Dietary intake of folate, vitamin B$_2$, B$_6$, B$_{12}$ and colorectal adenoma recurrence: a Dutch follow-up study

By Gabriëlla van Starrenburg and Ida Man
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Radboud University Nijmegen Medical Centre, department of Epidemiology and Biostatistics and HTA

Hogeschool van Amsterdam

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Dietary intake of folate, vitamin B₂, B₆, B₁₂ and the incidence of colorectal adenoma recurrence: a Dutch follow-up study

Authors
Gabriëlla van Starrenburg, Gabriella.van.Starrenburg@hva.nl
Frescobaldistraat 50 1447 NJ Purmerend
Ida Man, Ida.man@hva.nl
Springfontein 35 1448 RG Purmerend

Supervisors
Audrey Jung, MSc, Audrey.Jung@hva.nl
Minse de Bos Kuil, MD, Minse.deBosKuil@hva.nl

(Number thesis)
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

Preface

The following pages present the Bachelor thesis ‘Dietary intake of folate, vitamin B₂, B₆, B₁₂ and the incidence of colorectal adenoma recurrence: a Dutch follow-up study’. Both of us wanted to explore the research environment and we became fascinated by the interesting characteristics that accompany the work as a researcher. Before we began to work on our thesis, we both had our minds set on going abroad and this research question and the topic that was attached to this thesis elected our interest quickly as we learned more, and more about the international aspect of the assignment. We had a few ups and downs, although we swiftly learned that this is also part of the process of developing a thesis. Nevertheless, we are very grateful to have received this opportunity to gain more experiences and insight in the field of research and we enjoyed our time working on this thesis. We would like to express our gratitude to both our supervisors Audrey Jung and Minse de Bos Kuil for their constructive comments that inspired us to think further and motivated us to continue, as did their praise. Their supervision contributed remarkably to the quality of this thesis. We would also like to thank both our families for their ongoing support and the reassuring words. Without their support, our journey would not have been as joyful and memorable as it is now.

Thank you for reading,

Gabriëlla van Starrenburg and Ida Man

Amsterdam, January 2010
Abstract

**Background:** A high prevalence of colorectal cancer is found in the Western world. Colorectal adenoma is a well-established precursor for this disease and therefore plays an important role in colorectal cancer prevention. Recently, folate and B-vitamins (B<sub>2</sub>, B<sub>6</sub>, and B<sub>12</sub>, also known as riboflavin, pyridoxine and cobalamin, respectively) have emerged as important dietary factors that may influence colorectal neoplasia. Although the specific mechanism responsible for this assumption is not clearly identified yet, the folate mediated one-carbon metabolism appears to have a central role in this matter. This mechanism comprises of interrelated biochemical reactions that are involved in DNA synthesis and DNA methylation. Disturbances in these processes are thought to be involved in the pathogenesis of colorectal neoplasms. Despite of folate, other B-vitamins, functioning as cofactors, are critical in the one-carbon metabolism as well. Both epidemiological and interventional studies have reported about the possible preventive role of folate on colorectal neoplasia, while fewer studies about the role of other related B-vitamins on this topic have been conducted.

**Objective:** This thesis investigated the association between dietary B-vitamins intake (i.e., folate, vitamin B<sub>2</sub>, vitamin B<sub>6</sub> and vitamin B<sub>12</sub>) derived from foods and the incidence of colorectal adenoma recurrence in a Dutch population.

**Design:** A prospective follow-up study was carried out in consequence of a case-control study (the POLIEP study) designed to investigate the association between dietary intake of folate, riboflavin, MTHFR C677T genotype and colorectal adenoma risk. Subjects of the current study (n=683) were cases from the POLIEP study with a history of colorectal adenoma and all underwent polypectomy. Dietary intake and lifestyle information was obtained through a food-frequency questionnaire and follow-up medical data was collected from gastroenterology and pathology reports. Baseline characteristics were then analyzed between the whole study population, the low, medium and high intake groups (designated by tertile cut points) of the four corresponding B-vitamins. A two-sided chi-square test (P-value less than 0.05 was considered significant) was conducted to assess the association between intake of these B-vitamins and the incidence of colorectal adenoma. Statistical analysis was performed using SPSS, version 16.0.
Results: More men were prevalent among the study population (n= 361) and the overall average age was 59.1 (SD=10.1). The mean BMI of the total population was 26.2 kg/m$^2$ (SD=3.8 kg/m$^2$). The distribution of incidence of colorectal adenoma recurrence seems to be normal between the low, medium and high intake groups for all considering B-vitamins. No significant association has been found.

Conclusions: The results from this follow-up study suggest that dietary intake of folate, vitamin B$_2$, B$_6$ and B$_{12}$ has no association with the recurrence of colorectal adenomas. Further studies concentrating on the relation between dietary B-vitamins derived from food sources and the recurrence of colorectal adenomas are needed to draw a consistent conclusion.

Keywords: colorectal adenoma, one-carbon metabolism, folate, B-vitamins
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1.0 Introduction

There is a high prevalence of colon cancer in the Western world. It has been much more common in high-income countries but is now increasing in middle- and low-income countries. Cancer of the colon and the rectum, also known as colorectal cancer, is the fourth most leading cause of death from cancer.\(^1\) It is widely accepted that colorectal cancer originates from colorectal adenomas in humans. The progression of this precursor has been called the adenoma-carcinoma sequence, which describes the stepwise progression from healthy tissue to carcinogenic tissue.\(^2\) A highly important role in the causation and prevention of colorectal cancer is placed with dietary intake of specific macro- and/or micronutrients.\(^1\)

The vitamin folate and other related B-vitamins B\(_2\), B\(_6\) and B\(_{12}\), also known as riboflavin, pyridoxine and cobalamin, respectively, are several specific components of diet that have been of considerable interest because of the suggestion that they may modulate the risk of colorectal neoplasia. This is the abnormal proliferation of cells that may lead to the formation of both benign and malignant tissues, including adenomas as well as carcinomas.\(^3\) The above-mentioned vitamins play a role in this matter, given the fact that these B-vitamins are involved in the so-called folate mediated one-carbon metabolism. This mechanism is associated with the pathogenesis of colorectal neoplasm, as its biochemical reactions are important for maintaining normal DNA synthesis and DNA methylation. Disturbances in these cellular processes may enhance neoplasia.\(^4,5\) Foundations for this suggestion are reported in animal and human studies.\(^6-13\)

Several studies, both epidemiological\(^11,14-16\) and interventions,\(^17-25\) have reported about the possible preventive role of folate on colorectal neoplasm, while fewer studies about the other related B-vitamins have been conducted. A majority of studies concern the relation between the B-vitamins and the risk of cancer\(^26-30\), whereas fewer studies reported about the risk of developing new adenomas.\(^12,24\) Even less studies have used adenomas recurrence as the primary endpoint.\(^11,23\)

In 1997, the POLIEP study was started to investigate the associations between dietary intake of folate, riboflavin, methylenetetrahydrofolate reductase C677T genotype and colorectal adenoma risk. In this case-control study, all cases and controls underwent polypectomy. To determine the prognostic value of clinical and lifestyle factors in adenoma recurrence, the
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POLIEP follow-up study was designed, which is the focus of this thesis. This thesis, as part of the POLIEP follow-up study, describes the following research question:

*What is the association between dietary B-vitamins intake (i.e., folate, vitamin B$_2$, vitamin B$_6$ and vitamin B$_{12}$) derived from foods and colorectal adenoma recurrence in a Dutch population?*

To answer this research question, this thesis starts with a literature review, which includes the explanation of the potential mechanism, general information about the corresponding B-vitamins and scientific evidence of this topic. Subsequently, the study design is described, followed by the results, discussion and conclusion of this thesis.
2.0 Literature review

2.1 Colorectal adenoma

An adenomatous polyp, also termed as an adenoma, is a type of pre-malignant polyp and can be found anywhere along the entire large bowel.\(^2, \text{31}\) Colorectal adenomas are generally considered to be precursor lesions for most cases of colorectal cancer, defined by the term adenoma-carcinoma sequence. This term suggests that colorectal adenomas and colorectal cancer have common environmental and genetic risk factors. As a result, identification of the risk factors related to the development of colorectal adenomas has been established as an important goal in the prevention of colorectal cancer.

