Intake of fat-soluble vitamins (A, D, E, K) by food supplements and fortified foods in Belgian toddlers

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Master dissertation submitted in partial fulfilment of the requirements for the degree of Science in Nutrition and Rural Development
Main subject: Human Nutrition – Major: Public Health Nutrition
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Ghent University, June, 2016

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Abstract

**Background:** In the growing market of functional foods, a new concern has risen – the excessive intake of fat-soluble vitamins. Toddlers have the highest exposure per kg of body weight and they are considered to be one of the groups at risk. Despite this, the problem of inadequacy is still not solved (e.g. vitamin D). Subsequently an assessment of the current situation was needed, in order to develop the right public health policy.

**Objective:** The main objective of this thesis was to determine the proportion of Belgian, Flemish toddlers who have the intake of fat-soluble vitamins under the Recommended Daily Intake (RDI) and above the Tolerable Upper Intake Level (UL). The specific objective was to assess the contribution of enriched foods, supplements and basic diet to the total intake of fat-soluble vitamins in the case of Flemish toddlers.

**Design:** The study performed was a cross sectional study. The quantitative internet based food frequency questionnaire was used as the assessment method. The recall period of the intake was one month and/or the specific food items (e.g. liver) and supplements the recall period was one year. The estimated usual intake of each vitamin was calculated per person per day. We have assessed the proportion of toddlers that are under the Estimated Average Requirement (EAR) or Adequate Intake (AI) and the UL. The RDI was not used because of possible overestimation of inadequacy. The contribution of supplements, enriched food and basic diet to the total intake of fat-soluble vitamin was determined, as well.

**Results:** The intake of vitamin A for 78.6% of toddlers was between the EAR and the UL. The users of vitamin A supplements were having significantly higher intakes compared to non-supplement users. The 39.8% of toddlers had the intake of vitamin D under the AI and 37.9% were exceeding the UL. The supplement users were the one in the highest percentiles of the distribution. The intake of vitamin E in Flemish toddlers was low, counting that 76.7% had the intake below the AI. For vitamin K intake, all the toddlers in the survey had the intake above the AI. For vitamins A, E and K basic diet was the highest contributor to the total intake of specific vitamin. In the case of vitamin D, supplements were the ones contributing the most, with 54% of the intake. Enriched food had the biggest impact on the intake of vitamin E (14%).

**Conclusion:** Belgian supplementation program had impact on the intake of fat-soluble vitamins. However, the double problem of inadequacy and excessive intake exists in Flanders, Belgium. This survey represents a good starting point for the future researches in Europe concerning this topic.

**Keywords:** fat-soluble vitamins, toddlers, intake study, UL, RDI, FFQ
Acknowledgements

First of all, I would like to express my gratitude to the person that helped me the most, my tutor Isabelle Moyersoen. I know that without her help and support this thesis would not be possible. We will always remember our really long and stressful days, but we made it through stronger and smarter. I would also like to thank my colleague Olivia Kanora, for pushing me to work harder and helping me to cope with the language barrier. Likewise, I would like to express my gratefulness to my promoter professor dr. ir. John Van Camp for accepting, guiding and encouraging me in the execution of this study, from the day one.

I will use this opportunity to acknowledge the kind support and readiness to help of the academic and administrative coordinators of this Master program, ir. Anne-Marie Remaut-DeWinter and Mrs. Marian Mareen. I was more than happy to have you as my support in these past two years.

My gratitude is also devoted towards the ERASMUS MUNDUS - Basileus scholarship program for funding my studies and making this opportunity available for me.

I would also like to thank my friends and family who were personally part of this goal. First, I thank my family and friends in Serbia, for listening to me and giving me the necessary strength to bring this study to the end. My special thanks goes to my boyfriend, who was unconditionally supporting and understanding me in every moment of these studies.

I would like to extend my gratefulness to my dear classmates and friends Bruna, Sewwandi, Anais, Anna-Sophia, Milka, Lucas, Peter, Nima and Tiago for shining up these grey Belgium days. Thank you for making these two years incredible journey, without you nothing would be the same.

Monika Varga, June, 2016
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List of abbreviation

VITADEK – Vitamin Intake Study (A,D,E,K)
FFQ – Food frequency questionnaire
FCS – Food consumption survey
EFSA – European Food Safety Authority
RDI – Recommended daily intake
EAR – Estimated Average requirement
AI – Adequate Intake
PRI – Population Reference Intake
UL – Tolerable Upper Intake Level
NHANES – National Health and Nutrition Examination Survey
FITS – Feeding Infant and Toddler study
ESPGHAN- The European Society for Paediatric Gastroenterology, hepatology and Nutrition
NOAEL – No Observed Adverse Effect Level
LOAEL – Lowest Observed Adverse Effect Level
DONALD – Dortmund Nutritional and Anthropometric Longitudinally Designed Study
PUFA – Poly Unsaturated Fatty Acids
RE – Retinol Equivalent
1. Introduction

Prior to the year 2004, when the first national consumption survey was conducted, Belgium was one of the few countries lacking a systematic survey on the subject of the consumption patterns and food habits of its population. (Vandevijvere et al., 2008; De Vriese et al., 2005). The new national food consumption survey is about to be finished and published in the year 2016. Furthermore, as well in Belgium, as on the European level in general, we are confronted to a poor availability of food intake studies which estimate the intake of fat-soluble vitamins from the regular diet, fortified foods and food supplements. Both in Belgium and in Europe there have been very few studies performed on the susceptible group of children - infants and toddlers.

The group of fat-soluble vitamins (D, E, A, K and β-carotene) is considered to be of special importance for public health because of the vitamins’ unique characteristics. On the one hand, they have beneficial effects. On the other hand, since they are soluble in fats, if consumed in higher amounts, they will be stored in the adipose tissue (Huybrechts et al., 2010). The adverse health effects caused by the excessive intake of fat-soluble vitamins are: liver and bone damage, neurotoxicity (vitamin A), a reduction in blood clotting ability, bleeding (vitamin E), neurological disorders and kidney stones (vitamin D) (EFSA, 2006). For instance, antioxidants such as β-carotene were earlier considered to have beneficial effects on human health. However, the research conducted by Druesne-Pecollo et al. (2010) has shown that consumption of β-carotene in the case of smokers causes higher risk of lung cancer. The seriousness of the adverse health effects developed out of fat-soluble vitamins emphasizes the importance of the intake study in this field. Besides the risk of excessive intakes of fat-soluble vitamins, the population is facing risks of constant inadequate intakes (e.g. vitamin D), leading to severe health effects (Siro and Buttriss, 2012). The double burden exists, pointing out the need for the assessment of the current situation in Belgium, and around the world.

According to Rasmusen et al. (2006), there exist an urgent need for developing a model in order to set the ULs for all the population groups and all the micronutrients. For example, there is no UL for vitamin K, while the UL regarding vitamin A, vitamin D and vitamin E for children, are derived from the value for adults (EFSA, 2006). The increase of the risk of exceeding the UL results from the fact that the functional food market is constantly growing (Hilliam et al., 1998). It is shown that the population groups under the highest risk are: for vitamin A and β-carotene children age 1-10, for vitamin D children age 7-10, for vitamin K and vitamin E children age 1-3 (Rasmusen et al., 2006).

In line with all aforementioned, this study will present new data about the total intake of fat-soluble vitamins in Belgium and the contribution of the fortified foods and dietary supplements to the total intake. To perform good policy in the field of public health (effectiveness to improve nutritional status of the population) and food safety (protection against excessive intakes) more information is required about the consumption of functional foods and the intake of nutrients which are added to these foods. Moreover, this particular thesis will focus on the total intake of fat-soluble vitamins in
the case of toddlers, since they belong to the vulnerable groups of the population. It will also concentrate on estimating the risk of exceeding the safe intakes of fat-soluble vitamins (UL) and inadequate intakes of fat-soluble vitamins. The sample used in the thesis represents just a part of the original study (VITADEK), derived from the public health institute in Brussels. This thesis is focusing on the sample which is coming from Flanders, Belgium.

1.1. Objectives

1.1.1. Main objectives

The aim of the study is:

- To identify the proportion of Belgian, Flemish toddlers with an intake of fat-soluble vitamin <RDI
- To identify the proportion of Belgian, Flemish toddlers with an intake of fat-soluble vitamins> UI.

1.1.2. Specific objectives

More specifically, the study aims to:

- Collect data on the consumption of dietary supplements, fortified foods, and "regular" foods rich in fat-soluble vitamins;
- Make an estimate of the total intake of fat-soluble vitamins in the population and the contributions of the different groups of "regular" foods, fortified foods and supplements to the total intake;
- Separate the results for users and non-users of dietary supplements and fortified foods;

1.2. Hypothesis

**Null Hypothesis 1:** Intake of supplements and fortified foods will not contribute to exceeding Tolerable Upper Intake Levels of fat-soluble vitamins (A, D, E, K) in the specific population group – toddlers in Flanders, Belgium.

**Null Hypothesis 2:** Intake of supplements and fortified foods will not contribute to reaching the Recommended Daily Intake of fat-soluble vitamins (A, D, E, K) in the specific population group-toddlers in Flanders, Belgium.

1.3. Limitations

The major limitation of this research is the use of the FFQ as an intake assessment method. The limitation originates in the fact that the FFQ contains a substantial amount of measurement error. This is due to the following aspects (Rodrigo et al, 2015):

1. Lack of details: foods are divided into food groups and the food list does not contain all the food items which are being consumed.
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- Quantification of the intake is not accurate as when using dietary recalls or records (Rodrigo et al, 2015)
  - due to the use of incomplete listing of all possible foods
  - due to the error in estimation of frequencies and portion sizes
- Difficulties for the respondents to estimate the intake if consuming various foods from the same food group;
- Estimating the average intake may be difficult for the foods that may have highly variable portion sizes depending on the eating occasions (Rodrigo et al, 2015);

2. Difficult for respondents to handle and reflect to long recall period.

The second important limitation is related to the target group-toddlers. Assessing the food intake in the case of children is a complicated process due to several important factors. First, child’s growth from birth through infancy, toddlerhood and adolescence is an extremely complex process (Ortiz-andrellucchi et al, 2009). It occurs often that a child, due to the preferences, does not eat all the food served, which results in food disposal. Furthermore, parents often share the responsibility over their child with other adults (grandparents, day-care personnel), thus the information about the food eaten is biased (Ortiz-andrellucchi et al, 2009).

Another important limitation is correlated with the selection procedure. By selecting Health care centres for reaching toddlers, we have excluded from the sample toddlers visiting paediatric clinics. By choosing Dutch and French as languages of the questionnaire, we have excluded all registered immigrants who do not speak these languages. Furthermore, using a web-based questionnaire implies that the population who is internet and computer illiterate will not be reached. By conducting telephone interviews, we have tried to cope with the previously mentioned limitation. Respondents who had problems to fill in the questionnaire by themselves, were contacted by dieticians. Dieticians filled in the questionnaire while talking with the respondent on the phone.

Besides the general limitations, the original study was facing, the additional limitations this thesis was confronted are:

- The sample size used is smaller than what is planned
  - The data collection is still on-going;
  - Toddlers are the group which is difficult to assess, thus more time is needed to reach wanted sample size;
- Not all the information on the content of the fat-soluble vitamin was available
  - The laboratory analysis of specific toddler’s food will be performed in September 2016;
  - The values of vitamin content of toddler’s food were taken from the general items available on internet;
- Not all the intake of fat-soluble vitamins was assessed;
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- Due to the time limitations, open ended questions were not considered when the total intake of the vitamins was calculated;

It is important to state that above mentioned limitations are not going out of the scope of this thesis. However, the final outcome can be highly influenced by these shortcomings.
2. Literature review

2.1. Fortification and supplementation – past and present

The main goal of the fortification and supplementation programs in the past was to treat and prevent diagnosed nutrient deficiencies (e.g. beri-beri, goiter, pellagra, and rickets). Fortification of rice, wheat, maize, salt, sugar, gave good results in many countries - United States, Canada, Latin America (Chile, Guatemala, Mexico), Dominican Republic, and many others (Dwayer et al, 2015). These programs still exist in some developing countries and they are helping individuals defeat diseases (e.g. Vitamin A supplementation for pregnant women and lactating women and children; Iron supplementation, etc).

