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Faculty of Medicine, University of Oslo

Changes in health risk profile after a 5-months dietary intervention focusing on increased intake of fruits, vegetables and whole grain bread among young male adults

Tonje Holte Stea

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Abbreviations

BMI	Body mass index
CVD	Cardiovascular disease
CHD	Coronary heart disease
CI	Confidence Interval
CV	Coefficient of variation
DM	Dry matter
E%	Percentage of energy
FAD	Flavin adenine dinucleotide (coenzyme form of vitamin B2)
FMN	Flavin mononucleotide (coenzyme form of vitamin B2)
FFQ	Food frequency questionnaire
FW	Fresh weight
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
LDL	Low-density lipoprotein
NNR	Nordic nutrition recommendations
p-Cys	Cysteine in plasma
p-tHcy	Total homocysteine in plasma
SES	Socio-economic status
TG	Triacylglycerol
Vitamin B2	Riboflavin
Vitamin B6	Pyridoxine
Vitamin B12	Cobalamin
WHO	World Health Organization

Summary

The consumption of fruits, vegetables and whole grain bread and subsequently the intake of folate among young men is generally low. Low concentrations of folate, vitamin B_2 (riboflavin), vitamin B_6 and vitamin B_{12} have been related to an elevated concentration of total homocysteine in plasma (p-tHcy) which is a well-known risk factor for cardiovascular disease (CVD).

The aim of this project was to investigate the health risk profile of young male adults from different socio-economic groups, and possible changes in this profile after a dietary intervention with focus on an increased intake of fruits, vegetables and whole grain bread. The effect of the dietary intervention was studied with a special focus on changes in dietary intake of B-vitamins and changes in the concentration of p-tHcy and related metabolites. In addition, weight, height and serum lipids were measured before and after the intervention. In order to interpret the data on folate intake in the dietary intervention study, another objective was to study the retention of folates in foods after treatment commonly used in Norwegian military messes.

The dietary intervention study with duration of 5 months included 541 male recruits from the Norwegian National Guard, Værnes and 209 male recruits from the Norwegian Army, Heggelia. A combination of strategies was used in order to increase the intake of fruits, vegetables and whole grain bread during the intervention period, including nutrition information and increased availability of these food items.

The baseline results of the dietary intervention study demonstrated a high prevalence of overweight/obesity (34.8%) and low physical fitness among young male adults (Paper I). The baseline data also showed that a total of 24.8% of the young men had p-tHcy concentration >15 μmol/l (Paper II). On the other hand, serum lipid concentrations were within the normal range among most (98.8%) of the young men participating in the study (Paper I). This study also showed that paternal education was inversely associated with BMI and the concentration of total cholesterol and LDL. In addition, this study showed that fathers with higher education have a positive influence on young male adults' behaviour pattern and subsequently a positive

effect on BMI and lipid profiles. On the other hand, the educational level of the mothers was not related to any of the selected health parameters in young male adults.
An inverse relationship between the running performance and the concentration of triacylglycerol was shown. Furthermore, men with low BMI (both high and low fitness) had a better lipid profile than those with high BMI/low fitness. Men with high BMI/high fitness had a better lipid profile than those with high BMI/low fitness. Our results indicate that young men, especially those with low parental education and high BMI, should be considered as an important target group for health promotion efforts.

The dietary intervention resulted in a significant increase in the total consumption of vegetables, fruits, berries and juice (FV; +24%) and of whole grain bread (+29%) among the young male adults (Paper III). In accordance with these findings, the estimated total intake of dietary fibre and folate, as well as the relative contribution of folate intake from vegetables, fruits and whole grain bread, increased significantly during the study period. The dietary intervention also resulted in a significantly reduction in the concentration of p-tHcy (-10%), cysteine (p-cys; -6%) and flavin mononucleotide (FMN; -11%), and an increased concentration of vitamin B_2 (+23%) and flavin adenine dinucleotide (FAD; +10%). The change in p-tHcy concentration was inversely related to the serum concentration of folate and positively related to the concentration of p-cys and FMN in plasma (Paper II). Further, a significant association was found between an estimated increase in dietary intake of folate from bread and a decrease in p-tHcy concentration (Paper III).

The retention of folates was measured in vegetables after processing methods commonly used in the Norwegian military messes, including boiling, steam boiling, sous-vide, oven-baking, microwaving and blanching of vegetables (Paper IV). Only blanching of peas, boiling of potatoes and oven-baking of unpeeled potatoes caused a significant reduction in folate content. Further, this study showed that there were no significant losses of folates after subsequent storage and reheating of vegetables. When estimating the intake of folate during the intervention period, losses of folate due to blanching of vegetables was taken into consideration. The other methods of processing that caused significant reduction in folate content were rarely used during the intervention period. Thus, it is not likely that the reported intake on folate during the dietary intervention study is overestimated due to losses of folates after processing.

In conclusion, the results from the present study demonstrated that a 5-months dietary intervention with focus on increased intake of vegetables, fruits and whole grain bread had favourable effect on the concentration of p-tHcy and its metabolites.

List of papers

Paper I

BMI, lipid profile, physical fitness and smoking habits of young male adults and the association with parental education.

Stea TH, Wandel M, Mansoor MA, Uglem S, Frølich W.

Eur J Publ Health. 2009;19(1):46-51.

Paper II

Changes in predictors and status of homocysteine in young male adults after a dietary intervention with vegetables, fruits and bread.

Stea TH, Mansoor MA, Wandel M, Uglem S, Frølich W.

Eur J Nutr. 2008;47(4):201-209.

Paper III

Association between folate intake from different food sources in Norway and homocysteine status in a dietary intervention among young male adults.

Stea TH, Wandel M, Uglem S, Mansoor MA, Frølich W.

Br J Nutr. (in press).

Paper IV

Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems.

Stea TH, Johansson M, Jägerstad M, Frølich W.

Food Chem. 2007;101(3):1095-1107.

1. Introduction

1.1 Young adults as a target group for health promotion

Although cardiovascular diseases (CVD) generally manifest themselves in adulthood, risk factors for CVD are present in children and persist into adulthood (1-3). During recent years, the focus on health promotion and disease prevention has increased. Strategies for reduction of modifiable risk factors of CVD have been applied to a variety of population groups. Since dietary patterns formed during early years may have implications for development of certain non-communicable diseases later in life, children and adolescents have become target groups for several health promoting strategies (1, 4). Young adults could also be seen as an important target group, since the period of living away from home for the first time often involves several changes in health-related lifestyle factors (5). Despite the knowledge of this vulnerable stage of the young adults, only few studies have focused on reducing modifiable risk factors in this group. In particular young male adults have been considered a difficult group to reach with conventional dietary information.

1.2 Modifiable risk factors for cardiovascular disease

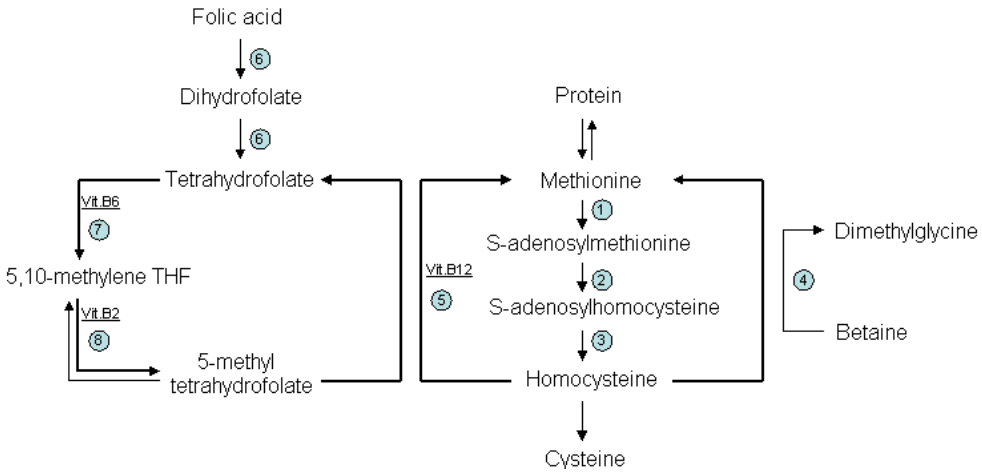
Several studies have shown that the most important modifiable risk factors for CVD include smoking, low physical activity, obesity, unhealthy eating habits, lipideamia and high blood pressure (6-10). Studies also suggest that even moderate elevations of total homocysteine in plasma (p-tHcy) are associated with increased risk of developing CVD (11, 12), inflammation (13) and endothelial dysfunction (14, 15), but the relation between cause and effect remains to be proven (16). Homocysteine is a sulphur-containing amino acid that is mainly derived from dietary methionine (Figure 1-1). Normally, about 50 % of the homocysteine formed is remethylated to methionine. This process requires three key enzymes; methionine synthase (MS), methylenetetrahydrofolate reductase (*MTHFR*), and betaine-homocysteine methyltransferase (BHMT). Folate, vitamin B_2, vitamin B_6 and vitamin B_{12} are involved as cofactors in the remethylation process. Important factors for causing elevations in p-tHcy concentrations in adults are deficiencies of folate, vitamin B_2, vitamin B_6, vitamin B_{12}, or a combination of these B-vitamins (14, 17-19). Several studies have demonstrated that the concentration of p-tHcy is responsive to intake of B-vitamins required for its metabolism, in particular folate and to a lesser extent vitamin B_{12}, vitamin B_6 and vitamin B_2, in that order (14, 17-19). Due to genetic variations, dietary changes may have a differential impact on a

genetically heterogeneous population (20). For example, some individuals have mutations in the *MTHFR* gene which impair the ability to metabolize folate, with an increase in p-tHcy concentration (20%) and higher risk of CVD as a result (21, 22).

Elevated concentrations of p-tHcy have also been positively associated with age, male sex, BMI, blood pressure, cholesterol level, smoking, coffee consumption, and inversely associated with alcohol drinking and exercise (23-27).

The exact role of homocysteine is, however, not yet established. The molecule may just be an indicator of cardiovascular disease rather than actually causing the disease, i.e. a risk determinant (28). Verhof (29) has suggested that an elevated p-tHcy concentration is a marker of an unhealthy diet, which may in fact explain its association with a higher risk of CVD.

Figure 1-1: The role of B-vitamins in homocysteine metabolism



Enzymes: 1 Methionine adenosyltransferase, 2. Methyltransferase, 3. S-adenosylhomocysteine hydrolase, 4. Betaine-homocysteine methyltransferase, 5. Methionine synthase, 6. Dihydrofolate reductase, 7. Serine hydroxy methyltransferase, 8. 5,10-methylene tetrahydrofolate reductase.

1.3 Protective effects of vegetables, fruits and cereals

Population studies have shown a significant association between high intake of fruits and vegetables and low prevalence of different types of cancer (30-32) and cardiovascular diseases (33-35).

Cereals are the best source of dietary fibres and several bioactive components. An increased intake of whole grain cereals seems to prevent the development of some types of cancer, including colon and rectal cancer (36, 37) and cancer in the prostate (38, 39). Furthermore, a study by Soler *et al.* (40) indicated that an increase in dietary fibre may have a protective role for oral, pharyngeal and oesophageal cancer. Whole grain products have also been shown to be protective against cardiovascular diseases (41-43).

However, the second expert report from the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research concluded that very few specific foods or drinks have convincingly shown to increase or reduce the risk of cancer (44). In addition to study specific food items, it is important to consider the total diet of individuals as it consists of many different foods, nutrients and chemicals that affect their risk of both cancer and CVD. A network of biological active substances may work in concert.

The combination of a high intake of whole grain cereals, fresh fruit, olive oil, mushrooms, cruciferous vegetables, wine, and nuts with a low intake of fried potatoes has been associated with a favourable biomarker profile of homocysteine metabolism and reduced risk of CVD (45). Furthermore, a high intake of vegetables, fruits and whole grain products has been related to weight loss and better weight maintenance as these food components may replace food with high fat content in the diet (46). Follow-up studies have shown that a food pattern associated with a stable weight was inversely related to the risk of diabetes type 2 and fatal CVD (47, 48).

