



Inga Elksne

Early eating habits in infants and their association with iron metabolism

Summary of the Doctoral thesis
for obtaining a doctoral degree (*Ph.D.*)

Sector – Health and Sports Sciences
Sub-sector – Nutrition

Rīga, 2020



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Abbreviations

ID – iron deficiency

IDA – iron deficiency anaemia

DNA – deoxyribonucleic acid

E% – total energy percent

EFSA – European Food Safety Authority

ESPGHAN – European Society for Paediatric Gastroenterology Hepatology and Nutrition

FFQ – Food Frequency Questionnaire

Hb – haemoglobin

IBM SPSS – programmatūras pakete statistiskajai analīzei (angļu val.

International Business Machines Statistical Package for the Social Sciences)

IQR – interquartile range

Lf – lactoferrin

MCH – mean corpuscular haemoglobin

MCV – mean corpuscular volume

WHO – World Health Organisation

SN – standard deviation

SPSS – Statistical Package for the Social Sciences

sTfR – soluble transferrin receptors

Tf – transferrin

TfR – transferrin receptors

TIBC – total iron-binding capacity

Introduction

There is very rapid growth and development in the first year of life. In this period, the need of the infant's body for nutrients per 1 kg of body weight is much higher than that of an adult, and therefore the qualitative composition of food is particularly important. The nutrients taken can be too much and too little, and they can have irreversible effects on the infant's body. During this period of life, eating habits for the whole life are also developed. It is important to study whether the eating habits of infants living in Latvia comply with both the guidelines set out in Latvia and the guidelines of other leading organisations, such as the World Health Organisation, the European Food Safety Authority (EFSA) and the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN). It is interesting to observe how infant's eating habits change over time.

Early-age eating habits are associated with diseases in later life, such as type 2 diabetes, obesity and cardiovascular diseases, more and more often (National Institute for Health and Welfare in Finland, 2016; Tarry-Adkins and Ozanne, 2017).

It is very important to respect the basic principles of healthy eating habits in the first year of life: exclusive breastfeeding in the first months of life, the introduction of complementary food in a timely manner, the use of food from different food groups and their timely introduction, the introduction of iron-fortified products into the diet, the exclusion of cow's milk from the diet.

Out of microelements, it is iron deficiency that is identified most frequently in the first year of life (Domellöf et al., 2014). Meanwhile, lack of iron in the body or iron deficiency can cause irreversible impairment of cognitive development (Baker and Greer, 2010). Errors in an infant's diet are one of the main reasons for iron deficiency. Therefore, compliance with the above-

mentioned healthy eating habits is not only necessary for the prevention of diseases in later life, but, above all, for the prevention of iron deficiency. Timely detection of iron deficiency through biomarkers may reduce the risk of anaemia.

It is important to understand the eating habits of infants living in Latvia in order to identify potential risks of shortage or excess of nutrients, as well as to make improvements if necessary.

It is also important to investigate the association between eating habits and iron metabolism in infants who live in Latvia, taking into account their specific eating habits. If an association between eating habits and iron metabolism cannot be found, other factors should also be studied which may affect iron metabolism and are characteristic to the population of Latvia, such as the eating habits of pregnant women and the prevention of iron deficiency using medicines (Dewey, 2013; Vaivada et al., 2017).

Aim of the study

The aim of the study was to investigate early eating habits in Latvian infants and their association with iron metabolism.

Objectives of the study

1. To investigate eating habits of infants in Latvia using the interview method.
2. To determine the main iron metabolism indicators in infants' blood samples.
3. To investigate correlations between early eating habits and iron metabolism disorders.

Questions of the study

1. What are the eating habits of infants in Latvia, and do they comply with the recommendations of the Ministry of Health (2003)?
2. What is the prevalence of iron deficiency and iron deficiency anaemia in infants in Latvia as defined by the following criteria:

a) iron deficiency:

- serum ferritin $< 12 \mu\text{g/l}$ and
- $\text{MCV} < 74 \text{ fl}$, and
- $\text{sTfR} > 2.4 \text{ mg/l}.$?

b) iron deficiency anaemia:

- haemoglobin $< 110 \text{ g/l}$ (WHO 2001) and
- serum ferritin below $< 12 \mu\text{g/l}$ or $\text{MCV} < 74 \text{ fl}$ (according to the recommendations of the European Paediatric Association and the World Health Organisation (WHO)).

3. What is the association between the early eating habits of infants and changes in iron metabolism in the body? Main nutritional factors: exclusive breastfeeding period, breastfeeding, early introduction of cow's milk in the diet, excessive consumption of cow's milk (over 500 ml per day), later introduction of meat in the diet, insufficient intake of iron with food, insufficient intake of iron with products of animal origin.

Scientific novelty of the study

1. Eating habits of infants living in Latvia have been studied in detail, deficiency and excess of nutrients has been evaluated.
2. The association between changes in iron metabolism and main nutritional factors: consumption of cow's milk, legumes, products of animal origin, as well as exclusive breastfeeding and breastfeeding without starting complementary feeding in a timely manner has been proven.
3. The research has identified the most frequent errors in infants' diet, as well as recommendations for general practitioners to prevent them have been introduced.

1. Materials and methods

The research consists of two parts – “Study A: research on eating habits of infants living in Latvia” and study B “Research on the association between eating habits of infants living in Latvia and iron metabolism”. Part A of the study was conducted within the scope of a project supported by the European Food Safety Authority (EFSA). A cross-sectional study has been conducted for the purposes of identifying the situation and obtaining justified national data on the food and beverages consumed by the Latvian population aged 0–74, eating habits and potential risks and threats related to food (started in 2012, completed in 2014).

Study B was conducted based on the methodology of Study A to collect data on diet, and blood counts to determine iron metabolism, indicators were carried out in addition in order to study their association with food, to determine the prevalence of iron deficiency and iron deficiency anaemia.

1.1. Study A: Research on eating habits of infants living in Latvia

1.1.1. Study subjects

This study intended to involve 260 infants (0–12 months), including equal numbers of girls and boys.

Such a respondent count has been chosen based on EFSA recommendations (EFSA, 2009), where the minimum number of respondents for the determination of eating habits in a population is 260 respondents: 130 girls and 130 boys.

Selection criteria of study participants for inclusion in the survey sample:

- appropriate age;
- sex;

- parental consent to participating in the study.

Participants from all regions of Latvia in proportion to the birth indicators specified in the database of the Central Statistical Bureau (CSP) participated in the study.

The procedure for the selection of study participants consisted of two stages. General practitioners and paediatricians registered in the Latvian Medical Associations were invited to participate in the first stage (the call was sent to all medical practitioners, and all the volunteers were accepted). In accordance with the survey plan, if the survey did not include some categories, a repeated call was sent to medical practitioners working in the respective field to promote participation in this survey. In order to motivate medical practitioners to participate, certification points were awarded for participation in the study (continuing education points awarded by the Latvian Medical Association). All children born in Latvia are registered with a general practitioner or paediatrician's office. At the second stage, the medical practitioners were asked to randomly invite part of their patients to participate in the survey. A selection plan was issued to each medical practitioner – a certain number of male and female participants to be invited. Since the medical practitioners had already been broken down by regions, no additional measures for determining places of residence of the study subjects were carried out. A general practitioner/paediatrician is usually located close to the place or residence or accommodation of the study subject (Siksna et al., 2017). If the parent had signed the study subject's consent form, the interviewer contacted the parents.

When analysing the age of initiation of complementary feeding, 334 respondents were included in the study group, including those who were in the age of initiation of complementary feeding indicated. When analysing the duration of breastfeeding, as well as the amount and frequency of intake of food groups, a sample of 266 infants was created, where the inclusion criteria were

the age from birth to 12 months old, inclusive, as well as correctly indicated eating habit data in questionnaires.

1.1.2. Research methods

Surveys within the study were conducted using the following methods:

- 24-hour dietary recall for 2 non-successive days;
- food frequency questionnaire.

24-hour dietary recall

The 24-hour dietary recall questionnaire was available on paper and electronically in 2 languages: Latvian and Russian. The questionnaires filled out on paper were transferred to the group leader or coordinator.

A food record method was used to collect data on the children attending a kindergarten. In order to be able to indicate more complete data in the food diary, the carers from kindergartens were asked to fill out the questionnaire on the food consumed by a child in the kindergarten.

At the end of the interview, special attention was devoted to learning about the consumed amount of water, vitamins and food supplements, sugar, fats or other ingredients added into food and taken between meals. Questions relating to the aforementioned were used as screening questions to ascertain that the obtained information was sufficient and correct (Siksna et al., 2017).

Food frequency questionnaire

The food frequency questionnaire was available on paper and electronically in 2 languages: Latvian and Russian. The questionnaires filled out on paper were transferred to the group leader or coordinator.

The decisions on the food to be included in the food frequency questionnaire were taken based on EFSA scientific reports (EFSA, 2010; EFSA, 2011; EFSA, 2012). The food groups were evaluated according to consumption

and adapted to the specific needs of the target group of the study. The same principles were used when selecting other foods for inclusion into the food frequency questionnaire. Based on the above-mentioned information, 115 foods have been included in the food frequency questionnaire for the target group of infants and young children (Siksna et al., 2017).

In order to learn the information related to complementary food habits, this questionnaire included basic information about the child's sex, age, birth and current weight, place of residence and education, employment of the child's parents.

Only data from food frequency questionnaires were used within this study paper, while 24-hour dietary recall diaries were used for validation of the data.

1.1.3. Statistical analysis of the study

Definitions of breastfeeding and complementary feeding were defined based on the criteria set by the World Health Organisation (WHO, 2001).