It is reported that approximately 40% of the Western population will develop colorectal adenomas. After polypectomy (colonoscopic resection of polyps) 30-35% will have an adenoma recurrence within 3-4 years and 3% will develop colorectal cancer. Thus, a relative low percentage of adenomas will progress to malignant tumours. In addition, polypectomy is reported to significantly reduce the incidence of colorectal cancer.\(^32\)

2.2 Risk factors of colorectal adenoma

Looking at most studies conducted about colorectal adenomas, it is remarkable that males are more likely to develop colorectal adenoma than females.\(^32\) Moreover, age also seems to be a factor associated with the incidence of colorectal adenoma. A larger proportion of colorectal adenomas are found among the older population.\(^33\)

In addition to demographic factors, there are also genetic risk factors that play a role in colorectal adenoma development. A history of colorectal cancer in first-degree relatives is found to be associated with elevated risk of colorectal adenoma. Besides, genetic variations of enzymes involved in the one-carbon metabolism may modify DNA methylation and DNA synthesis, two mechanisms modulating carcinogenesis.\(^31\)

Moreover, the development of colorectal adenomas may be preceded by presence of ulcerative colitis, a medical risk factor.\(^34-36\) At last, abdominal obesity and the metabolic syndrome are considered risk factors as well.\(^37-39\)

There are indications that several lifestyle factors increase the risk of colorectal adenoma. Cigarette smoking may have an adversely effect on the biological functions of folate and is therefore presumed to be stimulating in colorectal adenoma development, especially in early stages of adenoma formation.\(^15, \text{29, 40, 41}\) Although convincing evidence suggest that
higher physical activity reduces the risk for colorectal neoplasm, data that support the underlying mechanism remain scarce. A possible explanation for the preventive effect of physical activity lays behind its role in modulation of energy balance, just like total energy intake and body size characteristics. Finally, an interesting role is placed with the dietary factors in the prevention and causation of colorectal adenomas. Dietary factors such as alcohol drinking, high intake of (saturated) fat, red meat and calories are thought to elevate the risk of colorectal cancer. Low intake of vegetables, fruits and fibres is associated with an elevated risk of colorectal cancer. The hypothesis that alcohol may increase colorectal adenoma risk is based on its anti-folate effect. Namely, alcohol lowers the bioavailability of folate for one-carbon metabolism. Consequently, alcohol is suspected to have a negative effect on DNA methylation. Moreover, alcohol contributes to the destruction and loss of vitamin B₆, which is also critical for the proper functioning of the one-carbon metabolism. There are indications that high intake of both total and saturated fat is associated with an increased risk of colorectal adenoma. Some studies concerning the association with colorectal adenomas reported a positive association as well, but because of the small quantity of studies that have been conducted about colorectal adenomas, evidence seems less convincing. Scientific data suggest a preventive role of vegetables, fruits and fibre against colorectal cancer and adenomas, but results do not implicate a consistent conclusion. Despite inconsistent results, the World Cancer Research Fund suggests an adequate intake of vegetables, fruit and fibre as cancer preventive eating habits. Regarding micronutrients, calcium and antioxidants (e.g., carotenes, vitamins A, C and E) may be protective against colorectal adenomas. In addition, iron appears to have a promoting effect on colorectal adenoma and cancer causation. Finally, B-vitamins (i.e., folate, vitamin B₂, B₆, B₁₂) have recently garnered attention in regard to the risk and the development process of colorectal neoplasm. This concept is based on the involvement of these B-vitamins in folate-mediated one-carbon metabolism. This is also the initial principle of this study. See table 1.0 for an overall view of the proposed risk factors of colorectal neoplasia.
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Table 1.0 Proposed risk factors of colorectal neoplasia

<table>
<thead>
<tr>
<th>Diet- and lifestyle factors</th>
<th>Demographic factors</th>
<th>Genetic factors</th>
<th>Medical factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Cigarette smoking</td>
<td>• Gender (male)</td>
<td>• Family history of colorectal cancer</td>
<td>• Ulcerative colitis</td>
</tr>
<tr>
<td>• Alcohol drinking</td>
<td>• Increased age</td>
<td>• Genetic variations in one-carbon metabolism involved enzymes</td>
<td>• Abdominal obesity and/or metabolic syndrome</td>
</tr>
<tr>
<td>• High intake of (saturated) fat, red meat, calories</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Low intake of vegetables, fruits and fibres</td>
<td></td>
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</tbody>
</table>

2.3 The one-carbon metabolism

The one-carbon metabolism is a system that comprises of interrelated biochemical reactions that are involved in DNA synthesis and DNA methylation. These two mechanisms are proposed to be responsible for colorectal cancer.27, 63-65 Folate has a central role in the one-carbon metabolism cycle. In addition to folate, other related B-vitamins (B2, B6 and B12) are also essential components.27 Folate is responsible for arranging the transfer of methyl groups via this cycle. A change in folate status can lead to alteration in DNA methylation, or cause defective DNA synthesis. For decades, it has become clear that folate deficiencies result in chromosome breaks and that such breaks may result in increased risk of several cancers in humans. There are two potential mechanisms suspected to be responsible for DNA breaks in a folate deficient condition, namely uracil misincorporation into DNA and impaired DNA repair.66-68 Also, accumulating evidence has indicated interruption of DNA methylation as an important cause of carcinogenesis.8

The diet can provide three forms of folate; folate, dihydrofolate (DHF, which is also known as folic acid) and tetrahydrofolate (THF). Dietary folate has to be fully reduced to THF before it can carry a one-carbon unit; this conversion is carried out by the enzyme dihydrofolate reductase (DHFR). THF receives a methyl group from either the breakdown of glycine or serine, which results in 5,10-methyleneTHF. The enzyme serine hydroxymethyltransferase (SHMT) is responsible for this conversion. 5,10-methyleneTHF plays a central role in the one carbon metabolism, as it makes this part of the metabolism responsible for DNA synthesis, complete by again converting in dihydrofolate (DHF) with the help of enzyme thymidylate synthase (TS). And, simultaneously, helps in the conversion of deoxyuridylate (dUMP) to thymidylate (dTMP). This is the only source of thymidylate (dTMP), an essential compound for nucleotides synthesis, a precursor of DNA synthesis,68 which can be seen in figure 1. Moreover, 5,10-methyleneTHF can also be converted to oxidised and reduced forms for other biochemical purposes, such as 5-methylTHF and 10-formylTHF,
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which are needed for the biosynthesis of methionine and purine, respectively. Purine, as like thymidylate, is essential for nucleotides synthesis and methionine plays an important role in DNA methylation as part of the one-carbon metabolism presented in figure 2.

Figure 1: Simplified scheme of the DNA synthesis part of one-carbon metabolism and the role of folate

Abbreviations: DHF: dihydrofolate, DHFR: dihydrofolate reductase, THF: tetrahydrofolate, SHMT: serine hydroxymethyltransferase, TS: thymidylate synthase, dUMP: deoxyuridylate, dTMP: thymidylate

The central role of folate goes on when 5,10-methyleneTHF forms into 5-methylTHF by enzyme methylenetetrahydrofolate reductase (MTHFR), where it can be converted again to tetrahydrofolate (THF) by the enzyme methionine synthase (MTR). Simultaneously, methionine synthase (MTR) converts homocysteine into methionine. The metabolism cycle continues on when methionine is converted into S-adenosyl methionine (SAM) and then into S-adenosyl homocysteine (SAH) and then again into homocysteine. The essential role of folate in the one carbon metabolism is clear given that S-adenosyl methionine (SAM), which serves as a methyl donor for numerous biochemical reactions, is responsible for DNA methylation.\(^5,27\)
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Figure 2: Simplified scheme of the DNA synthesis and DNA methylation parts of the one-carbon metabolism and the role of folate.