As the diet of the developing world has made a transition, the purpose of fortification and supplementation has been modified. Nowadays these programs are used to help the population meet their daily requirements (Dwayer et al, 2015). The supplementation and fortification programs are developed to achieve the recommended daily intake of vitamins and minerals. However, due to the difficulties in finding a good food vehicle and population groups at risk of inadequate intake, it is a great challenge to establish satisfying programs (Dwayer et al, 2015).

The United States of America is considered to be the leader when it comes to the fortified food market. Accordingly, it is the centre of research issuing this topic (Datta and Vitolins, 2014), which result in diverse opinions about the actual effects of fortified foods on the regular diet of the population (Dwayer et al, 2015).

On the other hand, concerning fortification and functional foods in general, the European market is stricter than the one of the USA. EFSA (European Food Safety Authority) has high standards in regards to placing and allowing new products on the market. It has been also shown that European consumers are more conservative in terms of accepting and approving new foods on the market (Ozen et al, 2014).

Despite the strict policy of EFSA, it has been shown that in the last 20 years the trend of using supplements and fortified food in Europe is rising (Ozen et al, 2014). Northern European countries (above 40N) are using more supplements and fortified food than the countries in Southern Europe. This can be explained not only by the lower sun exposure, which leads to a higher usage of vitamin D supplements, but also by the fact that the distribution and diversification of fruits and vegetables conducted in the past in the northern countries, was not enough to meet the population needs. Consequently, supplements and fortified food have become a part of the daily diet in Northern Europe. In Belgium, consumption of fortified margarines and cereals is found to be high (Ozen et al, 2015).It is believed that, if micro-nutrients, such as fat-soluble vitamins, were to be added to food products, they could result in a positive health effect. Therefore, those sub-population groups in which the RDA are not met, are the ones most likely to be using these products (Huybrechts et al, 2010).
Furthermore, a study conducted in Germany (Sichert-Hellert et al., 2006) has found out that consumption of vitamin supplements is higher than consumption of minerals supplements. It has been shown that multivitamin supplements have the recommended daily amount of each vitamin in their composition (Rasmusen et al., 2006). On the other hand, despite the high availability and supplements consumption, there are not too many people in Germany who exceed the ULs. For folic acid and vitamin A, a substantial proportion of the population is exceeding ULs (Sichert-Hellert et al., 2006).

The fortified foods and supplementation effect on the vitamin intake remains unclear, and different studies show highly distinctive results. According to Dwayer et al (2015), the impact depends on the food vehicle and vitamin added. In the systematic review of Dwayer et al (2015) it is stated that after the fortification with folic acid, 10% of the children between 8-12 years exceed the Tolerable Upper Intake Levels only from food. Moreover, among children taking supplements, 70% exceeded the UL. On the other hand, post-fortification studies have shown that between the years 1995 and 1999, folic acid fortification reduced the prevalence of neural tube defect by half (Dwayer et al., 2015). It has also been shown that the usage of supplements does not alter the intake of vitamin K, because it tends to be in very low doses when it comes to children supplements (Sichert-Hellert et al., 2006).

Despite the inconsistent results of studies about positive impact of fortified foods and supplements, long term studies have shown that fortification and supplementation can pose a risk of excessive nutrient intakes (Rompelberg et al., 2006). As such, the public health sector imposed their attention on potential risks caused by global use of fortified foods and supplements.

### 2.2. Potential concerns

The risk of overconsumption of certain nutrients has increased due to different factors. For example, fortified food is distributed to the whole population and not just to the targeted groups (Dwayer et al., 2015). Moreover, changing culinary and social habits has led to low intakes of vitamins and minerals from food compared to the recommendation for certain groups of the population. Additionally, consumers believe that optimal health depends on a higher intake of vitamins than those recommended in order to avoid deficiencies and that requirements cannot be reached by normal diets (Hilliam et al., 1998). Consequently, the market of products contributing to a higher vitamin and mineral intake is growing. With such expansion, authorities need to set maximum levels of vitamins and minerals in order to ensure that the potential sum from all the sources does not threaten human health (European Commission, 2006).

In the year 2006, EFSA established ULs for micronutrients inside the European Union, in order to avoid potential harmful health effects (EFSA, 2006). The UL is an estimate of the highest level of a habitual nutrient intake. In other words, this nutrient amount does not increase the risk for adverse health effects (Verkaik-Kloosterman et al., 2012). There exist the legislation for micronutrients addition to food (Regulation 1995/2006) and food supplements (Directive 2002/46). Despite this, setting maximum levels for vitamins and minerals addition to foods and dietary supplements is still under discussion in the EU (Verkaik-Kloosterman et al., 2012).
Establishing ULs is based on a risk assessment, which includes three steps: i.e. food intake, dose-response relation and possible adverse health effects (Hathcock and Shao, 2008). However, data regarding food intake is not available for all vitamins - especially regarding age groups. Consequently, setting an UL without making an error is quite difficult (Zlotkin S, 2006). For example, for vitamin K there is no data available and the experiments conducted on animals are uncertain. Therefore, the UL could not be established (EFSA, 2006). It is also familiar the fact that for vitamin A there is a narrow difference between the RDI and the UL, which can be misleading for the researchers and health care professionals, driving them to wrong conclusions (Zlotkin S, 2006).

Population groups which have intakes higher than the UL do not necessarily have too high of an intake of that specific micronutrient, however they are potentially at risk for adverse effects on health based on the intake levels of that micronutrient (Rasmusen et al, 2006).

People more susceptible to adverse effects of high vitamins intakes belong to the vulnerable groups, such as - pregnant women, lactating women, infants, toddlers and the elderly. It is known that these population subgroups tend to use more fortified food and supplements in order to improve their health (Hilliam et al, 1998).

Additionally, the increasing awareness of the importance of following a healthy lifestyle is one of the key factors for the growth in the market of fortified foods and dietary supplements and, as such, the increasing consumption of these products. There is a large and growing market of products which can help with specific modifications to a healthy intestinal flora, a healthy cardiovascular system (vitamins A, C and E), strong bones (vitamin D), and promotion of the immune system (vitamin A) (Hilliam et al, 1998).

Furthermore, there is a higher risk of exceeding the UL when combining supplements with fortified foods (Dwayer et al, 2015). No matter the legislation, there are still manufacturers adding unknown amounts of vitamins in food products (Verkaik-Kloosterman et al, 2012). It is also proven that industry adds higher concentrations of nutrients than what is written on the label. This is a common practise in production line due to the need of industry sector to assure that the product will have labelled concentration of vitamin at the end of its shelf life (De Lourdes Samaniego-Vaesken et al, 2012).

De Lourdes Samaniego-Vaesken et al (2012) have shown concern about different portion size and consumption of fortified food. The consumers of breakfast cereals are using two times bigger portions than the recommended amount. Consequently, the ingested amount of vitamin doubled compared to the labelled one (De Lourdes Samaniego-Vaesken et al, 2012).
2.3. Toddlers

Toddlers (age 1-3 years), make a transition from breastfeeding (infant formula) to independent feeding and omnivorous diet. This age is crucial for developing basic habits and a child’s behaviour towards food (Green, 2015). Furthermore, as the prevalence of obesity is rising, many parents are over caring about their children’s food intake (Allen et al, 2006, Green, 2015). Worries that arise in this age are preference for sweets, undisciplined table behaviour and refusal of fruits and vegetables (Allen et al, 2006). It is observed that due to these factors parents tend to give vitamin supplements to the children. However, it is shown that vitamin intake from supplements can be reached with small amount of fruit and vegetables consumed, and usually supplements do not even contain all the vitamins and minerals needed (Allen et al, 2006).

Moreover, despite the fact that a balanced diet provides the necessary macro and micro-nutrients, one-third of the school children consume dietary supplements (Huybrechts et al, 2010). This leads to a conclusion that many children take unnecessary supplements and that the combination of vitamin intakes from normal foods, fortified foods and supplements, leads to a risk of over-intake of micronutrient, such as fat-soluble vitamins (Huybrechts et al, 2010). In addition, young children often have the greatest exposure per kg of body weight, while their gastric capacity is just one-third compared to the adults’ one. Therefore it is clear that toddlers need a small, but energy and nutrient dense meals, to meet their basal requirements (Hilger et al, 2015).

According to Briefel et al (2006), in the United States the first born child or picky eaters tend to use more supplements. Furthermore, the factors which are the independent predictors of supplementation are location of living, sex, age, and household composition. For example, the consumption of supplements increases during the years, while the more children live in a household, the more the tendency of using supplements decreases (Briefel et al, 2006). It is shown that the use of supplements increases with age. In particular, the prevalence of supplement use was 8% among infants from 4 to 5 months, 19% among infants from 6 to 11 months and 31% among toddlers (Briefel et al, 2006). Most of the supplements infants and toddlers were using were mineral and vitamin supplements (91% of the users) (Briefel et al, 2006). In the group of vitamin supplements the most consumed ones were multivitamins, including vitamins, A, C, B-6, B-12, D, E, thamine, riboflavin. Single vitamin preparation was rarely used (Briefel et al, 2006). Compared to the supplement users, non-users had higher prevalence of inadequate intake of vitamins A, C, D and E across all age groups (Bailey et al, 2012).

Fortification of food varies among countries, especially when comparing the USA and Europe. However, when analysing the contribution of fortified food to the vitamin intake, just a few countries were able to provide this data (Flynn et al, 2009). Data about fortified food was collected from the labels or companies, so when assessing contribution of fortified food in the diet, the added amount of nutrient is not the only one considered, but the total amount of nutrient in fortified products. According to Flynn et al (2009), we can conclude that the contribution of fortified food to the total
vitamin intake is too low, and for the most of fat-soluble vitamins, it does not make an impact except for vitamin D. In this research, the mandatory fortified food was taken into account when calculating intakes from the general diet, implying that just the voluntary fortified food was considered when calculating contribution of fortified food to the total intake.

However, data regarding food intake is not available for all vitamins, and particularly not for all age groups. It is therefore not easy to draw general conclusions about consumption patterns present among children. The United States of America has a leading role in researches in this field. They are conducting the study FITS (Feeding Infant and Toddler Study) every 4 years. In the setting of the USA, it is shown that 31 % of toddlers are supplement users (Fox et al, 2006).

In North America, Canada is the country following the USA in the research field. Shakur et al (2010) came to the conclusion that supplement users do not have higher prevalence of inadequate intakes in the case where just the basic diet is observed. It is also shown that supplement users have a higher vitamin intake from their basic diet, when compared to non supplement users. It is shown that 38% of toddlers are using vitamin supplements and 35% of them are using particular multivitamin supplements (Shakur et al, 2010). Looking at the figures, it can be said that Canadian toddlers consume more supplements than American ones (38% and 31% respectively). Therefore, Canadians have to be cautious when using vitamin supplements because of the risk of exceeding Upper Intake levels.

In Europe, national food consumption surveys often do not cover the age group of toddlers, but just children of 15 years and above (Huybrechts and De Henauw, 2007; Van Rosum et al, 2011; Ruston et al, 2004). Based on the results of EPIC, the supplements usage differentiates among European countries, where the biggest difference is to be seen between North and South of the continent (Flynn et al, 2009). At the same time, tradition and habits are shown to be contributors to the use of vitamin supplements. According to these factors, recommendations for certain vitamins differ among countries, resulting in different patterns of the consumption (Flynn et al, 2009). When it comes to Europe, the highest consumption is to be found in the Nordic countries, in Denmark especially (Sisse et al, 2012). In the south of Europe, it is observed that in Italy many paediatricians prescribe supplements to toddlers, making them the group of population with the highest usage of supplements (Sette et al, 2010). Anyhow, it can be said that the basic diet of children-supplement users is healthier than of children who do not use supplements for most of the micronutrients (Bailey et al, 2012). More specifically, in the Dortmund Nutritional and Anthropometric Longitudinally Designed study (DONALD), it has been shown that 7% of toddlers are using supplements. This percentage increased compared to the study done back in 1992 (Sichert-Hellert et al, 2006).