In order to evaluate the obtained data on eating habits, several guidelines developed by the leading specialists were used as references, for example, WHO, EFSA, Finnish national recommendations and German national recommendations (WHO, 2009; EFSA, 2013; National Institute for Health and Welfare in Finland, 2016; Prell and Koletzko, 2016).

When analysing the association between the mother's age and the age of initiation of complementary feeding or the duration of breastfeeding, the mother's age was characterised with an arithmetic average and a standard deviation (SD). The analysis of variance (ANOVA) was used to compare subgroups. In order to analyse data on the age of initiation of complementary feeding, children were broken down into 4 groups: group 1 – up to 4 months

(< 4), group 2 – more than 4 months, but less than 6 months ($\geq 4, < 6$), group 3 – from 6 to 7 months ($\geq 6, < 7$), group 4 – from 7 months (≥ 7).

Since the frequency of consumption of different food groups and portion sizes were not normally distributed (Shapiro-Wilk test), they were indicated as median values with an interquartile range (IQR). The age was specified as an arithmetic average value with a standard deviation (SD). The Pearson's chi-squared test was used to compare proportions. P value below 0.05 was accepted as statistically significant.

1.2. Study B. Research on eating habits of infants living in Latvia and the association with iron metabolism

This part of the study was conducted according to the methods already described in section A. Medical practitioners were not selected repeatedly, the selection from study A was used again.

1.2.1. Study subjects

The study intended to involve 100 infants from birth to 12 months of age (inclusive), including equal numbers of girls and boys. The sample size was calculated in OpenEpi, based on the expected prevalence of iron deficiency anaemia in Europe – 3 % and 2.5 % of errors (Hernell et al., 2015) or also based on the differences in prevalence of iron deficiency anaemia between infants who are and are not breastfed: 4 % to 25 % (Eussen et al., 2015).

Participant inclusion and exclusion criteria

1. The study involved infants:
 - a) aged 9–12 months old (inclusive);
 - b) birth starting from the 38th week of gestation and with birth weight no less than 2.5 kg.

2. The study does not involve infants who:
 - a) have had infectious diseases within the last 2 weeks;
 - b) have required treatment of anaemia using medicines in the last 3 months;
 - c) have a congenital pathology, chromosome disorders or other serious disease (for example, a transoesophageal fistula, tracheomalacia, congenital heart diseases, Down syndrome, HIV, cancer);
 - d) health disorders, diseases causing restrictions in the menu (for example, diagnosed food allergy, coeliac disease, etc.);
 - e) known haemoglobinopathy or thalassemia;
 - f) have had a blood transfusion in the last 6 months;
 - g) parents have not signed the study consent form.

In order to determine eating habits, data from a survey of 87 respondents were obtained, and laboratory blood indicators were obtained from 65 respondents. Those respondents, who had both questionnaires and laboratory blood data – 65 respondents in total, were included in the study.

1.2.2. Research methods

The data necessary for the study were collected using interviews. 2 types of questionnaire were used for the interview: the food frequency questionnaire and 24-hour dietary recall diaries for three non-consecutive days, which were filled out within one week.

Only data from 24-hour dietary recall diaries were used within this study paper, while food frequency questionnaires were used for validation of the data. The main aim was to study data related to the intake of iron with food, and, in accordance with publications, such data may be correctly obtained from 3-day food diaries (Erkkola et al., 2011).

A printed and also an electronic validated catalogue of food portions with images was used to determine the size of the portion; a cup with volume marks was issued to determine the amount of liquid.

Definitions of breastfeeding and complementary feeding were defined based on the criteria set by the World Health Organisation (WHO, 2004).

In order to evaluate the obtained data on eating habits, recommendations on the recommended daily dose of nutrients by the Latvian Ministry of Health, EFSA and WHO were used as a reference (EFSA, 2013; WHO, 2004; Ministry of Health, 2017).

Blood samples were taken from infants to collect data on complementary food, where parameters for determining iron metabolism, ID or IDA were determined. The parameters to be determined in a blood sample:

- a) full blood count (including MCV, MCH, MHCH);
- b) iron;
- c) transferrin and iron-binding capacity;
- d) ferritin;
- e) soluble transferrin receptors.

1.2.3. Laboratory analysis

In order to determine the prevalence of iron deficiency anaemia, the following indicators were determined:

- haemoglobin < 110 g/l (WHO, 2001) and
- serum ferritin below < 12 $\mu\text{g/l}$ or MCV < 74 fl (according to the recommendations of the European Paediatric Association and the World Health Organisation).

The following criteria were used to determine iron deficiency:

- 1) serum ferritin < 12 $\mu\text{g/l}$ and

- 2) MCV < 74 fl, and
- 3) sTfR > 2.4 mg/l.

1.2.4. Statistical analysis of the study

The Kruskal–Wallis test and the Wilcoxon–Mann–Whitney test were used in the case of continuous variables to analyse the association between eating habits and other parameters and laboratory indicators of iron metabolism. The Pearson χ^2 test was used for the analysis of the same associations, but in the case of discrete variables.

The frequency of consumption of different food groups and portion sizes were not normally distributed (Shapiro–Wilk test), they were indicated as median values with an interquartile range (IQR). The age was specified as an arithmetic average value with a standard deviation (SD). P value below 0.05 was accepted as statistically significant. The results with statistical reliability below 0.1 are also described. The level of statistical significance is indicated in the description of results and is expressed as a percentage, 5 % or 10 % respectively.

1.3. Ethical aspects

The study protocol was approved by the Latvian Central Medical Ethics Commission on 12 September 2013 and reapproved when the name of the study was updated on 8 September 2016. Parents of all the children included in the study signed a written consent form for the participation of their child in the study.

2. Results

2.1. Research on eating habits of infants living in Latvia

The general description of the sample group is shown in Table 2.1. Overall, the prevalence of boys and girls, as well as the distribution of respondents by regions is comparable to the demographic distribution of the Latvian population.

Table 2.1

General description of respondents and comparison with the Latvian demographic statistics 2012

Parameter		Study group (n = 266)	Statistics on Latvian infants and mothers, 2012
Infant's sex, n (%)	Girl	126 (47)	9640 (48)
	Boy	140 (53)	10257 (52)
Infant's place of residence, n (%)	Riga	88 (33)	6739 (34)
	Riga region	36 (13)	3982 (20)
	Other city	43 (16)	3171 (16)
	Rural area	99 (37)	6005 (30)
Mother's age, n (%)	Average, years (SD)	29 (6)	29
	17–25	71 (27)	6414 (32)
	26–35	156 (58)	10946 (55)
	36–45	39 (15)	2418 (12)
Mother's education, n (%)	Higher	149 (56)	-
	Secondary	93 (34)	-
	Basic	19 (7)	-
	None	3 (1)	-
	No data	2 (1)	-
Eating habits, n (%), categories are not conflicting	Breastfeeding	63 %	-
	Artificial formula	37 %	-
	Use of complementary food initiated	65 %	-

2.1.1. Prevalence of breastfeeding

Prevalence of breastfeeding was determined within the interval of one month (Figure 2.1). Almost all infants (89 %) were breastfed in the first month of their life. When they reached the age of 6 months old, the prevalence of breastfeeding reduced to 68 %. The number of breastfed infants reduced along with the infant's age ($p = 0.002$). The lowest indicators of prevalence of breastfeeding were observed in months 11 and 12, 33 % and 45 %, respectively.

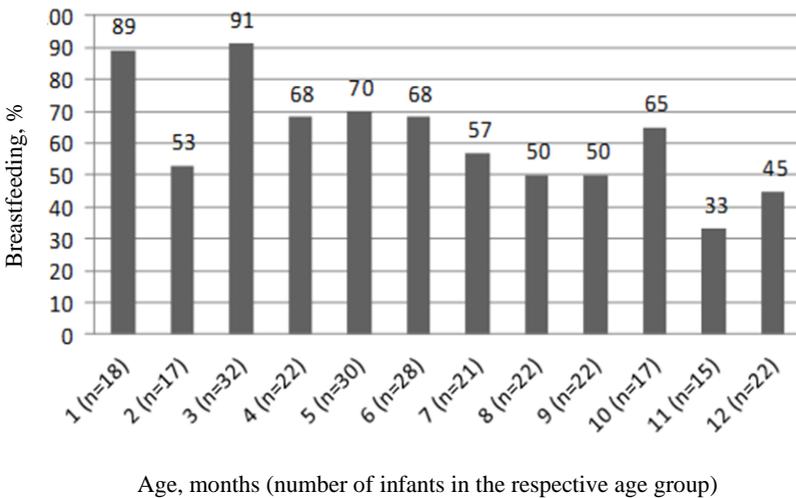


Figure 2.1. **Proportion of breastfed infants in the age group**

2.1.2. Initiation of complementary feeding

Complementary feeding in the current sample had been initiated for 164 infants in total. The average age of initiation of complementary feeding was 5 months old (SD = 1). Before the age of 4 months old, 9 % (n = 14) of infants received complementary food. Most parents (85 %) introduced complementary

food for infants at the age of 4–6 months old. For six percent of infants (n = 10) complementary food was introduced starting from the age of 7 months old. The most common choice of first complementary food was porridge (64 %); the second most common choice of food were vegetables (21 %). The most popular vegetables were pumpkin and potatoes. Fruit puree was chosen as the first complementary food in 10 % of cases and other food (for example, yoghurt) in 5 % of cases.