However, other B-vitamins also act as co-factors in the one-carbon metabolism, which resembles more and more a Shakespearean theatre play with every added complicated relationship, visible in figure 3. Flavin adenine dinucleotide (FAD), a metabolite of vitamin B2, serves as a cofactor for MTHFR. Vitamin B6 is a cofactor for serine hydroxymethyltransferase (SHMT), which converts tetrahydrofolate (THF) to 5,10-methyleneTHF. Furthermore, vitamin B12 acts as a cofactor for methionine synthase (MTR). Thus, without these corresponding B-vitamins, the one-carbon metabolism cannot function well.5,27
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Figure 3: Simplified scheme of the one-carbon metabolism and the role of folate and related B-vitamins


2.4 General features of the related B-vitamins

2.4.1 Folate

The function of folate – Folate is a water-soluble B-vitamin also incorrectly known as folic acid. Although these terms are frequently used in the same context, accurately, folate refers to tetrahydrofolate derivatives and folic acid to dihydrofolate monoglutamate, the fully oxidised monoglutamyl form of folate. Folic acid is the form used in supplements and fortified food. Folate in foods is often presented in its bound form, in which a string of amino acids (glutamate) is connected, also known as polyglutamate. Folate is absorbed by
the second part of the small intestines, the jejunum, but not before polyglutamate is hydrolyzed by enzymes, found on the intestinal cell surface, to monoglutamate. A methyl group is attached to monoglutamate, which then enters the bloodstream and is transported to the liver and other body tissues. The most prominent coenzyme of folate is THF, which arranges the transfer of methyl groups through the one carbon metabolism. The bioavailability of folate found in foods is consistently less than that of folic acid.\textsuperscript{47, 69}

\textit{Folate food sources} – Rich food sources of folate are legumes and vegetables, especially green leafy vegetables. Furthermore, folate is abundant present in tripe meat, beans, nuts and dairy products. During cooking and storage, nearly half of the amount of folate present in foods can be destroyed by heat and oxidation.\textsuperscript{49, 71}

\textit{Folate deficiency and recommendations} – Folate deficiency plays a role in the pathogenesis of anaemia, atherosclerosis, neural tube defects and several cancers.\textsuperscript{8, 9, 71} The Dutch guideline for folate recommends an intake of 300 $\mu$g/day for people aged 19 and older.\textsuperscript{72} Moreover, an amount of 400 $\mu$g/day is recommended for women periconceptionally to prevent neural tube defects. The latest Dutch National Food Consumption Survey however, reported an insufficient intake of folate in the Dutch population.\textsuperscript{71}

\textit{Folate fortification abroad} – Because of the assumed positive role of folate and the fact that folate deficiency is relatively common in Western countries, the United States of America made it mandatory to fortify grain products with folic acid to raise the folate intake of the population. However, fortification with folic acid raises safety concerns as well. Intake of folate, naturally occurring in foods, appears to cause no harm. Nevertheless, high intake of folic acid can mask the development of megaloblastic anaemia, an anaemia that results from inhibition of DNA synthesis in red blood cell production, caused by a vitamin B\textsubscript{12} deficiency. Recent evidence implicates that folic acid supplementation stimulates the progression of established neoplasms.\textsuperscript{6, 9, 49, 71} Moreover, questions are raised about what high intakes of folic acid will have as long-term effect.

\textbf{2.4.2 Vitamin B\textsubscript{2}}

\textit{The function of vitamin B\textsubscript{2}} – Vitamin B\textsubscript{2}, also known as riboflavin, is a water-soluble B-vitamin that fulfils numerous functions in a wide variety of reactions. The prominent role of this vitamin resides in the energy-yielding metabolism. FMN (flavin mononucleotide), also
known as riboflavin monophosphate, and FAD (flavin adenine dinucleotide) are coenzymes of vitamin B₂. FAD serves as a coenzyme in the one-carbon metabolism.⁴⁹,⁷¹

**Vitamin B₂ food sources** – In foods, the greatest amount of vitamin B₂ is found in the form of coenzymes FAD and FMN, where they are bound to enzymes. Milk and other dairy products, meat and fish contain large amounts of vitamin B₂, as well as dark green leafy vegetables. As food colour (E-101), vitamin B₂ has another purpose because of its natural strong yellow colour. Vitamin B₂ is lost by irradiation and exposure to ultraviolet light, which is why milk is stored in opaque plastic or cardboard containers. During cooking, vitamin B₂ is preserved because of its stableness to heat.⁴⁹,⁷¹

**Vitamin B₂ deficiency and recommendations** – A vitamin B₂ deficiency is quite common, although there is no clear defined disease or syndrome as a result of a riboflavin deficiency. In most cases, a vitamin B₂ deficiency chaperones other nutrient deficiencies. For example, a riboflavin deficiency can also result in a deficiency of vitamin B₆. During deficiency, vitamin B₂ is very effectively stored and recycled in the body. Consequently, a vitamin B₂ deficiency never leads to fatal health complications.⁴⁹,⁷¹ The Dutch guideline recommends for every male who is 19 years and older an intake of 1.5 mg vitamin B₂ daily. For females of 19 years and older, this recommendation is set at 1.1 mg/day.⁷³

### 2.4.3 Vitamin B₆

**The function of vitamin B₆** – The water-soluble vitamin B₆ appears in three forms, namely pyridoxal, pyridoxine and pyridoxamine. Vitamin B₆ serves a main role in the amino acid metabolism, but is also involved in the modulation of steroid hormone action and the regulation of gene expression. Similar to vitamin B₂, vitamin B₆ also acts as a cofactor in the energy-yielding metabolism. The three forms of vitamin B₆ can be converted to the vitamin’s coenzyme PLP (pyridoxal phosphate), which is the active form of vitamin B₆ and has numerous roles of its own. Through its ability to transfer amino groups, PLP makes it possible to create nonessential amino acids. Furthermore, the synthesis of DNA and RNA is PLP dependent. Vitamin B₆ is, unlike other water-soluble vitamins, primarily stored in the muscle tissues of the body.⁴⁹,⁷¹

**Vitamin B₆ food sources** – All three forms of vitamin B₆ have equal bioavailability. However, about 75% of this vitamin is present as glycosides in plant derived foods and is limited in its availability. Foods such as meat, fish, poultry and dairy products are rich
sources of vitamin B$_6$. Green vegetables and purple fruits also contain significant amounts. Nevertheless, a large part of the vitamin is lost during heating.$^{49,71}$

_Vitamin B$_6$ deficiency and recommendations_ – A vitamin B$_6$ deficiency is relatively uncommon, mainly because this vitamin appears in a broad variety of foods. Although most water-soluble vitamins are thought to be harmless, very high doses of vitamin B$_6$ supplements can cause neurological damage.$^{47,69}$ The Dutch guideline for vitamin B$_6$ intake is set at 1.5 mg/day for males as well as for females aged 19 and older.$^{74}$

2.4.4 Vitamin B$_{12}$

_The function of vitamin B$_{12}$_ – Vitamin B$_{12}$ houses the common name for cobalamins. The synthetic form, namely cyanocobalamin, is used in supplements and food fortification, because this form of the vitamin is chemically the most air-stable. This water-soluble vitamin is found mainly in foods as 5’-deoxy-5’adenosine (adenosylcobalamin) and hydroxocobalamin.$^{49,71,75,74}$ Vitamin B$_{12}$ plays an important role in the regeneration of the essential amino acid methionine and the synthesis of DNA and RNA. Furthermore, a special role of vitamin B$_{12}$ lies with maintaining the protective sheath that surrounds the nerve fibres and stimulates their normal growth.$^{49,71}$

_Vitamin B$_{12}$ food sources_ – Vitamin B$_{12}$ is unique because this vitamin is only found in foods that are derived from animals. Rich sources are meat, fish and milk. People who adapt a vegan diet and eliminate all foods that originate from animals, should therefore consume another reliable source of vitamin B$_{12}$ such as fortified soymilk or a vitamin B$_{12}$ supplement. As most other water-soluble B-vitamins, vitamin B$_{12}$ is vulnerable during cooking.

_Vitamin B$_{12}$ deficiency and recommendations_ – A normal western diet is rarely associated with a vitamin B$_{12}$ deficiency. Besides insufficient intake, the main cause of a vitamin B$_{12}$ deficiency is disturbed absorption. The lack of hydrochloric acid or a lack of intrinsic factor is the reason that inadequate absorption occurs. The symptoms of a vitamin B$_{12}$ deficiency seem to proceed only after a period of several years. The Dutch daily recommendation of vitamin B$_{12}$ intake for male and females is 2.8 µg.$^{49,71,75,74}$
2.5 Scientific evidence

2.5.1 Folate and colorectal adenoma risk

Of the vitamins involved in the one-carbon metabolism, most studies have investigated the relationship between folate and the risk of colorectal neoplasia. Clinical and epidemiological evidence suggest that folate deficiency might be a risk factor that predisposes to the development of neoplasia. Low dietary folate, either from foods or supplements, is associated consistently with a lower recurrence of colorectal adenoma. In some studies, a folate deficient diet is thought to increase the risk of colonic neoplasia, while folate supplementation may be chemopreventive. Inverse association between folate status and colorectal neoplasia has also been shown. The mechanism responsible for these findings has not been completely clarified yet, but the association of folate and colorectal neoplasia may be a consequence of aberrations in methylation, synthesis and repair of DNA.