It can be concluded that North American toddlers have higher consumption of vitamin supplements and therefore, they are more exposed to the risk of exceeding UL. However, based on the data available, it can be said that supplements consumption is increasing all over Europe.
Overall, there are 3 vitamin supplement-intake scenarios identified in the case of toddlers (Sichert-Hellert et al, 2006):

1. There is no benefit of supplement intake; this is the case for vitamin A, E and K. For those vitamins it is shown that the intake from the usual diet and fortified food reaches more than 80% of the US FDA reference value, so there is no need for additional supplementation.

2. There is a benefit of supplement intake to a single age group. In order to achieve at least 80% of US FDA reference values, intake of the supplements is necessary.

3. There is a benefit of supplement intake to almost all the age groups; the example is vitamin D. Even though supplements increased significantly the vitamin D intake, overall did not help to meet the recommended daily intakes of all the population groups.

In addition, children who are supplement users have a lower prevalence of nutrient inadequacy when compared to the supplement non-users. This situation can come as a result of the supplement use or the fact that the supplement users tend to have a higher fruits and vegetables intake. However, it should be also taken into consideration the fact that the supplement users are posed to the risk of exceeding upper intake levels.

2.4. Fat-soluble vitamins and their intake in toddlers

2.4.1. Vitamin A

Vitamin A is a fat-soluble vitamin which can be found in food of animal origin, in the form of retinyl esters, mainly as retinyl palmitate and retinol (Blomhoff et al, 1991). In fruits and vegetables, vitamin A appears in the form of provitamin A carotenoids, e.g β-carotene, α-carotene and β-cryptoxanthin (EFSA, 2006). Therefore, when calculating the vitamin A intake, it is essential to be familiar with the form of the vitamin present in the food.

The intake of vitamin A varies all over the world: while on the one side there is a problem of deficiencies, on the other side there is a potential risk of exceeding the UL. The vitamin A deficiencies are really rare in the developed countries, while in the developing world these deficiencies represent one of the major public health problems (Latham et al, 2010; Palmer et al, 2012).

The hypovitaminosis of vitamin A causes visual problems like night blindness and xerophthalmia. Further complications are the retardation of growth, skin disorders, impaired immune function, and congenital malformations of the eyes, lungs, cardiovascular and urinary systems, if the deficiency occurs during pregnancy (WHO & FAO report, 1998; EFSA, 2006). Many nongovernmental organizations invested their time and funds in solving these problems by providing supplements of vitamin A to children, pregnant and lactating women (Palmer et al, 2012). However, the final outcome is not succeeding in having a high impact on the situation. There is a statistical difference between the vitamin A status before and after the supplementation, but in the real setting, these
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changes are minor (Latham et al., 2010). Moreover, they do not have influence on the lives of the affected population (Latham et al., 2010).

The hypervitaminosis of vitamin A is, on the other hand, a potential risk in the developed world (EFSA, 2006). The potential hazards followed by an excess in retinol intake are: skin disorders, nausea, vomiting, bone pain, plus teratogenicity. The concern of exceeding ULs has risen because of the constant intake of fortified food in combination with supplements.

According to the EFSA (2015), the estimated mean requirement for children is 205 µg RE/ day. Furthermore, it is noticed that the vitamin A intake has a skewed distribution because the amount of retinol in foods varies significantly. This should be taken into account during the statistical analysis (EFSA, 2006).

The values of the ULs are based on different risks and different ages. The teratogenicity received a lot of attention in the evaluation of risk, because of the severe and irreversible nature of the toxicity. Consequently, ULs for the vitamin A are established to a value of 3000 µg/day which is a threshold for the teratogenicity caused by an excessive intake of this vitamin in the case of adults (EFSA, 2006). For children age 1-3, the value of 3000 µg RE/day is corrected for different basal metabolic rate according to the body weight and it is set to 800 µg RE/day (EFSA, 2006). However, any suggestion based on the ULs should take into consideration the narrow margin between the recommended daily intake and the intake associated with the adverse health effect.

According to the NHANES (National Health and Nutrition Examination Survey) 2005-2012 the food contributing the most to the vitamin A intake in the case of toddlers are: milk (37.4%), ready to eat cereals (8.1%), vegetables excluding potatoes (7.5%), baby food (4.7%), infant formula and cheese (4%) (Grimes et al., 2015). Cooked cereals, cheese, fruit juices, beverages, fats and oils, fruits and other food groups are contributing less than 4%. Briefel et al. (2006) have analysed the contribution of the supplements in the diet of infants and children, based on the FITS from the year 2002. According to this study, supplements were representing 42% of the intake of vitamin A.

It is also shown that the supplement users have a higher prevalence of excessive intake of retinol comparing to the non-users (97% and 15%, respectively). Using the data from FITS 2008, Butte et al. (2010) made the conclusion that 31% of the toddlers are exceeding the ULs. In this study there is no distinction between the supplement users and the non-users. Despite the differences in the analysis, the trends from FITS 2002 and 2008 show an increased prevalence of excessive intake in the case of toddlers using the supplements. This trend is also confirmed both in NHANES 1999-2000 and NHANES 2001-2002 (Butte et al., 2010). In addition, the data from NHANES 2003-2006 (Bailey et al., 2012), confirms the above mentioned trend with the 72% of the supplement users exceeding the UL for the vitamin A. The higher prevalence in this study compared to Butte et al (2010) can be due to the larger age range (2-8 years).

In Europe, different trends and patterns are being observed. According to Flynn et al (2009), while observing the group from 4 to 10 years, it is noticed that this group is under the risk of exceeding the
ULs for vitamin A. For example, in Poland this can appear as a result of the basic diet (high consumption of liver and other retinol rich food). In Denmark, toddlers exceed the ULs in vitamin A intake (Sisse et al, 2012). In the United Kingdom and Ireland, supplements are contributing to an increased intake of the vitamin A (Flynn et al, 2009). Data about intake from fortified foods is not available in every country. However, according to the limited data, the voluntary fortified foods have low influence on the total retinol intake. Mandatory fortified food was included in the analysis of the basic diet.

### 2.4.2. β-carotene

Carotenoids are compounds biosynthesised by plants and micro-organisms (EFSA, 2006). About 10% of all the carotenoids can be found in the general diet of the population, and about 20 compounds have been found in the plasma and animal tissues. The most common carotenoids which can be found in the food are: β-carotene, lycopene, lutein, β-cryptoxanthin. Carotenoids are known as the sun-light absorbing pigments during the photosynthesis and as the protectors of the cells against the photosensitization (EFSA, 2006). Furthermore, they give a significant coloration to the plant and animal tissues. Being a source of vitamin A, carotenoids are important for human consumption (e.g, β-carotene) (Scott and Rodriguez-Amaya, 2000). It is shown that in the developed countries, 40% of vitamin A intake is obtained through the carotenoids intake (EFSA, 2006).

In 1974, the NAS/NRC *Recommended dietary allowance* suggested that vitamin A should be presented in the form of retinol equivalent (RE), defined as follows (Scott and Rodriguez-Amaya, 2000):

1 RE = 1µg retinol;
1 RE = 6 µg β-carotene;
1 RE = 12 µg other carotenoid vitamin A precursor.

Retinol equivalents are being used today. The retinol potential of food is presented as a retinol equivalent, defined as the sum of retinol and the corresponding retinol equivalent of the provitamin (Scott and Rodriguez-Amaya, 2000). Despite the worldwide use of the RE approach, it is highly criticized by the researchers (Scott and Rodriguez-Amaya, 2000; Faulks and Southon, 2004). According to Scott and Rodriguez-Amaya (2000), the efficiency of the absorption and conversion of the carotenoids is highly dependent on the amount of the carotenoid ingested, food processing, cooking, other nutrients which may stimulate (e.g. type and amount of dietary fat) or inhibit the absorption, food matrix, carotenoids interaction, inter-individual variation, parasitic infestation and current nutritional status. Emphasising the fact that current RE factors are generalised, any conclusion driven from these calculations has to be taken with precaution.

The main food sources of β-carotene are carrots, oranges, tomatoes and dark green vegetables. This intake is contributing to the total intake of β-carotene with about 2-5 mg/person/day (EFSA, 2006). However, according to previous research, β-carotene intake varies between seasons. The availability
of specific fruits and vegetables and different food preparation techniques play a critical role in β-carotene intake and absorption (EFSA, 2006).

The second most important source of β-carotene is the food additives which contribute 1-2 mg/person/day. The intake of β-carotene both from foods and food additives reaches the value of 3-10 mg/person/day, depending on the season. Supplementation is the third source, but equally important. As a supplement, β-carotene is used because of its antioxidant activity (EFSA, 2006).

According to the EFSA, there is no dose-response relationship established for β-carotene (EFSA, 2006). This is due to the fact that most of the studies were single dose studies, and the conditions between them were differentiating. Thus, establishing ULs for isolated β-carotene is quite hard and uncertain. An additional important remark is that the composition of natural β-carotene and synthetic β-carotene differs in the relative proportion of trans/cis isomers (Woodside et al., 2005). According to the literature, the adverse health effects are seen with the concentration of 20 mg/day in the case of smokers (Druesne-Pecollo et al., 2010). Moreover, in the last decade research shows that all the trans-β-carotenes increase both lung cancer incidence and mortality in the case of human smokers. On the other hand, literature says that increased consumption of fruit and vegetables rich in carotenoids, reduces the risk of lung cancer (Woodside et al., 2005). This effect cannot be attributed to β-carotene itself because of the effect of other carotenoids and other compounds present in the food matrix (Woodside et al., 2005). Anyhow, it can be assumed that the effects of β-carotene are dependent on the specific source of food due to a different composition of other antioxidants and different proportion of β-carotene isomers (EFSA, 2006).

Having in mind all the sources of β-carotene, it should be concluded that the intake of food itself cannot reach doses that are associated with lung cancer in human studies (20 mg/day). However, it should be noticed that the supplements should be used with a big precaution because of the amount and the type of β-carotene present (EFSA, 2006).

In many national food consumption surveys, the β-carotene intake is not considered. However, in the German and Italian survey, intake of this carotenoid is much lower than 20 mg/day (Flynn et al., 2009; Lambert et al., 2004). For Italian toddlers intake is estimated to be around 1.5 mg/day (Sette et al., 2010).

### 2.4.3. Vitamin D

Under the name of vitamin D two closely related compounds are considered - animal based vitamin D₃ (cholecalciferol) and its synthetic analogue – plant based vitamin D₂ (ergocalciferol). The D₃ is a physiological form of vitamin D while the D₂ form is produced by irradiation of ergosterol, a common sterol present in the plants (Christakos et al., 2012). The synthetic form was used to fortify foods and as a supplement, but nowadays its use is declining and it has been substituted by the natural form of vitamin D (EFSA, 2006). Therefore, in the following text only the natural form of vitamin D will be considered when talking about vitamin D.
All over the world butters, margarines, milk and some fruit juices are enriched with vitamin D₃. In addition, vitamin D is rarely present in food at an amount impacting the dietary intake (EFSA, 2006). Some of the foods contain significant amount of cholecalciferol like fish liver, fish liver oils, fatty fish and egg yolks. Therefore, consumption of supplements and fortified food is highly present all over the world (Black et al, 2015). However, the form of vitamin D present in the food is inactive and needs to go through two hydroxylations in order to reach the active form – 1,25-dihydroxyvitamin (1,25 (OH)₂D). The exposure of the skin to the solar ultraviolet light catalysis the conversions of the inactive form to 1,25 (OH)₂D (Christakos et al, 2012).