2.1.3. Inclusion of foods from different food groups in the menu

Some complementary foods were included in the menu of only a few infants before the age of 4 months old. The biggest diversity of food was introduced at the age of 4 to 6.9 months old; those were vegetables, potatoes, fruit and berries, as well as cereals. From the age of 7 months old, each infant's diet included foods from nearly all food groups, such as cereals, vegetables and fruit, meat and fish, eggs, legumes, dairy products and others.

For further analysis all the infants were broken down into two groups: 0–5.9 months and 6–12.9 months.

The menu of 86 % (n = 126) of infants from the age of 6 months old (n = 126) included vegetables. The average consumption was once a day and the average size of one portion was 80 g (Table 2.2). Vegetables were included in the menu of only 10% (n = 12) of infants before the age of 6 months old.

In the vegetables group, the consumption of potatoes was analysed separately. The menu of 85 % (n = 124) of infants older than 6 months of age included potatoes with the average frequency of consumption being once a day. The average amount consumed per meal was 17 g. Potatoes were included in the menu of only 8 % (n = 9) of infants before the age of 6 months old.

Table 2.2

Median frequency of consumption of food groups and median amount of food per meal in infants aged 6–12.9 months old

Food group	Frequency of meals		Portion size	
	Per day or week	IQR	Per meal, g	IQR
Vegetables	1 / day	0.6	80	100
Potatoes	1 / day	0.6	17	4
Fruit, berries	1 / day	0.6	54	60
Cereals	6 / week	11	20	30
Meat	1.7 / day	1.5	46	40
Fish	1 / week	2.5	30	40
Milk	6 / week	7	46	91
Dairy products	1 / day	1	45	30
Eggs	1 / week	1.75	1 egg	0
Legumes	1 / month	1.75	30	40
Fats	5 / week	11	6	10

All complementary food groups were included in the menu, mainly from the age of six months old. The menu of infants older than six months of age in 81 % (n = 118) of cases included fruit and berries. The median frequency of consumption was once a day and the median consumed amount was 54 g per meal. The consumption of cereals was also popular (88 %); however, its median frequency of consumption was less than once a day (6 times per week), and the median amount of consumed cereals per meal was 20 g. Meat was also introduced in the diet of most of the infants (73 %, n = 107). The median frequency of consumption of meat was 1.7 times a day and the portion size was 46 g per meal. The menu of about one third of infants (36 %, n = 53) in this age group included fish. Fish consumption was relatively rare; the median frequency

of consumption of fish was once a week and the amount of fish per meal was 30 g.

Consumption of milk and dairy products was rather popular among infants older than six months of age. Milk and dairy products were introduced in the menu of 66 % (n = 96) and 78 % (n = 114), respectively. The median frequency of consumption of milk was less than once a day and that of dairy products – once a day; the amount of food per meal was 45 ml or 46 g of milk and 45 g of dairy products.

The diet of almost half of infants (45 %, n = 65) from the age of six included eggs; the median frequency of consumption of eggs and the median consumed amount was 1 egg per week. Consumption of legumes was less common. They were introduced in the menu of 28 % (n = 41) of infants in this age group. The average consumption of legumes was only once a month and the average amount per meal was 30 g.

Fats were also included in the menu of most (73 %, n = 107) infants from the age of six months old. The median frequency of consumption of fats was 5 times a week and the amount per meal was 6 g.

The menu of infants younger than six months of age included fruit and berries in only 6 % (n = 7) of cases, and cereal – in 5 % (n = 6). Meat and fish were introduced in the menu of only some infants (3 %, n = 3 and 1 %, n = 1, respectively).

Milk and dairy products were introduced in the menu of 3 % (n = 3) of infants younger than six months of age. The menu of only 1 % (n = 1) of infants of this age included legumes and the menu of 3 % (n = 4) included fats.

There is no statistically significant difference in the consumption of different food groups depending on the mother's education (Annex 5).

In order to compare the data of the eating habits in study A and B, the main sources of energy nutrients were viewed separately in the age group from

9 to 12 months. The main sources of energy in infants in this age group were still breast milk and artificial milk formulae, together constituting 36 % of total energy. The next largest source of energy were plant products and cereals, together constituting 30 %. Products of animal origin, except milk, constituted 11 % of all energy, while milk and dairy products – 14 %. Cakes, biscuits, chocolate and dough were the smallest sources of energy in infants in this age group and together constituted 8%.

2.2. Research on eating habits of infants living in Latvia and the association to iron metabolism

Overall, 73 respondents from different Latvian regions participated in study B. There was a similar distribution among girls and boys, 56 % and 44 %, respectively. Unlike in study A, most of the respondents in the sample for this study were from cities other than Riga, amounting to almost half or 44 %. Likewise study A, most of the mothers were aged 26 to 35 years old, which is 75 % in the study B and 58 % in the study A. The level of education of mothers in both studies was almost identical, higher education dominated in both.

2.2.1. Nutrition data analysis

The nutrition data analysis showed that iron is taken in the amount of 7.4 mg per day on average, while the median value is even lower – 6.5 mg per day, which does not correspond to the recommended daily amount. Only 38 % of infants (n = 27) consume at least 8 mg of iron per day.

Iron is only taken with products of animal origin in the amount of 13 % of the total intake of iron with food (Table 2.3). A deeper analysis showed that less than 10 % of iron is taken with products of animal origin by 35 % (n = 25)

of respondents, 10–20 % is taken by 39 % (n = 28) of infants, but 20 % or more is taken by 26 % (n = 19) of infants. It should be noted that out of the total consumed meat, red meat only constitutes 56 %, poultry – 35 %, sausages – 9 % and offal – 1%.

Table 2.3

Description of iron intake with food by infants

Parameter	Median (n = 72)	IQR
Average daily intake of iron, mg/d	6.5	3.9
Intake of iron from products of animal origin, mg/d	0.9	0.8
Intake of iron from other products, mg/d	5.1	3.8
Intake of iron from products of animal origin, % of total iron amount	13.1	10

21 % (n = 15) of respondents followed the exclusive breastfeeding recommendations – to continue it in the first 6 months of life, 29 % (n = 21) of breastfed infants from 4 to 5.9 months old, less than half (40 % (n = 29)) of the respondents did not breastfeed or breastfed infants for less than 4 months.

In response to the questions about breastfeeding at the time of the survey, 44 % (n = 32) of infants received breast milk, while 53 % (n = 39) of infants received artificial milk formula.

The use of complementary food in this sample was initiated for 69 infants in total, and there is no information about the age of initiation of complementary feeding for 4 infants. The average age of initiation of complementary feeding was 5 months (SD = 1), similar to study A. Before the age of 4 months old, only 1 infant (1 %) received complementary food. Most of the parents (93 %, n = 64) introduced complementary food for infants at the age of 4–6 months old. For six percent of infants (n = 4) complementary food was introduced starting from the age of 7 months old, which matches the results of study A.

At the time of the survey, cow’s milk was introduced for 38 % of infants, while meat for 34 % of infants at the age of 4–6 months old, and for 53 % at the age after 7 months old.

An important fact is that 79 % of mothers used iron medicines during pregnancy.

A diversity that was observed is food groups, which was evaluated later in relation to iron metabolism disorders. Meat, dairy products and cereals were used in considerable amounts, while cow’s milk was used in limited amounts. The use of legumes was very low, and the median value even did not exceed 0 grams per day (Table 2.4).

Table 2.4

Consumption of food from different food groups in infants

Food group	Median (n = 72)	IQR
Amount of meat per day, g/day	38	39
Amount of cow’s milk per day, g/day	45	75
Amount of dairy products (except milk) per day, g/day	90	100
Amount of milk and dairy products per day, g/day	151	135
Amount of cereals per day, g/day	77	67
Amount of legumes per day, g/day	0	4.9
Amount of products of animal origin per day, g/day	200	153

In order to compare eating habits data with study A, sources of nutrients in infants were analysed. Similar to the study A, in this study as well the main sources of energy in infants were breast milk and artificial milk formulae, together constituting 42 % of total energy. The next largest source of energy were plant products that together with cereals constitute 31 %. Products of animal origin, except milk, constituted 9 % of all energy, while milk and dairy products – 12 %. Cakes, biscuits, chocolate and dough were the smallest sources of energy in infants in this age group and together constituted 7 %.

2.2.2. Prevalence of iron metabolism disorders

Iron deficiency was stated in 9.6 % (n = 7; 95 % TI: 4.7–18.5 %) of infants and iron deficiency anaemia in 4.1% of infants (n = 3; 95 % TI: 1.4–11.4 %).

Overall, more than half of the infants had their iron metabolism indicators within normal limits: MCV, serum ferritin, iron and haemoglobin. However, only 36 % (n = 26) had their soluble transferrin receptors within normal limits. An increased level of sTfr in blood might be the evidence of reduced iron reserves in the body.

2.2.3. Early eating habits in association with iron metabolism

MCV, serum ferritin, iron, iron binding capacity, soluble transferrin receptors, haemoglobin were used as the main laboratory indicators of iron metabolism. Exclusive breastfeeding, the use of artificial formulae, cow's milk, legumes and meat, general intake of products of animal origin were considered as main nutritional factors when studying the association with iron metabolism. Other important factors were also considered for complementary food habits: the use of iron medicines during or after pregnancy, mother's age and education, child birth order. Discrete and continuous data were used to find the association between food and blood count data. Several statistically significant associations have been found as a result of both analyses (Table 2.5.).

Exclusive breastfeeding and iron metabolism

When analysing data as continuous variables, it was discovered that breastfeeding has the biggest association with iron metabolism. The association of exclusive breastfeeding and breastfeeding with the following iron metabolism indicators was observed: iron, MCV and serum ferritin.