1.3.1 Nutritional Recommendations and dietary habits in Norway

In 1975 the first national food and nutrition policy was adopted: Report to the Storting no. 32 (1975-76) *On Norwegian Nutrition and Food policy* (revised in 1982 and in 1992) (49). The nutrition policies and strategies that have been implemented, especially in the 1980s, have resulted in healthier food habits and reduced incidence of CVD (50). Important positive changes in dietary intake during the period 1975-2005 include a reduced intake of saturated fats and trans-fats and an increased consumption of fruits and vegetables (51). However, the consumption of cereal products, including bread, has been reduced during this period of time

(51). The Norwegian Directorate of Health (52) has recommended a dietary intake of minimum 3 portions of vegetables and potatoes and 2 portions of fruits and berries (including juice). For adults, the recommended intake corresponds to a total of 750 grams of fruits and vegetables, including potatoes, per day. In addition, bread and cereals should preferably be whole grain according to the Norwegian dietary recommendations (52). However, studies have shown that the diet of Norwegian children, adolescents and adults still consists of too much saturated fats and too little whole grain bread, potatoes, vegetables and fruits compared with the national dietary recommendations (53, 54). In 2000 and 2001, a nationwide dietary survey (UNGKOST-2000) showed an average intake of 250 grams of fruits and vegetables, including potatoes, per day among students in the fourth and eighth grades (53). Another national survey, Norkost, showed an average intake of fruits, berries, juice and vegetables, including potatoes, of 480 grams per day among adults (54). The latter study showed that the intake of these food items was particularly low among young adults that are between 16 and 29 years old (54).

1.4 Physical activity related to health

The World Health Report 2002: *Reducing Risks, Promoting Healthy Life*, has highlighted physical activity as a common and preventable risk factor for chronic diseases (55). Regular physical activity is recommended for reducing the risk of premature CVD, cancer of the colon, complications with overweight and obesity, and to improve emotional well-being (56, 57). Physical activity has also been reported to be associated with other types of positive health behaviour, like to eat healthy and to refrain from smoking (58). A recent study showed that the concentration of total cholesterol and LDL concentration decreased, whereas no significant changes was found in the concentration of p-tHcy, HDL and triacylglycerol after aerobic training (59). On the other hand, Durstine *et al.* (60) reported that an increase in physical capacity was associated with a reduced concentration of triacylglycerol.

1.4.1 Physical fitness and recommendations for physical activity in Norway

Five repeated cross-sectional studies from 1950 to 1997 have concluded that there seems to be a trend of less physical activity among Norwegian children and youth (61). From 1980 to 1985, maximal oxygen uptake ($VO_{2\,max}$) for 183 610 18-year old Norwegian men (91% of the male Norwegian population in this age group) was 8% higher than in a representative sample of the same age population (N=1028) in 2002 (62). This development is of particular concern since the prevalence of lifestyle-related diseases is higher in groups with low $VO_{2\,max}$ (63).

During compulsory military service in Norway, Dyrstad *et al.* (64) showed that the level of high-intensity endurance and strength training is too low to improve the soldiers' endurance and muscular strength.

According to the Nordic Nutrition Recommendations 2004 (65), children are advised to a minimum of 60 minutes of physical activity every day, whereas adults are advised to have at least 30 minutes of daily physical activity of at least moderate intensity. More physical activity (about 60 minutes daily) with a moderate and/or vigorous intensity may be needed for prevention of weight gain among adults.

1.5 Overweight and obesity related to health

Overweight and obesity have been shown to be associated with adverse levels of lipids and increased risk of cardiovascular disease and cancer (66, 67). A study by Hubert *et al*. (68) showed that an increase in one unit of BMI (<3 kg) increased plasma LDL cholesterol by 3 mg/dl. Studies have also observed an increased prevalence of adiposity and clustering of CVD risk factors, even among children and adolescents (69, 70).

Troiano *et al.* (71) demonstrated that overweight children and particularly adolescents, tend to remain overweight and, in general, have a 1.5- to 2.0-fold increased risk of being overweight as adults. The increased prevalence of obesity in early life indicates a need for primary prevention.

Because BMI reflects body mass rather than fatness, some individuals may have a high BMI because of a relatively large lean body mass, rather than excess body fat. Fat has a distinct different density than muscle and bone, and muscular athletes and bodybuilders in particular, may therefore be misclassified as overweight due to high BMI (72). When interpreting individual BMI levels, it is also important to consider that the prevalence of obesity increases with age and is higher among women than among men (73). In addition, race and nationality may affect body composition and BMI (74). Although the BMI does not account for and individual’s fat to muscle ratio, an observational study in 5 different European centres demonstrated have shown a valid overall prediction of body fat percentage (BF%) from BMI on a population level (75).

1.5.1 Changes in weight among the Norwegian population

Due to a general trend of excessive energy intake and physical inactivity, population studies have shown that the prevalence of overweight and obesity has increased over several decades (76-79). This alarming pattern is also pronounced in Norway (80). Dyrstad *et al.* (64) have demonstrated that body weight among 18-year old Norwegian men has increased by 7% and the BMI has increased by 6% over an approximately 20-year period. A recent published study of Norwegian adolescents (14-18 years old) showed that height and weight increased significantly in both sexes and all ages from 1966-69 to 1995-97 (81). In both sexes and in all ages there was a significant increase in the upper BMI percentiles, but also a trend towards a decrease in the lowest percentiles.

1.6 Socioeconomic status related to health

Socioeconomic status (SES) has been inversely associated with factors related to cardiovascular risk (82). The most commonly used indicators of SES in epidemiological studies have been education, occupation and income or combinations of these indicators (83, 84). Education has been the most frequent measure, which can be obtained easily. Population studies have demonstrated socioeconomic inequalities in analysis of health indicators and prevalence of common chronic diseases, including CVD and cancer (85-87).

Studies have reported that low SES may be a risk factor for smoking, unhealthy dietary habits, high BMI and adverse lipid and glucose levels (88-92). Among children and adolescents, those from lower SES groups have also reported poorer health profiles, including lower intakes of healthy foods and lower levels of physical activity, compared with children and adolescents from higher SES groups (93, 94). Few studies, however, have reported consistent results of different health variables related to SES among young adults between 18 and 26 years.

1.7 Strategies for dietary interventions

Interventions that increase the intake of whole grain products, fruits and vegetables among adolescents and young adults may improve health status and prevent development of chronic diseases (95, 96). Various methods have been used to change dietary habits of different groups. In order to make dietary changes, nutrition messages must be seen as relevant from a personal point of view in the near future. It is doubtful whether potential health benefits later in life

from diets rich in vegetables, fruits and whole grain bread are sufficiently appealing and motivating for young people to change their eating habits.

A review of intervention models and programs for promoting healthy eating habits reported that the previous studies have mainly focused on written and oral nutrition information (97). These types of interventions have, however, proven to be relatively ineffective. As a result, Frazao & Allshouse (98) have emphasized the importance of combining different strategies depending on the target groups. During the 9th European Nutrition Conference of the Federation of the European Nutrition Societies (FENS), intervention researchers emphasized the need for intervention strategies which combine health information with increased availability of healthy foods (99).

A study among Boy Scouts and their parents focused both on an increased availability of healthy food and different activities to increase the interest in vegetables, including a program on how to make easy, tasteful and healthy meals (100). A school trial to encourage fruit and vegetable consumption among children also used a combination of environmental strategies. A significant increase in intake of fruits and vegetables was shown after educating food-service staff and cook managers in preparing healthy meals and informing the children of the positive effects of a healthy lifestyle (101).

An intervention among Norwegian children showed that providing a free piece of fruit or vegetable was an effective strategy to increase fruit and vegetable intake for school children (102). A follow-up study confirmed a further significant effect after 3 years of a free school fruit program (103). Another study evaluated the effectiveness of multiple interventions targeted at school lunches with the aim of increasing the consumption of fruits, vegetables, whole grain bread, non-fried potatoes and non-cream cakes. Despite positive changes at an early stage, no significant overall changes in eating habits were reported after two years of considerable input (104). A study among adults (over 18 years) concluded that dietary intervention programs to increase the intake of fruits and vegetables should emphasize the "5-A-Day message", increase self-efficacy for consuming fruits and vegetables, and show ways to make vegetables more palatable and easily accessible (105). An environmental intervention showed that increasing the number of nutritious food choices and reducing the price of both fruit and salad by 50% in cafeterias resulted in a threefold increase in the consumption of these foods during the intervention period (106).

Several studies have described and tried to influence the dietary habits of children and adults. On the other hand only few dietary interventions have focused on young adults living away from home for the first time (107). Young male adults have been considered as a particularly difficult target group to reach when promoting a healthy lifestyle.

1.8 Biomarkers

Accurate registration of dietary intake is a demanding and expensive process. In addition, variations and diverse errors are associated with the most common registration methods of dietary intake. In 1983 Solomons and Allen (108) described the fundamental role of nutritional biomarkers as alternative measures of dietary intake. A nutritional biomarker can be a biochemical, functional or clinical indicator of nutritional status with respect to intake or metabolism of dietary constituents. An important advantage of biomarkers is the assessment of objective measurements of the dietary intake. It is important to note that a single biomarker may reflect both the status of a single nutrient or several nutrients, their interaction and metabolism. Homocysteine falls into this latter category, as elevated concentrations of p-tHcy due to defects in the intracellular metabolism may be nutritional (an inadequate intake of folate, vitamin B_2, vitamin B_6 or B_{12}) or have a genetic reason (109). Approximately two thirds of the cases of elevated concentrations of p-tHcy have been estimated to be due to low or a moderate concentration of these vitamins, of which folate is considered the most important (110, 111).

When choosing a biomarker, it is important for the researcher to understand how a nutritional biomarker relates to both dietary intake and the chronology of exposure. Furthermore, it is important to consider whether the biomarker will be used to evaluate long-term nutritional status, recent dietary intake, effectiveness of dietary modification or the efficacy of an intervention.

The concentration of serum cholesterol is for example, highly affected by the intake of different fatty acids and is used to assess short term dietary compliance (112, 113).

Erythrocyte folate, plasma folate and serum folate are frequently used as indicators for folate intake (114). The question of which of these provides the best measure of folate status is under discussion. Plasma or serum levels only reflect the short-term intake of folate prior to blood sampling, but can be analyzed quickly and cheaply, and are considered to be acceptable

for use in large epidemiologic studies and in clinical practice (115, 116). On the other hand, erythrocyte folate concentrations are indicative of the folate consumption of the preceding months. Laboratory analysis of erythrocyte folate is, however, more complicated and the results may therefore be affected by analytical biases. Several laboratory centres have therefore agreed that serum assay is the most appropriate screening test for folate deficiency (116).

In order to study prospective changes in dietary habits, the present study used biomarkers which are considered to reflect the intake of fruits, vegetables and whole grain bread (i.e. serum folate) and biomarkers which are risk factors of common chronic diseases (i.e. p-tHcy and serum lipids).

1.8.1 Stability of folate in vegetables after processing and storage

Rich dietary sources of folate are green vegetables, nuts, grain product and liver, whereas vegetables, fruits, potatoes and milk are the most important sources of folate in the European countries (117). Naturally occurring food folate is, however, rather unstable when exposed to oxygen, light and high temperatures (118). Food processing, such as cooking, warm holding, storage and re-heating might therefore considerably lower the concentration of folate in food components compared with raw food. Thus, it is important that the food industry consider how different methods of processing and storage in various food service systems may affect the content of folate in foods.

Determining the actual folate intake has proven difficult because existing folate data in food-composition tables are scarce, especially the folate data on processed foods. To establish a valid estimate of the folate intake, it is important to have reliable information about the folate content in food products in the form in which they are consumed. Thus, further studies are needed to provide more information on how different methods of processing and storage affect the folate content in various food items.

2. Aims and objectives

The aim of this project was to investigate the health risk profile of young male adults from different socio-economic groups, and possible changes in this profile after a 5-months dietary intervention. A combination of strategies, including nutrition information and increased availability of fruits, vegetables and whole grain bread was used in the dietary intervention in order to increase the intake of these foods. An objective was to study the effect of the intervention by a combination of dietary and biochemical data, with a special focus on changes in dietary intake of B-vitamins and changes in plasma homocysteine and related metabolites. Since naturally occurring food folates have been reported as rather unstable, another objective was to study the retention of folates after treatments commonly used in Norwegian military messes. This part of the project provided information that was useful in the interpretation of the folate data from the dietary intake.

