of a 12% (v/v) ethanol solution per ml of buffer solution), and in both cases we verified a significant inhibition of uptake. Red wine was more potent than white wine, and the effect of white wine seemed to be concentration-dependent (Table 1). A similar inhibition of ^{3}H-folic acid uptake was observed with alcohol-free wines. Interestingly enough, ethanol 6% (v/v) alone did also decrease ^{3}H-folic acid uptake (Table 1).

All tested beers (lager, stout and alcohol-free) significantly inhibited ^{3}H-folic acid and ^{3}H-methotrexate uptake in Caco-2 cells to the same extent (Table 2).

Green tea and black tea had a very potent inhibitory effect on ^{3}H-folic acid uptake in Caco-2 cells, which was more potent for black tea. These teas did also inhibit the apical uptake of ^{3}H-methotrexate, but to a lesser extent, green tea being significantly more potent than black tea (Table 2).

Table 1 Effect of wines on ^{3}H-folic acid uptake by Caco-2 cells

Beverages	^{3}H-Folic acid uptake (% of control)	
	500 μl/ml	250 μl/ml
Control	100 ± 3.4	100 ± 1.6
E6%	62.7 ± 3.2*	–
E3%	–	92.1 ± 3.7
RW	7.7 ± 1.6*,†	7.6 ± 0.5*,†
WW	25.8 ± 2.8$^{*,\dagger,\perp}$	40.5 ± 1.8$^{*,\dagger,\perp}$
afRW	7.7 ± 2.1*	6.6 ± 3.3*
afWW	24.1 ± 1.3$^{*,\#}$	44.3 ± 6.1$^{*,\#}$

Confluent monolayers were preincubated for 60 min and incubated at 37°C with 5 nM ^{3}H-folic acid for 3 min, in the presence or absence (control) of these wines or alcoholic solution. Shown are arithmetic means ± SEM. Solution with the same alcoholic content of the wines (E6%/E3%, n = 4), red wine (RW, n = 4), white wine (WW, n = 4), alcohol-free red wine (afRW, n = 4) and alcohol-free white wine (afWW, n = 4). *$P < 0.05$ versus respective control. $^{\dagger}P < 0.05$ versus E6%/E3%. $^{\perp}$Significantly different from RW. $^{\#}$ Significantly different from afRW.

Finally, orange juice also concentration-dependently inhibited the uptake of both ^{3}H-folic acid and ^{3}H-methotrexate, to the same extent (Table 2).

Polyphenol content of beverages

The total polyphenol content of the studied beverages was measured and is shown in Table 3. Red wines have the higher polyphenolic content; in contrast white wines have the lower content. High phenolic content can also be found in tea, particularly green tea, and in orange juice.

Discussion

Both red and white wine inhibited ^{3}H-folic acid uptake in Caco-2 cells. Furthermore, alcohol-free wines had almost the same effect, suggesting that other components of these beverages must play a role in this effect. These results support the notion that care should be taken in drawing conclusions for alcoholic beverages from results obtained with ethanol alone.

Wine, especially red wine, contains many biologically active compounds such as various polyphenolic compounds, which might be responsible for the beneficial effect of red wine consumption on heart disease, cancer, and inflammatory diseases [20, 34, 35]. The stilbene resveratrol, the flavonols quercetin, myricetin, kaempferol and rutin, and the flavanols catechin and epicatechin, are some of the phenolic compounds present in wines, in concentrations ranging from 1 μM to more than 300 μM [34]. Some of these phenolic compounds (resveratrol, quercetin, myricetin, kaempferol) significantly inhibited the uptake of ^{3}H-folic acid and ^{3}H-methotrexate by Caco-2 cells. These results suggest that phenolic compounds present in wines are, at least in part, responsible for the inhibitory effect of these beverages upon the intestinal uptake of ^{3}H-folic acid. This hypothesis is supported by the fact that red wine, that has a much higher content in phenolic compounds (Table 3), had a more potent inhibitory effect than white wine.