The level of iron in the blood of the infants, who did not receive breast milk at the time of the survey, was higher than in those who were breastfed ($p = 0.0125$) (Table 2.5).

Table 2.5

Association of eating habits and mother's factors with iron metabolism

	MCV	Sf	Fe	sTfr	Hgb	TIBC
Age until which the child was exclusively breastfed	N*	5%	N*	N*	N*	N*
Was the child exclusively breastfed at least until the age of 4 months?	5 %	0.5 %	N*	N*	N*	N*
Is the child breastfed?	N*	0.5 %	5 %	N*	N*	N*
Is the child fed with an artificial milk formula?	N*	5 %	N*	N*	N*	N*
Has cow's milk been introduced into the menu?	N*	N*	5 %	N*	N*	N*
At what age was meat introduced?	N*	N*	N*	N*	N*	N*
Consumed daily amount of iron ¹	N*	N*	N*	N*	N*	N*
Consumed daily amount of iron ²	N*	1 %	N*	N*	N*	N*
What share in the total amount of consumed iron was taken with products of animal origin?	N*	5 %	N*	N*	5 %	N*
Frequency of intake of meat (from three days)	N*	N*	N*	N*	N*	N*
Frequency of intake of cow's milk (from three days)	N*	N*	N*	N*	N*	N*
Frequency of intake of dairy products (from 3 days)	N*	N*	N*	N*	N*	N*
Frequency of intake of legumes (from 3 days)	N*	5 %	N*	N*	N*	N*
Amount of consumed meat	N*	N*	N*	N*	N*	N*
Amount of consumed cow's milk	N*	N*	N*	N*	N*	N*
Amount of consumed cereals	N*	N*	N*	N*	N*	N*
Amount of consumed products of animal origin per day	N*	N*	N*	N*	N*	5 %
Did the mother use iron medicines during the pregnancy?	N*	N*	N*	N*	N*	N*
Did the mother use iron medicines after giving birth?	N*	N*	N*	N*	N*	N*
Did the mother use iron medicines during/ after the pregnancy?	N*	N*	N*	N*	N*	N*
Mother's age	N*	N*	N*	N*	N*	N*
Mother's education	N*	N*	N*	N*	N*	N*
Child birth order	N*	N*	N*	5 %	N*	N*

1. Breakdown into groups: < 4 mg/d; 4–7.99 mg/d; 8–9.99 mg/d; > 10 mg/d

2. Breakdown into groups: < 8 mg/d; at least 8 mg/d

* There is no statistically significant association.

Table 2.6

Association of the level of iron ($\mu\text{mol/l}$) in the blood sample with breastfeeding at the time of the survey

Parameter	Median, $\mu\text{mol/l}$	IQR, $\mu\text{mol/l}$	N
Is not breastfed	10.7	5.7	30
Is breastfed	8.5	3.1	26

A similar correlation with exclusive breastfeeding was also observed for serum ferritin, where the level of serum ferritin was high in the infants who were not exclusively breastfed or were breastfed for a short period of time ($p = 0.0042$). A lower level of serum ferritin was observed with high statistical significance in infants who were breastfed at the time of the survey ($p = 0.0010$). And the opposite, the level of serum ferritin was higher in infants who received an artificial milk formula ($p = 0.0157$) (Table 2.7).

Table 2.7

Association of serum ferritin (ng/mL) with exclusive breastfeeding, breastfeeding and use of artificial formulae

Parameter		Median ng/mL	IQR ng/mL	N
Was the child exclusively breastfed at least until the age of 4 months?	Was not excl. breastfed for at least 4 months	29.5	24.1	24
	Was excl. breastfed for at least 4 months	17.8	14.3	31
Was the child breastfed during the survey?	Is not breastfed	30.5	22.9	30
	Is breastfed	16.1	10.7	27
Is the child fed with an artificial milk formula during the survey?	Is not fed with a formula	17.8	17.3	27
	Is fed with a formula	26.2	25.9	33

An association of exclusive breastfeeding with MCV was observed, where the infants who were not exclusively breastfed for at least 4 months, had lower MCV compared to infants who were not exclusively breastfed for at least 4 months, whose MCV was 76 fl (n = 31, IQR 7.2 fl) and 81 fl (n = 22, IQR 4 fl) (p = 0.0116), respectively.

Intake of cow's milk and iron metabolism

The introduction of cow's milk in the diet statistically significantly correlated with the level of iron in the blood (p = 0.0171). Iron in the blood sample was within normal limits in almost all (n = 30, 94 %) the infants whose diet did not include cow's milk and was lowered in 30 % (n = 8) of the infants in whose diet cow's milk had already been introduced (Table 2.8).

Table 2.8

Association of the level of iron in blood with the introduction of cow's milk in the diet

Consumption of cow's milk, n (%)	Lowered	Normal	Total
Not introduced	2 (6 %)	30 (94 %)	32
Introduced	8 (30 %)	19 (70 %)	27

When analysing the level of iron as a continuous variable, it was observed that the level of iron in the blood was lower in infants whose diet included cow's milk, the median level of iron in blood was 8.3 µmol/l (IQR 5.5 µmol/l, n = 27) in infants whose diet included cow's milk, and 10.3 µmol/l (IQR 4.8 µmol/l, n = 32) in those infants whose diet did not include cow's milk (p = 0.0234).

Intake of legumes and iron metabolism

A statistically significant association has been observed between the level of serum ferritin in blood and intake of legumes. The level of serum ferritin in the infants whose diet did not include legumes was within normal limits – 93 %, of which in those whose diet included legumes – 72 % (p = 0.0425) (Table 2.9).

Table 2.9

Association of serum ferritin with frequency of intake of legumes

Frequency of intake of legumes (from three days)	Lowered	Normal	Total
Not consumed	2 (7 %)	26 (93 %)	28
Consumed at least one day	8 (28 %)	21 (72 %)	29

Intake of products of animal origin and iron metabolism

Correlation of serum ferritin with the origin of iron showed that when iron was consumed with products of animal origin, its level in the blood was lower ($p = 0.0405$) (Table 2.10).

Table 2.10

Association of serum ferritin with intake of products of animal origin

What share in the total amount of consumed iron was taken with products of animal origin?	Lowered	Normal	Total
Less than 10 %	0 (0 %)	20 (100 %)	20
10–19 %	5 (22 %)	18 (78 %)	23
20 % or more	5 (29 %)	12 (71 %)	17

Contradictory results were observed in the association of the level of haemoglobin in blood with the intake of iron with products of animal origin. The less iron the infant consumed with products of animal origin, the higher the level of haemoglobin observed in the blood ($p = 0.0188$) (Table 2.11).

Table 2.11

Association of haemoglobin in blood sample with intake of iron with products of animal origin

What share in the total amount of consumed iron was taken with products of animal origin?	Median g/L	IQ R g/L	N
Less than 10%	124	10	21
10-19%	117	10	25
20% or more	118.5	12	18

Contradictory data have also been obtained in the association with the iron binding capacity. The highest level of iron binding capacity was observed, when infants received 150–250 g of products of animal origin per day, while it was lower when less than 150 g of products of animal origin per day, or more than 250 g per day, were consumed ($p = 0.0436$) (Table 2.12).

Table 2.12

Association of iron binding capacity in blood sample with the amount of consumed products of animal origin per day

Amount of products of animal origin consumed per day, g/day	Median $\mu\text{mol/L}$	IQR $\mu\text{mol/L}$	N
< 150	74	12	14
150–250	80	10	13
>250	70	7	21

Association of other factors with iron metabolism

Observations have confirmed that the use of iron medicines during pregnancy has an association with MCV. The strongest association ($p = 0.0061$) was observed, if iron medicines were used during pregnancy or after giving birth, where MCV was within normal limits in 84 % of the infants whose mothers used iron medicines, while MCV was lowered in 63 % of infants whose mothers did not use iron medicines ($p = 0.0061$) (Table 2.13).

Table 2.13

Association of the level of MCV in blood of infants with the use of iron medicines by the mother

Did the mother use iron medicines during the pregnancy or after giving birth?	Lowered	Normal	Increased	Total
Not used	5 (63 %)	3 (38 %)	0 (0 %)	8
Used	7 (14 %)	43 (84 %)	1 (2 %)	51

It was learnt that soluble transferrin receptors were within normal limits in 62 % of the infants who were first-born children, and only in 32 % in the infants who were not first-born children ($p = 0.0275$) (Table 2.14).

Table 2.14

Association of soluble transferrin receptors with the order of birth of the child

Which child according to the order of birth	Normal	Increased	Total
First child	18 (62 %)	11 (38 %)	29
Not first child	8 (32 %)	17 (68 %)	25

An association has been found between the order of birth of the child and soluble transferrin receptors and iron binding capacity. First born children had a lower level of soluble transferrin receptors and higher iron binding capacity in their blood sample ($p = 0.0179$) (Table 2.15).

Table 2.15

Association of soluble transferrin receptors in blood sample with the order of birth of the child

	Median mg/L	IQR mg/L	N
First child	1.7	0.5	29
Not first child	1.8	0.5	25

3. Discussion

The aim of this research was to study the association of eating habits of infants with breastfeeding, complementary food, selection of foods, as well as the association of eating habits with iron metabolism in infants in Latvia in the first year of life.

It is worth mentioning that it is hard to compare breastfeeding and eating habits, and this is particularly true for different studies, as definitions, observation and data collection methods are different. It is also difficult to determine iron deficiency and iron deficiency anaemia in infants, because of no uniform guidelines.

