

Original article:

EFFECT OF CRANBERRY DIETARY SUPPLEMENTS WITH DIFFERENT BRANDS ON HUMAN CYP3A4 ENZYME

Sompon Wanwimolruk^{1*}, Supaluk Prachayasittikul¹, Virapong Prachayasittikul², Bouchra Bernichi³

¹ Center for Innovation Development and Technology Transfer, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand

² Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok 10700, Thailand

³ School of Pharmacy, Loma Linda University, Loma Linda, CA 92350, USA

* Corresponding author: Dr. Sompon Wanwimolruk, E-mail: mtsompon@mahidol.ac.th; Tel.: +66 2 441 4370, Fax: +66 2 441 4380

ABSTRACT

The use of dietary supplements has increased dramatically, making drug interactions with those supplements a major concern. Because dietary supplements are not subject to the same regulations as prescription drugs, we hypothesize that the content of their active ingredients may vary among manufacturers, potentially causing a large variation in therapeutic outcome. The current study aimed to test this hypothesis on commonly used cranberry dietary supplements. Activity of human CYP3A4 enzyme was used as a parameter to determine the effect of cranberry supplement from nine manufacturers. The content of a cranberry product, equivalent to one capsule, was extracted with methanol. Aliquots of the extract were tested for their ability to inhibit the metabolism of the human CYP3A4 substrate quinine, using an *in vitro* liver microsomal technique. Human liver microsomes and quinine were incubated with or without (i.e. as control) cranberry extract. Formation of quinine's metabolite 3-hydroxyquinine, generated by the CYP3A4-mediated reaction was measured by a HPLC method. Of nine cranberry products tested, eight products had little or no effect but only one brand (Nature's Herbs 600 mg) caused very strong inhibition (67.2 %) of CYP3A4. The reason for this inhibition is unknown. The effect of cranberry was varied and ranged from 4.4 % activation by Ride Aid 800 mg to 67.2 % inhibition by Nature's Herbs 600 mg. Lack of effect on human CYP3A4 activity suggests that use of cranberry dietary supplement is unlikely to cause significant interactions with drugs metabolized by CYP3A4.

Keywords: Dietary supplements, cranberry, CYP3A4, drug interactions

INTRODUCTION

It has been recognized for many years that the medicinal use of herbs plays an important role in nearly every culture, including Asia, Africa, Europe and the United States of America. Recent surveys indicate that one of three Americans use dietary supplements daily (Kelly et al., 2005; Tindle et al., 2005). It is remarkable that

the rate of herbal usage is much higher in cancer patients (Carmady and Smith, 2011; Carpenter et al., 2009; Pierce et al., 2002). Many of these supplements are herbal in nature (Wargovich, 2001). Herbal supplements are normally taken for two main reasons. In the first situation they are used to improve symptoms of illness. For example, the widespread use of St. John's wort for relief of depression, use of Ginkgo bi-

loba for improvement in cognition and the use of Echinacea for relief of cold symptoms (Wargovich, 2001). In the second case, herbal supplements are used particularly with the hopes of preventing disease or reducing the risk for certain diseases. For example, the utilization of green tea, grape seed extract and other flavonoid-rich botanicals to take advantage of the natural antioxidants in these products (Nassiri-Asl and Hosseinzadeh, 2009; Wargovich, 2001). The use of garlic and its supplement preparation is another example, as it has been demonstrated to prevent and reduce risk to cancer (Zhou et al., 2011).

Cranberry (*Vaccinium macrocarpon*) is among the top 10 herbs sold in the United States (Blumenthal, 2003). Cranberry juice concentrate has been formulated into capsules and sold as dietary supplements. There are many products of cranberry dietary supplements available in various US markets. Consumption of cranberry juice is useful for the treatment and prevention of urinary tract infections (Guay, 2009; Jepson and Craig, 2007). Cranberry juice extracts have also been suggested to possess anticancer effects (Bomser et al., 1996; Kresty et al., 2011; Neto, 2011). Cranberries contain significant levels of vitamin C, total phenols, catechins and anthocyanins with associated antioxidant activity (Howell, 2007; Krenn et al., 2007). Though cranberry juice and cranberry dietary supplements have become very popular, its potential interaction with drugs has not been well documented. It has been shown that cranberry juice did not alter the disposition of cyclosporine, suggesting it does not interact with other drugs metabolized by CYP3A4 (Grenier et al., 2006). There has been an increasing number of case reports of life-threatening interactions between cranberry and warfarin (Abdul et al., 2008; Haber et al., 2012).