Knowing that vitamin D is produced in the skin when exposed to sunlight, there is no nutritional need for vitamin D when there is enough sunlight. However, vitamin D becomes an important nutritional factor in the absence of sunlight and in the elderly (Black et al, 2015). It is known that in some European countries there is no enough sunlight during the whole year, especially during the winter season. Despite the geographical and the seasonal factors, a modern lifestyle also defines the exposure to the sunlight, e.g. clothing and indoor life (EFSA, 2006). Thus, many countries have their own recommendation for vitamin D intake, having in mind the sunlight exposure and the vulnerable population groups (Black et al, 2015).

Despite the fortification of food and supplementation, vitamin D deficiencies are present in almost all the developed countries. The potential risk of not having enough vitamin D is closely related to the Ca mechanism and the bone development in the case of children (EFSA, 2006).

In the USA, the food contributing the most to the vitamin D intake, besides the supplements, is milk with 74% of the daily vitamin D intake. Followed by infant formula (4.6%), eggs (3%), flavoured milk (2.9%), ready to eat cereals (2.8%), dairy drinks (2%), yoghurt (1.5%) (Hill et al, 2012).

Comparing with the USA, the situation in Europe is different with following food groups contributing to vitamin D intake: milk and milk products (24%), meat and meat dishes (20%), fat spreads (20%) and cereal products (14%). Eggs and eggs products and fish and fish products contribute both with around 8% (Grimes et al, 2015).

In Flanders, Belgium, the highest amount of vitamin D was provided from the protein food group (34.2%, with 14.6% from fish, 7.7% from meat and meat products and 4.2% from eggs), followed by fats (25.9%) and milk and milk products (24.9%, where fortified milk contributed with 19.7%) (Huybrechts et al, 2008).

On the graph (figure 1), it is shown how diverse food groups contribute differently among various countries. In the USA, milk and milk products are the biggest contributors to vitamin D intake. In Europe and Flanders the same trend is observed. The food group of milk, meat and fats contribute the most to the intake, compared with eggs and cereals. The difference between the USA and Europe may come up as a result of the differences in the fortification legalization.

Briefel et al (2006) have shown that supplements are contributing up to 54% of the daily vitamin D intake. According to NHANES 2003-2006, the majority of the non supplement users, children 2-8
years old, did not meet the recommendations for vitamin D (Bailey et al, 2012). However, even among the supplement users, more than 30% of the children 2-8 years old, failed to meet the vitamin D recommendation. This study suggests that attention should be given to the reformulation of the supplements for this nutrient (Bailey et al, 2012).

Based on the European review done by Mensink et al (2013), the mean intake of vitamin D in the case of children is really low. Despite the fact that many countries have a high intake of the vitamin D supplements, children aged 1-3 failed to meet the daily recommended intakes. In the Netherlands, this percentage is going up to 84% of the children, while in the UK it is reaching 74%. In Flanders, similar situation has been observed, where just 4% of the all children aged 1-3 years met the RDI (Spiro and Buttriss, 2014). Moreover, vitamin D deficiency is common in southern Europe, in the Middle East, and in many Asian countries. It is caused by the low sunshine exposure, skin pigmentation, air pollution, skin coverage and a low vitamin D intake (Lips, 2007). No matter the limited literature in this particular age group, Braegger et al. 2013 confirmed the opinion of many authors that the intake is too low in the case of the European toddlers (ESPGHAN). However, no clinical symptoms of deficiencies are shown. The usage of supplement and fortified food is therefore encouraged, as well as the sun exposure and the consumption of food rich in vitamin D (Braegger et al. 2013).

On the other hand, there is a lot of attention on the excess intake of vitamin D in the vulnerable groups of the population, e.g. infants. At the International Congress of Nutrition in Granada in 2013, health care professionals and nutritionists have shown their concerns about the Dutch infants and the overconsumption of vitamin D (Verkaik-Kloosterman et al, 2015).
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The toxic effects of vitamin D are related to the role of free 1,25 (OH)$_2$D$_3$ in the regulation of plasma calcium (EFSA, 2006). It is shown that a higher production of 1,25 (OH)$_2$D$_3$ may lead to increased levels of plasma calcium - hypercalcaemia. This can happen due to the over-stimulated intestinal absorption and due to the excessive calcium mobilization from the bones (EFSA, 2006). The hypercalcaemia also can lead to hypercalciuria, an increased calcium excretion into urine.

However, setting the UL for the intake of vitamin D is very challenging, as the data obtained from the animal studies were uncertain. These studies were defined as inappropriate for the identification of the critical endpoints and establishment of the NOAEL and the LOAEL (EFSA, 2012). The effect which is being considered when setting up the ULs for vitamin D is hypercalcemia (EFSA, 2012). This effect is observed in the group of adults, infants, children and adolescents.

It is shown, however, that the susceptibility towards vitamin D changes with age (EFSA, 2006). The EFSA repeated a risk assessment in 2012 with the studies available for children age 11-17. No adverse effects were noticed when 50µg of vitamin D was ingested. Thus, the UL is set to 100 µg/day for adolescents aged from 11-17. For children aged 1-10 years, the value is set to 50 µg/day because of the smaller body size (EFSA, 2012). Comparing to the risk assessment from 2006, the UL for children drastically changed (from 25 µg/day to 50 µg/day).

The mean intake of vitamin D from the base diet in European countries is about 5 times lower than the UL. It should be underlined that this comparison has been done without considering the intake from supplements and fortified food, which can make a big difference when it comes to the intake of this vitamin (Ovesen el at, 2003; EFSA, 2006). Moreover, according to Hirvonen et al (2011), it is necessary to estimate the association between fortification levels and the risk of exceeding safe intake levels. According to the Nordic dietary survey (2012), Nordic countries (Denmark, Finland, Sweden, Norway) have different levels of voluntary and mandatory fortification, but not even in one country were the ULs exceeded due to the consumption of fortified food (Sisse et al, 2012). Flynn et al (2009) estimated that supplements are responsible for the largest differences in the intake of vitamin D. For instance, in Norway vitamin D is the most commonly used supplement, and Denmark is the country with the highest supplements consumption.

Despite concerns being raised in the world and the high usage of supplements and fortified food, the levels of vitamin D among toddlers are well below the ULs, both in Europe and the USA. Moreover, even with the usage of supplements, many toddlers fail to achieve the requirements.

2.4.4. Vitamin E

Vitamin E is a term used for a group of naturally occurring components. These components are known for their antioxidative activity and for their nutritional essentiality. The term vitamin E was used as a generic term for four tocopherols (α,β,γ,δ) and four tocotrienols(α,β,γ,δ) (Wu and Croft, 2007). Factors have been used to convert food content of tocopherols and tocotrienols to α-tocopherol equivalence (EFSA, Scientific Opinion on Dietary Reference Values for vitamin E as α-tocopherol, 2015). However, it is shown that just naturally occurring RRR-α-tocopherol and its synthetical isomer
(all-rac- α-tocopherol) have an antioxidative activity in humans, putting α-tocopherol equivalence out of use. Nowadays, just α-tocopherol is considered under the term of vitamin E (EFSA, Scientific Opinion on Dietary Reference Values for vitamin E as α-tocopherol, 2015).

The major food sources of vitamin E are vegetable oils, whole grain cereals and nuts. In smaller amounts, vitamin E can be found in fruits, vegetables and meat (fatty portion) (Wu and Croft, 2007). However, the concentration of vitamin E in the blood is highly correlated with the total lipid intake (Wu and Croft, 2007; EFSA, 2006). Furthermore, it is shown that the intake of α-tocopherol is connected with the intake of polyunsaturated fatty acids (PUFA). Therefore, it is complicated to set a right recommendation for vitamin E. It is necessary to take into account population’s intake of PUFAs, which differentiate among different population groups (EFSA, 2006). As well, there is insufficient data about markers of α-tocopherol, intake, status and function (EFSA, Scientific Opinion on Dietary Reference Values for vitamin E as α-tocopherol, 2015). The Panel of EFSA reconsidered the requirements for vitamin E in year 2015, and draw the conclusion that ARs and PRI cannot be set for vitamin E. Consequently, an AI has been derived. For the children aged from 1 to 3 years, based on observed dietary intake of healthy subject, the AI is set on 6mg/day (EFSA, Scientific Opinion on Dietary Reference Values for vitamin E as α-tocopherol, 2015).

As vitamin E is a nutrient present in different foods, a deficiency resulting from a low dietary intake is uncommon. On the other hand, a number of literature and research dealing with the toxicity of this vitamin in humans is pretty high. The principal negative effects are related to blood clotting (EFSA, 2006). It is shown that oral intakes with a high concentration of vitamin E can increase blood coagulation among individuals with a low vitamin K status. Thus, ULs are established using factors for interpersonal deviation and uncertainty of the data. Therefore, the UL is set at 270 mg/day for adults and it is rounded to 300 mg/day (EFSA, 2006). As there is no data related to children and adolescents, the UL for this population group is derived from the UL for adults based on the body weight. The UL is set to 100 mg/day for toddlers (1-3 years).

In the USA, the foods contributing the most to the vitamin E intake in the case of toddlers are mixed dishes-grain base (8.7%), infant formulas (8.4%), milk (7.3%), plant-based protein food (5.9%), baby foods (5.8%), fruits (5.5%), eggs (4.9%), while vegetables contribute with less than 4% (Grimes et al, 2015). Supplements are contributing with up to 45% to the total intake (Grimes et al, 2015). It has been shown that in Canada every age group is under the UL set for this vitamin, despite using supplements (Shakur et al, 2010).

In a study conducted in Japan, it has been shown that in the case of toddlers, supplements and fortified foods contribute just with 1% to the overall vitamin E intake (Tsubota-Utsugi et al, 2013). Additionally, not even one population group exceeded the UL. The trend of using supplements in Japan is stagnating, while the consumption of fortified food products is increasing (Tsubota-Utsugi et al, 2013).
The overall picture is pretty much the same around Europe: a higher vitamin E consumption is correlated with the higher intake of oil, fruits, vegetables and cereals (Flynn et al, 2009). Following the Mediterranean diet, this trend is present in the South of Europe. According to Lambert et al (2004), the highest vitamin E intake is to be seen in Central and Eastern Europe, because of a high PUFAs consumption. However, the ULs are not being exceeded from the base diet, neither when combining it with supplements. Furthermore, the voluntary and mandatory fortification appears not to have an influence on the total intake of this vitamin (Flynn et al, 2009). Therefore, the vitamin E intake in the case of toddlers worldwide, is mainly under the recommended daily intakes.

2.4.5. Vitamin K

There are more than 100 compounds with vitamin K activity to be found in the nature, but only three are of a physiological importance (In X, 1994; Newman and Shearer, 1998):

- vitamin K\(_1\)(\(\alpha\)-phyllloquinone), the only one synthesized by the plants. The predominant form in the dietary sources is phylloquinone, but its concentration in foods is really low (<10 µg/100g). The majority of vitamin K\(_1\) is coming from the green leafy vegetables and vegetable oils (soybean, cottonseed, canola and olive oil).

- vitamin K\(_2\) (menaquinones), synthesized by the bacteria. Vitamin K2 is mainly present in dairy products, like cheese. Other food sources of this vitamin are cereal and bread products, fats and margarines.

- Menadione, a synthetically produced compound which will not be further discussed, because it is not present in the normal diet (Newman and Shearer, 1998).

Vitamin K is essential for the biosynthesis of the proteins involved in blood coagulation and the metabolic pathways in the bone and other tissues (Newman and Shearer, 1998).

Evaluating the population intake of vitamin K has always been a challenging task, due to a lack of knowledge about this vitamin’s concentration in the food. However, as the science has been developing, new methods have been obtained. They contributed to moving forward with the research in this field (EFSA, 2006). Vitamin K is still the one with the least amount of evidence and researches comparing to other fat-soluble vitamins. Recommended daily intake is set to 1 µg /kg of body weight/day, both for children and adults. More specific recommendation was not possible to be set, due to a lack of the valuable data (EFSA, 2006).