Eating habits in infants

Healthy eating habits of infants should start with breastfeeding. According to the recommendations of the World Health Organisation and the Latvian Ministry of Health, exclusive breastfeeding should continue in the first 6 months, then complementary food should be introduced combining it with breastfeeding, and breastfeeding should continue up to the age of 2 years old (WHO, 2009; Ministry of Health, 2003). The results of this study show that 89 % of mothers start breastfeeding infants living in Latvia in the first month of their life. If we compare the data of this study on the duration of breastfeeding in the first half a year of life with studies from Nordic countries, then this indicator is one of the highest in Latvian infants: Finland (58 %), Sweden (63 %), Latvia (68 %), Iceland (74 %) and Norway (80 %) (Hornell et al. 2013). The breastfeeding indicator until the age of 6 months old is the highest in Latvia among the Baltic states; it is 31 % in Lithuania and 40 % in Estonia (Save the Children 2012). The duration of breastfeeding has improved, if we compare these results with the latest published data. The initiation of breastfeeding of new-borns has reduced from 94 % to 89 %, but breastfeeding until the age of 6 months old, in accordance

with the data provided by surveyed persons, has increased from 58 % to 63 %, but until the age of 12 months old it has increased from 39 % to 45 % (Oginska, 2008). The results of the second study (B) evidence that almost half of the infants involved in the study (44 %) continue breastfeeding at the age of 9 to 12 months old, which also matches the results of part A of the study. Artificial formula was received by slightly more than half of infants – 53 %.

Study B distinguishes and studies exclusive breastfeeding. As to the recommended duration of exclusive breastfeeding in the first months of life, contradictions are observed in the results of several studies. Until 2001, the World Health Organisation recommended to continue exclusive breastfeeding in the first 4 to 6 months, respectively introducing complementary food (Fewtrell et al., 2007). A systematic review of literature leads one to the conclusion that this opinion has changed, and since 2001, as has already been mentioned before, it has been recommended to continue exclusive breastfeeding in the first 6 months of life (Kramer and Kakuma, 2002; World Health Organisation and UNICEF, 2003).

In 2004 and 2012, the authors Kramers M. S. and Kakuma R. performed systematic reviews of literature to learn the optimal duration of exclusive breastfeeding. In all the reviews, the authors come to the same conclusions that exclusive breastfeeding in emerging and developed countries should continue in the first 6 months and that this duration of exclusive breastfeeding does not cause a shortage of any substances in the infant's body. Although, in particular with regard to iron, the authors recognise that the results of reviewed studies are contradictory, and one of the conclusions is that the level of iron in infants in emerging countries is lowered (Kramer, 2002; Kramer and Kakuma, 2004). The order "Healthy nutrition recommendations for the feeding of infants" issued by the Latvian Ministry of Health on 25 July 2003 also recommends continuation of breastfeeding in the first 6 months of life (Ministry of Health, 2003). The

results of study B evidence that 21 % of the respondents follow the recommendation for exclusive breastfeeding – its continuation in the first 6 months of life, but 29 % of the respondents follow it in the first 4–5.9 months; less than half (40 % (n = 29)) of the respondents do not breastfeed at all or breastfeed for less than 4 months.

There are different opinions about the age of initiation of complementary feeding, and therefore recommendations also vary. The Latvian national nutrition recommendations have been developed in accordance with WHO guidelines, where the initiation of complementary feeding is recommended from the age of 6 months old, starting with a small amount of food and increasing it as the child becomes older, while at the same time continuing breastfeeding (WHO, 2009; Dewey, 2013; Ministry of Health 2003). To be noted, the European Food Safety Authority indicates that complementary food should be initiated in infants not earlier than at the age of 4 months old and no later than at the age of 6 months old (EFSA 2013).

EFSA recommendations match the latest recommendations of Finland. All infants from the age of 6 months old require complementary food. Infants not getting breast milk, should start complementary feeding at the age of 4 to 6 months old. The German recommendations on infant nutrition specify that starting from the age of 4–5 months old most infants are capable of taking complementary food (Prell and Koletzko, 2016). Similar results with regard to the age of introduction of complementary food can be found in the Italian study, where complementary food for infants is initiated at the age of 4 to 6 months old, 34.2 % and 85.5 %, respectively (Giovannini et al., 2004).

The results of both studies (A and B) evidence that complementary food for infants was mainly initiated at the age of 4 to 6 months old, in 85 % and 93 % of cases, respectively. Early initiation (before the age of 4 months) was only

observed in some infants: 9 % in study A and 1 % in study B, but later, after the age of 7 months old, in 6 % of cases in both studies.

Contrary to accurate indications in the guidelines on recommended daily doses for infants in the first year of life, there are no definite or harmonised guidelines regarding the time of introduction of different foods in the diet, frequency and amount of their consumption.

The guidelines express a single opinion in relation to the introduction of vegetables in the diet stating that vegetables should be one of the first foods to be introduced as complementary food. The study data evidence that vegetables and potatoes are a very popular choice of food, and they are introduced into food after reaching the age of 6 months old; they are taken by 86 % and 85 % of infants, respectively; before the age of 6 months old, 10 % and 8 %, respectively. Similar to vegetables, fruit and berries are eaten by 81 % of infants older than 6 months of age on a regular basis. The recommendations in Latvia provide that fruit and berries may be introduced at the same time as vegetables (Ministry of Health, 2003). Such recommendations are supported by the Finnish nutrition guidelines (National Institute for Health and Welfare in Finland, 2016).

WHO, Finnish and Latvian nutrition guidelines provide that porridge may be chosen as one of the first components of complementary food (WHO, 2009; National Institute for Health and Welfare in Finland, 2016; Ministry of Health, 2003). The EFSA and German nutrition guidelines recommend to start porridge after the first complementary food has been introduced in the infant's diet (EFSA, 2013; Prell and Koletzko, 2016). This study showed that cereal products are consumed mainly after 6 months of age; they are consumed by 88 % of infants. This study shows that cereal products are used less than once a day in the amount of 20 g per meal. This could be too little.

Cow's milk is not recommended for use in children before they reach the age of one year old (Department of Education and Early Childhood

Development, 2014; Ministry of Health, New Zealand, 2008; Crawley and Westland, 2015). However, the European Society for Paediatric Gastroenterology Hepatology and Nutrition indicates that small amounts of cow's milk and milk alternatives may be introduced after iron-fortified products have been included in the menu. Early introduction of cow's milk and its use in amounts exceeding 500 ml is related to the development of iron deficiency in the body (Agostoni et al., 2008). Study data evidence that 66 % of infants consume cow's milk in Latvia before they reach the age of one year old; however, it is used less than once a day, including 45 ml of milk in one meal. The results of study A also confirm the amount of cow's milk being used, and it is used in the amount of 45 ml per day in the age group from 9 to 12 months old. Fermented dairy products are mainly introduced after 6 months of age (78 % of infants). In accordance with Latvian nutrition recommendations, dairy products should only be introduced into the infants' diet after they reach the age of one year old (Ministry of Health, 2003). Finnish recommendations state that dairy products may be introduced starting from the age of 10 months old (National Institute for Health and Welfare in Finland, 2016). Other recommendations do not include clear instructions regarding the use of dairy products.

Meat is an important source of haem iron, where iron is inherently of high bioavailability compared to sources of non-haem iron, which can be found in meat alternatives (for example, legumes), as well as iron-fortified cereals (Critch, 2014). The data of this research on the introduction and use of meat in infants provide good results. Meat is introduced into the diet of children in 73 % of cases, when they reach the age of 6 months old, and later its consumption per meal increases considerably when calculated per meal, reaching its intake almost twice a day (median = 1.7). In 2003, the WHO and the Pan American Health Organisation published uniform, scientifically justified guidelines on the initiation of complementary feeding for breastfed infants. The guidelines

recommend that “meat, poultry, fish or eggs should be eaten daily or as often as possible” to satisfy the child’s need for nutrients (WHO and Pan American Health Organisation, 2003). EFSA and Finnish recommendations provide that vegetable, potato and meat puree may be offered as the first complementary food (replacing meat with oily fish once or twice a week) to provide them with biologically high value iron and zinc (EFSA, 2013; National Institute for Health and Welfare in Finland, 2016). The German guidelines indicate that the use of meat, liver and fish at an early age is associated with good further growth and cognitive development (Prell and Koletzko, 2016). The Latvian recommendations provide that meat should be introduced at the age of 8 months old (Ministry of Health, 2003). Despite the fact that intake of fish is recommended by replacing fish with meat once or twice a week, study data evidence that fish is only taken by 36 % of children after they reach the age of 6 months old, and this is done once a week.

In accordance with Latvian recommendations, eggs should only be introduced after they reach the age of one year old. These study results show that eggs are taken after infants reach the age of 6 months old, and 45 % of children eat 1 egg per week. Contrary to the Latvian recommendations, WHO and Finnish nutrition guidelines permit the intake of eggs before infants reach the age of one year old (WHO, 2009; Ministry of Health, 2003; National Institute for Health and Welfare in Finland, 2016).

The reviewed guidelines do not describe the intake of legumes, unlike Latvian nutrition guidelines which provide that legumes may be introduced at the age of 8 months old (Ministry of Health, 2003). This study has shown that only 28 % of infants who are 6 months old or older consume legumes, and they do this rarely and in small amounts. Other similar nutrition studies often do not distinguish legumes and study them together with vegetables or cereals.