Cytochrome P450 (CYP) is a family of isoenzymes, mainly found in the liver and gut wall. There are more than 50 human CYP isoforms, but CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 en-

zymes metabolize 90 % of clinically used drugs (Guengerich, 1999; Lynch and Price, 2007). CYP plays an important role in detoxification and the elimination of many drugs and other xenobiotics including carcinogens. Human CYP3A4 is the most important human CYP isozyme because it is involved in the metabolism of approximately 50 % marketed drugs and some environmental toxicants (Guengerich, 1999; Zhou, 2008). Human CYP3A4 is expressed in the gut, colon, small intestine, breast and prostate. However, its expression is most abundant in the liver which accounts for 30 % of the total CYP protein content (Guengerich, 1999; Lown et al., 1997). Activity of CYP3A4 can be inhibited or induced by drugs, herbs, pesticides and carcinogens (Jiang et al., 2012; Zhou, 2008). Also human CYP3A4 metabolizes most prescription drugs. This makes drug interactions with CYP3A4 extremely important (Guengerich 1999; Shi and Klotz, 2012). Natural ingredients in grapefruit juice, primarily furanocoumarin derivatives, have the capacity to inhibit intestinal CYP3A4 enzyme, accounting for a number of clinically important drug interactions (Bailey et al., 1998; Greenblatt et al., 2001; Ho et al., 2000). Therefore the use of dietary supplements such as cranberry is of concern with regard to herbal drug interactions.

Since dietary supplements including cranberry dietary supplements, are not regulated by the same FDA regulations as prescription drugs and over the counter medications, they normally lack quality control and the regulatory oversight of therapeutic products (Frankos et al., 2010; Sanzini et al., 2011). Thus, we hypothesize that the content of active ingredients in cranberry dietary supplements may vary among different manufacturers. This variation in the active ingredients may result in variation in therapeutic outcomes and extent of herbal drug interactions. This hypothesis is based on the following previous findings. First, with regard to antioxidant activity of grape polyphenols, there was a

correlation between the antioxidant activity and the content of polyphenols in grape seed extracts (Zhao et al., 1999). Second, it has been testified with a few herbal supplements, including ephedra, ginseng and St. John's wort, that the content of active ingredients varied largely between brands and in some instances the content variation was also found between batches of the same herbal products (Draves and Walker, 2003; Gurley et al., 2000; Harkey et al., 2001). Therefore, this study was conducted to test this hypothesis on commonly used cranberry dietary supplements. Activity of human liver CYP3A4 was employed as a parameter to determine the effect of cranberry dietary supplements purchased from various manufacturers.

MATERIALS AND METHODS

Chemicals and dietary supplements

All chemicals and reagents used were of analytical grade. Quinine hydrochloride dehydrate, NADPH and sodium dodecyl sulfate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). 3-Hydroxyquinine was a gift from Dr. Peter Winstanley, Department of Pharmacology and Therapeutics, University of Liverpool, UK. Pooled human liver microsomes were

purchased from In Vitro Technologies (Baltimore, MD, USA) and were kept at -80°C until required. The CYP content of the microsomes was 0.29 nmol of CYP/mg of protein. Nine different brands of cranberry supplements were randomly purchased from local health product stores in California, USA, during the year 2009. Details of manufacturers, content of each capsule and indications are listed in Table 1. All experiments were carried out well before the expiration dates of the cranberry dietary supplement products investigated.

Preparation of cranberry supplement methanolic extract

The content of cranberry dietary supplement studied was measured by weighing the actual content in each capsule. This was done with six replicates ($n = 6$ capsules). The contents from these six capsules were combined. The mean (average) content of the capsule was determined. Content of each cranberry supplement equivalent to the average content in one capsule was extracted with methanol using procedures as previously described (Wanwimolruk and Prachayasittikul, 2012).