Deficiencies of vitamin K appear really rarely. Anyhow, newborns have a low vitamin K status which comes as a result to the limited intestinal synthesis. They are thus confronting an increased risk of developing a haemorrhagic disease. This is why vitamin K is given routinely to the newborn babies (EFSA, 2006).

Furthermore, toxicity and carcinogenicity were not found to be in a correlation with vitamin K. There was also not enough data to make a conclusion about genotoxicity of this vitamin. Looking at the
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limited amount of human data with increased consumption of vitamin K (up to 10 mg/day), it can be said that no adverse effects are to be observed. These studies were supported with animal studies, performed with even higher amount of phylloquinone (2000 mg/kg body weight). Therefore, there has been no UL set for vitamin K. However, its interaction with vitamin E, should be taken into account (EFSA, 2006). The Institute of Medicine (IOM, 2001), Expert Group on Vitamins and Minerals (EVM, 2003) and Council for Responsible Nutrition (CRN) came up with the same conclusion as the EFSA.

Having in mind all the above-mentioned facts, it was quite troublesome assessing the data about the population’s intake of vitamin K. One of the few studies which have included phylloquinone is the German observational DONALD study. It has been shown that supplements and fortified food products contribute little to the vitamin K intake (Sichert-Hellert et al, 2006). This can be explained by the fact that the concentration of this vitamin is really low, both in supplements and fortified foods. On the other hand, in the DONALD study, it has been shown that the normal diet contributes with 95% to the vitamin K intake (Sichert-Hellert et al, 2006).

2.4.6. Summary

According to the above-reviewed literature, these are the dietary reference values which will be used in this master thesis (table 1):

Table 1- Dietary reference values for fat-soluble vitamins for age group 1-3 years

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Adequate intake</th>
<th>Tolerable Upper Intake Levels for toddlers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol Equivalent</td>
<td>205 µg/day*</td>
<td>800 µg/day</td>
</tr>
<tr>
<td>D</td>
<td>10 µg/day</td>
<td>50 µg/day</td>
</tr>
<tr>
<td>E</td>
<td>6 mg/day</td>
<td>100 mg/day</td>
</tr>
<tr>
<td>K</td>
<td>1 µg/kg BW/day</td>
<td>NA**</td>
</tr>
</tbody>
</table>

*EAR is used for Retinol Equivalent

**vitamin K intake will be just compared with AI values

Researchers should, however, be very cautious when making conclusions using the above-stated values, since they are derived from the calculations and values for adults. Consequently, limitations exist and they have to be taken into consideration. For example, EFSA’s report from 2006 sets the vitamin D UL for toddlers on 25 µg/day. Nevertheless, by doing more research (EFSA, 2012), it has been shown that the UL should be higher, since no adverse health effect has been noticed when having consumed such a low concentration. There is a true risk for certain vitamins to exceed the ULs (vitamin A). In order to make environment healthier for the consumers, programs of supplementation and fortified food should be monitored in a better way.
Materials and methods

3. Materials and methods

3.1. Sample size, sample frame and selection methodology

The sample size is calculated to be 385 respondents. Putting the emphasis that this thesis is a part of the VITADEK survey, the final sample size was smaller, counting 103 respondents. This thesis included in the analysis only the Dutch speaking participants in Belgium (Flanders).

The following steps are used for selecting the sample of the target group:

1. Stratification on the level of the provinces;
2. Selection of the number of communities proportional to the size of the province (size = number of births per province);
3. Systematic sampling of the communities within the province;
4. Selection of the Health consultation centres within communities;
5. All toddlers visiting Health consultation centres were invited to participate.

The toddlers have been reached through the Child Health Consultation centres (GHC). The coverage of toddlers via GHC’s is 62.8% on annual basis (Gezin K, 2006). In this way, toddlers going to the paediatric clinics are missed and as well as those whose parents are not speaking Dutch. Computer and internet illiterate respondents are reached through telephone interviews.

3.2. Assessment method – Food frequency questionnaire

Epidemiologists are using FFQ when they want to estimate the usual food and nutrient intake of the population. The FFQ is a highly cost-effective method with a wide spread use in large epidemiological studies (Rodrigo et al, 2015). From the statistical point of view, the FFQ is the only dietary assessment method that minimizes the intra-personal variability and day to day variability of nutrient intake without relying on multiple day assessment (Kristal et al, 1992). The VITADEK study is a cross-sectional study, i.e. the overall intake of fat-soluble vitamins was assessed at a given moment in time by evaluating the consumption of food, fortified foods and dietary supplements. The quantitative food frequency questionnaire was chosen with a recall period of one month (for most of the food items) and one year (for supplements and specific food items, e.g. liver). Via Quantitative FFQs we tried to estimate the portion size consumed for each food item present in the questionnaire (Rodrigo et al, 2015). The survey mode used in this study was internet based.

“Food frequency questionnaires are an advanced form of the diet history method aimed to assess habitual diet by asking how often and how much of selected food items or specific food groups included in a list are consumed over a reference period.” (Rodrigo et al, 2015)

The main components of the FFQ are the food list, the frequency of the consumption and the portion sizes consumed of each item (Rodrigo et al, 2015).
Materials and methods

The food list should be clear, concise and specific for the target group. Furthermore, it can be developed for the specific setting of the study (Rodrigo et al., 2015). For the purpose of the current survey, the informative food list was formed, assessing all the food groups which are the largest contributors to the intake of vitamin D, A, K, E. In order to select those food groups, the database approach was used. More specific, the Netherlands national food consumption survey (FCS) was assessed. Indeed the results of the recent new Belgian national FCS (2014-15) were not available yet and the previous FCS (2004) was not contributive as it did not have data about intake fat-soluble vitamins. Based on Netherlands national FCS, 90% of the food groups and subgroups contributing to the intake of fat-soluble vitamins were chosen to be part of the questionnaire. The differences in eating patterns that exist between Belgium and the Netherlands did not allow the determination of foods on the level of food items. However, raw data from the 2014 Belgian FCS were used to determine the food items and brands mostly consumed for different food subgroups.

The market of functional foods is a fast changing market and not all the products are available in the food composition tables. Therefore, for this survey it was required to do a market research of the enriched foods and supplements available in Belgium. All the shops and large retailers were visited and the food items, subdivided in specific brands, which are fortified with fat-soluble vitamins, were included in the questionnaire. Thus, an inventory table was available with all the brands and enriched food items.

The supplements used mostly by the target population were assessed using pharmacies as a source of information. An inventory table was made with the most consumed supplements of fat-soluble vitamins. Following this approach, not all the supplements from the market were included in the questionnaire. Thus, there is an open ended section where respondents can record the consumption of the other supplements not included in the food list.

3.3. Preparation and analysis of the food frequency questionnaire

Assessing the intake of the population is a complex process based on two major steps.

Before starting to process, there is the preparation step which is crucial for getting valid results from the analysis. In this stage it was important to make all the questions and the answers recognizable to the statistical program. This is achieved through the steps of linking, coding and merging. These actions will be discussed in details in the following chapters.

Parallel to the preparation step, there is a data collection which also needs to be followed up and inserted carefully in the statistical program. It is important that the data is further cleaned and prepared for analysis. By finishing the above mentioned actions, the statistical analysis can be conducted. The whole process is described on the flow chart below (figure 2).
Figure 2 - Flow diagram of the steps performed in the thesis

**Preparation Step**
- Food frequency questionnaire
- Food items (informative list + enriched food + supplements)
- National nutrition databases (Nubel, Nevo, etc)
- Inventory tables for enriched food and supplements
- Assessment of the fat-soluble vitamin content in food items

**Data Collection**
- FFQ filled in by the respondents
- Answer options
- Portion sizes and portion quantities
- Frequencies

**Coding**
- Frequencies as numerical values
- Portion sizes and portion quantities as numerical values
- Coding valid responses
- Cleaning the data and deleting invalid responses

**Linking**
- Open-ended questions

**Merging**
- Food Composition Tables
- Food Consumption Tables
- Merging Food Composition Tables with Food consumption Tables
- Cleaning and coding of merged files

**Data Analysis**
- Calculation of the estimated usual intake of vitamins
- Comparison with UL and RDI
- Contribution of supplements, enriched food and normal diet vitamin intake
- Proportion of Flemish toddlers above UL
- Proportion of Flemish toddlers under RDI
3.4. Preparation step

3.4.1. Forming the food composition data base of the survey (linking)

Per food item, we need to assess the amount of fat-soluble vitamin present in the food. Through different food composition data bases, fat-soluble vitamin content of the food will be determined.

The basic actions in the linking step of the food items from the informative food list are:

1. Making the database with all the food items, organized per food group, food subgroup and brand names (if available);
2. Firstly, information about the fat-soluble vitamin content was searched in the food composition data bases:
   - Nubel – Belgium nutritional data base,
   - Nevo – Netherlands nutrition data base;
   This food composition data bases are chosen because of the local setting of the survey. The Netherlands and Belgium have close food markets.
3. Checking the frequencies of the consumption of the different food items in the files of the Belgian National Food Consumption survey;
   - Information about the vitamin content was taken for the food items in the form that is mostly consumed by the population (frequencies of the consumption from the Belgian National Food Consumption Survey);
4. Just two forms of food – raw and prepared, were taken into account
   - Under the term ‘prepared’ we have considered any type of the food preparation – boiling, steaming, frying, baking, etc;
   - If the information about cooked food was not found, we took the information from the raw form of the same food item. In the end we will need to use a retention factor to consider the losses which are occurring during preparation of the food;
5. Use of fat when preparing food was considered as fat uptake per 100 g of food item prepared;
6. Deciding which is the best suitable information (vitamin values) according to the previously assessed information and inserting the information next to the food item;
7. If information was not found in Nubel and Nevo, other food composition databases were used in the following order:
   - McCance and Widdowson’s The Compositions of Foods integrated dataset (United Kingdom),
   - Ansces French food composition table (France),
   - Danish Food Composition databank (Denmark);
8. Due to the lack of information about vitamin K, for some food items, with missing values, it was necessary to calculate the amount of vitamin K (e.g. mayonnaise based spreads):
   - Using recipes and vitamin K content of main ingredients of the food item(e.g. eggs, oil);
9. The missing values were assessed using median values of the similar food items;
Materials and methods

10. For specific toddlers food items, the general information of vitamin content is assessed;
   - The laboratory analysis will be done in September 2016.

For the enriched foods and supplements, the ingredient list present on the food label is used as the main source of information. The food items were organized per brand followed by the data about the fat-soluble vitamin content. If the content of fat-soluble vitamins was not available for specific brand names then information was taken from the general food item. For example, if information for the cornflakes brand Golden Horn was not available, the data is taken from the general enriched cornflakes (generic database).

3.4.2. Coding

Per person, we need to calculate for each food item:
   - the frequency of daily intake and
   - total daily grams of food consumed.

First of all, each question receives a name which will be further used as a variable in the statistical program. In addition, reply categories and portion sizes of each question also get a numerical value, which will be later on used in the analysis.

When calculating the daily intake of the person, the following formula is used:

\[
\text{Daily consumption (g/person/day)} = \text{Frequency of the food eaten (person/day)} \times \text{Portion size of the food eaten (g)}.
\]

3.4.2.1. Coding of the questions

All the questions were assessed and different codes were assigned to the each question. Different type of code was assigned depending on the type of the question – general questions, food items questions, frequency questions, portion size and portion quantity questions.

3.4.2.2. Coding of the frequency of the consumption

When coding frequencies, all the food items and the subgroups were taken into account. The frequency of the consumption varies among the food items. Accordingly different codes are calculated.