As to sources of nutrients for 9 to 12-month-old infants, the results of study A and B are very similar despite the fact that the data of study A have been analysed from food frequency questionnaires, but nutrient data in study B have been analysed from food diaries (24-hour dietary recall). It is not surprising that breast milk or an artificial formula are the main source of energy at this infant age, followed by plant products together with cereals as the next largest source of energy. Products of animal origin, except milk, provide 10% and 9% of energy in study A and B, respectively. Milk and dairy products are also a considerable source of energy in study A and B – 14 % and 12 %, respectively. The largest sources of iron nutrients are breast milk and artificial formulae; however, if we consider breast milk and an artificial milk formula separately, breast milk as a source of iron amounts to 5 % in study A and B, but artificial formulae in study B is a larger source of iron, 23 % to 32 %, respectively. The iron from plants (including cereals) taken with food dominated over iron from products of animal origin in both studies (including milk and dairy products), 37 % to 17 % in study A and 40 % to 13 % in study B, respectively. Similarities between sources of nutrients in both studies suggest an interpretation that studies of part A and B include very similar data on sources of nutrients, and all the other results might be applicable to a larger population group, like in study A, referencing the results of the study of the association of iron metabolism disorders with eating habits to a larger Latvian population group, which was used in study A.

Prevalence of iron deficiency and iron deficiency anaemia

Prevalence of iron deficiency and iron deficiency anaemia in infants in emerging countries and in developed countries is still recognised as a topical problem. In 2015, the authors of the systematic review of literature confirmed that iron deficiency is a common problem in European countries. Iron deficiency was found in 2 % to 25 % of infants aged 6–12 months old. In older children

(from the age of 12 months to the age of 36 months old) iron deficiency was stated in 3 % to 48 % of cases.

The results might be several percent worse if a uniform methodology is used. In order to determine iron deficiency, some studies permitted a lower level of serum ferritin ($< 10 \mu\text{g/l}$ rather than $< 12 \mu\text{g/l}$). The authors mention the association of iron deficiency with several eating habits such as: intake of cow's milk as the main drink in the first year of life, intake of iron-fortified foods as complementary food, intake of red meat, intake of fruit and vegetables. Apart from nutrition factors, the authors also mentioned the socioeconomic factor and ethnic origin. Similar to the prevalence of iron deficiency, the incidence of iron deficiency anaemia also varies considerably among countries. Iron deficiency anaemia is mainly below 5 % in Nordic countries and Western Europe, while it reaches up to 50 % in some countries and populations in Eastern Europe (Eussen et al., 2015).

Unfortunately, other sources on the prevalence of iron deficiency and anaemia in Latvian infants is not available. Data within this study evidence that 9.6 % of infants have iron deficiency and 4.1 % have iron deficiency anaemia. Since we failed to include the planned 100 infants in the study due to low responsiveness, this affected the accuracy of the calculation of prevalence. However, the 95 % credibility interval shows that iron deficiency might affect 4.7 % to 18.5 % of infants in Latvia, while iron deficiency anaemia – 1.4 % to 4.4 % of infants.

Although eating habits are not the only factors that might affect the risk of iron deficiency and anaemia, the conclusion of several studies evidence that they are associated with the increase in the risk of iron deficiency and anaemia; therefore, eating habits may also be used in the prevention of ID/IDA.

It is very important to study eating habits of infants, because such data may provide information on a potential deficit of nutrients. As confirmed by the results of this study, infants living in Latvia do not take sufficient amounts of iron with food (median 6.5 mg per day). The recommended daily dose of iron (in accordance with the order of 24 November 2017 reviewed by the Ministry of Health) in the period from 7 to 12 months of age is 8 mg. Most European countries, including France, Germany, Italy, Spain, the Netherlands and England, recommend an intake of 7 or 8 mg of iron with food per day for infants and young children aged 6–36 months old (Eussen et al., 2015). The authors of the same systematic review published summarised data on the amount of iron consumed with food in these countries. Similar to Latvia, up to 50 % of infants also do not receive the recommended dose of iron in most countries (Figure 3.2).

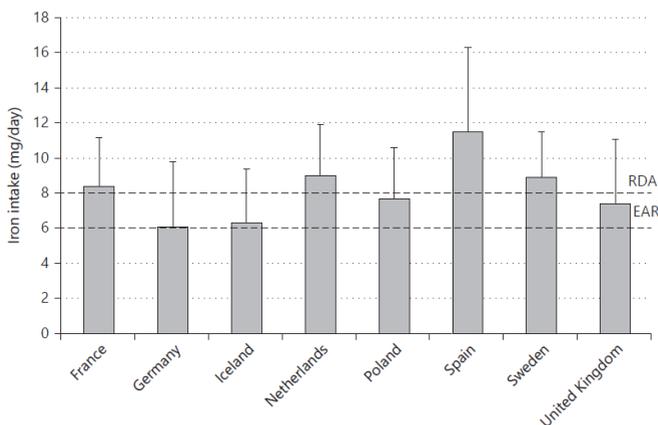


Figure 3.2. Average iron intake with food in 6–12-month-old infants in different European countries compared to the Estimated Average Requirement (EAR) and the Recommended Dietary Allowance (RDA)

(Eussen et al., 2015).

(The error bar shows the standard deviation of included studies.)

Data of another prospective longitudinal cohort study surveying 11 European countries evidence that 2.3 % of 12-month-old European infants have iron deficiency anaemia. Cow's milk was mentioned as the most important factor affecting eating habits; its exclusion from food in the first year of life may serve as a preventive measure for the development of iron deficiency (Male et al., 2001).

The results of the British prospective cohort study have shown that the intake of iron with food in the group of 8-month-old infants is sufficient, except for those infants who did not eat meat; their median value was 6.3 mg of iron per day (SD 3.8) (Taylor et al., 2004).

It is an interesting fact that the results of several studies show that the total amount of iron in the diet does not differ in the children who consume more or less meat (Taylor et al., 2004).

Exclusive breastfeeding in association with iron metabolism

One of the most important factors in eating habits, which might be closely related to the lack of iron in the body, is exclusive breastfeeding. Despite convincing conclusions of systematic overviews of literature on the continuation of exclusive breastfeeding in the first 6 months of life, which does not cause any risk to growth, the results of this study evidence that exclusive breastfeeding may have a negative effect on iron metabolism.

Statistically significant results have confirmed that infants, who were exclusively breastfed, had a lower level of serum ferritin and MCV in the blood, which might evidence early iron metabolism disorders. In 2014, Rosa F. S. V. et al. conducted a cohort study for the purposes of determining the prevalence of iron deficiency and iron deficiency anaemia among exclusively breastfed infants aged one to six months old and determining related risk factors. Haemoglobin and serum ferritin were used as iron metabolism indicators. Overall, 102 healthy

infants born on time participated in the study. The results have shown that the number of infants at the age of 6 months old with iron deficiency has grown more than four times, reaching 26.1 %, where iron deficiency anaemia was found in 23.9 % of infants. At the age of six months old iron deficiency significantly correlated with growth rates. The authors have concluded that exclusive breastfeeding protects infants from iron deficiency and iron deficiency anaemia in the first 4 months of life. Study results on infants after the age of 4 months old lead one to conclude that the risk of anaemia and iron deficiency in infants increases. The authors recommend control of iron metabolism indicators in exclusively breastfed infants with excessive weight after the age of 4 months old (Rosa et al., 2014). Furthermore, Baker and Greer believe that starting from the age of 4 months old, infants should receive iron medicines of additional 1 mg per 1 body kg per day, because exclusively breastfed infants face an increased risk of iron deficiency after they reach the age of full 4 months old (Baker and Greer, 2010).

American researchers describe results regarding exclusive breastfeeding that are similar to those of this Doctoral Thesis. The purpose of their study was to learn whether breastfeeding in the first 6 months of life increases the risk of iron deficiency in infants compared to breastfeeding during a 4-months period. This cross-sectional study was rather extensive and included 2268 children aged 6–24 months old, creating a nationally representative sample. The results have shown that the prevalence of anaemia among infants that are exclusively breastfed was only 10 % compared to 2.3 %, which was the percentage in the group where infants received only breast milk for 4 to 5 months ($p = 0.007$). When the data were adapted to birth weight and demography, it was stated that infants who were breastfed in the first 4–5 months had a lower risk of anaemia, lowered serum ferritin and lowered haemoglobin in the history compared to the infants who were breastfed for 6 months. The authors have concluded that the

American children, who are exclusively breastfed in the first 6 months, may have an increased risk of iron deficiency (Chantry et al., 2007).

The results of another prospective study conducted in Mexico also evidence an increased risk of iron deficiency in the infants who were exclusively breastfed in the first 6 months of life. The aim of the study was to learn the association between eating habits, iron metabolism and probability of gastrointestinal infections in the first 6 months of life. Only healthy women and healthy infants born on time participated in the study. The study results have shown that breastfeeding in the first 6 months of life has a positive impact on gastrointestinal infections – reducing their risk, but increasing the risk of iron deficiency. However, the prevalence of anaemia in comparable groups does not differ (Monterrosa et al., 2008).

The correlation between the duration of exclusive breastfeeding and iron deficiency is also described in a study which was conducted in Bolivia. The study included infants who were born on time and had a normal amount of iron in the body. The results have shown that infants who were breastfed for 4 months or longer had a strong correlation with iron deficiency, but not anaemia, compared to the group of infants who were exclusively breastfed for less than 4 months. The level of iron and haemoglobin considerably reduced along with the growing duration of exclusive breastfeeding (Burke et al., 2018).