Table 1: Product information of the cranberry supplements used in this study

Brand Name	Manufacturer	mg/capsule	Indication*
GNC	General Nutrition Corp.	500 mg	Supports a healthy urinary tract
Kroeger	Kroeger Herb Products Co Inc.	200 mg	No claim
Natural Balance (Cran Balance)	Natural Balance	500 mg	Provides nutritive support for urinary tract health
Natural Factors (Cran Rich)	Natural Factors, Canada	500 mg	Supports urinary tract health
Nature's Answer	Nature's Answer, Inc.	800 mg	As a traditional treatment for supporting healthy urinary tract function
Nature's Herbs	Nature's Herbs	600 mg	Nutritionally supports healthy urinary tract function
Nature's Way	Nature's Way Products, Inc.	400 mg	Supports urinary tract health
Pharm Assure	Pharm Assure, Inc.	500 mg	Helps maintain a healthy urinary tract
Rite Aid	Rite Aid Corp.	850 mg	Supports urinary tract

* These statements have not been evaluated by the Food and Drug Administration (FDA). This product is not intended to diagnose, treat, cure, or prevent disease.

CYP3A4 assay procedure

Aliquots (5 μ L) of the cranberry methanolic extracts were tested for their ability to inhibit the metabolism of a CYP3A4 marker substrate using an *in vitro* liver microsomal technique. All experiments were performed in four replicates. Quinine was used as marker substrate for human CYP3A4 (Zhang et al., 1997). Human liver microsomes (0.5 mg/mL) were incubated with quinine (100 μ M) in the presence or absence (i.e. control) of cranberry methanolic extract with 1 mM NADPH in phosphate buffer (0.067 M, pH 7.4) at a final volume of 0.5 mL. Each incubation was performed at 37 °C in shaking water bath for 30 min. The reaction was stopped by the addition of cold methanol solution (1 mL). The samples were vortexed briefly and centrifuged at 2500 g for 10 min. The resultant supernatant (30 μ L) was injected onto the HPLC column. The formation of quinine's metabolite (3-hydroxyquinine) was assayed by a reversed-phase HPLC method (Wanwimolruk et al., 1996). The detection limit of this assay was 0.1 μ M (0.034 μ g/mL).

Statistical analysis

Results are expressed as mean \pm standard deviation (S.D.). Data were analyzed by one way ANOVA, followed by multiple comparisons using a Tukey's test for post hoc comparison. Statistical analysis was assessed by SPSS statistical software (Version 18.0, SPSS Inc., Chicago, IL, USA). For all tests differences were considered to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Preliminary study has demonstrated that the cranberry dietary supplement products of interest did not have any compounds which can interfere with the HPLC assay of the CYP3A4 metabolite (3-hydroxyquinine). This was confirmed by the results obtained from incubation of each cranberry supplement extract with human liver microsomes under the same experimental conditions used, without

CYP3A4 substrate quinine. After incubation, none of the cranberry supplement extracts had peaks that interfere with 3-hydroxyquinine in the HPLC assay.

Figure 1 shows the effect of cranberry dietary supplement extract on the activity of human CYP3A4. The results showed that the majority of cranberry products tested had no or very little inhibitory effect on the activity of CYP3A4. Three of the cranberry product extracts, i.e. Kroeger 200 mg, Pharm Assure 500 mg and Rite Aid 850 mg, caused minor activation of CYP3A4 activity. However, the extent of CYP3A4 activation by these three products was very small, ranging from 4.2 % for Pharm Assure 500 mg to 6.1 % for Kroeger 200 mg.

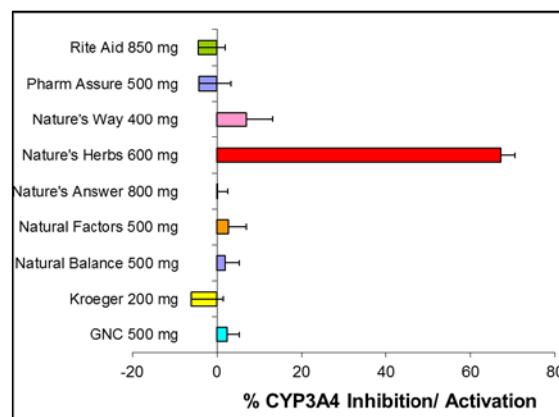


Figure 1: Effect of cranberry supplement extracts on human CYP3A4 activity. Results were expressed as mean values and S.D. bars, obtained from 4 measurements.