Table 2 Effect of beers, teas and orange juice on ^{3}H-folic acid and ^{3}H-methotrexate uptake by Caco-2 cells

Beverages	^{3}H-Folic acid uptake (% of control)		^{3}H-methotrexate uptake (% of control)	
	500 μl/ml	250 μl/ml	500 μl/ml	250 μl/ml
Control	100 ± 3.4	100 ± 1.6	100 ± 2.3	100 ± 3.0
E2.8%	77.7 ± 3.1*	–	89.8 ± 2.8*	–
E1.4%	–	96.8 ± 4.2	–	99.0 ± 4.2
LBeer	16.8 ± 0.9*,†	29.0 ± 1.1*,†	7.8 ± 0.9*,†	26.3 ± 0.8*,†
SBeer	15.6 ± 2.8*,†	28.5 ± 1.5*,†	10.6 ± 1.1*,†	18.6 ± 3.1*,†
afBeer	21.2 ± 1.1*	35.2 ± 2.0*	12.8 ± 1.3*	31.0 ± 2.1*
GT	5.5 ± 1.1*	8.2 ± 1.2*	57.6 ± 2.7*	38.3 ± 3.8*
BT	4.2 ± 1.2*	2.9 ± 0.9$^{*,\#}$	92.2 ± 5.3$^{\#}$	72.5 ± 6.8$^{*,\#}$
OJ	21.5 ± 0.4*	43.0 ± 8.2*	18.2 ± 1.0*	33.1 ± 1.5*

Confluent monolayers were preincubated for 60 min and incubated at 37°C with 5 nM ^{3}H-folic acid or ^{3}H-methotrexate for 3 min, in the presence or absence (control) of these beverages or alcoholic solution. Shown are arithmetic means ± SEM. Solution with the same alcoholic content of the beers (E2.8%/E1.4%, n = 4), lager beer (LBeer, n = 4), stout beer (SBeer, n = 4), alcohol-free beer (afBeer, n = 4), green tea (GT, n = 4), black tea (BT, n = 4) and orange juice (OJ, n = 4). $^{*}P < 0.05$ versus respective control. $^{\dagger}P < 0.05$ versus E2.8%/E1.4%. $^{\#}$Significantly different from GT.

All tested beers significantly inhibited ^{3}H-folic acid and ^{3}H-methotrexate uptake in Caco-2 cells, the effect of beer and alcohol-free beer being very similar. Again, these results strengthen the notion that care should be taken in drawing conclusions for alcoholic beverages from results obtained with ethanol. Xanthohumol is a prenylflavonoid present in the hop plant, which adds bitterness and flavour to beer. Beer constitutes the main dietary source of xanthohumol and other prenylflavonoids like isoxanthohumol [36]. These hop-derived constituents of beer are potential cancer chemopreventive agents [36–38]. Both xanthohumol and isoxanthohumol, which is more abundant in beer [36], inhibited ^{3}H-folic acid uptake, suggesting that they may be responsible for the inhibitory effect of beers on the intestinal uptake of this vitamin. However, we have to consider that folate is present in beer and may also contribute to the inhibition of ^{3}H-folic acid and ^{3}H-methotrexate uptake. Similarly, the high folate content of orange juice [39] may be responsible for the inhibition of both ^{3}H-folic acid and ^{3}H-methotrexate uptake in Caco-2 cells.

Green and black teas inhibited uptake of both ^{3}H-folic acid and ^{3}H-methotrexate by Caco-2 cells, but the latter less potently. The effect of both teas is possibly due to different polyphenols; flavonols commonly

Table 3 Total polyphenol content of studied beverages

Beverages	Total phenolic content (mg/l catechin equivalents)
RW	1716.7 ± 49.6
AfRW	1992.1 ± 58.0
WW	163.0 ± 20.5
AfWW	179.6 ± 10.4
Lbeer	315.0 ± 9.2
Sbeer	690.7 ± 41.0
AfBeer	179.5 ± 5.1
GT	871.5 ± 24.2
BT	589.2 ± 20.3
OJ	928.6 ± 19.5