The thesis consists of four papers presenting the results from the different subprojects. The specific objectives were:

- To investigate some of the main risk factors of CVD, such as body mass index, lipid profile, physical fitness and smoking habits, as well as to explore the relationships between these parameters in young men entering the military service. Furthermore to study the associations between these risk factors of CVD in young men and the educational level of their parents as an indicator of socioeconomic status. (Paper I).

- To investigate the baseline plasma concentrations of tHcy and examine changes in plasma concentrations of tHcy, cysteine, vitamin B_2, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and the serum concentration of folate and vitamin B_{12} after the 5-months dietary intervention study (Paper II).

- To examine the changes in intake of vegetables, fruits and whole grain bread and the subsequent changes in nutrient intake after the dietary intervention study. Furthermore, to investigate the association between changes in folate intake from different food groups and changes in the plasma concentration of homocysteine after the 5-months dietary intervention period (Paper III).

- To examine the retention of folates in selected vegetables after the different methods of processing, storage and reheating, that are commonly used in Norwegian military messes (Paper IV).

3. Materials and methods

This project is made up of three different sub-projects; (1) study of the baseline data in the 5-months dietary intervention, (2) study of the effect of the 5-months dietary intervention and (3) study of the folate content in vegetables and effects of food processing and storage (Table 3-1).

Data collection in the dietary intervention included information about physical fitness, BMI, smoking habits, parental education, dietary intake and collection of blood samples for analyses of selected biomarkers. The blood samples collected at baseline reflected dietary habits before entering military service. Data collection in the study of folate retention in vegetables included duplicate samples of raw and processed broccoli, peas and potatoes (Table 3-1).

Table 3-1: Overview of the studies included in the present thesis

Type of study	**Sample**	**Measurements**	**Statistical analyses**
Baseline in the dietary intervention study	750 healthy men, 18-26 years old	Parental education Smoking habits Physical fitness BMI Serum lipids	Regression analyses ANOVA Bonferonni post-hoc test
Dietary intervention study	Intervention group 415 healthy men, 18-26 years old	Plasma tHcy, cys, vit.B_2 Serum Folate, vit.B_{12}	Correlations, Regression analyses
	Control group 105 healthy men, 18-26 years old	Nutrient intake Energy components, fibre, folat and vit.B_2 Plasma tHcy Serum Tot.chol., TG, HDL, LDL	Regression analyses
Effects of food processing	Peas, broccoli and potatoes	Folate retention	Tukey's pairwise comparison

3.1 Dietary intervention study

3.1.1 Study design

This study was part of a larger project with the aim to provide a healthy and appealing assortment of foods served in military messes, and to investigate changes in dietary habits and CVD risk factors after a dietary intervention period. Two doctoral students were responsible for the development of survey instruments and the collection of data; Solveig Uglem for the development of the food diary and the collection of dietary data and Tonje Holte Stea for the development of healthy and appealing recipes and the collection of clinical data. During the data collection the doctoral students worked together extensively.

At baseline and after a 5-months study period, information was collected on the dietary intake, weight, height, lipid profile, serum concentration of folate and vitamin B_{12}, p-tHcy, cysteine and vitamin B_2 in plasma, physical fitness, smoking habits and parental education.

The strategies used in the intervention study to promote healthier food habits among the young men in the intervention group included: (a) nutritional education, (b) a reduced fat content in meals served in the military mess, and (c) increased accessibility and availability of vegetables, fruits and whole grain bread in the military mess.

Nutritional information included meetings where all the participants were informed about the study and the positive effects of a healthy diet. A series of nutrition information posters were made for the intervention group and posted in the military mess hall. During the study period, the posters were regularly replaced with new versions. Other materials that were available in the military mess hall included folders, leaflets and charts with health and nutrition information provided from the Norwegian Directorate of Health and various Norwegian health organizations. For specific questions about health and nutrition, two project workers were available, and a post box was also placed in the military mess hall for additional questions.

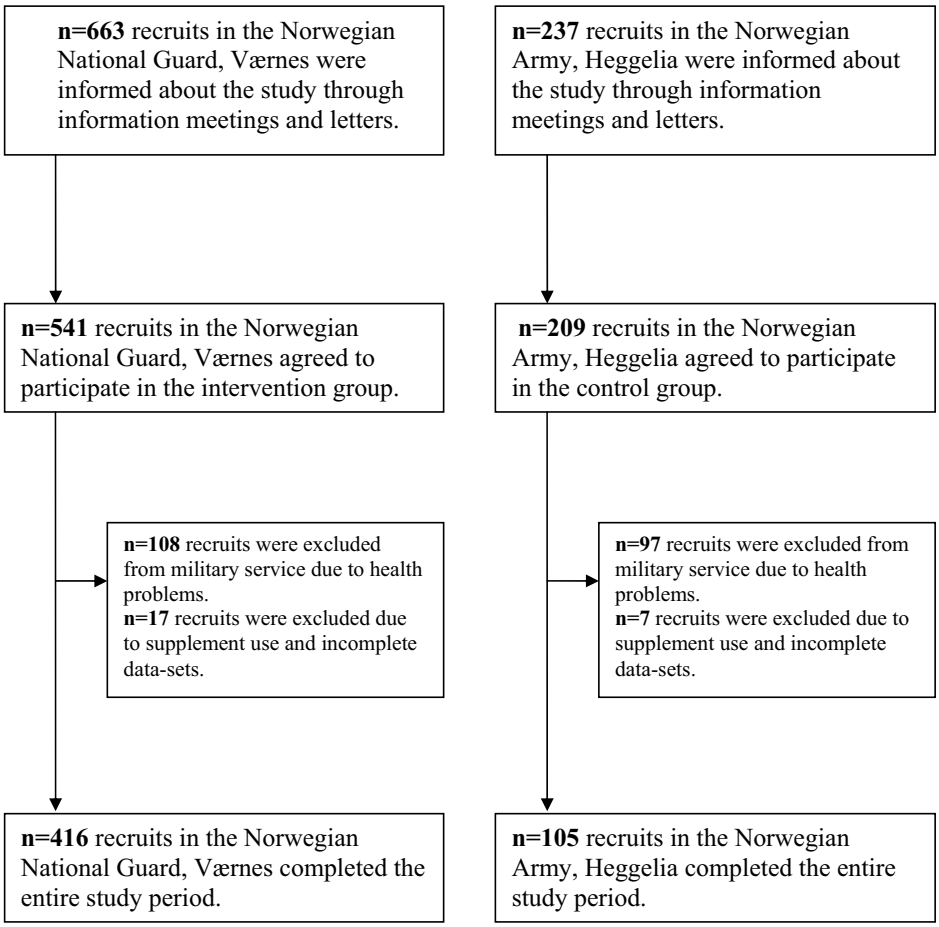
In the planning process for the study, new recipes were prepared and the nutritional content was controlled using a food database developed at the Department of Nutrition, University of Oslo (Mat på Data, version 5.0). Thus, the intervention groups was offered meals which were composed according to the Nordic Nutritional Recommendations, whereas the control group received meals based on the traditional military recipes. Prior to the intervention period,

military cooks at Værnes military camp were trained to use methods of food processing that result in a high retention of nutrients, i.e. procedures that involve a minimum amount of water. Further, they were trained in preparing meals and how to make appealing food presentations. The meals that were offered to the intervention group were characterized by a lower fat content, especially of saturated fat, and more vegetables, fruits and whole grain bread than the traditional military meals. All recruits were offered free breakfast, lunch, dinner and an evening meal. In the military mess hall, the recruits chose their own food and decided their own serving sizes. Alternative places to eat included a commercial military canteen, restaurants/cafeterias, and grocery stores outside the military camp. These places were available for all recruits at their own expenses.

3.1.2 Subjects

Enrolled male recruits in the Norwegian National Guard, Værnes (n=663) and the Norwegian Army, Heggelia (n=237) were the source population in this study. These military camps were chosen due to similar levels of physical activity during military service. Totally 750 (83.3%) young men agreed to participate in the baseline data collection, which was carried out by trained research staff during the first week after enrolment in the military service. As the military administration did not accept any randomizing of the participants, recruits in the Norwegian National Guard were invited to participate in the intervention group and recruits in the Norwegian Army were invited to participate in the control group. Among these, 416 (62.7%) participant in the intervention group and 105 (44.4 %) participants in the control group completed the entire study period. Most of the recruits (89.6%) that did not complete the entire study had been excluded from continuing military service due to health problems. Further, use of dietary supplements and incomplete data-sets were exclusion criteria (Figure 3-1).

Figure 3-1: Chart diagram of participants through the dietary intervention study



3.1.3 Physical examination

Body weight was measured to the nearest kilogram and body height was measured to the nearest half centimetre, with subjects wearing light standard military clothing without shoes. Mean body mass index was calculated (kg/m^2).

Physical fitness was measured with two physical examinations: time, in minutes, for running 3000 metres and a muscular strength test (Table 3-2). According to the military requirements, 15 minutes was the maximum time limit in order to obtain an approved result from the 3000 metres run.

Table 3-2: Muscular strength measured as number of push-ups, sit-ups and lift ups

Grade	**Push-ups (number)**	**Sit-ups (number)**	**Lift-ups (number)**
6.0	45	70	14
5.5	42	64	13
5.0	39	58	12
4.5	36	52	10
4.0	32	46	9
3.5	28	40	7
3.0	24	34	6
2.5	20	27	5
2.0	16	20	4
1.5*	12	15	3
1.0*	9	10	2
0.5*	6	5	1
0*	<6	<5	0

* Not passed test of muscular strength according to the military guide lines.

3.1.4 Blood samples

Blood samples were obtained after minimum twelve hours of fasting. For plasma analyses of homocysteine, cysteine, vitamin B_2, FAD and FMN levels, peripheral venous blood was drawn with a minimum of stasis into evacuated glass tubes containing EDTA, total volume 27 mL. The vials were immediately cooled on ice and protected from daylight throughout the whole processing. Tubes without additives, total volume 10 mL, were used for the determination of total cholesterol, triacylglycerol and HDL concentration in serum.

All samples were centrifuged within 20 minutes of blood collection. Plasma or serum was obtained by centrifugation at 2000g for 10 minutes at 4°C. All samples were aliquoted in 1 mL blood collected tubes and frozen at -20°C at Værnes and Heggelia Military Camp, respectively. Within four days, all blood samples were transferred to -80°C for storage until analysis. The samples were de-identified and analysed at the same time.

3.1.5 Biochemical analyses

Serum and plasma specimens were analysed in groups of 10-20 cases and 2 referents, with the position of the cases varied at random with each referent sample to avoid systemic bias and inter assay variability. The investigators and laboratory staff were blinded to case and referent status. Paired samples collected at baseline and post-intervention were analysed collectively within the groups of 20 cases to avoid inter assay variability.

Serum concentrations of total cholesterol, triacylglycerol and HDL were analysed at Section of Medical Biochemistry, Stavanger University Hospital. The level of total cholesterol and triacylglycerol was measured with enzymatic procedures using kits provided by Roche Diagnostics, Mannheim, Germany (GPO-PAP, CHOP-PAP). Likewise, the concentration of HDL was measured after precipitation with dextran sulphate and magnesium chloride. The concentration of LDL was estimated by using the Friedewald formula (119). The coefficients of variation (CVs) for analysed serum lipids were <2%.

Serum concentrations of folate and vitamin B_{12} were determined by immunoenzymatic assay (Access, Beckman Instruments, Inc.1998, Chaska, MN, USA). CVs for folate and vitamin B_{12} were 8.8% and 5.2% respectively.

Plasma concentrations of tHcy and cysteine were measured by reduction of disulfides with sodium borohydride, derivatization with monobrmobimane, separation with HPLC and quantification with fluorescence detection (120) and plasma concentration of vitamin B_2, FAD and FMN were determined by a modified HPLC method (Mansoor *et al*., manuscript in preparation). The instrumentation used for HPLC analyses included a Spectra-Physics SP 8700 solvent delivery system coupled to a Perkin-ElmerISS 100 autosampler. The detector was a Shimadzu RF-535 fluorimeter equipped with concave diffraction grating excitation and emission wavelength 475/15 nm bandpass. Plotting and integration of peaks were performed

by a Model 4290 computing integrator from Spectra-Physics- CVs for tHcy and cysteine were <6.8% and CVs for vitamin B_2, FAD and FMN were 4.9%, 4.1% and 7.5% respectively.