The actual activity of CYP3A4 in the presence of all cranberry extract was not significantly different ($p > 0.05$) from that observed in the controls. One exception was the Nature's Herbs 600 mg brand which caused a strong CYP3A4 inhibition of 67.2 ± 2.5 %. The actual CYP-3A4 activity in the presence of the Nature's Herbs 600 mg brand was significantly less than (7.1 ± 0.7 pmol/min/mg vs 21.5 ± 1.4 pmol/min/mg, $p < 0.05$) that observed in the controls. The reason for the inhibition of CYP3A4 enzyme caused by the Nature's Herbs product is unknown. The possible interference of the cranberry supplement extract on HPLC analysis of 3-

hydroxyquinine was excluded. This is because our preliminary results have demonstrated that after the human microsomal mixture was incubated with the cranberry supplement extract without the CYP3A4 substrate (quinine), no interfering peak was found. The difference could not be due to difference in content of cranberry dietary supplement products as there was no significant correlation ($r = 0.012$, $p > 0.5$) between % inhibition of CYP3A4 caused by different brands and cranberry contents claimed in the capsule. This is also supported by the fact that the Nature's Answer (800 mg) brand product contained higher cranberry content than that of Nature's Herbs brand (600 mg), but it caused virtually no inhibitory effect (0 ± 2.5 % inhibition) on the activity of CYP3A4 (Figure 1). The marked inhibitory effect produced by the Nature's Herbs brand (600 mg) may be caused by other phytochemical compounds in this cranberry product, which are not present in other cranberry brands. There is also a possibility of contamination during manufacturing processes. A product may also contain, as contaminants, parts of other herbs not mentioned on the label, which may not accurately reflect the herbal content of the product (Sanzini et al., 2011). If these are the cases, it points to the existing problem of quality control that is one of the main issues in the use of dietary supplements (Frankos et al., 2010; Sanzini et al., 2011).

The present study has shown that most of the cranberry dietary supplements tested had no significant effect on human CYP3A4 activity. Our *in vitro* finding supports previous observations which were reported by other investigators. For example, a clinical study has shown that drinking a glass of cranberry juice did not appear to alter the pharmacokinetics of cyclosporine, suggesting it does not interact with other drugs metabolized by CYP3A4 (Grenier et al., 2006). Similarly, a separate clinical study reported that cranberry juice had no effect on the pharmacokinetics of the CYP3A4 substrate midazolam (Lilja et

al., 2007). In contrast, an *in vitro* study found that component(s) in cranberry juice caused a mechanism-based inhibition during CYP3A-mediated oxidation of nifedipine both in human liver and rat intestinal microsomes (Uesawa and Mohri, 2006). The authors suggested that a component or components present in cranberry juice inhibit human CYP3A-mediated metabolism of nifedipine. Inhibition of another human CYP3A4 drug, midazolam by commercial fruit juices, including cranberry juice, grapefruit juice, pomegranate, black raspberry, black mulberry and wild grape juice during preincubation with NADPH, was also reported (Kim et al., 2006). A recent *in vitro* study had led to isolation of three triterpenes (maslinic acid, corosolic acid, and ursolic acid) from cranberry (Kim et al., 2011). These triterpenes were shown to be strong inhibitors of enteric human CYP3A4, with inhibitory potencies being within the range of those reported for CYP3A4 inhibitory components in grapefruit juice (Kim et al., 2011). Contradiction of results found from these studies could be related to the fact that some studies were conducted *in vivo* (healthy volunteers) whereas others were performed *in vitro*. It may also be accounted for by differences in experimental conditions such as concentrations of cranberry juice or extracts used in those *in vitro* experiments.