Frequencies were calculated in the following way (table 2). Since the usual intake will be expressed as an amount of vitamin consumed per day, all the other frequencies were calculated according to that statement. The month has an average of 30.5 days (February not included) and year 365 days.
### Table 2 - Process of coding replies

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 times per day</td>
<td>2</td>
</tr>
<tr>
<td>1 time per day (every day)</td>
<td>1</td>
</tr>
<tr>
<td>5-6 times a week</td>
<td>5.5/7=0.78</td>
</tr>
<tr>
<td>3-4 times a week</td>
<td>3.5/7=0.5</td>
</tr>
<tr>
<td>1-2 times a week</td>
<td>1.5/7=0.21</td>
</tr>
<tr>
<td>3-4 times a month</td>
<td>3.5/30.5=0.12</td>
</tr>
<tr>
<td>1-2 times a month</td>
<td>1.5/30.5=0.05</td>
</tr>
<tr>
<td>3-4 times a year</td>
<td>3.5/365=0.009</td>
</tr>
<tr>
<td>5-6 times a year</td>
<td>5.5/365=0.015</td>
</tr>
</tbody>
</table>

#### 3.4.2.3. Portion sizes and amount of food eaten

Different ways exist of how to assess the portion sizes of food consumed by respondents during the previous day, week, month or year. In this survey, three approaches were used:

1. GloboDiet mnemonic pictures
2. Household measures
3. Food units

##### 3.4.2.3.1. GloboDiet mnemonic picture

GloboDiet (former EpicSoft) is a computerized 24h-recall program, used in Europe as the software for national consumption surveys (Bel *et al*, 2016). Aside from the program, the picture book is used when performing the 24h recall interviews. The photo book contains country-specific food items obtained from Epic-Soft picture book PANCAKE study picture book, the menu CHSwiss picture book and the French picture book from national food consumption survey (Bel *et al*, 2016). For the purpose of the VITADEK study, GloboDiet picture book is used.

Food items are presented through pictures which are representing different portion sizes. One example is presented on the figure 3.
Materials and methods

To each portion size a correct mass in grams is assigned. Along side with portion sizes, it was required by respondent to define portion quantity of the food consumed. The following portion quantities were used:

- Quarter of the portion;
- Half of the portion;
- One portion;
- One and the half of the portion;
- Two portions;
- Three portions;
- Four portions;

If the portion quantity was available, the final value of the portion size was calculated in the following way:

\[ \text{Portion size (g)} = \text{mass of food item from the picture (g)} * \text{portion quantity}; \]

Portion sizes are then calculated for each food items or food group.
Materials and methods

3.4.2.3.2 Portion sizes and food units

For the food items which are presented as units in the questionnaire, a specific protocol was used to assess the actual portion sizes. These food items belong to the food groups of fruits, vegetables, cookies, chocolate, meat and meat products. It was important to determine the exact mass, in grams, of each food item and to further calculate according to the given answers. The information about most of the food items was found using Maten en Gewichten (2005).

In this part of study, we have made the following assumptions:
1. When more than one value for the mass is available in Maten en Gewichten (2005), the average is calculated and this value was used in the further steps (example breakfast cookies);
2. For toddlers, all the values for fruits are average values between fruit with skin and without skin (example apple);
   - For toddlers, there was no available information about the consumption pattern of apples, therefore taking the average value was the best choice to minimize possible mistakes;
3. When information was available for different sizes of food items (small, medium, large), medium value was taken;
4. When information was not available for the cookies and waffles with chocolate, the mass was calculated by adding 5 grams to the mass of the same food item without chocolate.

For certain food items information was not available. Thus, the information was assesses by using label checking. If not available, missing food items were searched in the retail shops and their mass was measured.

For the fruits and products which are never eaten as single items, a range of values was offered as an answer. Therefore, we have used a median value when calculating the exact amount. The example is presented in table 4.

Table 3 - An example of coding food items portion sizes

<table>
<thead>
<tr>
<th>Food item</th>
<th>Answer offered</th>
<th>Median value</th>
<th>Single item (g)</th>
<th>Portion size= Median value*Single item (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackberry</td>
<td>1-5</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td>8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>11-15</td>
<td>13</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>16-20</td>
<td>18</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>21-25</td>
<td>23</td>
<td></td>
<td>23</td>
</tr>
</tbody>
</table>
Materials and methods

3.4.2.3.3 Portion sizes assessed through Household measures (HHM)

Household measures were used as a third way to define the portion size used in the questionnaire. The assumption made in this step is: When a spoon (tea and table spoon) is used as HHM, the volume of a normal level spoon is taken, if not stated differently.

3.5. Forming the final data set of the survey (merging and cleaning of the data)

Merging and cleaning was performed in SAS 9.3. In this part of the study, we needed to merge information obtained from preparation steps with the food consumption database (data collection) (figure 2). By forming macro variables, we have organized consumption data in 6 variables:

- Email;
- Food item;
- Frequency of the consumption;
- Portion size;
- Portion quantity.

The linking files, with all the information available on the content of fat-soluble vitamins in food items, represented the food composition database of the survey (figure 2). This step was followed by merging of the composition data base with the food consumption data base (figure 2). These two data sets were merged based on the variable Food item. The full data set obtained was coded and cleaned using the information gathered in the coding step.

3.6. Data analysis

Data management was performed in SAS 9.3 Statistical analysis were completed in SPSS 2.2. The demographic variables were analysed implementing Linear Regression Analysis. The differentiation between supplement users and non-users was assessed.

The distribution of the estimated usual intake was assessed and compared with cut off points (reference values). The variable Email was used for identification on the level of each respondent and for the calculation of the usual intake per person per day. According to the literature, the overestimation of inadequate micronutrient intake can occur when comparing intakes of the population with the RDI (EFSA, 2010; IOM, 2000). Therefore, reference values used in this thesis are the EAR and the UL. If the EAR was not available the AI was taken. The skewness of the distribution was taken into the consideration when observing the results. According to EFSA, the overestimation of inadequacy can occur when observing positive skewed distribution (EFSA, 2010).

The analysis was performed in the following way. The continuous variable (usual intake of fat-soluble vitamin) was transformed into a categorical variable. The categories were made using reference values.
Materials and methods

Thus three categories were obtained for each vitamin:

- Under the EAR or AI
- Meeting recommendation (between the EAR or the AI and the UL) and
- Above the UL

According to EFSA report (2010), the proportion of the population with inadequate habitual intakes can be estimated when using the EAR. In this way, the percentage of the population with habitual intake lower than EAR has the inadequate intakes. However, when using the AI as the reference value, the proportion of inadequacy cannot be assessed, due to the fact that the AI is not enough precise estimate of recommended intake. Therefore, just the probability of inadequacy of the habitual intake can be estimated.

When comparing the habitual intakes with the UL, it should be taken into consideration the method used for setting this reference value. For vitamin A, D and E the risk assessment method was used to set the UL for adults (EFSA, 2006). For toddlers, all the values were derived from the value set for adults, basing the calculations on the smaller body weight. For this reason, the final conclusions should not be considered as the absolute values (Zlotkin, 2006; EFSA, 2006).

For the assessment of the contribution of basic diet, fortified foods and supplements to total intake of fat-soluble vitamins, the distribution of the estimated usual intake was reorganized. It was presented as cumulative sum, where three categories were present:

- Basic diet
- Basic diet + enriched foods
- Basic diet + enriched foods + supplements

Using the distribution, the contribution was calculated using simple calculations:

\[
\text{Contribution (\%/person/day)} = \frac{\text{Intake}_\text{Source} (\text{mg/person/day}) \times 100}{\text{Total intake} (\text{mg/person/day})}
\]

Intake\_Source – the vitamin intake coming separately from fortified foods, supplements and basic diet per person.

After calculating the contribution per person, the average value was assessed. The important remark is that mandatory fortified foods were assessed as a part of the basic diet, while voluntary fortified foods were considered as the separate group, e.g. enriched foods.

For all the analysis performed confidence intervals of 95% were used (level of significance p<0.05).
4. Results and discussion

4.1. Demographic characteristics of the population

The final number of respondents for this survey was 103, from which 51.5% are boys and 48.5% are girls. In 97% of the cases, the questionnaire was filled in by the mother of the child. 97% of the children’s mothers are from Belgian origin. The country of birth of the mother’s partner is Belgium in 95% of cases. The ethnical distribution of the sample it is not a true representation of the diversified Belgium population. For instance, 23% of the population is non-Belgian ethnicities (National Institute for Statistics, 2015). This can be explained with the fact the language of the FFQ was Dutch. This distribution should be further assessed when French based questionnaire is analysed.

Taking into account that the survey was conducted in Flanders, the Dutch speaking region of Belgium, the distribution of respondents through the provinces of Flanders was determined (figure 4). Where the province of Antwerp (25.2%), East Flanders (23.3%), West Flanders (24.3%) and Flemish Brabant (23.3%) are having almost the same share, while Brussels is contributing less (3.9%). The Flemish Child Health Consultation canters in Brussels were solicited, but the response rate was really low.

![Figure 4 Distribution of respondents through different provinces of Flanders, Vitadek study, Belgium, 2015](image)

The education of both parents was assessed and the results are presented in figure 5. Different categories of education are present: No Diploma, Secondary Education, Bachelor degree and Master or higher degree.
Results and discussion

The most common education type for both parents is master degree, with 49.5% and 40.8%, respectively. The results demonstrate that in our survey, mainly parents with higher education were participating. This can be partially explained with the fact that our survey was internet based, requiring knowledge about internet use.

The variables gender, education of the parents and province of Flanders were tested in order to verify their relationship with usual intake of fat-soluble vitamins. It was performed using the Linear Regression Analyses. The final output showed no statistically significant difference of the intake for the three variables (95% confidence intervals). The outputs of the analysis are shown in Appendices.

4.2. Assessment of supplement users and non-users

In this thesis, among 103 respondents, 82.5% used some kind of supplements. The results obtained are presented in the table

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total supplement users</td>
<td>85</td>
<td>82.5</td>
</tr>
<tr>
<td>Total non-supplement users</td>
<td>18</td>
<td>17.5</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>100</td>
</tr>
</tbody>
</table>
Results and discussion

The supplement users in the USA, count for 31%, which is more than two times lower than the results we obtained (Briefel et al, 2006). The figures presented should be further checked once the all sample is available.

The detailed differentiation among supplement users was performed, and table 5 presents the final result.

Table 5 – Distribution of different supplement used, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Total percentage¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td>71</td>
<td>83.5</td>
<td>69</td>
</tr>
<tr>
<td>Multivitamin</td>
<td>2</td>
<td>2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Vitamin D + Vitamin mix A+D</td>
<td>2</td>
<td>2.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Vitamin D + Vitamin K</td>
<td>6</td>
<td>7.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Vitamin D + Multivitamin supplements</td>
<td>3</td>
<td>3.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Vitamin D + Multivitamin + Vitamin mix A+D</td>
<td>1</td>
<td>1.2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total supplement users</strong></td>
<td><strong>85</strong></td>
<td><strong>100</strong></td>
<td><strong>82.5</strong></td>
</tr>
</tbody>
</table>

¹ – Percentages calculated based on total number of respondents (n=103)

Based on table 5, the majority of the respondents used just one type of supplement (69 %), while 7.7% used two products combined. Multivitamin products were used as a single supplement by 1.9%, and 3.9 % of toddlers used it in the combination with other items. Just one of the respondents used three supplements combined. Analysing in more specific, vitamin D was the most used supplement (69 %), followed by the usage of two products - vitamin D and vitamin K supplements (5.8 %).

The trend observed is in line with the literature. The American toddlers mostly consumed just one type of supplements (29.8%) (Briefel et al, 2006). The second most received supplement was multivitamin complex (Briefel et al, 2006). In Norway, like in Belgium, the most used supplement is supplement of vitamin D (Flynn et al, 2009). The high consumption of vitamin D supplements, in northern European countries, is correlated with the low sun exposure. Based on that, national public health institutes are encouraging the supplementation of this vitamin, making the prevalence of supplement use high (Breagger et al, 2013; Lips, 2007).
4.3. Estimated usual intake of fat-soluble vitamins

The estimated usual intake (UI) of fat-soluble vitamins is calculated using the following formula:

\[ \text{UI} = \text{FR} \times \text{PS} \times \text{PQ} \times \text{vitamin content (mg/person/day)}; \]

- FR = Frequency of the consumption,
- PS = Portion size,
- PQ = Portion Quantity.