A recent meta-analysis that included five randomised placebo-controlled trials showed that folic acid supplementation had no potential benefits in preventing recurrence of colorectal adenomas. The doses of folic acid supplementation used in the involved trials varied from 0.5 mg/day to 5 mg/day. However, two trials that used doses of 1 mg/day showed a significant greater risk of adenoma recurrence in the placebo group, with an overall odds ratio of 0.62 (95% CI; 0.48, 0.80; \(P<0.001\)). However, the overall effect of all involved studies was non-significant, with an odds ratio of 0.78 (95% CI; 0.49, 1.24; \(P=0.30\)). Two more intervention trials, not included in this meta-analysis, were conducted in order to investigate the effect of folic acid supplementation on colorectal adenoma risk. Both used 1 mg/day of folic acid and found no significant effect. While interventional studies, overall, failed to report a positive effect of folic acid supplementation on the risk of colorectal adenoma, nevertheless, epidemiological studies showed an inverse association of dietary folate intake and the risk of colorectal adenoma. Four case-control studies reported an average reduction of 35% in the risk of developing colorectal adenoma in subjects with the highest dietary folate compared with those with the lowest intake. Two prospective studies also showed that dietary folate intake has an inverse association with the risk of colorectal adenoma. In one of the two studies, folate only from food sources was less likely to be associated with colorectal adenoma than supplementary folate, while
the other study reported that folic acid supplementation was not associated with a reduced risk of colorectal adenoma.\textsuperscript{41}

However, the chemopreventive role of folate must be applied with prudence since timing and doses of folic acid supplementation seem to be crucial in providing safe and effective chemoprevention. Namely, several animal studies reported that high doses of folic acid supplementation stimulate the progression of established pre-malignant and malignant lesions.\textsuperscript{69, 80, 84, 85} With the latter finding in mind, anti-folate agents have been involved in chemotherapy. This application of anti-folate agents rests on the theory that folate is essential for the biosynthesis of purine and thymidylate and therefore, plays an important role in DNA synthesis and replication. Consequently, it is expected that an interrupted folate metabolism in neoplastic cells would result in inhibition of DNA synthesis and therefore leading to tumour growth suppression.\textsuperscript{8, 65, 86}

2.5.2 Other corresponding B-vitamins and colorectal adenoma risk

As mentioned earlier, the other B-vitamins, B\textsubscript{2}, B\textsubscript{6} and B\textsubscript{12} play a role in the one-carbon metabolism, which is important for DNA synthesis and DNA methylation. These vitamins have also been hypothesised to be associated with a reduced risk of colorectal neoplasia. However, a relatively small amount of studies are present to confirm this association.

FAD, a metabolite of vitamin B\textsubscript{2}, functions as cofactor for the enzyme MTHFR, which converts 5,10-methyleneTHF forms into 5-methylTHF, after which it can be converted to THF, the active form of folate to carry a one-carbon unit. Therefore, riboflavin has a main role in the folate metabolism and has been hypothesised to be associated with a reduced risk of colorectal neoplasia. For riboflavin, five studies examined this association, three of them found no association,\textsuperscript{12, 16, 87} one reported a statistically non-significant association,\textsuperscript{88} whereas one showed that riboflavin was inversely associated with colorectal adenoma risk, especially among subjects with the MTHFR TT genotype or a relatively higher dietary folate intake.\textsuperscript{13}

Vitamin B\textsubscript{6} functions as coenzyme in the synthesis of purines and thymidylate for DNA. Deficiency of this vitamin may lead to misincorporation of uracil into DNA, which eventually leads to a lower production of DNA and disruption of DNA repair.\textsuperscript{89} Namely, uracil is needed to produce thymine, which protects DNA and enhance the efficiency of DNA replication.\textsuperscript{90, 91} Recently, vitamin B\textsubscript{6} has been hypothesised to be preventive for cancer development via other mechanisms, i.e., suppression of cell proliferation, oxidative stress,
nitric oxide synthesis, and angiogenesis.\textsuperscript{92} Regarding studies focused on the association between dietary B\textsubscript{6} and the development of colorectal adenomas, six observational studies\textsuperscript{11, 13, 16, 87, 88, 93} and one randomised trial were carried out.\textsuperscript{12} Four of these studies found a statistically significant inverse association,\textsuperscript{11, 16, 87, 88} between B6 and colorectal adenoma risk, one found a suggestive but not significant inverse (P-trend=0.08) association\textsuperscript{93} and two found null associations.\textsuperscript{13} Only one of these studies concerned adenoma recurrence.\textsuperscript{11} Two studies concerning plasma B\textsubscript{6} and the risk of colorectal adenoma were done, both showed a borderline non-significant inverse association (P-trend=0.08) with the risk of colorectal adenoma.\textsuperscript{12, 93} In one of these studies, the protective effect of plasma PLP, the main active form of vitamin B\textsubscript{6}, was present only among subjects who did not drink alcohol.\textsuperscript{12}

In comparison to other B-vitamins involved in the one-carbon metabolism, the association between vitamin B\textsubscript{12} and the risk of colorectal adenoma remains less clear. Evidence that support this association is rare, of the five observational studies investigating this,\textsuperscript{11-13, 16, 88} only one succeeded to show an association between plasma vitamin B\textsubscript{12} and the risk of adenoma recurrence. However, even this was a statistically non-significant association.\textsuperscript{13}
3.0 Methods

3.1 Subjects and population
The participants of this study are enrollees of the case group of the POLIEP study, a Dutch case-control study designed to investigate the associations between dietary intake of folate, riboflavin, MTHFR C677T genotype and colorectal adenoma risk. The POLIEP study recruited cases and controls through endoscopy in the outpatient clinics of ten hospitals in the Netherlands between 1997 and 2002. Inclusion criteria for the subjects were Dutch speaking, from European origin and in the range of 18 – 75 years at the time of endoscopy. Exclusion criteria were hereditary forms of colorectal cancer (i.e., hereditary nonpolyposis or familial adenomatous polyposis colorectal cancer), history of chronic inflammatory bowel disease or colorectal cancer and bowel resection. The analysis consisted of 768 cases and 709 controls. The overall design and results of this study has been previously reported. To determine the prognostic value of clinical and lifestyle factors in adenoma recurrence the POLIEP follow-up study was designed. This study consisted of 683 cases that were being followed prospectively and were selected from those enrollees of the study population (case group) where food-frequency questionnaire (FFQ) data as well as follow up medical information is present. All cases underwent surgery where adenomatous polyps were removed. A colorectal adenoma recurrence was determined when an adenoma was discovered where existing adenomas had been removed previously during repeat colonoscopies.

3.2 Data collection: Questionnaires and medical information
For the POLIEP study, participants filled out a self-administered semi-quantitative questionnaire on diet. The FFQ, which was originally developed for the Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC), was used to assess dietary intake. Questions assessed the average consumption of 178 food items over the past month. The selection of these food items was based on the Dutch National Food Consumption Survey 1987-1988 dataset, the chosen food items covered at least 90% of the population mean intake of the food groups and nutrients. Consumption frequency for main food items was categorised in times per day, per week, per year, or never. The FFQ also contains additional questions about preparation methods, the use of sub items or additions. This could be indicated by always/mostly, often, sometimes and seldom/never. Coloured
photographs were added to several questions in order to assist subjects in indicating portion size. Furthermore, a detailed explanation about the questions could be found in the appendix of the questionnaire. Although the Dutch EPIC Food Frequency Questionnaire has not been specifically validated for B-vitamin intake, reproducibility and relative validity of this FFQ has been determined for intake assessment of food groups that provide these vitamins. Administering the questionnaires three times at 6-month intervals assessed the reproducibility, whereas a 24-hour recall method was used as a reference to define the relative validity. The relative validity, expressed as a Spearman correlation coefficient, for the food groups fish and vegetables is relative low (0.32 and 0.38 for men, and for women 0.37 and 0.31 respectively). However, in general, the questionnaire seems to be a competent method to assess dietary intake of food groups.