3.1.6 Smoking, dietary supplements and parental education

Information on smoking habits, vitamin- and drug consumption and parental education was collected using a self-administered questionnaire.

Current smoking status (yes/no), number of cigarettes per day and number of years of smoking was reported, as well as information on the number of former smokers.

Recruits who used dietary supplements were excluded from the study. Participants were also excluded due to use of medicines that were expected to affect the concentration of selected biomarkers in the present study.

Parental education, an indicator of socio-economic status, was reported as a four-level categorical variable: 1) elementary school, 2) high school, 3) college/university, 1-3 years, 4) college/university, >3 years. As Norwegian men usually complete their military service just after high school, recruits' own educational level was not considered useful for the purposes of this study. After recruits are dismissed from service, they continue their education or start to work. Therefore, the main proportion of the recruits had the same level of education (85% at high school level) at the time of the study.

3.1.7 Dietary assessment

For dietary data, a validated food diary developed at Department of Nutrition, University of Oslo (sef.no/assets/11002260/vedlegg1_ungkost.PDF) was adapted to cover most of the food available in the military mess (121, 122). The food diary was tested in a pilot study with 12 recruits in a military camp at Lutvann, Norway, and revised on the basis of the results and comments from this group. The revised survey instrument were tested and retested with fairly acceptable responses over a 3-week period among 63 recruits in another military camp in Norway (Heistadmoen).

The food diary gave choices of food items, which covered their intake of vegetables and fruits, bread and breakfast cereals, drinks, butter, milk products and hot meals for lunch and dinner, in addition to fast food, snacks and beverages. The food diary included pre-printed

questions where the participants recorded their consumption during the day. All pre-printed choices for hot dishes in this diary were removed and replaced by open-ended questions where the participants filled in a number for the day's special served at lunch and dinner. The diary included open-ended questions about consumption, frequency of consumption and portion sizes of food available outside the military mess, e.g. in the commercial canteen at the camp or at restaurants outside the camp.

Although the food diary covered most food items available in the military mess, it has limitations due to less accurate registration of food intake outside the military mess. Most of the food that the recruits purchased outside the military mess was high in energy content; e.g. fast food, snacks and soft drinks. To some extent there may therefore be an underestimation of energy intake in the food diary.

Portion sizes were units such as slices, glasses and pieces. To obtain estimates of the consumption in grams, most of these food items that were printed in the food diary were also pre-weighed. The estimates were the average of 10 weighed pieces. In order to make the questionnaire short and simple, hot dishes served at lunch and dinner were included in the diary as open-ended questions in which the recruits filled in a number for the menu of the day. For hot dishes and some other items, like salad, the participants stated their intake as number of servings and serving sizes (small, medium, large or extra large). For determination of serving size for each recruit, they were asked to refer to photographs and/or weighed portion-sizes placed in the military mess during the meals. In addition, two research workers were present at every meal to guide in determining serving sizes. To estimate the consumption of different food items included in the hot dishes, recipes and estimates of the amounts of ingredients used in every dish were collected. An average content of food items in all dishes (small, medium, large and extra large serving sizes) was calculated from these data. For registration of side dishes served at lunch and dinner, the recruits also stated the number of servings and serving sizes. Serving sizes for these food items were decided with guidance from the kitchen personnel and from other Norwegian surveys (123).

The intake of different food items (g/day) was calculated by combining the frequency of intake and serving sizes. Nutrient intakes were calculated with the aid of specific portion questions in the food diary, Norwegian standard measures (124) and the Norwegian Food database (Mat på Data, version 5.0) linked to the official food composition table in Norway

(125). The average daily consumption for each recruit in the survey period was obtained by adding up their total consumption of different food items or estimated nutrients and divide by the number of days on which the young men kept records. Data were collected with a food diary over a 4 days period. Recruits who registered total food intakes less than 3 days were excluded from the analysis.

3.2 Folate retention in vegetables

3.2.1 Study design

This part of the study investigated how different methods of processing, warm holding, storage and re-heating used in food service systems affect the content of folate in broccoli, potatoes and green peas. These vegetables were selected since they are amongst the major contributors of folate in the Nordic diet, and main ingredients in recipes commonly used in modern small- and large-scale service systems, e.g. in the Norwegian military. Thus, broccoli, potatoes and green peas were also main ingredients in several of the recipes in the dietary intervention study.

Following industrial handling at Findus ‘‘green pea line’’ in Bjuv, Sweden, green peas were boiled, steam boiled and microwaved at the Swedish University of Agricultural Sciences in Uppsala, Sweden. Raw samples of broccoli were boiled, steam boiled, blanched and sous-vide processed, whereas potatoes were boiled, oven baked and sous-vide processed at Norconserv AS in Stavanger, Norway. These methods of processing were chosen as they are commonly used in Norwegian military messes. This part of the study thereby provided information that was used to interpret the folate data from the dietary intake.

3.2.2 Chemical analyses

Materials

All chemicals were of HPLC quality or analytic grade and purchased from E. Merck (Darmstad, Germany or Sigma-Aldrich (St. Louis, USA). Termostable α-amylase (E-BLAAM) was purchased from Megazyme International, Ireland. Rat serum was obatained fraom Scanbur, Sollentuna, Sweden and dialysed for 2 h before use. Folate standards (6S)-H_4folate, (6S)-5-CH_3-H_4folate, (6S)-5-HCO-H_4folate were donated by Eprova AG, Schaffhausen, Switzerland. De-ionised water was of Milli-Q grade.

Bovine folate-binding proteins for affinity chromatography (Sigma Chemicals Co., St.Louis, USA) was purchased from Scripps Laboratories (San Diego, USA), and agarose affi gel 10 and poly prep chromatography columns from Bio-Rad Laboratories (Richmond, USA). Different silica-based sorbents of the trademark Isolute from International Sorbent Technology (Mid-Glamorgan, UK), including SAX (strong anion exchange), PH EC (phenyl-endcapped) and CH EC (cyclohexyl-endcapped) have been used for sample purification by solid-phase extraction.

Methods

All processes of broccoli, peas and potatoes were performed in duplicates. To prevent folate oxidation, samples were protected by nitrogen and subdued light and cooled on ice throughout sample preparation. The samples were further treated with dialysed rat serum for deconjugation of folate polyglutamates to monoglutamates. Preparation of potato samples also included treatment with α-amylase prior to the deconjugation step.

We used solid-phase extraction (SPE) and SAX cartridges for purification of food samples. Aliquots of extract were applied to the preconditioned cartridges and eluted with 0.1 M sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid and 0.1% 2,3-dimercapto-ethanol (BAL) for sample clean-up.

HPLC analysis was carried out using an AGILENT 11000 HPLC system. A Zorbax SB C_8 column (5 µm, 150 ×4.6 mm) and a matching C_8 guard column (5 µm, 12.5 ×4.6 mm) were used to separate the folate. During HPLC quantification, retention times were used for peak identification. The ration of sample peaks from fluorescence and diode-array detectors to ratio of standard peaks as well as fluorescence and diode-array spectra was used for verification of peaks. Quantification was based on an external standard method with multilevel calibration curve (n=7) for each folate form.

3.3 Statistical analyses

The data were statistically analysed and graphically presented using SPSS version 15.0/16.0 (Chicago, IL, USA) and software Minitab release 14 (Minitab Ltd., Coventry, UK).

Linear regression models were performed to determine the explained variance of physical performance, BMI and serum lipids as a function of parental education, age and physical

health factors. Differences in the concentration of serum lipids across four cross-tabulated groups (low BMI/high fit, low BMI/low fit, high BMI/high fit and high BMI/low fit) was assessed by ANOVA. Post-hoc analyses were analyzed with the Bonferonni multiple comparison test.

The concentration of aminothiols and vitamins did not follow a gaussian distribution as raw data; they therefore were log-transformed before calculations and presented as geometric means with 95% confidence intervals. Multiple regression models calculated the intervention effects on all selected variables. Multiple regression models were also used to calculate the predictors of changes in p-tHcy concentration.

The retention of folates from different methods of processing, storage and reheating were compared using Tukey's post-hoc test.

4. Results

I. BMI, lipid profile, physical fitness and smoking habits of young male adults related to parental education.

The objective was to study cardiovascular risk factors among young Norwegian men, including BMI, lipid profile, physical fitness and smoking habits, and to explore the relationship between these variables. Another objective was to investigate the association between these cardiovascular risk factors in young male adults and the educational level of their parents.

This baseline study showed that young male adults with high-educated fathers had a lower BMI level ($p=0.035$) and a lower concentration of total cholesterol ($p=0.003$) and LDL ($p=0.014$) than male adults with low-educated fathers. On the other hand, the educational level of the mothers was not related to any of the selected health parameters in young male recruits.

As expected, low physical fitness (time used in a 3000 metres run) was positively related to current smoking status (yes/no; $p=0.022$) and the concentration of triacylglycerol ($p=0.001$) among the male recruits. A high BMI of the recruits was associated with a high concentration of LDL ($p=0.002$) and total cholesterol/HDL ratio ($p<0.001$). Contrary, the BMI of the recruits was inversely associated with the concentration of HDL ($p<0.001$) and with their scores on both tests of physical fitness, which included running performance ($p<0.001$) and muscular strength ($p=0.011$). Another result showed that recruits with low BMI, both those with high and low fitness, had a significantly better lipid profile than recruits with high BMI and low fitness ($p\leq0.016$). A better lipid profile was also shown among recruits with high BMI/high fitness compared to recruits with high BMI/low fitness.

II. Changes in predictors and status of homocysteine in young male adults after a dietary intervention with vegetables, fruits and bread.

The purpose of this paper was to investigate the baseline concentration of p-tHcy and examine the changes in plasma concentrations of tHcy, cysteine, vitamin B_2, FAD and FMN, and the serum concentration of folate and vitamin B_{12} during the 5-months dietary intervention study. After the 5-months intervention period, a reduced concentration of p-tHcy ($p=0.002$) and p-cys ($p<0.001$) was shown in the intervention group compared with the control group. The intervention group showed an increased concentration of vitamin B_2 ($p<0.001$) and FAD ($p=0.008$) compared with the control group. These results show that the multiple intervention strategies had a favourable effect on the concentration of p-tHcy and its metabolites. The findings also suggested that the reduction in the concentration of p-tHcy seems to be predicted by an increase in the concentration of p-cys ($p<0.001$), serum folate ($p=0.021$) and a reduction in FMN ($p=0.035$).

III. Association between folate intake from different food sources in Norway and homocysteine status in a dietary intervention among young male adults.

This study investigated changes in intake of vegetables, fruits, whole grain bread and selected nutrients during the 5-months dietary intervention study. Furthermore, the study examined the relationship between folate intake from different food sources and changes in p-tHcy during the dietary intervention period.

The multiple intervention strategies resulted in an increased consumption of vegetables, fruits, berries and juice ($p=0.014$) and of whole grain bread ($p<0.001$). At the same time, the estimated intake of dietary fibre and folate increased during the study period ($p<0.001$ for both). The study also showed a higher intake of folate from vegetables, fruits and whole grain bread in the intervention group compared with the control group ($p<0.001$ for all). The increased intake of dietary folate from whole grain bread was shown to be inversely associated with a reduced concentration of homocysteine ($p=0.017$).

IV. Retention of folates in cooked, stored and reheated peas, broccoli and potatoes for use in modern large-scale service systems.

The objective of this study was to examine the retention of folates in vegetables after different methods of processing, warm holding, storage and reheating which are commonly used in military messes.

Blanching of green peas (medium size) resulted in a significantly reduced retention (-64%) compared with medium size peas before processing, both on fresh weight (FW) and dry matter (DM) basis ($p<0.05$). For green peas, no method used for processing in service systems (boiling, steam-boiling or microwave heating) caused any significant losses of folates. Boiling significantly reduced folate retention in both peeled potatoes (-59% on DM basis) and unpeeled potatoes (-72% DM) when compared to raw potatoes ($p<0.05$ for both). A significantly reduced folate content was also shown after oven baking of unpeeled potatoes (-63% on DM basis) when compared with raw potatoes ($p<0.05$). No significant ($p < 0.05$) differences in folate content could be observed in broccoli processed by traditional cooking systems such as boiling or by minimal processing such as steam boiling. Subsequent storage at various temperatures and length of times followed by reheating caused no further significant losses of total folate.