In summary, the current study has demonstrated that cranberry dietary supplements with different brands caused no significant inhibitory effect on human CYP3A4. Lack of inhibitory effect on human CYP3A4 activity implies that use of cranberry dietary supplement is unlikely to cause significant interactions with drugs metabolized by CYP3A4 (e.g., felodipine, nifedipine, midazolam, cyclosporine, atorvastatin and saquinavir, etc.). Nevertheless, without good quality control implemented for manufacturing dietary supplements, there may be an inconsistency of products. This may cause variation in phytochemical compounds which can generate unexpected herbal drug interactions.

ACKNOWLEDGEMENTS

This project was funded by the Office of the Higher Education Commission, Mahidol University under the National Research Universities Initiative and Loma Linda University School of Pharmacy, California, USA.

REFERENCES

Abdul MIM, Jiang X, Williams KM, Day RO, Roufogalis BD, Liauw WS, et al. Pharmacodynamic interaction of warfarin with cranberry but not with garlic in healthy subjects. *Br J Pharmacol* 2008;154:1691-700.

Bailey DG, Arnold JMO, Spence JD. Grapefruit juice-drug interactions. *Br J Clin Pharmacol* 1998;46:101-10.

Blumenthal M. Herbs continue slide in mainstream market: sales down 14 percent. *HerbalGram* 2003;58:71.

Bomser J, Madhavi DL, Singletary K, Smith MA. *In vitro* anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med* 1996;62:212-6.

Carmady B, Smith CA. Use of Chinese medicine by cancer patients: a review of surveys. *Chin Med* 2011;6:22.

Carpenter CL, Ganz PA, Bernstein L. Complementary and alternative therapies among very long-term breast cancer survivors. *Breast Cancer Res Treat* 2009;116:387-96.

Draves AH, Walker SE. Analysis of the hypericin and pseudohypericin content of commercially available St. John's wort preparations. *Can J Clin Pharmacol* 2003;10:114-8.

Frankos VH, Street DA, O'Neill RK. FDA regulation of dietary supplements and requirements regarding adverse event reporting. *Clin Pharmacol Ther*. 2010;87:239-44.

Greenblatt DJ, Patki KC, von Moltke LL, Shader RI. Drug interactions with grapefruit juice: an update. *J Clin Psychopharmacol* 2001;21:357-9.

Grenier J, Fradette C, Morelli G, Merritt GJ, Vranderick M, Ducharme MP. Pomelo juice, but not cranberry juice, affects the pharmacokinetics of cyclosporine in humans. *Clin Pharmacol Ther* 2006;79:255-62.

Guay DR. Cranberry and urinary tract infections. *Drugs* 2009;69:775-807.

Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol* 1999;39:1-17.

Gurley BJ, Gardner SF, Hubbard MA. Content versus label claims in ephedra-containing dietary supplements. *Am J Health Syst Pharm* 2000;57:963-9.

Haber SL, Cauthon KA, Raney EC. Cranberry and warfarin interaction: a case report and review of the literature. *Consult Pharm* 2012;27:58-65.

Harkey MR, Henderson GL, Gershwin ME, Stern JS, Hackman RM. Variability in commercial ginseng products: an analysis of 25 preparations. *Am J Clin Nutr* 2001;73:1101-6.

Ho P-C, Ghose K, Saville DJ, Wanwimolruk S. Effect of grapefruit juice on pharmacokinetics and pharmacodynamics of verapamil enantiomers in healthy volunteers. *Eur J Clin Pharmacol* 2000;56:693-8.

Howell AB. Bioactive compounds in cranberries and their role in prevention of urinary tract infections. *Mol Nutr Food Res* 2007;51:732-7.