An additional questionnaire was used to gather information about socio-demographic factors (e.g., gender and age), lifestyle factors (e.g., smoking history and physical activity), information about adenoma characteristics and other medical history data (e.g., history of colorectal disorders and family history of colorectal cancer).

Both energy and B-vitamin (B$_2$, B$_6$, B$_{12}$) intake was calculated using the Dutch composition table (NEVO table 2001). Folate intake was calculated using a recently updated article on folate consumption.

Follow-up medical information was obtained from gastroenterology and pathology reports. These reports were collected in nine of the ten hospitals. Subjects were followed from baseline (which was in 1997–2002) to 2008. The follow-up time of the study population (n=683) is 53172 person-months.

3.3 Statistical analysis

Tertile cutoff points were used to designate low (lowest tertile), medium (middle tertile) and high (highest tertile) dietary intake of folate and other involving B-vitamins (i.e., B$_2$, B$_6$, B$_{12}$), based on the distribution of the total study population. The distribution of baseline demographic factors and risk factors of colorectal adenomas were compared between intake tertiles. The means of the variables age, BMI, and all nutrients were compared, except alcohol. Alcohol intake was compared using the median, the 25th and 75th percentile given that the distribution was left-skewed. Non-dietary variables and gender were compared by using percentages. Physical activity was dichotomised by the median of the physical activity score, in the categories ‘low’ and ‘high’. Educational level was categorised in low, medium
and high, which were defined as ≤LBO (Lower Vocational Education), MBO (Intermediate Vocational Education) and ≥HBO (Higher Vocational Education) respectively. Family history of colorectal cancer was considered ‘yes’ when at least one first-degree relative had a history of colorectal cancer. Analysis of the association between B-vitamin intake and the incidence of colorectal recurrence was carried out using the chi-squared test. This test was two-sided, and a P-value less than 0.05 was considered significant. Again, tertile cut points were used to divide dietary B-vitamin intake into low, medium and high. Statistical analyses were conducted using the Statistical Package for Social Science (SPSS), version 16.0.
4.0 Results

The baseline characteristics of the study population are summarised in table 1.1, 1.2, 1.3, 1.4, stratified by dietary intake of folate, vitamin B₂, B₆ and B₁₂ respectively. The study population consisted of more males (n= 361). The overall mean age was 59.1 (SD=10.1). The average BMI of the total population was 26.2 kg/m² (SD=3.8 kg/m²), while the international reference for healthy bodyweight is a BMI of ≥18.5 and <25 kg/m². Mean dietary folate, fibre and saturated fat intake were 200 µg (SD=61 µg), 24 g (SD=7) and 35 g (SD=13) respectively. Furthermore, the mean intake of saturated fat was 15 energy%. The median intake of alcohol was 8.1 g/day (1st quartile 0.8 g/day, 3rd quartile 23.4 g/day. Low and high physical activity is equally divided (50.1% low and 49.9% high). 60.8% of the study population reported to have a smoking history. 23.8% were high educated, which is considered tertiary education. At last, 23.4% had a family history of colorectal cancer. In all four baseline characteristics tables, the high intake groups consisted of fewer females compared with the low intake group, and were likely to be younger. All low intake groups had a lower mean BMI than the high intake groups. In all four tables, the low intake group had also the lowest intake of all nutrients; the medium intake group followed the trend of increasing intake of these nutrients, whereas the high intake group had the highest intake of all nutrients. Except for the alcohol intake in table 1.2, this was higher in the medium intake group than the high intake group. More subjects with a low physical activity occurred in all low intake groups, except for table 1.2 (low intake group 50.2%, high intake group 49.5%). Regarding to smoking history, less people reported to have a smoking history in the low intake group, except for table 1.2 (low intake group 63.6%, high intake group 58.8%). Although not significant, subjects who were higher educated tended to have a higher intake of B-vitamins. The occurrence of family history of colorectal cancer is less in the low intake group in table 1.2 and 1.4. Contrary to these tables, a higher occurrence was found in the low intake group in table 1.1 and 1.3.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

Table 1.1 Characteristics of the study population at baseline, stratified by dietary folate intake (low, medium, high)

<table>
<thead>
<tr>
<th>Demographic factors</th>
<th>n=683</th>
<th>Dietary folate intake (tertiles)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low (n=229)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>47.1</td>
<td>56.8</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>59.1 ±10.1</td>
<td>59.7 ±9.6</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>26.2 ±3.8</td>
<td>25.8 ±3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dietary Intake</th>
<th></th>
<th>Low (n=229)</th>
<th>Medium (n=227)</th>
<th>High (n=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/day)*</td>
<td>8691 ±2517</td>
<td>6958 ±1582</td>
<td>8458 ±1554</td>
<td>10673 ±2672</td>
</tr>
<tr>
<td>Total fat (g/day) *</td>
<td>82 ±30</td>
<td>65 ±19</td>
<td>79 ±21</td>
<td>103 ±34</td>
</tr>
<tr>
<td>Saturated fat (g/day) *</td>
<td>35 ±13</td>
<td>30 ±9</td>
<td>33 ±9</td>
<td>43 ±16</td>
</tr>
<tr>
<td>Alcohol (g/day) †</td>
<td>8.1 (0.8;23.4)</td>
<td>6.0 (0.4;21.6)</td>
<td>9.2 (0.7;20.2)</td>
<td>10.1 (1.4;27.3)</td>
</tr>
<tr>
<td>Fibre (g/day)*</td>
<td>24 ±7</td>
<td>18 ±5</td>
<td>24 ±5</td>
<td>29 ±6</td>
</tr>
<tr>
<td>Vitamin B₉ (mg/d) *</td>
<td>1.6 ±0.6</td>
<td>1.3 ±0.4</td>
<td>1.6 ±0.4</td>
<td>2.0 ±0.6</td>
</tr>
<tr>
<td>Vitamin B₆ (mg/d) *</td>
<td>1.7 ±0.5</td>
<td>1.3 ±0.3</td>
<td>1.6 ±0.3</td>
<td>2.1 ±0.5</td>
</tr>
<tr>
<td>Folate (µg/d) *</td>
<td>200 ±61</td>
<td>143 ±24</td>
<td>193 ±13</td>
<td>265 ±55</td>
</tr>
<tr>
<td>Vitamin B₁₂ (µg/d) *</td>
<td>4.8 ±2.7</td>
<td>3.6 ±1.2</td>
<td>4.4 ±1.3</td>
<td>6.5 ±3.7</td>
</tr>
<tr>
<td>Calcium (mg/day) *</td>
<td>1086 ±430</td>
<td>882 ±289</td>
<td>1086 ±310</td>
<td>1292 ±541</td>
</tr>
<tr>
<td>Supplementary B-vitamin use (% yes)</td>
<td>6.6</td>
<td>9.2</td>
<td>4.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Supplementary multivitamin use (% yes)</td>
<td>17.7</td>
<td>16.6</td>
<td>16.3</td>
<td>20.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-dietary factors</th>
<th></th>
<th>Low (n=229)</th>
<th>Medium (n=227)</th>
<th>High (n=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity (% low)</td>
<td>50.1</td>
<td>56.7</td>
<td>50.0</td>
<td>59.5</td>
</tr>
<tr>
<td>Smoking history (% ever)</td>
<td>60.8</td>
<td>60.7</td>
<td>55.1</td>
<td>66.5</td>
</tr>
<tr>
<td>Educational level (% high)</td>
<td>23.8</td>
<td>21.8</td>
<td>25.0</td>
<td>24.5</td>
</tr>
<tr>
<td>Family history of CRC (% yes)</td>
<td>23.4</td>
<td>23.4</td>
<td>24.7</td>
<td>21.9</td>
</tr>
</tbody>
</table>

* Mean ± SD
† Median (25th percentile; 75th percentile)
‡ Cut points for tertiles of daily dietary intake: low < 171 µg/day; medium 172-218 µg/day; high > 218 µg/day
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

Table 1.2 Characteristics of the study population at baseline, stratified by dietary vitamin B<sub>2</sub> intake (low, medium, high)