Values represent means ± SEM (n = 4). The concentration is expressed as mg/l catechin equivalents.

known as catechins (e.g. epicatechin, epicatechin gallate, epigallocatechin, EGCG) predominate in green tea, usually accounting for 30–42% of the dry weight. However, black tea manufacture involves fermentation, leading to the conversion of catechins to theaflavins and thearubigins, the major polyphenols found in black tea [17, 19]. The effect of green tea might be explained by its high content of EGCG [17], which was one of the most potent inhibitors on the uptake of both ^{3}H-folic acid and ^{3}H-methotrexate. Other phenolic compounds like myricetin, quercetin and kaempferol can also be found in teas [19] and may also contribute to inhibition of ^{3}H-folic acid and ^{3}H-methotrexate uptake.

Ethanol inhibited, in a concentration-dependent way, uptake of both compounds by Caco-2 cells. However, ^{3}H-folic acid inhibition was observed only with the highest concentrations of ethanol (7.5 and 10% [v/v]). In a recent study [40] we reported that acute exposure of rat jejunum to ethanol (0.05 or 2.4% [v/v]) did not change the jejunal permeability to ^{3}H-folic acid. This is in agreement with the present study, despite the fact that different models were used (human Caco-2 cells versus rat jejunum). Furthermore, chronic (48 h) treatment of Caco-2 cells with ethanol had no effect on intestinal uptake of ^{3}H-folic acid; this result is also in accordance with our previous study [40] where chronic ethanol consumption by the rats did not change jejunal permeability to ^{3}H-folic acid.

Although ethanol alone doesn't seem to cause a great inhibition of ^{3}H-folic acid uptake in the intestine, it is shown in this study that many beverages, including alcoholic beverages, can significantly reduce the intestinal uptake of this vitamin. These results suggest that, in human alcoholism, folate deficiency can result from a decrease in its intestinal absorption.

Myricetin, epigallocatechin gallate and isoxanthohumol were potent inhibitors of ^{3}H-folic acid uptake in the acute studies; however, when tested chronically (48h) myricetin and epigallocatechin gallate lost their inhibitory effect and isoxanthohumol showed a much less potent effect. Folate depletion in Caco-2 cells induces an increased expression of RFC [27]. A similar process may occur in our case, where folate depletion caused by acute exposure to phenolic compounds may lead to an increased expression of folate transporter(s) that could compensate, in chronic treatments, the effect of phenolic compounds.

Previous studies from our group have shown that the tested beverages and phenolic compounds don't affect the viability of Caco-2 cells [29, 30]. Thus, we can conclude that the modulation of ^{3}H-folic acid and ^{3}H-methotrexate uptake reported in this study is independent of any cytotoxic effect.

In this study, ^{3}H-folic acid and ^{3}H-methotrexate uptake in Caco-2 cells were similarly modulated by most of the beverages and phenolic compounds, suggesting that these compounds share the same transport system in these cells. Methotrexate and natural reduced folates are substrates of RFC [41, 42]; however it is not clear whether RFC is the major transporter involved in the intestinal transport of folates. Although many studies suggest a role for RFC in the transport of folates in intestinal cells [26, 27, 43–45], there are significant differences between the intestinal transport of these compounds and the RFC-mediated transport in other cells. One of those differences concerns optimum pH activity; RFC-mediated transport in leukaemia cells has an optimum pH of 7.5 [46]. However, the intestinal transport of folates shows a quite low optimum pH (around 5.0–5.5) [23, 24], as was also shown in our study. This indicates that a low pH folate transporter independent of RFC might be responsible for the intestinal transport of folates and methotrexate [42]. Folic acid is a poor substrate for RFC at pH 7.4, underlying the presence of a separate transporter. Further studies must take place to address this question.