5. Discussion of methodology

In the present study, scientific methods have been used to evaluate the effect of the study. In general, a valid experiment is one that fairly tests the hypothesis. In the following discussion, some major threats to validity posed by our study are discussed.

5.1 Random error

Rothman and Greenland (126) define "random error" as "that part of our experience that we cannot predict". Regression to the mean (RTM) occurs because values are observed with random error and this statistical phenomenon can make natural variation in repeated data look like real change. It happens when unusually large or small measurements tend to be followed by measurements that are closer to the mean. Repeated data as described in the present intervention study are rarely observed without random error. Thus the data in the present studies are exposed to errors due at least in part to regression to the mean. To reduce the effect of random error, the sample size has been estimated which results in 95% power to reveal significant differences between selected variables.

5.2 Calculation of sample size and power

A major problem in research trial is having an inadequate sample size (127). The main idea behind the sample size calculation is to have a high chance of detecting a clinically significant effect if it exists, and thus to be reasonably sure that no such benefit exists if it is not found in the trial. Freiman *et al.* (128) have demonstrated that many published clinical trials that find a non-significant difference between treatments had little chance of detecting major treatment effects due to small sample sizes.

The sample size calculations in the present dietary intervention study were based on previous results published by researchers at Stavanger University hospital (129). Apparently healthy subjects were given 0.6 mg folic acid vs. placebo (n=29) and the concentrations of serum folate were measured before and after treatment. The log (ln) concentrations of serum folate were at baseline 2.369±0.263 vs. 2.573±0.268 (mean±SD) after treatment 3.147±0.336 vs. 2.530±0.301 (mean±SD). In the following table, a standard deviation of 0.36 of log serum folate is assumed. The computations are based on a two sample t-test for comparing log serum folate between an intervention group and a control group, a two-sided 5% test is assumed, and

the power is set at 80%, 90% and 95% respectively. The power is for detecting a mean difference of 0.16, and also smaller (0.10) and a larger (0.20) difference. Computations are based on the program Power and Precision (Biostat Inc., Englewood, NJ, USA):

Table 5-1: Two-sample t-test for difference in log scale serum folate

SD	Difference (log scale)	80% power, number	90% power, number	95% power, number
0.36	0.20	52	70	86
	0.16	81	108	133
	0.10	205	**274**	**338**

Prior to the data collection in the present intervention study, we calculated that 338 participants in both the intervention group and the control group (totally 676 participants) would be required for a power of 95% to reveal significant changes in folate intake during the study period (Table 5-1).

Several introductory accounts fail to consider the very common situation in which two groups (e.g. cases and controls) of unequal size are to be compared (130). This may be a fundamental problem in studies with small sample sizes. In this study, 541 recruits participated in the intervention group and 209 recruits participated in the control group at baseline. A total of 376 recruits in the intervention group and 105 recruits in the control group completed the entire study. Generally, the control group sample should be at least as large as the target group sample. The initial design of the present study included therefore equally large groups, which would have resulted in a higher power. Unfortunately, extensive changes in the structure of the military camps made it impossible to continue the study period and to invite more recruits to participate in the control group.

Despite the limitations in the present study, few other studies have investigated the effect of multiple strategies to increase the intake of fruits, vegetables and whole grain bread among young men. Thus, the present study has provided information about an important, but difficult, target group to reach when promoting healthy diets.

5.3 Generalizability

External validity (generalizability) involves the extent to which the results of a study can be generalized (applied) to other circumstances. In particular, lack of generalizability may be a concern if the exclusion of participation is non random. An important question in the present study was whether the lifestyle of young male recruits enrolled in the Norwegian National Guard, Værnes and in the Norwegian Army, Heggelia were representative for young Norwegian male adults in other situations.

The generalizability of the results may have been limited due to the fact that the study included only young men from two different military camps in Norway. However, Norwegian military service is compulsory and young men are supposed to be randomly distributed to different military camps across the country. Thus, different social classes and geographical areas were represented in the study sample. This relationship, in addition to a high baseline participation rate, had positive implications for the generalizability of the results.

When interpreting the results of the study, it should also be noted that the recruits had some restrictions regarding food choices in the military camps. Since the recruits eat most of their meals in the military mess hall, their food choices depended on the availability of different foods here. On the other hand, the recruits had a choice of hot or cold foods, and they decided their own serving sizes, and they had the opportunity to buy food in a commercial military canteen or in restaurants/cafeterias or grocery stores outside the military camps. There was a large variation among the recruits in their intake of vegetables, fruits and whole grain bread. For example, their intake of vegetables at baseline varied according to eating habits at home, weight beliefs, taste preferences, and number of hot meals eating in the military mess hall (121).

Most young Norwegian men outside the military are also restricted in their choices concerning food e.g. under restrictions set by parents before leaving home and restrictions by school and canteens at work. The food choices of young adults may also be restricted by economy and limited knowledge about how to prepare food.

The present intervention study successfully changed the dietary habits of young male adults participating in the study and subsequently increased the estimated intake of folate and decreased the concentration of p-tHcy. Based on the results from this study, it may be

expected that a similar intervention study among young Norwegian men in other situations would result in positive dietary changes. However, whether these intervention studies would show the same clinical effect size remains an open question.

5.4 Selection bias

Selection bias refers to a systematic error resulting from procedures used to recruit subjects that consequently influence the study participation. Selection bias also refers to systematic differences between comparison groups in prognosis/health status or responsiveness to treatment.

In the present dietary intervention study, baseline data indicated a better health status among the recruits in the Norwegian National Guard (intervention group) compared with the recruits serving in the Norwegian Army (control group). Regression models however, have controlled for baseline differences in health status between the groups and thus reduced the effect of the identified selection bias (131). After baseline data collection, dismissal of several recruits from military service due to the different health problems resulted in exclusion from the study and consequently a reduced participation rate. Results from baseline showed that several of the selected variables that represent health risk factors, were significantly lower among those who completed the entire study compared with those who dropped out of the study. The results indicated a selection of recruits who had a healthier lifestyle than those who were excluded from the study. However, this selection of the participants was observed both in the intervention group and the control group and may therefore be a minor problem.

5.5 Information bias

Information bias arises from systematic errors in measuring exposure or disease and may result in misclassification of study cases. Instrumentation, for example, can be functioning in an unreliable or erroneous fashion and thereby affect the validity of the results, in particular when biomarkers are collected and analysed over extended periods of time (132). Biological variation in markers and time characteristics of the exposure marker- disease relationship may also cause measurement error. In studies using interviews or questionnaires to obtain data, the respondents may misunderstand questions, be unable to recall the requested information, be unable to articulate the information precisely or understandably, be unwilling to disclose the information, or may give a response they regard as more socially desirable than the unvarnished truth. Data collections which are administrated by different persons may also

increase the risk of information bias. In conclusion, studies are prone to information bias if groups participating in the study are being treated differently. In some trials, blinding of both participants and researchers may be an effective method to reduce information bias.

Information bias related to preanalytic handling of data

The baseline study and the dietary intervention study may have been exposed to information bias through data collection of dietary intake or preanalytic handling of blood samples. To obtain information of food intake, a diary was considered to give a better estimate of the dietary intake than a food frequency questionnaire. The problems with misreporting and recall bias are smaller when subjects record their daily intake and do not have to estimate their usual intake. This is particularly the case for groups with low concern about nutrition. Furthermore, the risk of distortion of data due to misreporting or recall bias is expected to be the same for participants in both the intervention group and the control group. A further strength of the dietary intake data is that all the recipes were known and it was possible to calculate specific ingredients in the lunch and dinner courses as well as a more reliable estimate of nutrient intake. However, this type of data collection is time consuming and it takes a good deal of effort to motivate young men to record their food intake over four days. To minimize the burden on the respondents they were asked to record selected food groups (main courses, vegetables, fruits, drinks, bread and other cereals) in the diary which was kept as short as possible. Thus, the food diary covered most food items available in the military mess but had some limitations due to less accurate registration of food intake outside the military mess. As most of the foods purchased outside the military mess were high in energy content, there may to some extent have been an underestimation of absolute energy intake.

Another source of information bias could be that the recruits may have had problems in reporting the accurate education of their parents. In the study, educational level was reported as a four-level categorical variable: 1) elementary school 2) high school 3) college/university, 1-3 years, 4) college/university; >3 years. Due to unequal group sizes, parental education was merged into a 2-level categorical variable for statistical analyses; 1) elementary and high school (low), 2) college/university (high). Thus, potential bias due to misreporting of parental education may have been reduced.

During the intervention period, the intervention group received nutritional information of healthy food choices besides an easy access to low fat food, vegetables, fruits and whole grain

bread. Alternative places to eat included a commercial military canteen, restaurants/cafeterias and grocery stores outside the military camp. These places were available for all recruits at their own expenses. Thus, the present study was designed to generate conditions regarding food choices similar to life outside the military camp. This aim was not possible to combine with a strictly controlled design.

Proper pre-analytic handling of blood samples for analysis of p-tHcy concentration is crucial. To reduce the risk of potential measurement bias, EDTA-samples were cooled on ice and protected from daylight prior to centrifugation. The maximum period before centrifugation was then delayed to 4 hours (133). Without this pre-analytic handling, the release of homocysteine from erythrocytes would have continued as long as the erythrocytes were viable. The concentration of homocysteine would then have increased significantly before centrifugation (0.5-1 μmol/L per hour) irrespective of initial concentration (134).

The results of the study of folate retention in different vegetables may be affected by differences in selection of the raw samples or differences in preanalytic handling of the samples.

To avoid variation in folate content due to differences in cultivar, ripeness, time of harvest and storage, raw samples of both broccoli and potatoes were obtained at the same time from the same local distributor (BAMA, Stavanger, Norway). Samples of green peas were obtained from another distributor (Findus, Bjuv, Sweden), but selected from the same year of harvest according to size, colour and ripeness. During preanalytic and analytic treatment, duplicate samples of broccoli, potatoes and peas were further handled under the same conditions (temperature and light) to avoid uncontrolled losses of folate.

Information bias related to analytical handling of data

Considering folate's susceptibility to oxidation by heat, pH and oxygen, it was also important to use reliable and validated methods for determination of folate content in foods. The most commonly used techniques for food folate determination include microbiological, HPLC and protein-binding methods, all with several modifications. HPLC methods allow a more detailed characterization of the individual folate forms and both the intact folate polymers as well as the various monomer forms in samples after conjugase treatment can be quantified (135). It is important to be aware of these differences in accuracy in different analytical

techniques when comparing results from various studies. In our study, however, all samples were analyzed collectively under the same conditions using a validated HPLC method.

To reduce the risk of information bias in the dietary intervention study, blood samples from both the intervention group and the control group were analyzed collectively. Blinding was also used to hide information of group allocation when analyzing the blood samples. To further reduce the risk of measurement bias during analytical handling of blood samples, all results reported in the studies were analysed using properly characterized reference materials and validated reference methods.

High CVs may lead to an incorrect interpretation, i.e., the lack of an association between biomarker and outcome. It has been suggested that for epidemiological studies ideally the CV should not be > 5% (136). This level of accuracy is very difficult to attain for many nutrients; often it is not possible to attain a CV < 10%. At levels > 10% there may be concern about the utility of the assay. In all cases, inclusion of the CV for the assay in the report of the study results is important.

Results from interlaboratory comparison studies have shown a high overall CV for B-vitamins and in particular for serum folate (27.6 %) (137). Pfeiffer *et al*. (138) compared plasma total homocysteine measurements in 14 laboratories. The mean among-laboratory and within-laboratory (among-run) CV were 9.3 and 5.6%, respectively, for plasma samples.
This must be taken into consideration when comparing results from studies using different analytical methods. A comparison and evaluation of several techniques for the determination of p-tHcy in another Norwegian study resulted in an interassay CV between 3 and 11% (139). The CV after analyses of p-tHcy and p-cys in the present study is within this range (Table 5-2). The estimated CV after analyses of B-vitamins is also in line with the result from other studies and within the cut-off of critical concern (< 10%):

Table 5-2: The CV`s for the measured tHCy and related B-vitamins

Plasma variables	**CV (%)**
Homocysteine	6.4
Cysteine	6.8
Vitamin B_2	4.89
FAD	4.12
FMN	7.46
Serum variables	
Folate	8.8
Vitamin B_{12}	5.2

Serum lipids are an example of important biomarkers for which collaborative efforts have led to both understanding biological sources of variation and standardization of laboratory measurements (140). An accepted CV cut point for total cholesterol is 3%; low-density lipoprotein (LDL) cholesterol, 4%; high-density lipoprotein (HDL) cholesterol, 4%; triacylglycerol, 5% (140). The analyses of serum lipids in the present study resulted in low CV`s compared with the accepted cut points:

Table 5-3: The CV`s for the measured serum lipids

Serum variables	**CV (%)**
Total cholesterol	<0.3
Triacylglycerol	<0.7
High-density lipoprotein	<2

As the estimated CV after analyses of both B-vitamins and serum lipids are within the respective cut-off values of critical concern, the potential for information bias related to analytical handling of data is limited.