<table>
<thead>
<tr>
<th>Demographic factors</th>
<th>n=683</th>
<th>Dietary vitamin B&lt;sub&gt;2&lt;/sub&gt; intake (tertiles)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low (n=228)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>47.1</td>
<td>53.3</td>
</tr>
<tr>
<td>Age (years) *</td>
<td>59.1 ±10.1</td>
<td>59.8 ±9.5</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;) *</td>
<td>26.2 ±3.8</td>
<td>25.8 ±3.8</td>
</tr>
<tr>
<td>Dietary Intake</td>
<td></td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Energy (kJ/day) *</td>
<td>8691 ±2517</td>
<td>7120 ±1706</td>
</tr>
<tr>
<td>Total fat (g/day) *</td>
<td>82 ±30</td>
<td>66 ±19</td>
</tr>
<tr>
<td>Saturated fat (g/day) *</td>
<td>35 ±13</td>
<td>27 ±8</td>
</tr>
<tr>
<td>Alcohol (g/day) ◊</td>
<td>8.1 (0.8;23.4)</td>
<td>8.4 (0.4;24.5)</td>
</tr>
<tr>
<td>Fibre (g/day) *</td>
<td>24 ±7</td>
<td>20 ±6</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt; (mg/d) *</td>
<td>1.6 ±0.6</td>
<td>1.1 ±0.2</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt; (mg/d) *</td>
<td>1.7 ±0.5</td>
<td>1.4 ±0.4</td>
</tr>
<tr>
<td>Folate (µg/d) *</td>
<td>200 ±61</td>
<td>162 ±40</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (µg/d) *</td>
<td>4.8 ±2.7</td>
<td>3.3 ±1.2</td>
</tr>
<tr>
<td>Calcium (mg/dag) *</td>
<td>1086 ±430</td>
<td>760 ±224</td>
</tr>
<tr>
<td>Supplementary B-vitamin use (% yes)</td>
<td>6.6</td>
<td>8.8</td>
</tr>
<tr>
<td>Supplementary multivitamin use (% yes)</td>
<td>17.7</td>
<td>19.7</td>
</tr>
</tbody>
</table>

| Non-dietary factors                      |       |-------------------------------------------------|
| Physical activity (% low)                | 50.1  | 50.2        | 50.0           | 49.5         |
| Smoking history (% ever)                 | 60.8  | 63.6        | 59.9           | 58.8         |
| Educational level (% high)               | 23.8  | 22.2        | 20.4           | 28.3         |
| Family history of CRC (% yes)            | 23.4  | 20.5        | 26             | 23.4         |

* Mean ± SD

◊ Median (25th percentile; 75th percentile)

† Cut points for tertiles of daily dietary intake: low <1.3 mg/day; medium 1.4-1.8 mg/day; high >1.8 mg/day
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

Table 1.3 Characteristics of the study population at baseline, stratified by dietary vitamin B<sub>6</sub> intake (low, medium, high)

<table>
<thead>
<tr>
<th></th>
<th>Dietary vitamin B&lt;sub&gt;6&lt;/sub&gt; intake (tertiles)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=683</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Demographic factors</strong></td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>47.1</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>59.1 ±10.1</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)*</td>
<td>26.2 ±3.8</td>
</tr>
<tr>
<td><strong>Dietary Intake</strong></td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/day)*</td>
<td>8691 ±2517</td>
</tr>
<tr>
<td>Total fat (g/day) *</td>
<td>82 ±30</td>
</tr>
<tr>
<td>Saturated fat (g/day) *</td>
<td>35 ±13</td>
</tr>
<tr>
<td>Alcohol (g/day) ◊</td>
<td>8.1 (0.8;23.4)</td>
</tr>
<tr>
<td>Fibre (g/day) *</td>
<td>24 ±7</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt; (mg/d) *</td>
<td>1.6 ±0.6</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt; (mg/d) *</td>
<td>1.7 ±0.5</td>
</tr>
<tr>
<td>Folate (µg/d) *</td>
<td>200 ±61</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (µg/d) *</td>
<td>4.8 ±2.7</td>
</tr>
<tr>
<td>Calcium (mg/dag) *</td>
<td>1086 ±430</td>
</tr>
<tr>
<td>Supplementary B-vitamin use (% yes)</td>
<td>6.6</td>
</tr>
<tr>
<td>Supplementary multivitamin use (% yes)</td>
<td>17.7</td>
</tr>
<tr>
<td><strong>Non-dietary factors</strong></td>
<td></td>
</tr>
<tr>
<td>Physical activity (% low)</td>
<td>50.1</td>
</tr>
<tr>
<td>Smoking history (% ever)</td>
<td>60.8</td>
</tr>
<tr>
<td>Educational level (% high)</td>
<td>23.8</td>
</tr>
<tr>
<td>Family history of CRC (% yes)</td>
<td>23.4</td>
</tr>
</tbody>
</table>

* Mean ± SD

◊ Median (25th percentile; 75th percentile)
† Cut points for tertiles of daily dietary intake: low <1.4 mg/day; medium 1.5-1.8 mg/day; high>1.8 mg/day
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

Table 1.4 Characteristics of the study population at baseline, stratified by dietary vitamin B<sub>12</sub> intake (low, medium, high)

<table>
<thead>
<tr>
<th>Demographic factors</th>
<th>n=683</th>
<th>Dietary vitamin B&lt;sub&gt;12&lt;/sub&gt; intake (tertiles)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n=228)</td>
<td>Medium (n=228)</td>
</tr>
<tr>
<td>Female (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.1</td>
<td>61.0</td>
<td>47.4</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>59.1 ±10.1</td>
<td>59.3 ±9.9</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>26.2 ±3.8</td>
<td>25.8 ±3.7</td>
</tr>
</tbody>
</table>

| Dietary Intake                      |       |                                 |             |
| Energy (kJ/day)*                    | 8691 ±2517 | 7263 ±1843 | 8649 ±1952 | 10167 ±2763 |
| Total fat (g/day) *                 | 82 ±30 | 66 ±22 | 81 ±22 | 99 ±35 |
| Saturated fat (g/day) *             | 35 ±13 | 27 ±9 | 34 ±9 | 42 ±16 |
| Alcohol (g/day) ◊                   | 8.1 (0.8;23.4) | 4.3 (0.2;20.1) | 9.7 (1.1;21.6) | 12.3 (2.1;26.8) |
| Fibre (g/day) *                     | 24 ±7 | 22 ±6 | 24 ±6 | 25 ±7 |
| Vitamin B<sub>2</sub> (mg/d) *      | 1.6 ±0.6 | 1.2 ±0.3 | 1.6 ±0.3 | 2.0 ±0.6 |
| Vitamin B<sub>6</sub> (mg/d) *      | 1.7 ±0.5 | 1.4 ±0.4 | 1.6 ±0.4 | 1.9 ±0.5 |
| Folate (µg/d) *                     | 200 ±61 | 167 ±44 | 191 ±40 | 242 ±70 |
| Vitamin B<sub>12</sub> (µg/d) *     | 4.8 ±2.7 | 2.9 ±0.7 | 4.4 ±0.4 | 7.2 ±3.3 |
| Calcium (mg/dag) *                  | 1086 ±430 | 865 ±276 | 1092 ±312 | 1302 ±537 |
| Supplementary B-vitamin use (% yes) | 6.6 | 7.9 | 7.9 | 4.0 |
| Supplementary multivitamin use (% yes) | 17.7 | 21.1 | 14.0 | 18.1 |

| Non-dietary factors                 |       |                                 |             |
| Physical activity (% low)           | 50.1  | 49.3                                  | 49.3        | 49.8 |
| Smoking history (% ever)            | 60.8  | 61.4                                  | 57          | 63.9 |
| Educational level (% high)          | 23.8  | 17.2                                  | 23.9        | 29.9 |
| Family history of CRC (% yes)       | 23.4  | 22.4                                  | 22.6        | 25.0 |

* Mean ± SD
○ Median (25th percentile; 75th percentile)
† Cut points for tertiles of daily dietary intake: low <3.7 µg/day; medium 3.8-5.2 µg/day; high >5.2 µg/day