In table 6 the Usual Intake of four fat-soluble vitamins by Flemish toddlers is presented. The right tail skewed distribution is observed in all four intakes of vitamins (Figure 7 – 10, Appendices). This is explained by the fact that not all the toddlers are using supplements. Therefore extreme values are present in the distribution (Bailey, 2012).

Table 6– Estimated usual intake of fat-soluble vitamins in Flemish toddlers, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>N</th>
<th>Valid</th>
<th>Missing</th>
<th>UI_VitA^1</th>
<th>UI_VitD^2</th>
<th>UI_VitE^3</th>
<th>UI_VitK^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>103</td>
<td>0</td>
<td>420.216</td>
<td>42.38</td>
<td>5.09</td>
<td>10.646</td>
</tr>
<tr>
<td>Median</td>
<td>365.46</td>
<td>18.339</td>
<td>423.67</td>
<td>42.38</td>
<td>5.09</td>
<td>10.646</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>279.933</td>
<td>50.614</td>
<td>280.933</td>
<td>50.614</td>
<td>5.28</td>
<td>10.68</td>
</tr>
<tr>
<td>Variance</td>
<td>78362.270</td>
<td>2561.754</td>
<td>78362.270</td>
<td>2561.754</td>
<td>50.28</td>
<td>10.80</td>
</tr>
<tr>
<td>Skewness</td>
<td>2.955</td>
<td>1.188</td>
<td>2.955</td>
<td>1.188</td>
<td>2.334</td>
<td>1.588</td>
</tr>
<tr>
<td>Std. Error of Skewness</td>
<td>0.238</td>
<td>0.238</td>
<td>0.238</td>
<td>0.238</td>
<td>0.238</td>
<td>0.238</td>
</tr>
<tr>
<td>Minimum</td>
<td>27.450</td>
<td>.288</td>
<td>27.450</td>
<td>.288</td>
<td>2.28</td>
<td>1.048</td>
</tr>
<tr>
<td>Maximum</td>
<td>2,071.990</td>
<td>148.525</td>
<td>2,071.990</td>
<td>148.525</td>
<td>19.892</td>
<td>36.541</td>
</tr>
<tr>
<td>Percentiles</td>
<td>10</td>
<td>156.214</td>
<td>0.733</td>
<td>2.015</td>
<td>4.078</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>268.25</td>
<td>4.156</td>
<td>3.089</td>
<td>5.745</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>365.46</td>
<td>18.339</td>
<td>4.267</td>
<td>9.036</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>501.81</td>
<td>60.686</td>
<td>5.805</td>
<td>13.549</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>702.38</td>
<td>144.0450</td>
<td>8.639</td>
<td>20.356</td>
<td></td>
</tr>
</tbody>
</table>

1 Usual Intake of vitamin A (µg RE/person/day)
2 Usual Intake of vitamin D(µg/person/day)
3 Usual Intake of vitamin E (mg/person/day)
4 Usual Intake of vitamin K (mg/kg/day)

The Estimated Usual Intake presented here is the value derived from values estimated from basic diet, but supplements and enriched foods also. In this point of the research, part of the estimated usual intake is coming from the informative food list, excluding open ended section where respondent could add other food eaten. Therefore, results obtained in this thesis can differ from the results gathered at the end of the VITADEK survey. These results are fully discussed in the following chapter.

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4.4. Usual intake of fat-soluble vitamins in Flemish toddlers

4.4.1. Vitamin A

4.4.1.1. The comparison of the Usual Intake of Vitamin A with reference values

The summary of the data is presented in table 7. The usual intake of vitamin A is below the EAR just for 15% of Flemish toddlers, while 78.6% are meeting the recommendations. 7 respondents (6.8%) exceed the UL.

Table 7- The comparison of the Usual Intakes of Retinol Equivalents in Flemish toddlers with reference values, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>Proportion of toddlers</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below EAR(^1)</td>
<td>15</td>
<td>14.6</td>
<td>14.6</td>
</tr>
<tr>
<td>Meeting the recommendation</td>
<td>81</td>
<td>78.6</td>
<td>93.2</td>
</tr>
<tr>
<td>Above UL(^2)</td>
<td>7</td>
<td>6.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) EAR=205 µg/day
\(^2\) UL=800µg/day

According to the literature, there are not many toddlers in developed countries facing inadequacy of vitamin A due to high availability of the food rich in retinol and carotenoids (Latham \textit{et al}, 2010; Palmer \textit{et al}, 2012). This is confirmed with the results obtained in this thesis, where nearly 80% of the toddlers are meeting the recommendation.

Considering that the intake of vitamin A estimated here is coming from supplements, enriched foods and basic diet all together, the total intake is lower when compared with the literature (Butte \textit{et al}, 2010). In the USA, the total intake is above 800 µg RE/day for 31% of toddlers (Butte \textit{et al}, 2010); comparison between European countries is difficult because data is hardly available. The supplement users are more frequently the ones exceeding the UL than non-users (Bailey \textit{et al}, 2012; Butte \textit{et al}, 2010). Corresponding with the literature, in our sample the toddlers using supplements are those with the highest intakes (>2000µg RE/day). 5 toddlers are slightly exceeding the value of 800 µg RE/day, without using supplements. The general trends for supplement users cannot be driven from this data set, due to the small number of supplement users (n=2). Still, the usage of vitamin A supplements in Flanders, Belgium is lower than in the USA making the total intake of this vitamin lower as well.

The proportion of the population who have intakes below EAR, are the ones with probable inadequacy of the vitamin. In the case of Flemish toddlers, the proportion of the population with probable inadequacy is low (14.6%). This statement is further confirmed when comparing the median of the vitamin A distribution (median=420.216 µg RE/day) with the EAR: the median is indeed higher than the EAR.
Results and discussion

4.4.1.2. Retinol and β-carotene contribution to the usual intake of vitamin A

In this thesis, usual intakes of retinol and of β-carotene were assessed. In order to determine the contribution of retinol and β-carotene to the total intake of vitamin A, the usual intakes of these two compounds were compared with total usual intake of vitamin A. The contribution is calculated as percentages and it is presented on the following figure 6.

Figure 6 – Contribution of retinol (left) and β-carotene (right) to the total intake of vitamin A, toddlers, Vitadek study, Belgium, 2015

The data, presented on figure 6, are crucial for better understanding the vitamin A intake. According to the distribution presented above, it is observable that β-carotene contributes more to the intake of vitamin A (expressed as RE) than retinol. Thus, in our sample fruits and vegetables were contributing more to vitamin A intake compared to the animal based products. This is probably related to consumption of fruits and vegetables. This has already been observed in toddlers (Allen et al., 2006; Green, 2015). Further analysis is needed to determine the actual food sources of retinol and β-carotene.

4.4.1.3. Contribution of fortified foods, supplements and basic diet to total intake of vitamin A

The determination of intake of vitamin A coming from basic diet, enriched food and supplements was assessed. The distribution of the intake among basic diet, enriched food, and supplements is presented in table 8.
Table 8 – Distribution of the vitamin A usual intake between basic diet, enriched food and supplements, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th></th>
<th>Basic diet</th>
<th>Basic diet + enriched food</th>
<th>Basic diet + enriched food + supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Valid</td>
<td>103</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td>Missing</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>408.85</td>
<td>408.85</td>
<td>419.52</td>
</tr>
<tr>
<td>Median</td>
<td>363.38</td>
<td>363.38</td>
<td>364.58</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>256.862</td>
<td>256.862</td>
<td>279.56</td>
</tr>
<tr>
<td>Variance</td>
<td>65978.026</td>
<td>65978.026</td>
<td>78152.264</td>
</tr>
<tr>
<td>Minimum</td>
<td>27.45</td>
<td>27.45</td>
<td>27.45</td>
</tr>
<tr>
<td>Maximum</td>
<td>900.93</td>
<td>900.93</td>
<td>2068.93</td>
</tr>
<tr>
<td>Percentiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>266.63</td>
<td>266.63</td>
<td>267.91</td>
</tr>
<tr>
<td>50</td>
<td>363.38</td>
<td>363.38</td>
<td>364.58</td>
</tr>
<tr>
<td>75</td>
<td>491.15</td>
<td>491.15</td>
<td>500.65</td>
</tr>
<tr>
<td>90</td>
<td>664.088</td>
<td>664.088</td>
<td>699.746</td>
</tr>
<tr>
<td>95</td>
<td>836.556</td>
<td>836.556</td>
<td>876.918</td>
</tr>
</tbody>
</table>

Based on table 8, the general trends observed are:

- the 98% of vitamin A is coming from the basic diet
- for a few supplement users present in this survey (n=2), supplements contributed with really high percentage, making them exceed the ULs.
- Enriched food did not play an important role in intake of vitamin A.

According to Briefel et al (2006), in the USA, supplements were representing 42% of the intake of vitamin A. This contrast with the results observed here may occur because of the differences which exist between the two countries, as far as dietary habits and practices are concerned. The data about the contribution of supplements and basic diet to the vitamin A intake in European toddlers is hardly available, limiting further discussion. The focus of the research is based on the deficiencies present in developing countries.

4.4.2. Vitamin D

4.4.2.1. The comparison of the usual intake of vitamin D with reference values

In table 9, the obtained results are presented for vitamin D intake. Below AI and above UL are the groups representing the majority of the sample, with 39.8% and 37.9%. The proportion of toddlers that falls in between the AI and the UL counts for 22.3%.
Results and discussion

Table 9- The comparison of the usual intake of vitamin D of Flemish toddlers with reference values, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>Proportion of toddlers</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below AI(^1)</td>
<td>41</td>
<td>39.8</td>
<td>39.8</td>
</tr>
<tr>
<td>Meeting recommendation</td>
<td>23</td>
<td>22.3</td>
<td>62.1</td>
</tr>
<tr>
<td>Above UL(^2)</td>
<td>39</td>
<td>37.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) AI=10µg/day  
\(^2\) UL=50 µg/day

According to the analysis, different scenarios are observed when it comes to the usual intake of vitamin D. Firstly, the percentage of toddlers who are not meeting the recommendations. This group represents the largest category in our sample, corresponding to 39.8%. These percentages are quite high all over Europe. For instance, in the Netherlands this number reaches 84% of all toddlers, while in the UK it is 74% (Mensink et al, 2013). According to Spiro and Buttriss (2014), in Flanders just 4% of toddlers are meeting the RDI. In The USA, the share of toddlers not meeting the recommendation is similar to the results obtained in this thesis (30%) (Bailey et al, 2012). The results obtained in this survey showed that toddlers not meeting the recommendations are non-supplement users. Thus, the difference present among VITADEK study and Spiro and Buttriss (2014), can be due to the supplementation program present in Belgium, showing the positive impact on the population’s intake of vitamin D.

The second category are the toddlers meeting the recommendation. This group is representing 22.3% of our sample. Comparing to the surveys done in the other countries (Mensink et al, 2013), the share of the toddlers consuming recommended amounts of vitamin D is bigger in the present study. This result implies that recommendation of using supplements and fortified food had an effect. From 23 toddlers meeting the recommendation, 74% of them fulfilled the criteria by just taking into account supplements. The other 26% achieved desired levels of vitamin D, when including supplements and normal diet.

Having in mind that the AI was used as a cut off point, the prevalence of inadequacy cannot be estimated (EFSA, 2010). The mean intake of vitamin D (mean intake=42.381 µg/day) was higher than the AI. Correspondingly, it can be assumed that the group of Flemish toddlers has low prevalence of inadequacy of vitamin D.