- Jepson RG, Craig JC. A systematic review of the evidence for cranberries and blueberries in UTI prevention. *Mol Nutr Food Res* 2007;51:738-45.
- Jiang B, Cai F, Gao S, Meng L, Liang F, Dai X, et al. Induction of cytochrome P450 3A by Shexiang Baoxin Pill and its main components. *Chem Biol Interact* 2012;195:105-13.
- Kelly JP, Kaufman DW, Kelley K, Rosenberg L, Anderson TE, Mitchell AA. Recent trends in use of herbal and other natural products. *Arch Intern Med* 2005;165:281-6.
- Kim E, Sy-Cordero A, Graf TN, Brantley SJ, Paine MF, Oberlies NH. Isolation and identification of intestinal CYP3A inhibitors from cranberry (*Vaccinium macrocarpon*) using human intestinal microsomes. *Planta Med* 2011;77:265-70.
- Kim H, Yoon YJ, Shon JH, Cha IJ, Shin JG, Liu KH. Inhibitory effects of fruit juices on CYP3A activity. *Drug Metab Dispos* 2006;34:521-3.
- Krenn L, Steitz M, Schlicht C, Kurth H, Gaedcke F. Anthocyanin- and proanthocyanidin-rich extracts of berries in food supplements - analysis with problems. *Pharmazie* 2007;62:803-12.
- Kresty LA, Howell AB, Baird M. Cranberry proanthocyanidins mediate growth arrest of lung cancer cells through modulation of gene expression and rapid induction of apoptosis. *Molecules* 2011;16:2375-90.
- Lilja JJ, Backman JT, Neuvonen PJ. Effects of daily ingestion of cranberry juice on the pharmacokinetics of warfarin, tizanidine and midazolam - probes of CYP2C9, CYP1A2 and CYP3A4. *Clin Pharmacol Ther* 2007;81:833-9.
- Lown KS, Bailey DG, Fontana RJ, Janardan SK, Adair CH, Fortlage LA, et al. Grapefruit juice increases felodipine oral availability in humans by decreasing intestinal CYP3A protein expression. *J Clin Invest* 1997;99:2545-53.
- Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician* 2007;76:391-6.
- Nassiri-Asl M, Hosseinzadeh H: Review of the pharmacological effects of *Vitis vinifera* (Grape) and its bioactive compounds. *Phytother Res* 2009;23,1197-204.
- Neto CC. Cranberries: ripe for more cancer research? *J Sci Food Agric* 2011;91:2303-7.
- Pierce JP, Faerber S, Wright FA, Rock CL, Newman V, Flatt SW, et al. A randomized trial of the effect of plant-based dietary pattern on additional breast cancer events and survival: the Women's Healthy Eating and Living (WHEL) study. *Control Clin Trials* 2002;23:728-56.
- Sanzini E, Badea M, Santos AD, Restani P, Sievers H. Quality control of plant food supplements. *Food Funct* 2011;2:740-6.
- Shi S, Klotz U. Drug interactions with herbal medicines. *Clin Pharmacokinet* 2012;51:77-104.
- Tindle HA, Davis RB, Phillips RS, Eisenberg DM. Trends in use of complementary and alternative medicine by U.S. adults: 1997–2002. *Altern Ther Health Med* 2005;11:42-9.
- Uesawa Y, Mohri K. Effects of cranberry juice on nifedipine pharmacokinetics in rats. *J Pharm Pharmacol* 2006;58:1067-72.

Wanwimolruk S, Wong SM, Zhang H, Coville PF. Simultaneous determination of quinine and a major metabolite 3-hydroxyquinine in biological fluids by HPLC without extraction. *J Liquid Chromatogr* 1996; 19:293-305.

Wanwimolruk S, Prachayasittikul V. Variable inhibitory effect of herbal supplements of different brands on human P450 CYP1A2. *EXCLI J* 2012;11:7-19.

Wargovich MJ. Colon cancer chemoprevention with ginseng and other botanicals. *J Korean Med Sci* 2001;16 Suppl: S81-S86.

Zhang H, Coville PF, Walker RJ, Miners JO, Birkett DJ, Wanwimolruk S. Evidence for an involvement of human CYP3A in the 3-hydroxylation of quinine. *Br J Clin Pharmacol* 1997;43:245-52.

Zhao J, Wang J, Chen Y, Agarwal R. Antitumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidins B5-3'-gallate as the most effective antioxidant constituent. *Carcinogenesis* 1999;20: 1737-45.

Zhou SF. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. *Curr Drug Metab* 2008;9:310-22.

Zhou Y, Zhuang W, Hu W, Liu GJ, Wu TX, Wu XT. Consumption of large amounts of allium vegetables reduces risk for gastric cancer in a meta-analysis. *Gastroenterology* 2011;141:80-9.