5.6 Confounding

Confounding bias is a systematic error of interpretation that results from making an unfair comparison, i.e., from a failure to take into account important differences between exposed and unexposed groups. Confounding describes a situation in which two or more effects are not separated; the distortion of the apparent effect of an exposure on risk, brought about by the association with other factors that influence the outcome. Misclassification of confounders and omitting to control for them may lead to false positive or false negative results. In contrast to bias, confounding can be controlled at several levels of a study. Strategies to reduce confounding factors when planning and performing data collection include *randomisation* of participants, *restriction* of volunteers with confounding factors or matching of individuals or groups.

The administration at Værnes and Heggelia military camp accepted and supported the data collections during the study period. However, the administration of both military camps required that recruits at Værnes military camp were the source population for the intervention group and recruits at Heggelia military camp were the source population for the control group. As there was no possibility of randomizing the participants in the intervention group and the control group, respectively, potential (including unidentified) confounding factors may occur between the compared groups.

The effect of confounders in the present study was explored and reduced in two ways; firstly by *restriction* of participants to males enrolled in the military. This selection of participants resulted in a relatively homogeneous age group with acceptable health status (according to the military requirements). Despite restriction of participants, baseline results showed a favourable health status among recruits in the intervention group compared with the control group. Unidentified confounding factors may be responsible for the difference between the groups. Thus, the effect of potential confounders was reduced by using *multiple regression analyses*. Multivariate analyses then served to adjust for the confounding effects of the different explanatory variables, in order to evaluate the independent effects of the variables in consideration.

Although age is thought to be a likely confounding factor of both the concentration of serum lipids and p-tHcy, regression analyses in Paper II-IV showed that confounding by age may have been prevented by enrolling subjects who were nearly of the same age.

As previously mentioned, other studies have also shown that an elevated concentration of p-tHcy is associated with other major components of the cardiovascular risk profile, i.e. smoking, lack of exercise, high BMI and elevated concentration of serum lipids (30, 26, 27). Results from the present study however, did not show a significant association between these traditional risk factors and the concentration of p-tHcy. In addition, no significant relationship was shown between changes in the smoking habits, physical fitness, BMI and the concentration of serum lipids and the change in the p-tHcy concentration during the study period.

6. Discussion of main findings

Young male adults are an important, but difficult, target group to reach when promoting healthy diets. In most European countries, recent studies among young adults indicate a general trend towards an unhealthy lifestyle with increased prevalence of overweight and obesity as a result (141). Clinical data indicate that elevated concentrations of total cholesterol, triacylglycerol and low density lipoprotein (LDL) are risk factors for cardiovascular events, whereas high-density lipoprotein (HDL) is reported to have an anti-atherogenic effect (142, 143). A high concentration of p-Hcy is also associated with increased risk of developing CVD (9, 10). In the recent years, the interest in the study of the association between B-vitamins and homocysteine status has considerably increased due to the growing evidence that B-vitamin deficiency is linked to high concentrations of p-tHcy.

Most of the folate deficiencies are related to inadequate dietary intake of folate (144). Knowledge about folate intake is therefore of major importance when giving recommendations to prevent deficiencies. To reduce the gap between recommended and actual daily intake, introduction of mandatory fortification has been discussed in several European countries.

6.1 Baseline differences between the intervention group and the control group

A total of 750 recruits participated in the baseline data collection; 81.6% in the intervention group at Værnes military centre and 88.2% in the control group from the Norwegian Army, Heggelia. Despite high participation rates in both camps and despite the concept of random distribution of recruits to different military camps, baseline results showed some important differences in health status between the groups. A significantly lower BMI, a higher prevalence of smokers and a higher number of cigarettes smoked was measured in the intervention group compared with the control group (Paper I). Furthermore, the smokers in the intervention group reported having smoked for a longer period of time than smokers in the control group. In addition, a significantly lower concentration of homocysteine, cysteine and FAD and a significantly higher concentration of folate, vitamin B_{12}, vitamin B_2 and FMN were reported in the intervention group compared with the control group (Paper II). On the other hand, the results showed no significant baseline differences between the participants in the two military camps with regard to concentration of serum lipids, physical fitness levels, or parental

educational levels (Paper I). The study also showed that the total intake of vegetables, fruits, berries and fruit juice (FV) and the total intake of bread did not differ between the intervention group and the control group (Paper II).

The reported baseline differences between the intervention group and the control group might have been avoided with a study design that involved randomization of the participants. Unfortunately, randomization of the recruits was not possible in the present study.

6.2 Baseline risk factors for cardiovascular disease

Few studies have examined risk factors of CVD among young male adults. The present study showed a mean BMI of 23.61 among participants from the intervention group and the control group combined (Paper I). Despite a mean BMI within the normal range (18.5-25 kg/m^2), a total of 29.6% of the recruits were overweight (25-30 kg/m^2) and 5.2% were obese (>30 kg/m^2) according to the definitions of the World Health Organization (141). A positive association between BMI and both the concentration of LDL and the ratio total cholesterol/HDL was demonstrated, whereas an inverse association was shown between BMI and the concentration of HDL and the level of physical fitness (Paper I). Men with low BMI, both high and low fitness, showed to have a better lipid profile than those with high BMI/low fitness. These results indicated that a high level of BMI was most likely related to a high level of body fat rather than a high level of muscle mass. As expected, men with high BMI/high fitness had a better lipid profile than those with high BMI/low fitness. The present results are therefore in line with reports describing that excessive energy intake and physical inactivity have resulted in a subsequent increase in overweight and obesity among children and young adults during the last decades in Norway (61, 64, 80). Despite a rather high prevalence of overweight (34.8% with BMI≥25), the results showed that most of the recruits (98.8%) had a concentration of serum lipids within the Nordic reference values for subjects within this age group (Paper I) (145). A normal range of p-tHcy concentration has been suggested to be between 5 and 15 µmol/l (146). The results from the present study showed that 24.8% of the recruits had a concentration of p-tHcy> 15 µmol/l at baseline (Paper II). A strong relationship has been demonstrated between high concentration of p-tHcy and noncardiovascular as well as cardiovascular mortality in adults (147). The results from the present study should therefore encourage further studies to investigate the effect of health promotion strategies for reducing the p-tHcy concentration in young men.

6.3 Parental education and health status

In contrast to the present study, previous studies have mainly reported either mothers' educational level or parental educational level as a collective predictor of health status. Few studies have reported separate effects of parental education. A positive health relation, i.e. a lower BMI and a better lipid profile, was found between the young men and their fathers' educational level but not with that of their mothers (Paper I). These findings suggest that fathers' with higher education may act as positive role models for young men. Furthermore, the results emphasize the importance of health promotion efforts targeted toward young adults of low paternal educational level.

6.4 Baseline characteristics among those who completed the entire study

A total of 416 (62.7%) recruits in the intervention group and 105 (44.4%) of the recruits in the control group completed the entire study. At baseline, there were no significant difference in total intake of VF and bread, concentration of p-tHcy, vitamin B_{12}, and physical fitness between the young men in the intervention group and the control group who completed the entire study period (Paper II). The study showed no differences in serum lipids among the groups (Paper III). On the other hand, a significantly higher serum concentration of folate and plasma concentration of vitamin B_2 and FMN was reported among the participants in the intervention group that completed the entire study than among the participants in the control group (Paper I). The study also showed a lower concentration of p-cys, FAD and level of BMI in the intervention group than in the control group. A significantly lower energy intake at baseline was shown in the intervention group compared with the control group (Paper III). According to the Norwegian Directorate of Health, the reference value for energy intake is 12300 kJ per day among men between 18 and 30 years old with low physical fitness (52). The food diary that was used in the present study had limitations due to less accurate registration of food intake outside the military mess. As most of the food that the recruits purchased outside the military mess had higher energy content, e.g. fast food, snacks and soft drinks, the total energy intake may to some extent have been underestimated for some of the recruits.

In general, the variables measured in the present study showed a more favourable health profile among those who completed the entire study compared with those who only participated in baseline data collection. These differences in health status were expected since most of the recruits (89.6%) who did not complete the entire study had been excluded from continuing military service due to health problems (Paper III).

6.5 Effects of the dietary intervention among young male adults

The baseline intake of vegetables, fruits, berries and juice (VF) was fairly low among the participants who completed the intervention study, compared with the recommendations from the Norwegian Directorate of Health (52). The present study showed a total baseline intake of VF of 434 g/d in the intervention group and 450 g/d in the control group (Paper III), whereas the recommended intake corresponds to a total of 750 grams of fruit and vegetables (including potatoes) per day (52). Our results are in line with the national dietary survey, Norkost, which showed an average intake of 448 g fruits, berries, juice and vegetables, including potatoes, per day among 16- to 29-year-old men (54). The present dietary intervention resulted in an increased consumption of both VF and bread (Paper III). To our knowledge, no other studies have reported similar intervention effects on young male adults. The result from this study demonstrated that nutritional information and increased availability of vegetables, fruits and whole grain bread was an effective combination of strategies, which resulted in an increased intake of these food items among the young male adults. These data thereby suggest that the main aim of our nutritional intervention was achieved.

The results, however, showed an increased level of BMI at the end of the study period (Paper II). On the other hand, the study reported a positive change in estimated dietary intake of fatty acids in line with the recommendations from the Norwegian Directorate of Health (Paper III) (52). Changes in the concentration of serum lipids did not, however, reflect these positive changes in estimated intakes of fatty acids. This incoherence between changes in estimated dietary intake of fatty acids and changes in serum lipids may be due to the fact that the concentration of serum lipid at baseline reflect dietary intake of fatty acids before entering military service, whereas the registration of dietary intake was done after entering military service.

The marginal increases in the concentration of triacylglycerol were shown in both the intervention group and the control group, but were higher in the latter group. The recruits in both groups achieved a significant improvement in physical capacity during the study period (Paper II). At the end of the study, however, the intervention group showed a significantly improved result on the 3000-metre run compared with the control group. This latter result was unexpected, since to the fact that the military camps were selected due to a similar level of physical training during military service. A study by Durstine *et al*. (59) has demonstrated an inverse relationship between increase in physical capacity and the concentration of

triacylglycerol. The difference in triacylglycerol between the intervention group and the control group at the end of the study may therefore be explained by different levels of improvement in physical fitness between the groups. The recruits did not report any significant changes in smoking habits during the intervention period.

In agreement with the reported increased intake of VF and whole grain bread, the present study showed an estimated increase in the intake of dietary fibre and folate (Paper III and Figure 6-1). It was confirmed that the relative contribution of estimated folate intake from fruits, vegetables and whole grain bread was significantly higher in the intervention group compared with the control group. On the other hand, the results showed a minor decrease in the estimated intake of vitamin B_2 (Paper III and Figure 6-1).

To provide an objective measure of B-vitamin intakes, the present study reported changes in serum concentrations of folate and vitamin B_{12}, and plasma concentrations of vitamin B_2, FAD and FMN (Paper II). Although both the intervention group and the control group increased the concentration of serum folate during the study period, a larger increase was observed in the latter group. Nevertheless, the concentration of serum folate was higher in the intervention group than in the control group at the end of the study period. The inability to demonstrate a positive intervention effect on folate status may therefore be due to a significantly lower baseline concentration of folate in the control group compared with the intervention group. When interpreting the results, it is also important to take into consideration that the levels of serum folate decrease rapidly even after a few days with a low intake (72).