In conclusion, our results suggest that the effect of all tested beverages, significantly decreasing folic acid and methotrexate uptake, can be justified, at least in part, by the effect of their phenolic compounds. Dietary habits, especially those related to the consumption of the tested beverages or phenolic compounds, can modulate the intestinal uptake of both ^{3}H-folic acid and ^{3}H-methotrexate. The latter may reduce the therapeutic efficacy of methotrexate in patients taking it by the oral route. Furthermore, we conclude that care should be taken in drawing conclusions on alcoholic beverages from results obtained with ethanol alone.

Acknowledgments This work was supported by FCT (SFRH/BD/16883/2004), Programa Ciência, Tecnologia e Inovação do Quadro Comunitário de Apoio (POCTI/SAU-FCF/59382/2004) and iBeSa (Instituto de Bebidas e Saúde).

References

1. Stover PJ (2004) Physiology of folate and vitamin B12 in health and disease. Nutr Rev 62:S3–S12
2. Said HM (2004) Recent advances in carrier-mediated intestinal absorption of water-soluble vitamins. Annu Rev Physiol 66:419–446
3. Lucock M (2000) Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab 71:121–138
4. van der Put NM, van Straaten HW, Trijbels FJ, Blom HJ (2001) Folate, homocysteine and neural tube defects: an overview. Exp Biol Med (Maywood) 226:243–270
5. Ueland PM, Refsum H, Beresford SA, Vollset SE (2000) The controversy over homocysteine and cardiovascular risk. Am J Clin Nutr 72:324–332
6. Choi SW, Mason JB (2000) Folate and carcinogenesis: an integrated scheme. J Nutr 130:129–132
7. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, Yi P, Tafoya DL, Swenson DH, Wilson VL, Gaylor DW (1999) Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 70:495–501
8. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM (1998) Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 55:1449–1455
9. Rots MG, Pieters R, Kaspers GJ, Veerman AJ, Peters GJ, Jansen G (2000) Classification of ex vivo methotrexate resistance in acute lymphoblastic and myeloid leukaemia. Br J Haematol 110:791–800
10. McGuire JJ (2003) Anticancer antifolates: current status and future directions. Curr Pharm Des 9:2593–2613
11. Dijkmans BA, Jansen G (2004) Antimetabolites in the treatment of arthritis: current status of the use of antimetabolites. Nucleosides Nucleotides Nucleic Acids 23:1083–1088
12. Peters GJ, Jansen G (1996) Resistance to Antimetabolites. In: Schilsky RL, Milano GA, Ratain MJ (eds) Principles of antineoplastic drug development and pharmacology. Marcel Dekker, New York, pp 543–585
13. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ (2002) Metabolic interactions of alcohol and folate. J Nutr 132:2367S–2372S
14. Lieber CS (2000) ALCOHOL: its metabolism and interaction with nutrients. Annu Rev Nutr 20:395–430
15. Bode C, Bode JC (2003) Effect of alcohol consumption on the gut. Best Pract Res Clin Gastroenterol 17:575–592
16. Soleas GJ, Diamandis EP, Goldberg DM (1997) Wine as a biological fluid: history, production, and role in disease prevention. J Clin Lab Anal 11:287–313
17. Mukhtar H, Ahmad N (2000) Tea polyphenols: prevention of cancer and optimizing health. Am J Clin Nutr 71:1698S–1702S
18. van de Wiel A, van Golde PH, Hart HC (2001) Blessings of the grape. Eur J Intern Med 12:484–489
19. Yang CS, Maliakal P, Meng X (2002) Inhibition of carcinogenesis by tea. Annu Rev Pharmacol Toxicol 42:25–54
20. Middleton E Jr, Kandaswami C, Theoharides TC (2000) The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 52:673–751
21. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74:418–425
22. Lemos C, Calhau C, Martel F, Azevedo I (2004) Intestinal thiamine uptake: characterization and nutritional modulation. FASEB J 18:A708