The association between dietary B-vitamin intake and colorectal adenoma recurrence is shown in table 2. Adenoma recurrence in the same low, medium and high intake groups of folate, vitamin B<sub>2</sub>, B<sub>6</sub> and B<sub>12</sub> as in table 1.1, 1.2, 1.3 and 1.4 are shown in percentages. The number of patients per intake group is reported behind the percentages. The distribution of incidence of colorectal adenoma recurrence seems to be normal between the low, medium and high intake group for all considering B-vitamins. No significant association has been found.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

Table 2 Follow-up characteristics of the study population according to B-vitamin intake (N=683, follow-up time= 53172 person-months)

<table>
<thead>
<tr>
<th></th>
<th>Dietary B-vitamin intake (tertiles)†</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n=229)</td>
<td>Medium (n=227)</td>
<td>High (n=227)</td>
<td>P trend</td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>0 adenomas %</td>
<td>0.98</td>
<td>≥ 1 adenomas %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>70.7</td>
<td>71.4</td>
<td>70.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29.3 (229)</td>
<td>28.6</td>
<td>29.5 (227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>69.6 (228)</td>
<td>68.3 (227)</td>
<td>74.4 (228)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.4 (228)</td>
<td>31.7 (227)</td>
<td>25.6 (227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>69.2 (227)</td>
<td>74.6 (228)</td>
<td>68.7 (228)</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.8 (227)</td>
<td>25.4 (227)</td>
<td>31.3 (227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>72.2 (228)</td>
<td>70.2 (228)</td>
<td>70.0 (227)</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.8 (228)</td>
<td>29.8 (228)</td>
<td>30.0 (227)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Cut points for tertiles of daily dietary intake: folate 172 µg/218 µg, vitamin B₂ 1.4 mg/1.7 mg, vitamin B₆: 1.4 mg/1.8 mg, vitamin B₁₂: 3.7 µg/5.2 µg
5.0 Discussion and Conclusion

The results from this follow-up study suggest that dietary intake of folate, vitamin B\(_2\), B\(_6\) and B\(_{12}\) has no association with the recurrence of colorectal adenomas. These results do not support the initial hypothesis that dietary B-vitamin intake derived from foods is associated with the incidence of colorectal adenoma recurrence. Earlier studies have indicated a reduction in the risk of developing colorectal adenomas in subjects with the highest dietary folate intake compared to those with the lowest intake.\(^{14,16,59,77}\) For vitamins B\(_2\), B\(_6\) and B\(_{12}\), fewer studies have confirmed this possible preventive effect.\(^{11,13,16,87,88}\) Moreover, current articles concerning the relation between dietary B-vitamins intake and the recurrence of colorectal adenoma remain scarce. Most of the studies carried out, focused on one B-vitamin in particular, namely folic acid.

The study by Martínez et al.,\(^{11}\) which included folate, vitamin B\(_6\) and B\(_{12}\), reported a lower recurrence of colorectal adenomas in subjects with the highest intake of total folate and total vitamin B\(_6\) in comparison with those with the lowest intake. Furthermore, looking at the dietary folate intake only derived from foods, no significant association was found. These analyses were conducted in a study population of 1014 subjects, whereas the current study consisted of 683 subjects. Calculation of total intake of B-vitamins in this study included supplemental use of B-vitamins as well as folate intake from food sources. A notable difference between the study of Martínez et al. and the current study is that the current study only focused on the intake of B-vitamins derived from food sources. In addition, folate, vitamin B\(_6\) and B\(_{12}\) intake was relatively high in the study of Martínez et al. compared to the intake of the subjects in this present study. An explanation may be that Martínez et al. included supplemental use in their study and the mandatory fortification of folate in the United States of America.\(^{9}\) A remarkable point in this study is the low folate intake in the whole study population, even the highest intake group of folate is below the Dutch daily recommend intake of 300 µg. This is not surprising, considering the matter of the general inadequate intake of folate among the Dutch population.\(^{72}\) Furthermore, looking at the Dutch daily recommended intake of fibre, intake was inadequate. In addition, the mean saturated fat intake of 15 energy% of the study population exceeded the Dutch recommendation of 10 energy% of total energy intake. A positive aspect of this study is the
relative long follow-up time, namely, subjects were followed from baseline (which was in 1997 – 2002) to 2008.

For this study a food-frequency questionnaire is used. Although this type of questionnaires is not the most accurate way to assess dietary intake, especially for dietary folate, they are the most common tool used in large epidemiological studies of diet and health to assess dietary intake of nutrients.\textsuperscript{8, 97} The Dutch EPIC food-frequency questionnaire used in this study showed a weak correlation between dietary folate intake and plasma concentrations of folate, as do most other used food-frequency questionnaires.\textsuperscript{76, 13, 98}

Another limitation for this follow-up study is that the data used for nutrient intake derives from the food-frequency questionnaires administered at baseline. Meanwhile, eating and lifestyle habits of the subjects are susceptible to changes, which subsequently may alter the development of colorectal adenomas.

Furthermore, many studies, concerning the association between B-vitamins and colorectal adenoma risk, looked at potential confounding factors and usually controlled for it. As mentioned earlier, several variables such as age, gender, BMI and cigarette smoking are suspected or established risk factors for colorectal adenomas. Calculation of hazard ratios with adjustments for these covariates is a logical suggestion for further analysis, taking 53712 person-months of follow-up into consideration. Also, these studies commonly look at the possible effect modifiers, like variation in genotype, because of the indications that genetic variation in enzymes involved in the one carbon metabolism may also interfere with DNA methylation and DNA synthesis.\textsuperscript{27} As mentioned earlier, these mechanisms are established to play a crucial role in the development of colorectal neoplasia.\textsuperscript{4, 5} Analysis regarding the relation between B-vitamins intake and the risk of colorectal adenomas stratified by several genotypes might therefore also be interesting for further analysis.

In summary, dietary intake of folate, vitamin B\textsubscript{2}, B\textsubscript{6} and B\textsubscript{12} has not been found to have an association with the incidence of colorectal adenomas recurrence. Further studies concentrating on the relation between dietary B-vitamins from food sources and recurrence of colorectal adenomas are needed to draw a definite conclusion on this topic.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

References


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Appendix: Syntax of the statistical analysis

*This is to get the characteristics for the whole population (N=683) at baseline.
DATASET ACTIVATE DataSet1.
FREQUENCIES VARIABLES=sex FVITBP FMULTIP nfamca EDUC ALC_V active eversig
   /NTILES=4
   /ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V
    FOLIUM_V VITC_V
    VITE_V VITB12_V
   /STATISTICS=MEAN STDDEV MIN MAX.

*This is to define cutpoints for tertiles for intake of vitamin B2, B6, B12 and folate.
FREQUENCIES VARIABLES=VITB2_V VITB6_V VITB12_V FOLIUM_V
   /NTILES=3
   /STATISTICS=SKEWNESS SESKEW
   /ORDER=ANALYSIS.

*This is to make a new variable for vitamin B2 intake categorised in low, medium, high (by tertiles).
RECODE VITB2_V (Lowest thru 1.3577=0) (1.3578 thru 1.7472=1) (1.7473 thru Highest=2)
INTO B2_cat.
VARIABLE LABELS  B2_cat 'Vitamin B2 intake in tertiles'.
EXECUTE.

*This is to make a new variable for vitamin B6 intake categorised in low, medium, high (by tertiles).
RECODE VITB6_V (Lowest thru 1.4120=0) (1.4121 thru 1.7838=1) (1.7839 thru Highest=2)
INTO B6_cat.
VARIABLE LABELS  B6_cat 'Vitamin B6 intake n tertiles'.
EXECUTE.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

*This is to make a new variable for vitamin B12 intake categorised in low, medium, high (by tertiles).
RECODE VITB12_V (Lowest thru 3.7076=0) (3.7077 thru 5.2108=1) (5.2109 thru Highest=2) INTO B12_cat.
VARIABLE LABELS B12_cat 'Vitamin B12 intake in tertiles'.
EXECUTE.

*This is to make a new variable for folate intake categorised in low, medium, high (by tertiles).
RECODE FOLIU_M_V (Lowest thru 171.967=0) (171.968 thru 218.2550=1) (218.2551 thru Highest=2) INTO Folate_cat.
VARIABLE LABELS Folate_cat 'Folate intake in tertiles'.
EXECUTE.