Exclusive breastfeeding in the first 6 months of life unambiguously has more favourable effects on a woman's and child's health. As several authors believe, exclusive breastfeeding in the first 6 months of life reduces the risk of gastrointestinal infections, which is critical for emerging countries or low income population groups; however, such eating practices may have an unfavourable effect on iron metabolism and increase the risk of iron deficiency (Kramer, 2002; Kramer and Kakuma, 2004).

Although based on the results of this study it cannot be argued that breastfeeding may increase the risk of iron deficiency, statistically insignificant (at a level of 10 %) associations between breastfeeding and MCV and haemoglobin were found. The infants who received breast milk had a lower level of MCV and haemoglobin in the blood. Furthermore, a statistically significant association with iron and serum ferritin has been found – the level of iron and serum ferritin was higher in infants who did not receive breast milk. And, on the contrary, infants who received artificial milk formulae had the highest level of serum ferritin in the blood. Furthermore, a study conducted in China showed that breastfeeding had a statistically significant association with iron metabolism ($p < 0.001$). The study was conducted in several Chinese cities. For example, in one of the cities (Zhejiang) researchers found iron deficiency anaemia in 27.5 % of 9-month-old infants compared to 0 % of artificially fed infants. The authors have concluded that the risk of iron deficiency and iron deficiency anaemia at the age of 9 months old is higher in infants who receive breast milk or mixed feeding (breast milk and artificial milk formula). Although favourable effects of breastfeeding cannot be doubted, the authors emphasise that it may cause the risk of iron deficiency and anaemia in the early infant period; therefore, guidelines for the determination and prevention of iron deficiency and anaemia are necessary (Clark et al., 2017). Similar results are described by Korean authors where the prevalence of iron deficiency and iron deficiency anaemia correlated with the type of eating: in the infants who were exclusively breastfed in the first 4–6 months of life and whose breastfeeding continued in later months, iron deficiency was observed in 52.9 % and anaemia in 28.3 % of cases; in those infants who received both breast milk and artificial milk formula, iron deficiency was observed in 10.1 % and anaemia – in 4.2 % of cases, while in infants who received artificial milk formulae, iron deficiency and anaemia were observed in 3 % and 0 % of cases, respectively ($p < 0.001$) (Hong et al., 2017).

Cow's milk consumption habits in association with iron metabolism

One of the ideas of this study proposed for defence was: early introduction of cow's milk and its consumption have a negative effect on iron metabolism in infants.

The study has revealed a statistically significant association between the consumption of cow's milk and reduced level of iron in the blood, as well as the association with soluble transferrin receptors, which were increased in those infants for whom the intake of cow's milk had already been initiated. These results evidence a potential risk of iron deficiency in these infants. Similar to a Canadian study, where the main reason was to find an association between the consumption of meat and iron metabolism, it was revealed that consumption considerably increases the risk of iron deficiency (Cox et al., 2016).

Another study has revealed correlations between the intake of cow's milk as the main drink and increased risk of anaemia at the age of 12 months old, as well as the second correlation with a lowered level of ferritin in the blood in 8 and 12-month-old infants. This study has also not revealed an association with haemoglobin concentration. The conclusions of the study have certified that feeding of infants with cow's milk or artificial milk formula above 600 ml or breastfeeding more than 6 times a day, is associated with a smaller amount of complementary food in the diet. However, an increased risk of anaemia was stated in those infants who received breast or cow's milk. The infants who take artificial milk formula up to 600 ml or are breastfed no more than 6 times a day receive a sufficient amount of iron with complementary food. An important conclusion is that cow's milk should be strictly prohibited as the main drink until the age of 12 months old (Hopkins et al., 2007).

Risk factors in infants with severe iron deficiency anaemia, as well as potential preventive measures in the environment of developed countries were studied in Canada. Compared to the group of healthy infants (sufficient iron in

blood, average Hb 122.4 g/l), infants with severe iron deficiency anaemia took more cow's milk per day (median 1065 ml, compared to 500 ml, $p < 0.001$), as well as these infants used to drink cow's milk from a bottle during the day (78 % compared to 43 %) and in the evening before bedtime (60 % compared to 21 %). The researchers concluded that severe iron deficiency anaemia is related to a negative impact on health, and it could be prevented. The conclusions mentioned 3 eating habits which can be changed, and which are related to iron deficiency anaemia: intake of cow's milk in amounts of more than 500 ml per day; drinking of cow's milk from a bottle in infants younger than 12 months of age; use of a bottle before bedtime. General practitioners should specifically point out these recommendations in communication with young mothers, as well as these recommendations should be taken into account in the development of public health related diet recommendations (Parkin et al., 2016).

There is another interesting study, the purpose of which was to find an association between the intake of cow's milk and Vitamin D and iron reserves in healthy pre-school children (aged 2 to 5). As a result, such an association has been found among 1311 children – when the intake of cow's milk increased, the level of serum ferritin in blood is reduced ($p < 0.0001$). This allows the researchers to conclude that 2 cups or 500 ml of cow's milk per day is a sufficient amount to take the necessary daily dose of vitamin D and to keep adequate iron reserves in the body of most children (Maguire et al., 2013).

All the more so, the systematic review of literature and meta-analysis confirm the negative impact of cow's milk on iron metabolism in infants and young children. The purpose of this systematic review was to summarise the best evidence available related to the intake of cow's milk in the short term and the long term and the impact of these processes on the health of healthy infants born on time and children up to the age of 3 years old. The analysis included 23 studies (one randomised controlled study, 2 non-randomised controlled studies, 8 case

control studies and 10 cohort studies) for a complete evidence base. Overall, the results of four studies revealed that infants consuming cow's milk have a higher risk of iron deficiency anaemia compared to the group of infants who received an artificial milk formula (relative risk = 3.76). Authors of this systematic review of literature have concluded that the intake of cow's milk by infants is related to the increased risk of iron deficiency. The restriction of intake of cow's milk may be an important preventive measure to ensure sufficient intake of iron by infants and young children. Authors of this publication emphasise the need for high quality informative materials, where parents would be explained how infants can take sufficient amount of iron with food (Griebler et al., 2016).

Meat eating habits in association with iron metabolism

The study results showed that the introduction of meat correlated with the level of haemoglobin in blood. The level of haemoglobin was within normal limits in almost all infants (95 %) for whom the intake of meat was introduced at the age of 7 months old or later. It is an interesting fact that infants for whom intake of meat was introduced at an early stage, at the age of 4–6 months old, the level of haemoglobin was reduced in 20 % of cases and within normal limits – in 80 % of infants, respectively. Another intervention study revealed an association between the use of meat and haemoglobin level. The results of the study suggest that depending on the amount of meat in the diet, the HB level in the blood changed considerably ($p = 0.008$). The infants who consumed less meat had a lower Hb concentration in the blood compared to the group of infants who receive more meat in the diet. However, no considerable difference was observed in SF and Tfr indicators, similar to this study (Engelmann et al., 1998).

Another cross-sectional study, which searched for correlations between the intake of meat and meat alternatives and iron metabolism, and between the intake of red meat and iron metabolism in older children, 12 to 36 months old, has not revealed statistically significant correlations between the intake of meat

and meat alternatives and the level of serum ferritin in the blood. However, it has been proven that the intake of meat may reduce the risk of iron deficiency ($p = 0.03$) by 3 %, but if less meat is consumed, this may be a risk factor for iron deficiency. The study has not revealed a statistically significant correlation between the intake of red meat and iron metabolism indicators (Cox et al., 2016). A British study has also revealed an association between the intake of meat and better iron content in the body of older children (from 1.5 to 4.5 years old) (Thane et al., 2000).

These research results have not shown a statistically significant association between the frequency or amount of intake of meat and iron metabolism indicators in the blood. The statistically insignificant association at the level of 10 % was also contradictory. This might be explained by the fact that only slightly more than half (56 %) of consumed meat was red meat or meat rich in iron. Furthermore, another prospective cohort study studied whether more meat in the diet of children aged 4 to 24 months old would improve the metabolism of iron and other microelements in the body. The study cohort included 198 infants. A considerable association was observed in infants at the age of 12 months old between serum iron and meat intake ($p < 0.023$). A trend for an inverse association between haemoglobin concentration and the intake of meat was observed in the same age group ($p < 0.068$). The researchers have concluded that the intake of red meat has a positive effect on iron metabolism in infants at the age of 12 months old (Taylor et al., 2004).

A similar cross-sectional study was conducted in Jerusalem, including 263 healthy children aged 1.5 to 6 years old. The results of this survey showed that iron deficiency was observed 4 times more often in the group where children consumed very little meat than in children who consumed it 2 or more times per week ($p = 0.023$). However, such an association with iron deficiency was not observed in the case of the use of poultry. Researchers of this study have also

concluded that iron deficiency is related to the insufficient intake of red meat. Another important conclusion from this study is related to poultry. Developed countries consume poultry much more often than red meat, which may become a risk factor for iron deficiency and increase its prevalence (Moshe et al., 2013).

A randomised double-blind controlled study in Germany studied whether the low content of meat in complementary food, which as accepted by European Union law, may increase the risk of iron deficiency in the period of complementary feeding in infants receiving adequate nutrition. In the group of healthy infants, who were breastfed or fed with artificial milk formula and received complementary food in accordance with nutritional recommendations, average iron metabolism biomarker indicators were within normal limits before (4 months), during (7 months) and after (10 months) the involvement with different amounts of consumed meat. No significant differences in biomarkers have been found between the groups where meat was consumed in increased and low amounts as might be expected. This study has also not revealed any difference in the intake of iron with food between high and low meat consumption groups. The researchers emphasise that the most important discovery is that after the primary data analysis, no justified evidence of disturbed iron metabolism when infants receive meat as complementary food in lower amounts than recommended by the European Union has been found. However, secondary analysis of data allows one to conclude that such a small amount of meat in the diet of many infants consuming breast milk in the first 4 to 6 months of their life in accordance with recommendations may increase the risk of iron metabolism disorders in the second half of the first year of their life (Dube et al., 2010).