However, the vitamin intake of toddlers which is exceeding the UL is the major public concern. As a result of this survey, 37.9 % of toddlers are consuming more vitamin D than recommended. At the international nutrition summit in 2012, this concern was raised for infants and young children (Verkaik-Kloosterman et al, 2015).
Results and discussion

In our survey, the toddlers who are exceeding 50 µg/day are the ones who consumed supplements in higher doses. According to Flynn et al (2009), the supplements make the largest difference in the daily intake of vitamin D. Certain brands available on the market contain an amount which is higher than the UL. Of 46 toddlers using these supplements, 39 of them had usual daily intakes highly above 50 µg/day. The intakes were up to three times higher than the UL.

The Flemish toddlers exceeding the UL have higher probability of increased risk for adverse health effects. However, the precaution should be taken when comparing intakes with the UL which is derived from the value set for adults (EFSA, 2012). The toddlers should be monitored and reported if adverse health effects are shown (Zlotkin S, 2006).

4.4.2.2. Contribution of fortified foods, supplements and basic diet to total intake of vitamin D

The results obtained when analysing the contribution of fortified foods, supplements and basic diet to the total intake of vitamin D are presented in table 10.

According to table 10, the following trends are observed in Flemish toddlers:

- The enriched food contributed less than 1% of total intake of vitamin D. The mandatory fortified foods (margarines) were not considered under the group of enriched foods but in the group of basic diet.
- Supplements contributed to 62% of the total intake of vitamin D.
- The basic diet was counting for 38% of the total intake, while in non-supplement users this percentage was even higher (90%).

The obtained results can be compared with FITS (2002), where supplements are contributing to 54% of the daily intake of vitamin D (Briefel et al, 2006). In Europe, it is known that supplements make the largest contribution to the total vitamin D intake (Flynn et al, 2009). This is in line with the results gathered in this thesis.
Results and discussion

Table 10—The distribution of the usual intake between basic diet, enriched food and supplements, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th></th>
<th>Basic diet</th>
<th>Basic diet + Enriched food</th>
<th>Basic diet + Enriched food + Supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Valid</td>
<td>103</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mean</td>
<td>1.186</td>
<td>1.377</td>
<td>42.373</td>
</tr>
<tr>
<td>Median</td>
<td>0.90</td>
<td>0.94</td>
<td>17.870</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.131</td>
<td>1.297</td>
<td>50.409</td>
</tr>
<tr>
<td>Variance</td>
<td>1.281</td>
<td>1.683</td>
<td>2541.021</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Maximum</td>
<td>6.50</td>
<td>6.63</td>
<td>146.300</td>
</tr>
<tr>
<td>Percentiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.460</td>
<td>0.590</td>
<td>5.310</td>
</tr>
<tr>
<td>50</td>
<td>0.90</td>
<td>0.940</td>
<td>17.870</td>
</tr>
<tr>
<td>75</td>
<td>1.490</td>
<td>1.800</td>
<td>60.680</td>
</tr>
<tr>
<td>90</td>
<td>2.752</td>
<td>2.982</td>
<td>143.706</td>
</tr>
</tbody>
</table>

4.4.3. Vitamin E

4.4.3.1. The comparison of the usual intake of vitamin E with reference values

The results of comparing usual intake of Vitamin E with the reference values are displayed in table 11. The share of Flemish toddlers which are under the AI is 76.7%. The rest is in the group meeting the recommendation with an intake above 6 mg/day, but less than 100 mg/day.

The data presented in table 11 is following the trend present in Europe. According to Flynn et al (2009) and Lambert et al (2004), the South, East and Central Europe have higher intakes of vitamin E, due to higher intakes of PUFAs. This trend is not seen in West and North Europe. Therefore, the results obtained can be clarified with this geographically correlated course of vitamin E intake. According to the literature, the intake of vitamin E does not exceed UL in any European country, the USA, Canada and Japan. The outcome of this survey is strengthening the results gathered in the previous researches.
Results and discussion

Table 11 - Comparison of Usual Intakes of Vitamin E of Flemish toddlers with reference values, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>Proportion of toddlers</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below AI(^1)</td>
<td>79</td>
<td>76.7</td>
<td>76.7</td>
</tr>
<tr>
<td>Meeting recommendation</td>
<td>24</td>
<td>23.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Above UL(^2)</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

1 AI=6mg/day
2 UL=100mg/day

The usual intakes of vitamin were compared with the AI therefore the prevalence of inadequacy cannot be estimated (EFSA, 2010). The mean intake of vitamin E (mean=5.090 mg/day) is smaller than the AI (6mg/day). Therefore, no conclusion can be derived on inadequacy of vitamin E for group of Flemish toddlers.

4.4.3.2. The contribution of fortified foods, supplements and basic diet to the total intake of vitamin E

Table 12 summarizes the results obtained from the analysis of the contribution of enriched foods, supplements and basic diet to the total intake of vitamin E.

Table 12 - The distribution of the usual intake of vitamin E between basic diet, enriched food and supplements, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>The usual intake of vitamin E</th>
<th>Basic diet</th>
<th>Basic diet + enriched food</th>
<th>Basic diet + enriched food + supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Valid</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td>Mean</td>
<td>4.799</td>
<td>4.932</td>
<td>5.091</td>
</tr>
<tr>
<td>Median</td>
<td>4.230</td>
<td>4.270</td>
<td>4.270</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>2.845</td>
<td>3.026</td>
<td>3.308</td>
</tr>
<tr>
<td>Variance</td>
<td>8.096</td>
<td>9.155</td>
<td>10.946</td>
</tr>
<tr>
<td>Percentiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3.000</td>
<td>3.050</td>
<td>3.090</td>
</tr>
<tr>
<td>50</td>
<td>4.230</td>
<td>4.270</td>
<td>4.270</td>
</tr>
<tr>
<td>75</td>
<td>5.700</td>
<td>5.770</td>
<td>5.810</td>
</tr>
<tr>
<td>90</td>
<td>7.642</td>
<td>8.152</td>
<td>8.640</td>
</tr>
</tbody>
</table>
According to table 12, intake of vitamin E in Flemish toddlers was derived from enriched food, supplements, and basic diet:

- Not all toddlers were consuming vitamin E enriched foods. But in those ones who consumed (n=12), fortified food products contributed with 14%.
- Single vitamin supplements were not used, while multivitamin products (containing vitamin E) were consumed (n=6). Supplements presented 24% of the vitamin E intake.
- In most of the respondents, basic diet was the major contributor to the intake of this vitamin.

The results collected in this thesis are corresponding with the literature. Due to Flynn et al (2009), mandatory and voluntary fortified food products appear not to have major impact on the total intake. On the other hand, basic diet was the main source of vitamin E.

### 4.4.4. Vitamin K

#### 4.4.4.1. The comparison of the usual intake of vitamin K with reference values

Assessing the usual intake of vitamin K is an added value of the VITADEK survey and this thesis. The results gathered are shown in table 13.

Table 13- Comparison of Usual Intakes of Vitamin K of Flemish toddlers with reference values, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>Proportion of toddlers</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meeting the recommendation(^1)</td>
<td>103</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\(^1\)AI=1mg/day/kg

The findings are straightforward – all toddlers meet the recommendations. The only study available that have assessed the intake of vitamin K is the observational German study (DONALD) (Sichert-Hellert et al, 2006). The information obtained in this thesis is in line with DONALD.

The cut off point used is the AI, the same as for vitamin E and vitamin D. Under these circumstances, the prevalence of inadequacy cannot be estimated. When comparing the mean intake (mean intake=10.646 mg/day/kg) of vitamin K with the AI, it can be assumed that the group of Flemish toddlers has a low prevalence of inadequate intakes (mean intake>AI).
Results and discussion

4.4.4.2. The contribution of fortified foods, supplements and basic diet to the total intake of vitamin K

The contribution of fortified foods, supplements, and basic diet to the total intake of vitamin K is given in table 14.

Table 14 - The distribution of the usual intake of vitamin K between basic diet, enriched food and supplements, Vitadek study, Belgium, 2015

<table>
<thead>
<tr>
<th>Usual intake of vitamin K</th>
<th>Basic diet</th>
<th>Basic diet + enriched food</th>
<th>Basic diet + Enriched food + Supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Valid</td>
<td>103</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Missing</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>120.232</td>
<td>120.232</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>102.240</td>
<td>102.240</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td></td>
<td>74.934</td>
<td>74.934</td>
</tr>
<tr>
<td>Variance</td>
<td></td>
<td>5615.102</td>
<td>5615.102</td>
</tr>
<tr>
<td>Percentiles</td>
<td>25</td>
<td>66.060</td>
<td>66.060</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>102.240</td>
<td>102.240</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>151.760</td>
<td>151.760</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>227.016</td>
<td>227.016</td>
</tr>
</tbody>
</table>

Referring to table 14, the total intake of vitamin K in Flemish toddlers was coming from supplements and basic diet, while enriched food, in this case, did not have an influence:

- The single vitamin supplements were used, but just few respondents were taking supplements of vitamin K (n=5). Supplements were counting for 8% of total intake.
- For most Flemish toddlers basic diet is the most important source of vitamin K.

According to the literature, the intake of vitamin K is exclusively based on the basic diet. This is due to the fact that amounts of vitamin K present in the fortified foods and supplements are really low (Sichert-Hallert et al, 2006).
5. Conclusion

The public health sector focused on the fat-soluble vitamins and their intakes in the specific age groups. The double burden present when talking about these vitamins is showing the seriousness of the problem we are facing (Flynn et al., 2009). The health consequences correlated with both excessive and inadequate intakes are severe and represent the threat to the population’s health (Dwayer et al., 2015). Subsequently, the estimation of the usual intake is crucial for addressing the right public health policy.

The aim of this thesis was to determine the proportion of Flemish toddlers under the RDI and above the UL. The survey performed is a good starting point for the future studies in Belgium and Europe. It shows the actual fat-soluble vitamin intake distribution of the Flemish toddlers compared with reference values. The estimation of the level of inadequacy and of the probable risk of adverse effects on health when exceeding the ULs were done with a high precaution due to the study, analysis and the reference values limitations.

The final results showed the low prevalence of inadequacy in vitamin A intake and low probability of inadequacy of vitamin D, and vitamin K intake. The probability of inadequacy could not be identified just for vitamin E intake. The risk of exceeding the UL was observed in the estimated usual intakes of vitamin A, and vitamin D. In both cases, supplement users were toddlers with excessive intakes.

The specific objective was to determine the contribution of enriched foods, supplements and basic diet to the intake of fat-soluble vitamins. The enriched foods had low impact on the intake of all four vitamins. The basic diet was the biggest contributor to the intake of vitamin A, E and K. Concerning supplements, it can be concluded that the biggest differences in the intakes of fat-soluble vitamins were seen between supplement users and non-users (e.g. vitamin D). The intake of supplements made considerable change in vitamin D intake, showing the impact of Belgian supplementation program. Nonetheless, supplement users are exposed to the risk of exceeding the UL. The supplements that have higher concentrations than the UL are available on the market. Therefore, precaution should exist when buying and prescribing supplements to toddlers.

In order to address the right public health and food safety policy, further researches are needed. The contribution of different food groups to the intake of fat-soluble vitamins is essential for better understanding of obtained results. Furthermore, the outcomes of this thesis should be compared with the final results of the VITADEK survey. The final results of VITADEK still can differ significantly, due to a bigger sample size and the availability of key information. It is encouraging to link the obtained results from VITADEK with the Belgian FCS (2014), in order to minimize the bias and strengthen the conclusion.


References


References


References


Appendices

Table 15 – The output of linear regression analysis of demographic variables

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</table>

Figure 7 – The distribution of the estimated usual intake of vitamin A (expressed as Retinol equivalent) (µg/day)
Appendices

Figure 8 – The distribution of the estimated usual intake of vitamin D (µg/day)

Figure 9 – The distribution of the estimated usual intake of vitamin E (mg/day)
Figure 10 – The distribution of the estimated usual intake of vitamin K (mg/kg/day)