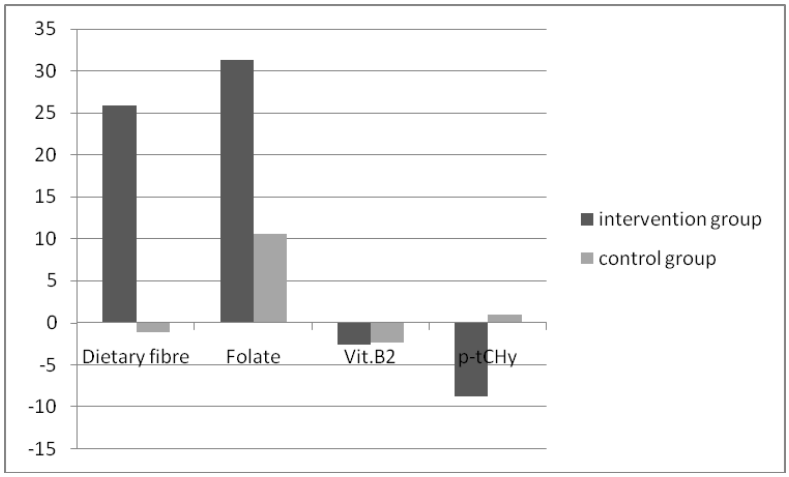
As dietary changes may have a differential impact on a genetically heterogeneous population, information on *MTHFR* polymorphism among the recruits could have contributed to interpret the dietary data on folate intake (20). However, genetic analyses were not included in the design of the present study.

A significant increase in the plasma concentration of vitamin B_2 was shown in the present study (Paper II). The increased concentration of vitamin B_2, however, did not correspond with the estimated decrease in vitamin B_2 intake (Paper III). The lack of association between changes in estimated intake from the diet, and the changes in riboflavin status may be due to an incomplete registration of food items that contributed to the total intake of vitamin B_2. The

principal dietary sources of vitamin B_2 include dairy products, poultry, meat, fish, asparagus, broccoli and spinach (72). The quantities of e.g. poultry and meat that several of the recruits purchased outside the military mess may most probably have been less accurately reported than the food served in the military mess.

The dietary intervention resulted in a significant decrease in the concentration of p-tHcy (Paper II and Figure 6-1). As expected, an inverse association was shown between the concentration of p-tHcy and the serum concentration of folate and vitamin B_{12} and the plasma concentration of vitamin B_2, FMN which are involved in the metabolism of p-tHcy. The present study also showed that the changes in p-tHcy were inversely related to the concentration of p-cys, serum folate and FMN in plasma. This is in agreement with the result presented in Paper III, which showed an inverse relationship between changes in p-tHcy concentration and changes in estimated total intake of folate and estimated intake of folate from whole grain bread.

Figure 6-1: Changes (%) in estimated intake of dietary fibre, folate and vitamin B_2 and changes in the concentration of p-tHcy during the intervention study among young men



6.6 Methods of food processing and retention of folates in vegetables

Few studies have investigated the retention of folate in foods after different methods of processing used in small- and large-scale service systems, e.g. in military messes. We investigated folate retention in peas, broccoli and potatoes after processing, warm holding, storage and re-heating (Paper IV). These vegetables were selected since they are among the major contributors of folate in the Nordic diet. Furthermore, peas, broccoli and potatoes were main ingredients in several of the dishes that were prepared for the intervention group in order to increase the dietary intake of folate during the 5-months intervention study. The results demonstrated the importance of using cooking procedures with a minimum amount of water in order to retain the folate content in vegetables (Paper IV). This result is in accordance with previous reported results which have shown that losses of folate during processing occur by leaching of folates to water used for washing, boiling, blanching or steam boiling (142, 149). In order to retain the nutritional benefits of the selected ingredients, recipes used in the intervention study were therefore developed in order to use cooking procedures with a minimum of water.

The military messes that were involved in the dietary intervention study used peas that were industrially blanched and frozen before further processing with steam-boiling. The study of folate retention in vegetables showed minimal losses of folate after steam-boiling, whereas blanching of peas resulted in significant losses of folate. In the dietary intervention study, the estimated intake of folate from peas was based on the folate content of blanched and frozen peas reported from the Norwegian Food database. Losses of folate due to blanching of peas were therefore taken into consideration when calculating the total estimated intake of folate during the study period.

Likewise, broccoli, potatoes and other vegetables were usually processed with steamed-boiling in the military messes of both the intervention group and the control group. The study of folate retention in vegetables showed minimal losses of folate during steam-boiling of broccoli, and since this method of processing involves a minimum amount of water, it is also expected to result in a high retention of folates in other food items.

In addition to steam-boiling, unpeeled potatoes were occasionally oven-baked during the intervention period. The present study of food processing and retention of folates in vegetables showed a significant loss of folate after oven-baking of unpeeled potatoes. Another

study by Augustin *et al*. (150) did not report significant losses of folate after oven-baking of unpealed potatoes. The difference in folate retention between these studies might be explained by a higher oven temperature and longer baking time in the present study. The oven temperature and baking time in the military messes was, however, similar to the method used in the study by Augustin *et al*. (150). Thus, results from the study of folate retention in vegetables indicate only minor losses of folate in food after processing methods used in the military messes.

Previously, warm holding has been suggested to ensure better folate retention than a cook/chill system (151). This could not be confirmed by this study since storage at various temperatures and times followed by reheating caused no further significant losses of total folate. To our knowledge, no other studies have investigated the retention of folates after storage of cooked vegetables at various temperatures and lengths of time followed by reheating. This result is of importance for an optimal conservation of nutrients as there is an extensive use of storage and reheating in several modern large-scale service systems. The methods of processing, storage and reheating that were investigated in the present study are also commonly used in Norwegian military messes. The military cooks that assisted in the dietary intervention study were, however, trained to use cooking procedures with a minimum amount of water. Thus, results from the study of folate retention in vegetables does not show losses of folate in food due to methods of storage and/or reheating of processed used in the military messes.

7. Conclusions

Baseline results from the present study have clearly demonstrated that young male adults are an important target group for health promotion efforts. The findings demonstrated a low level of physical fitness, a high rate of overweight and obesity and a high prevalence of hyperhomocysteinemia among the young adults. The results also showed that high paternal education has a positive influence on young male adults' lifestyle behaviour and a subsequent positive effect on BMI and lipid profiles. On the other hand, the educational level of the mothers was not related to any of the selected health parameters in young male adults. The results emphasize the importance of health promotion efforts targeted toward young adults of low socio-economic background, in particular those with high BMI and low paternal educational level.

The present study showed that the 5-months dietary intervention was successful in increasing the intake of fruits, vegetables and whole grain bread among young male adults. The dietary intervention study demonstrated that increased nutritional information and increased availability of vegetables, fruits and whole grain bread appear to be an effective combination of strategies for increasing the intake of these food items among young men. These findings are important as young men, in particular, have been considered a difficult group to reach with conventional dietary information. The effective combination of strategies used in the present intervention study should therefore be considered in the process of implementing official health promotion strategies for this important target group.

In addition, the increased intake of vegetables, fruits and whole grain bread has shown a favourable effect on the concentration of p-tHcy and its metabolites. The findings also suggest that the changes in estimated intake of folate from whole grain bread and changes in the plasma concentration of cysteine and FMN and serum concentration of folate seem to be predictors of changes in the p-tHcy concentration. In the current debate on folic acid fortification, this study has shown new evidence of an alternative policy approach based on nutrition education and a food-based alternative to supplementation and/or folic acid fortification.

Finally, this study has demonstrated that the retention and availability of folate in various food items is dependent on both the food in question and the method of processing. It has also confirmed the importance of cooking procedures that involve a minimum amount of water in order to retain the nutritional benefits of the vegetables. This study has provided new information about high retention of folates after subsequent storage and reheating of vegetables. These findings are of great importance due to an extensive use of storage and reheating of processed food in several modern small- and large-scale service systems, including military messes. The results from this study have also shown that methods of processing that were commonly used at the military messes during the study period caused minimal losses of folate. These findings indicate that the reported intake of folate during the dietary intervention period is not overestimated due to losses of folate after processing.

8. Perspectives and further research

There is a need for additional research to investigate if any effect like the one found in the present dietary intervention program among young men is found in other situations, and whether the changes in dietary behaviour are sustained over longer time frames. Additional information on *MTHFR* polymorphism among the participants would provide useful information when interpreting the dietary data on folate intake (20).

Given the complexities of eating behaviour, further work is required to identify the factors that influence the eating behaviour and the main barriers experienced among young men, in order to increase their intake of vegetables, fruits and whole grain bread.

Food composition databases provide detailed information on the nutrients contained in raw foods. However, there is a lack of information on nutrient content in processed food and mixed diets. To obtain more accurate data in nutritional surveys, there is a need for determining nutrient availability in various food items and mixed diets after different methods of processing and storage which are used in small- and large-scale service systems. Based on research findings, food industry and large-scale service systems should also implement methods of food processing and storage that result in minimal nutritional losses.

Unquestionably, nutrition is one of the primary environmental exposures that determine health status. However, it is also recognized that chronic diseases are complex in their etiology and include substantial genetic as well as epigenetic components (152, 153). Increased understanding of the function of the human genome will help to identify how the diet affects our genes and why individuals vary in their response to different nutrients and diets. In turn, this will help in the development of nutritious foods and special functional ingredients that offer the potential of enhanced health or reduced risk of disease of individuals.

To successfully change dietary habits on a national level, it is essential that the selected strategies address not only the individual but also the environment that influence the food choices of young men. The ability to reverse current trends in diet-related disease will depend on support by national leadership through policies, and by commitment from health professionals, industry and individuals.

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Appendix I

Information consent

INFORMASJON OM FORSKNINGSPROSJEKT

BAKGRUNN FOR PROSJEKTET. Hensikten med studien er å finne ut hvilke endringer som skjer med kostholdet ditt mens du er i det militæret og hvordan fysisk aktivitet innvirker på helsen.

HVA BETYR DETTE FOR DEG?
Ved forsøksstart og etter 6 måneder ber vi deg om å være behjelpelig med å **fylle ut et spørreskjema i fire dager** som måler inntak og holdninger/kunnskap om frukt/grønt/kornprodukter, bruk av spisemesse, kantine og kiosk. For å registrere eventuelle endringer av næringsopptak må vi også **ta 2 blodprøver** (en ved forsøksstart og en etter 6 måneder). Disse vil undersøkes med hensyn til fettstoffer og andre næringsstoffer i blod som har betydning for helsetilstanden. Vekt og høyde vil også registeres. For å kunne vurdere blodprøvesvarene er det nødvendig å vite alder, om du inntar vitaminer eller medisiner og til hvilket tidspunkt du inntok ditt siste måltid. Disse spørsmålene besvarer du ved avkryssing på et eget skjema.

BEHANDLING AV DATA. Innsamling og analyse av data vil være ferdig våren 2006 og prosjektet vil avsluttes 1.juli 2007. Resultatene fra forsøket vil publiseres i vitenskapelige artikler og i to doktorgrader. I disse vitenskapelige arbeidene er det ikke mulig å finne tilbake til hva du har svart eller til deg som person.

Alle data fra undersøkelsen vil bli lagret konfidensielt. Prosjektet er **meldt til Personvernombudet for forskning, Norsk samfunnsvitenskapelig Datatjeneste AS og til Regional komitè for medisinsk forskningsetikk**. Ved forsøksslutt vil prøvene bli anonymisert. Hvis du gir tillatelse til det, vil prøvene i løpet av en tiårsperiode etter prosjektslutt kunne brukes til eventuelle tilleggsanalyser, som vil kunne gi ytterligere informasjon om ernæringsstatus på det tidspunktet prøvene ble tatt. Prøvene vil oppbevares uten mulighet for personidentifikasjon i disse 10 årene før de destrueres.

SKRIFTLIG SAMTYKKE. For å kunne benytte datamaterialet trenger vi ditt skriftlige samtykke. Deltakelsen er frivillig og hvis du ikke ønsker å delta, får dette **ingen negative konsekvenser**. Hvis du velger å delta, kan du likevel når som helst trekke deg fra studien. Prøvene, helseopplysninger og personopplysninger vil utleveres eller destrueres.

