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ORIGINAL CONTRIBUTION

Complementary food with low (8%) or high (12%) meat content as source of dietary iron: a double-blinded randomized controlled trial

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Abstract
Background To investigate whether a low meat content of complementary food as accepted by EU law increases the risk of well-nourished infants to develop iron deficiency during the complementary feeding period.
Methods Term born, healthy infants were randomized into a 'High Meat' Group (HM, $n = 48$) receiving commercial baby jars with a meat content of 12% by weight (according to pediatric guidelines), and a 'Low Meat' Group (LM, $n = 49$) receiving meals as marketed (meat 8% by weight, the lowest level of EU law). Intervention was from 4 to 10 months of age. Dietary intake was recorded continuously, repeated blood samples were collected.
Results Estimated intake of bioavailable iron conformed to reference requirements. In the primary analysis of the total sample, iron status was adequate before (4 months), during (7 months), and after (10 months) the intervention. A secondary analysis in the subgroup of infants fully breast-fed for 4–6 months demonstrated an increased risk of low Hb values with 10 months of age in the LM group.
Interpretation Present day low meat content of complementary food does not significantly impair iron status in well-nourished infants but may increase the risk of developing marginal iron status in older infants after fully breast-feeding for 4–6 months, i.e., in the subgroup of infants with the lowest habitual iron intake.

Study registration number at http://www.clinicaltrials.gov: NCT00571948.

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Keywords Infants · Iron status · Complementary food · Meat · Hemoglobin · Breast milk

List of abbreviations

BA iron	Bioavailable iron
BM	Breast milk
DINO	Dortmund Intervention Trial for Optimization of Infant Nutrition
DRI	Dietary reference intake
Fer	Serum-ferritin
Hb	Hemoglobin
HM	High-meat
ID	Iron deficiency
IDA	Iron deficiency anemia
LM	Low-meat
MCH	Mean cell hemoglobin
MCV	Mean cell volume
RCT	Randomized controlled trial
SD	Standard deviation
TEE	Total energy expenditure
TfR	Soluble transferrin-receptor
ZPP	Zinc protoporphyrin

Introduction

Infants in the second 6 months of life are at high risk for iron deficiency (ID) and iron deficiency anemia (IDA) because of extraordinary requirements for growth. IDA and even ID alone in early life may exert long lasting negative effects on central nervous system development [1–3].

Due to prenatal iron stores and postnatal metabolism of fetal erythrocytes, healthy term infants are nearly independent from exogenous iron during the first months of life [4]. During the second 6 months of life, iron requirements rise rapidly, and dietary intakes between 8 mg/day [5] to 11 mg/day [6] are needed; however, data on the development of iron metabolism in this age group are rare. As human milk is a poor source of iron [7, 8], complementary foods which are providing highly bioavailable (BA) iron [9] should be introduced between 17 weeks of age at the earliest and 26 weeks at the latest [10].

In the 1996 European Commission Directive on complementary food, meat content was prescribed to be 10 or 8% by weight dependent on the ranking of meat in labeling [11]. At the same time, meat content of commercial complementary meals in Germany decreased from 12 to 8%, whereas pediatric recommendations remained at about 12%. So far, the scientific evidence to evaluate the significance of introduction and composition of complementary feeding is limited. Moreover, when giving recommendations for a group of infants, it is difficult to balance the risk of iron deficiency and iron overload (e.g., in single infants with hereditary haemochromatosis). These may be reasons for considerable variations found in recommendations and comments on complementary feeding given by several panels [10]. Most infants in Germany receive meat containing meals in the form of commercial baby jars.

These observations prompted us to investigate in a primary analysis (1) the potential impact of the decrease in meat content of commercial complementary meals on iron status in a group of healthy well-nourished infants, and (2) in a secondary analysis, the impact of low meat intake in the subgroup of infants with lowest dietary iron intake while fully breast-fed during the first 4–6 months of age. Both analyses were part of the Dortmund Intervention Trial for Optimization of Infant Nutrition (DINO), a double-blinded randomized controlled trial (RCT), focused on intake of iron and polyunsaturated fatty acids via complementary food during the second 6 months of life [12].

Patients and methods

Study sample

The primary outcome variable of this trial was the hemoglobin (Hb) concentration in venous blood samples after the intervention period at the age of 10 months. A sample size of at least 74 (37/37) was required to detect a 1SD difference of Hb between the study groups at the age of 10 months based on a SD of 7 (mean 117 g/L) [13] for $\alpha = 0.05$; $\beta = 0.01$. Considering study dropouts of 40% about 120 participants had to be included. A starting sample of 132 infants was recruited in delivery hospitals in Dortmund, Germany, between September 2005 and July 2006. Inclusion criteria were: a healthy term newborn infant (gestational age >37 weeks, birth weight >2,500 g); German speaking mother; the intention of the mother to breast-feed the child; to use the study-specific complementary food according to the study protocol. Furthermore, as a quality criterion for dietary recording, a limit of at least 75% out of the potential 112 record days was set.