*This is to get the characteristics for the LOW folate intake group at baseline.
USE ALL.
COMPUTE filter_$=(Folate_cat = 0).
VARIABLE LABEL filter_$ 'Folate_cat = 0 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V /NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the MEDIUM folate intake group at baseline.
USE ALL.
COMPUTE filter_\$_=(Folate_cat = 1).
VARIABLE LABEL filter_\$ 'Folate_cat = 1 (FILTER)'.
VALUE LABELS filter_\$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_\$ (f1.0).
FILTER BY filter_\$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDE

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V
VITC_V VITE_V

VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the HIGH folate intake group at baseline.
USE ALL.
COMPUTE filter_\$_=(Folate_cat = 2).
VARIABLE LABEL filter_\$ 'Folate_cat = 2 (FILTER)'.
VALUE LABELS filter_\$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_\$ (f1.0).
FILTER BY filter_\$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
          
DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
          
*This is to get the characteristics for the LOW vitamin B2 intake group at baseline.
USE ALL.

COMPUTE filter_$=(B2_cat = 0).

COMPUTE filter_$=(B2_cat = 1).

VARIABLE LABEL filter_$ 'B2_cat = 0 (FILTER)'.

VARIABLE LABEL filter_$ 'B2_cat = 1 (FILTER)'.

VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.

VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.

FORMAT filter_$ (f1.0).

FILTER BY filter_$.

EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
          
DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
          
DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
          
*This is to get the characteristics for the MEDIUM vitamin B2 intake group at baseline.
USE ALL.

COMPUTE filter_$=(B2_cat = 0).

COMPUTE filter_$=(B2_cat = 1).

VARIABLE LABEL filter_$ 'B2_cat = 0 (FILTER)'.

VARIABLE LABEL filter_$ 'B2_cat = 1 (FILTER)'.

VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.

VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.

FORMAT filter_$ (f1.0).

FILTER BY filter_$.

EXECUTE.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V /NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V /STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the HIGH vitamin B2 intake group at baseline.
USE ALL.
COMPUTE filter_$=(B2_cat = 2).
VARIABLE LABEL filter_$ 'B2_cat = 2 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V /NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the LOW vitamin B6 intake group at baseline.
USE ALL.
COMPUTE filter_Š=(B6_cat = 0).
VARIABLE LABEL filter_Š 'B6_cat = 0 (FILTER)'.
VALUE LABELS filter_Š 0 'Not Selected' 1 'Selected'.
FORMAT filter_Š (f1.0).
FILTER BY filter_Š.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
   VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the MEDIUM vitamin B6 intake group at baseline.
USE ALL.
COMPUTE filter_Š=(B6_cat = 1).
VARIABLE LABEL filter_Š 'B6_cat = 1 (FILTER)'.
VALUE LABELS filter_Š 0 'Not Selected' 1 'Selected'.
FORMAT filter_Š (f1.0).
FILTER BY filter_Š.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the HIGH vitamin B6 intake group at baseline.
USE ALL.
COMPUTE filter_$=(B6_cat = 2).
VARIABLE LABEL filter_$ 'B6_cat = 2 (FILTER)'.
VALUE LABELS filter_$_ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$_ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex everS Ig active nfamca EDUC FVITBP FMULTIP ALC_V
/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V
VITC_V VITE_V VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the LOW vitamin B12 intake group at baseline.
USE ALL.
COMPUTE filter_$_=(B12_cat = 0).
VARIABLE LABEL filter_$_ 'B12_cat = 0 (FILTER)'.

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The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V
VITC_V VITE_V
VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the MEDIUM vitamin B12 intake group at baseline.
USE ALL.
COMPUTE filter_$=(B12_cat = 1).
VARIABLE LABEL filter_$ 'B12_cat = 1 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V
VITC_V VITE_V
VITB12_V FOLIUM_V

46
/STATISTICS=MEAN STDDEV MIN MAX.

*This is to get the characteristics for the HIGH vitamin B12 intake group at baseline.

USE ALL.

COMPUTE filter_$=(B12_cat = 2).
VARIABLE LABEL filter_$ 'B12_cat = 2 (FILTER)'.
VALUE LABELS filter_$ 0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE.

FREQUENCIES VARIABLES=sex eversig active nfamca EDUC FVITBP FMULTIP ALC_V
/NTILES=4
/STATISTICS=SKEWNESS SESKEW
/ORDER=ANALYSIS.

DESCRIPTIVES VARIABLES=age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
  VITB12_V FOLIUM_V
/STATISTICS=MEAN STDDEV MIN MAX.

FILTER OFF.
USE ALL.
EXECUTE.

*This is to compare the characteristics at baseline between the low, medium and high folate intake group.

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V
  VITB12_V FOLIUM_V
  BY Folate_cat
/MISSING ANALYSIS.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
  BY Folate_cat
  /MISSING ANALYSIS
  /POSTHOC=BONFERRONI ALPHA(0.05).

RECODE nfamca (Lowest thru 0=0) (0.1 thru Highest=1) INTO fam_cat.
VARIABLE LABELS  fam_cat 'family history of CRC yes or no'.
EXECUTE.

RECODE active (Lowest thru 8.0592=0) (8.0593 thru Highest=1) INTO active_cat.
VARIABLE LABELS  active_cat 'physical activity low/high'.
EXECUTE.

RECODE FVITBP (Lowest thru 0=0) (0.001 thru Highest=1) INTO vitbsup_cat.
VARIABLE LABELS  vitbsup_cat 'vitamin b supplement yes/no'.
EXECUTE.

RECODE FMULTI (Lowest thru 0=0) (0.001 thru Highest=1) INTO multi_cat.
VARIABLE LABELS  multi_cat 'multivitamin supplement yes/no'.
EXECUTE.

CROSSTABS
/TABLES=Folate_cat BY sex EDUC fam_cat active_cat vitbsup_cat multi_cat eversig
/FORMAT=AVALUE TABLES
/STATISTICS=CHISQ
/CELLS=COUNT EXPECTED ROW COLUMN TOTAL
/COUNT ROUND CELL.

*This is to compare the characteristics at baseline between the low, medium and high vitamin B2 intake group.
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
   BY B2_cat
   /MISSING ANALYSIS.

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
   BY B2_cat
   /MISSING ANALYSIS
   /POSTHOC=BONFERRONI ALPHA(0.05).

CROSSTABS
   /TABLES=B2_cat BY sex EDUC fam_cat active_cat vitbsup_cat multi_cat eversig
   /FORMAT=AVALUE TABLES
   /STATISTICS=CHISQ
   /CELLS=COUNT EXPECTED ROW COLUMN TOTAL
   /COUNT ROUND CELL.

*This is to compare the characteristics at baseline between the low, medium and high vitamin B6 intake group.

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
   BY B6_cat
   /MISSING ANALYSIS.

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
   BY B6_cat
   /MISSING ANALYSIS
   /POSTHOC=BONFERRONI ALPHA(0.05).

CROSSTABS
The association between dietary intake of B-vitamins and the incidence of colorectal adenoma recurrence

/TABLES=B6_cat BY sex EDUC fam_cat active_cat vitbsup_cat multi_cat eversig
/FORMAT=AValue TABLES
/STATISTICS=CHISQ
/CELLS=COUNT EXPECTED ROW COLUMN TOTAL
/COUNT ROUND CELL.

*This is to compare the characteristics at baseline between the low, medium and high vitamin B12 intake group.

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
   BY B12_cat
   /MISSING ANALYSIS.

ONEWAY age BMI KJ_V FAT_V SF_V FIBRE_V ALC_V CA_V VITB2_V VITB6_V VITC_V VITE_V VITB12_V FOLIUM_V
   BY B12_cat
   /MISSING ANALYSIS
   /POSTHOC=BONFERRONI ALPHA(0.05).

CROSSTABS
/TABLES=B12_cat BY sex EDUC fam_cat active_cat vitbsup_cat multi_cat eversig
/FORMAT=AValue TABLES
/STATISTICS=CHISQ
/CELLS=COUNT EXPECTED ROW COLUMN TOTAL
/COUNT ROUND CELL.

*This is to get the follow-up characteristics of the study population according to B-vitamin intake.

DATASET ACTIVATE DataSet1.
CROSSTABS
/TABLES=recurrencepol BY B6_cat B2_cat B12_cat Folate_cat
/FORMAT=AValue TABLES