Med vennlig hilsen

Wenche Frølich (Prosjektleder) Margareta Wandel Tonje Holte Stea Solveig Uglem

Appendix II

Clinical questionnaire

1218

Kliniske opplysninger

Identifikasjon Kode fylles ut av den som behandler blodprøvene

Hvor gammel er du?

Høyde (cm) **Vekt** (kg)

Når inntok du ditt siste måltid? **Dato** d d . m m . å å **Klokkeslett** t t m m

Hva spiste og drakk du?

Kode fylles ut av den som behandler resultatene

Tar du vitamintabletter?

Ja ☐ Nei ☐ *Hvis ja, hvilke?*

Kode fylles ut av den som behandler resultatene

Bruker du medisiner?

Ja ☐ Nei ☐ *Hvis ja, hvilke?*

Kode fylles ut av den som behandler resultatene

Hvor mange sigaretter røyker du per dag? (antall)

Hvor lenge har du røykt? (antall år)

Kan vi fryse ned en prøve til senere bruk i prosjektet? (i henhold til den informasjon som er gitt om prosjektet)

Ja ☐ Nei ☐

Appendix III

Validated food diary

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SPØRRESKJEMA

OM FRUKT, GRØNNSAKER OG BRØD

Kjære soldat!

I dette spørreskjemaet spør vi om dine spisevaner, med særlig fokus på frukt, grønnsaker og brød.

Spørreskjemaet er todelt. Hver dag i en uke skal du fylle ut et skjema der vi spør om dine spisevaner. Det er viktig at du tenker nøye igjennom hva du har spist og hvor mye du har spist i løpet av dagen og at du fører det inn i skjemaet. Skjemaets andre del skal kun besvares en gang. Dette er spørsmål om din sosiale bakgrunn og om dine holdninger og kunnskaper om kosthold og helse. For hvert spørsmål er det viktig at du finner det svaralternativet som passer best for deg.

Spørreskjemaet består stort sett av spørsmål hvor du kan svare ved å sette ett kryss. Noen spørsmål krever imidlertid at du svarer med enstavelsesord eller korte setninger.

Følg godt med når prosjektmedarbeideren går igjennom spørreskjemaet. Er det noe du lurer på underveis er det bare å spørre.

Det er frivillig å svare på disse spørsmålene og du kan trekke deg når som helst. Opplysningene er fortrolige og ingen får vite hva du har svart.

Takk for at du tok deg tid til å svare på spørsmålene!

Tonje Holte Stea
Stipendiat

Solveig Uglem
Stipendiat

Margareta Wandel
Professor

Wenche Frølich
Professor

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Del 1

Dato ☐☐.☐☐.☐☐

Navn

Kode etter navneliste fylles ut av den som analyserer skjemaet

For hver dag skal du føre opp hvor mye og hva du har spist av de forskjellige matvarene som finnes i dette spørreskjemaet. Det er viktig at du tenker igjennom hva du har spist til alle måltider og mellom måltider før du fyller ut skjemaet.

1. Brødvarer (legg sammen det du har spist av brødvarer til alle måltider og fyll inn i de åpne rutene)

1/2 rundstykke = 1 skive

	Antall skiver i dag		Antall skiver i dag
Fint brød/rundstykker (loff, baguetter o.l)	☐☐	**Mørkt knekkebrød** (husman o.l)	☐☐
Mellomgrovt brød/rundstykker (kneip o.l.)	☐☐	**Lyst knekkebrød** (frokost o.l)	☐☐
Grovt brød/rundstykker (fiberkneipp o.l)	☐☐	**Flatbrød**	☐☐

2. Frokostblandinger og grøt (legg sammen det du har spist til alle måltider)

	Antall porsjoner i dag				Porsjonsstørrelse			
	1	2	3	4	A	B	C	D
Havregrøt	☐	☐	☐	☐	☐	☐	☐	☐
Kornblanding, usøtet (4-korn o.l)	☐	☐	☐	☐	☐	☐	☐	☐
Kornblanding, søtet (Cornflakes, Honnikorn o.l)	☐	☐	☐	☐	☐	☐	☐	☐

Annet

Fyll inn hva, antall porsjoner og porsjonsstørrelse

Fylles ut av den som analyserer skjemaet ☐☐ ☐

Husk! A er liten posjon, B er middels porsjon, C er stor porsjon og D er ekstra stor porsjon

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3. Poteter, ris og pasta (legg sammen det du har spist til alle måltider og mellommåltider)

Mengde i dag

	1	2	3	4	5	6	7	8	9	10	11	12
Poteter (stk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

	Antall porsjoner i dag				Porsjonsstørrelse			
	1	2	3	4	A	B	C	D
Pasta (spaghetti o.l)	☐	☐	☐	☐	☐	☐	☐	☐
Ris	☐	☐	☐	☐	☐	☐	☐	☐
Potetstappe	☐	☐	☐	☐	☐	☐	☐	☐

4. Grønnsaker (her registreres det du har spist av "synlige grønnsaker". Grønnsaker som er en del av en rett, for eksempel gryteretter, registreres under punkt 6 og 7. Legg sammen det du har spist til alle måltider og mellommåltider og anslå det tallet som er nærmest det du har spist)

Antall i dag

	1	2	3	4	5	6	7	8	9	10
Gulrot (stk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kålrot (skiver)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tomat (båter/skiver)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Agurk (biter/skiver)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Løk (spiseskjeer) stekt og rå	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Paprika (ringer)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Squash (biter/skiver)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Asparges (antall)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Reddik (stk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oliven (stk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Selleri (staver)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rosenkål (stk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brokkoli (dusker)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Blomkål (dusker)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

mer grønnsaker neste side

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	Antall porsjoner i dag				Porsjonsstørrelse			
	1	2	3	4	A	B	C	D
Kål (hodekål, hvitkål o.l)	☐	☐	☐	☐	☐	☐	☐	☐
Ertestuing	☐	☐	☐	☐	☐	☐	☐	☐
Surkål	☐	☐	☐	☐	☐	☐	☐	☐
Bønner	☐	☐	☐	☐	☐	☐	☐	☐
Grønnsaksblanding, friske eller frosne. *Ikke grønnsaksretter med nummer på*	☐	☐	☐	☐	☐	☐	☐	☐
Fennikel	☐	☐	☐	☐	☐	☐	☐	☐
Grønne erter	☐	☐	☐	☐	☐	☐	☐	☐
Spinat og andre kokte grønne blader	☐	☐	☐	☐	☐	☐	☐	☐
Salatblanding (grønn salat/kinakål, tomat, agurk, mais etc.)	☐	☐	☐	☐	☐	☐	☐	☐
Grønn salat	☐	☐	☐	☐	☐	☐	☐	☐
Sukkererter	☐	☐	☐	☐	☐	☐	☐	☐
Sopp, fersk, hermetisk, stekt (champignon o.l)	☐	☐	☐	☐	☐	☐	☐	☐

Annet

Fyll inn hva, antall porsjoner og porsjonsstørrelse

Fylles ut av den som analyserer skjemaet

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5. Frukt, bær, syltetøy og desserter bestående av frukt og bær (Her registreres det du har spist av frukt og bær. I tillegg registreres det du har spist av syltetøy og desserter hvor frukt og/eller bær inngår som ingredienser. Legg sammen det du har spist til alle måltider og mellommåltider.)

Mengde i dag

	1	2	3	4	5
Eple (stk)	☐	☐	☐	☐	☐
Pære (stk)	☐	☐	☐	☐	☐
Fersken/ Nektarin (stk)	☐	☐	☐	☐	☐
Kiwi (stk)	☐	☐	☐	☐	☐

Mengde i dag

	1	2	3	4	5
Banan (stk)	☐	☐	☐	☐	☐
Appelsin (stk)	☐	☐	☐	☐	☐
Mandarin/ klementin (stk)	☐	☐	☐	☐	☐
Melon (skive)	☐	☐	☐	☐	☐

Mengde i dag

	1	2	3	4	5	6	7	8	9	10
Plommer (stk)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Druer (klaser på ca 10)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Moreller/ kirsebær (stilker)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ananas (biter)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Syltetøy (spiseskjeer)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Frukt/bær-yoghurt (beger)	☐	☐	☐	☐	☐					
Go` morgenyoghurt	☐	☐	☐	☐	☐					

	Antall porsjoner i dag				Porsjonsstørrelse			
	1	2	3	4	A	B	C	D
Fruktsalat	☐	☐	☐	☐	☐	☐	☐	☐
Jordbær	☐	☐	☐	☐	☐	☐	☐	☐
Blåbær	☐	☐	☐	☐	☐	☐	☐	☐
Bringebær	☐	☐	☐	☐	☐	☐	☐	☐
Bjørnebær	☐	☐	☐	☐	☐	☐	☐	☐
Solbær	☐	☐	☐	☐	☐	☐	☐	☐
Hermetisk frukt (alle typer)	☐	☐	☐	☐	☐	☐	☐	☐

Annet

Fyll inn hva, antall porsjoner og porsjonsstørrelse

Fylles ut av den som analyserer skjemaet

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6. Ferdige lunsjretter (Her registreres det du har spist av ferdige lunsjretter med nummer. Poteter og andre "synlige grønnsaker" som ikke er en del av en rett registreres under punkt 3 og 4.)

	Nummer	Antall porsjoner i dag				Porsjonsstørrelse			
		1	2	3	4	A	B	C	D
Lunsjrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Lunsjrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Lunsjrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Lunsjrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Lunsjrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐

7. Ferdige middagsretter (Her registreres det du har spist av ferdige middagsretter med nummer, som gryteretter o.l som ikke er en del av en rett. Poteter og andre "synlige grønnsaker"registreres under spørsmål 3 og 4.)

	Nummer	Antall porsjoner i dag				Porsjonsstørrelse			
		1	2	3	4	A	B	C	D
Middagsrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Middagsrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Middagsrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Middagsrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐
Middagsrett	☐☐	☐	☐	☐	☐	☐	☐	☐	☐

8. Smør/margarin (her registreres hvilken type smør/margarin du spiser og hvor mye du smører på brødskiven.)

Hvilken type smør/margarin bruker du?

- ☐ Bruker ikke smør
- ☐ Meierismør
- ☐ Bremykt
- ☐ Brelett
- ☐ Myk margarin (soft o.l)
- ☐ Lett margarin (soft light o.l)

Hvor tykt lag smør/margarin smører du på brødet?

Tynt	Middels	Tykt
☐	☐	☐

Annet

Fyll inn hva og hvor mye

Fylles ut av den som analyserer skjemaet ☐☐ ☐

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9. Drikke (Her registrerer du hva du drikker av utvalgte drikkevarer, og hvor mye du drikker i løpet av en dag. Se bort i fra alt du ikke finner i listen som vann, kaffe, te etc.)

	Antall glass pr. dag									
	1	2	3	4	5	6	7	8	9	10
Helmelk, kefir, drikkeyoghurt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettmelk, cultura, biola	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ekstra lett melk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Skummet melk, skummet kulturmelk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Smakssatt melk (litago, sjokomelk o.l.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Juice (eple, appelsin o.l)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brus med sukker (cola, solo, mozell etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brus uten sukker (cola light, solo light etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Drikker som består av presset frukt og bær	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

10. Måltider og snacks som spises andre steder enn i spisemessen (Her registreres det du spiser av måltider og snacks i kantinen/kiosken og utenfor forlegningen som du ikke finner noe annet sted i skjemaet. Mat som kommer fra spisemessen noteres ikke her, men under spørsmålene ellers i skjemaet)

Har du spist utenfor forlegningen i dag?

Ja ☐ Nei ☐

Hvis ja, hvor spiste du?

- ☐ I kantinen/kiosken i militærforlegningen
- ☐ Hjemme på helgeperm o.l
- ☐ Hjemme hos venner og kjente
- ☐ På kafeer, restauranter etc utenfor forlegningen
- ☐ I kasernen (mat kjøpt i dagligvarebutikk, takeaway osv.)

Hvilket eller hvilke måltid spiste du i kantinen/kiosken eller utenfor forlegningen?

Frokost ☐ Lunsj ☐ Middag ☐ Kveldsmat ☐ Snacks/mellommåltid ☐

Hva spiste du i kantinen/kiosken eller utenfor forlegningen? (Angi hva og porsjonsstørrelse, for eksempel 1 hamburger, 3 stykker pizza o.l.)

Fylles ut av den som analyserer skjemaet