23. Selhub J, Rosenberg IH (1981) Folate transport in isolated brush border membrane vesicles from rat intestine. J Biol Chem 256:4489–4493
24. Said HM, Ghishan FK, Redha R (1987) Folate transport by human intestinal brush-border membrane vesicles. Am J Physiol 252:G229–G236
25. Said HM, Ma TY, Ortiz A, Tapia A, Valerio CK (1997) Intracellular regulation of intestinal folate uptake: studies with cultured IEC-6 epithelial cells. Am J Physiol 272:C729–C736
26. Martel F, Goncalves P, Azevedo I (2006) Absorption of folate by Caco-2 cells is not affected by high glucose concentration. Eur J Pharmacol 551:19–26
27. Subramanian VS, Chatterjee N, Said HM (2003) Folate uptake in the human intestine: promoter activity and effect of folate deficiency. J Cell Physiol 196:403–408
28. Delie F, Rubas W (1997) A human colonic cell line sharing similarities with enterocytes as a model to examine oral absorption: advantages and limitations of the Caco-2 model. Crit Rev Ther Drug Carrier Syst 14:221–286
29. Monteiro R, Calhau C, Martel F, Faria A, Mateus N, Azevedo I (2005) Modulation of MPP+ uptake by tea and some of its components in Caco-2 cells. Naunyn Schmiedebergs Arch Pharmacol 372:147–152
30. Monteiro R, Calhau C, Martel F, Guedes de Pinho P, Azevedo I (2005) Intestinal uptake of MPP+ is differently affected by red and white wine. Life Sci 76:2483–2496
31. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
32. Arnous A, Makris DP, Kefalas P (2001) Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J Agric Food Chem 49:5736–5742
33. Said HM, Blair JA, Lucas ML, Hilburn ME (1986) Intestinal surface acid microclimate in vitro and in vivo in the rat. J Lab Clin Med 107:420–424
34. German JB, Walzem RL (2000) The health benefits of wine. Annu Rev Nutr 20:561–593
35. Sun AY, Simonyi A, Sun GY (2002) The "French Paradox" and beyond: neuroprotective effects of polyphenols. Free Radic Biol Med 32:314–318
36. Stevens JF, Page JE (2004) Xanthohumol and related prenylflavonoids from hops and beer: to your good health! Phytochemistry 65:1317–1330
37. Gerhauser C, Alt A, Heiss E, Gamal-Eldeen A, Klimo K, Knauft J, Neumann I, Scherf HR, Frank N, Bartsch H, Becker H (2002) Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. Mol Cancer Ther 1:959–969
38. Gerhauser C (2005) Beer constituents as potential cancer chemopreventive agents. Eur J Cancer 41:1941–1954
39. Gregory JF III (1997) Bioavailability of folate. Eur J Clin Nutr 51 (Suppl 1):S54–S59
40. Lemos C, Azevedo I, Martel F (2005) Effect of red wine on the intestinal absorption of thiamine and folate in the rat: comparison with the effect of ethanol alone. Alcohol Clin Exp Res 29:664–671
41. Jansen G (1999) Receptor- and carrier-mediated transport systems for folates and antifolates: exploitation for folate-based chemotherapy and immunotherapy. In: Jackman AL (ed) Anticancer drug development guide: antifolate drugs in cancer therapy. Humana Press, Totowa, NJ, pp 293–321

42. Matherly LH, Goldman DI (2003) Membrane transport of folates. Vitam Horm 66:403–456
43. Chiao JH, Roy K, Tolner B, Yang CH, Sirotnak FM (1997) RFC-1 gene expression regulates folate absorption in mouse small intestine. J Biol Chem 272:11165–11170
44. Wang Y, Zhao R, Russell RG, Goldman ID (2001) Localization of the murine reduced folate carrier as assessed by immunohistochemical analysis. Biochim Biophys Acta 1513:49–54
45. Balamurugan K, Said HM (2006) Role of reduced folate carrier in intestinal folate uptake. Am J Physiol Cell Physiol 291:C189–C193
46. Sierra EE, Brigle KE, Spinella MJ, Goldman ID (1997) pH dependence of methotrexate transport by the reduced folate carrier and the folate receptor in L1210 leukemia cells. Further evidence for a third route mediated at low pH. Biochem Pharmacol 53:223–231