Intake of legumes in association with iron metabolism

Although the infants living in Latvia do not consume legumes in large amounts, their use has shown statistically significant associations with iron

metabolism. When studying the association of legumes with iron metabolism, it was observed that the intake of legumes reduced the level of serum ferritin in blood. This might be explained by the fact that the high content of phytates in legumes bind to iron and thus delay its absorption by the child's body (Dewey, 2013; Gibson et al., 2010; Lim et al., 2015).

Association of other factors with iron metabolism

Although eating habits are very important in the prevention of iron deficiency, it is important to study not only the impact of nutrition, but also the potential association of other factors with iron metabolism in infants. This study studies the associations with the use of iron medications during pregnancy or after giving birth, the mother's age, mother's education and the order of birth of the child. Statistically significant results were only evaluated for the association – the order of birth of the child and soluble transferrin receptors, where sTfR in first-born infants was within normal limits in more infants (62 %) compared to those who were not first-born (32 %).

Based on the data of this study as well as when examining the results of other studies, it is important to consider and study the association with the mother's age, the order of birth of the child, mother's nutrition and mother's health condition (Marques et al., 2016), mother's education, socioeconomic condition of the family (Thane et al., 2000), infant's sex (Soh et al., 2004), infant's weight, including excessive weight, obesity and other factors (Cox et al., 2016; Soh et al., 2004).

Study restrictions and challenges

During the study, several restrictions and challenges had to be faced. As to the assessment of data on nutritional value, there is always a possibility that the data provided by the parent or carer about the child's diet or daily nutrition doses are exaggerated or underestimated. Parents did not receive any remuneration for participation in the study (only dosing cups and food pyramid

stickers were issued). At the same time, the contribution of parents in terms of time had to be rather extensive. They needed about 1.5–2 hours to collect all the necessary data for food frequency questionnaires (FFQ) and 24-hour dietary recall diaries. This might cause additional errors when providing data on the infant's daily nutrition. However, similar results obtained in both studies using different measuring instruments is most likely the evidence of the correctness of the methods used. The study is restricted by small samples of respondents in different age groups.

Conclusions

1. Eating habits of infants living in Latvia partially correspond to the guidelines of the Latvian Ministry of Health: diversity of food is observed, complementary feeding is initiated at the recommended age period, cow's milk is not used in amounts exceeding 600 ml per day, meat is introduced in the diet in a timely manner and in sufficient amounts. However, only 21 % follow the exclusive breastfeeding recommendation – its continuation in the first 6 months of life, 29 % breastfeed in the first 4 to 5.9 months, less than half (40 %) of all respondents did not breastfeed or breastfed for less than 4 months.
2. 63 % of infants at the age of 9–12 months old do not consume a sufficient amount of iron with food and iron deficiency was observed in this group in 9.6 % of infants, while iron deficiency anaemia in 4.1 % of infants.
3. Associations with the following eating habits at an early age and other factors that affected iron metabolism in a favourable way have been found:
 - a. a higher level of serum ferritin in the blood was observed in infants who received an artificial milk formula;
 - b. a normal level of serum ferritin in the blood was observed in infants who consumed iron with food at least in the recommended dose (8 mg).
 - c. a normal MCV level in the blood in the second half of the first year of life was observed in those whose mothers used iron medicines during pregnancy and after giving birth.
4. Associations with the following eating habits at an early age and other factors that affected iron metabolism in an unfavourable way, and could promote the development of ID and IDA, have been found:

- a. lower level of iron in the blood was observed in infants for whom cow's milk was introduced at an early stage;
- b. lower MCV level was also observed in those infants whose exclusive breastfeeding continued for at least the first 4 months of life compared to infants who were not exclusively breastfed for at least 4 months;
- c. lower serum ferritin was observed in those infants who were breastfed and whose exclusive breastfeeding continued for at least the first 4 months of life, as well as in those infants in whose diet legumes have been introduced;
- d. lower level of iron in the blood was observed in infants who were breastfed;
- e. a higher level of soluble transferrin receptors in the blood was observed in infants who were not first-born compared to those who were first-born.

Nutrition recommendations for infants and young children

1. The recommended duration of exclusive breastfeeding is the first 6 months of life. If for some reason this is not possible, temporary exclusive breastfeeding is also desirable. When planning an exclusive breastfeeding, healthcare professionals should pay attention to the diet of pregnant and breastfeeding women, her iron metabolism, to ensure early prevention of iron deficiency or the risk of its occurrence during pregnancy and the postpartum period.
2. Breastfeeding should continue until 2 years of age.
3. It is recommended to introduce the first complementary food at the age of 4 to 6 months. It is important to consider that complementary food should not be introduced earlier than week 17 and not later than week 26.
4. Food consistency should change along with the child's age – from semi-liquid when starting complementary feeding to more solid food with pieces at about 8 months, at the age of 10 months at the latest.
5. Vegetables or porridge without gluten can be chosen as the first complementary food. When preparing puree, water or breast milk should be added as the liquid.
6. When starting complementary feeding, breastfeeding should continue, only in smaller amounts. The recommended number of complementary feedings is accepted assuming that 1 g of complementary food contains 0.8 kcal or more.
7. Complementary food shall start with one meal per day, offering some teaspoons of new food, then increase the number of meals and the size of the meal: 2 to 3 meals per day are recommended at the age of 6–8 months old, 3 to 4 meals for infants and children aged 9–24 months old. Offer 1 to

2 snacks between meals, depending on the child's appetite. It is assumed that an infant can eat about 30 ml of food per 1 kg of body weight.

8. Diversity should be observed when selecting food for complementary feeding in order to provide the infant's body with all the necessary nutrients. Meat, fish or eggs should be eaten every day. A vegetarian diet cannot provide all the necessary nutrients; therefore, the possibility to complement food with food supplements should be considered. Vegetables and fruit should be eaten every day. Food should contain a sufficient amount of fats. Drinks such as tea, coffee and sweetened drinks are not recommended. Juices should be used with caution; they can be replaced with other foods, which are richer in nutrients.
9. Vegetables, fruit and berries should be eaten every day. It is very important to observe diversity to provide the body with all the necessary vitamins. It is not recommended to add salt or sugar when preparing vegetables or fruit.
10. Meat should be introduced in the diet at an early stage – at the age of 6 months old, as one of the first complementary foods. Meat should be replaced with oily fish 1 to 2 times per week. Vegetable-potato-meat purees can be offered as one of the first meals (20–30 g of meat).
11. Dairy products should only be introduced as complementary food after iron-fortified foods, vegetables and fruit have been introduced in the period between 6 and 9 months old.
12. Cow's milk can be introduced from 6 months of age for the purposes of preventing allergy, adding it into meals in small amounts, and as a separate meal after the age of 12 months old. Milk and dairy products should have a high fat content, i.e. they should be full cream milk products.
13. Cereals are recommended as one of the first meals. Recommended cereals for meals are oat, rye, wheat, spelt, rice, buckwheat, maize, millet. The first offered cereal should be without gluten, for example, rice, buckwheat,

maize, millet. Later, cereals containing gluten can be used. Gluten should be introduced at the age of 4–12 months. Wholegrain products are preferable. Excessive use of cereals is not recommended, because fibre may negatively affect the absorption of iron by the body.

14. Eggs should be introduced at an early age, at the age of 4–6 months, to protect against the risk of allergy. Eggs may replace meat or fish from time to time. Eggs should be thermally processed before eating.
15. The inclusion of legumes in the diet depends on the individual peculiarities of the infant's body. The approximate age of inclusion of legumes starts from 7 months old. They should be taken at least once a week.
16. Exclusively breastfed infants do not usually need additional water, because breast milk provides a sufficient amount of liquid. Juices, both natural and produced, are not required in the first year of the infant's life.
17. During the second half of the first year of life, the total intake of liquid, including breast milk, should be 800 ml to 1000 ml.
18. At the age of 10 to 12 months old the infant should eat almost all food groups and gradually switch to family meals.
19. The energy distribution of nutrients in infants in the first half a year of life is as follows: proteins 10–15 E%, carbohydrates 40–45 E%, fats 50–55 E%. In the second half a year: proteins 5–15 E%, carbohydrates 45–55 E%, fats 40 E%.

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I. Širina, I. Strēle, I. Sikсна, D. Gardovska, “Association between infant’s feeding habits and iron metabolism in Latvia, Rīga Stradiņš University International Conference on Medical and Health Care Sciences, 1.04.–5.04.2019, Rīga.

Oral presentations in local Latvian congresses

I. Širina, I. Strēle, I. Siksna, D. Gardovska, “Piena un piena produktu lietošanas paradumi zīdaiņiem Latvijā (Eng. Milk and milk products usage habits for infants in Latvia)”, Rīgas Stradiņa universitātes Zinātniskā konference, 18.03.2016, Rīga.

I. Širina, I. Strēle, D. Gardovska, “Papildu uztura uzsākšanas vecums zīdaiņiem un tā saistība ar zīdīšanas ilgumu (Eng. Complementary nutrition start-up age in infants, length of breastfeeding and their relationship)”, Rīgas Stradiņa universitātes Zinātniskā konference, 26.03. –27.03.2015, Rīga.

Oral presentations in international congresses

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