In a secondary analysis focused on conditions of habitually low iron intake during the first 4–6 months, the subgroup of breast-fed infants (group BM >95% of energy intake from breast milk through months 3–4; at least partially breast-fed up to month 6) was examined.

Diet

At the time of parental consent within the first 2 months after birth, the participants were randomly assigned into the study groups high meat ("HM", verum) and low meat ("LM", standard). Group assignment was unknown to parents and study staff.

Study food was provided in the form of commercial vegetable–meat meals (VMM) in jars (produced alike by the companies Hipp, Pfaffenhofen, and Nestlé, Frankfurt, Germany). Jars were labeled with a numerical code blinded for the study staff and the parents. LM meals were products as marketed (meat content: 8% by weight, the lowest level allowed by EU law), HM meals had higher meat content (12% by weight, the amount recommended by the German pediatric dietary guidelines). 50% of the meals contained beef, 50% contained poultry. Energy density ($\approx$60 kcal/100 g) was kept constant by compensating energy from meat in HM meals by a lower proportion of vegetable oil (Table 1). Study jars conformed to portion sizes as marketed in the form of "baby" jars for the age of 4–7 months (190 g; mean meat content LM/HM: 15.2/20.0 g per portion) and "junior" jars for the age of 8–10 months (220 g; mean meat content LM/HM: 17.6/28.6 g per portion).

Parents were advised to start complementary feeding by using the study food between the age of 4 and 6 months (weeks 17–26), and to continue until the age of 10 months, with a frequency of 1 jar per day at least 5 times per week. Therefore, all infants received the study food regularly at least during months 7–10 and the greater meat portions (junior jars) during months 8–10. For the remaining diet, parents were counseled according to the pediatric guidelines in Germany [14, 15], but exact timing and choice of the remaining foods were left to the parents.

Table 1 Food proportions and mean nutrient content of the study food

	"Baby" menus		"Junior" menus	
	LM	HM	LM	HM
Vegetables (%)	39.0	38.7	34.6	34.6
Potatoes/pasta/rice[a](%)	51.7	50.0	55.6	49.3
Meat (%)	8.0*	10.5*	8.0*	13.6*
Oil (%)	1.3	0.8	1.8*	1.1*
Energy (kcal/100 g)	59.9	61.5	62.5	60.0
Fat (% of energy)	32	34	34	27
CHO (% of energy)	49	45	48	47
Protein (% of energy)	19	21	18	26
Iron (mg/100 g)	0.33	0.39	0.35	0.41

[a] Including water

*$P < 0.05$ (LM vs. HM, Wilcoxon two-sample test)

Diet recording

From the age of 2 months, parents kept a daily diet record of any food consumed by their child. They also collected food packages providing information about ingredients and nutrients of formula and complementary food other than the study food.

Parents were advised to follow package instructions for formula preparation and they read the estimated consumed formula amounts from the bottle. For quantifying complementary food, electronic food scales (±1 g) were used.

To enable calculation of the energy and nutrient intake also for breast-fed infants and to avoid the high burden of test weighing, we used a statistical approach to estimate breast milk quantity. For this, the individual intake of breast milk (69 kcal/100 g [16]) was based on the assumption that normally growing infants regulate their energy intake by their total energy expenditure (TEE). Data on TEE were taken from recent references. For partially breast-fed infants the difference between the calculated energy intake from formula or complementary food and estimated TEE was assumed as energy intake from breast milk. This method proved to be valid throughout the first year of life on a group level in infants fully breast-fed for at least 4 months [17].

Data on energy and nutrient content of basic food including breast milk were taken from standard food tables [16]; nutrient content of commercial food other than study food was calculated by recipe simulation based on the labeled ingredients [18].

Nutrient content of study meals was analyzed before and after the study period by the SGS Institute Fresenius GmbH, Taunusstein (Germany). Here, mean values of the two analyses were applied for dietary evaluation (Table 1).

Medical examination

Medical examinations were conducted at the age of 4, 7 and 10 months at the local Pediatric Clinic of Dortmund, including measuring body weight and length and venous blood sampling (venipuncture). Parents were interviewed about recent illnesses, febrile infections and other medical complications of the infant.

Laboratory analyses

Blood samples were analyzed for 16 parameters. Here, we report on variables with clinical or functional relevance to iron status: hemoglobin (Hb), which was the primary outcome variable, hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), serum-ferritin (Fer), soluble transferrin-receptor (TfR), zinc-protoporphyrin (ZPP) and serum-iron (Fe). Standard analytical methods were used.

Statistics

Statistical analyses were performed using SAS® (SAS Inc., Cary, USA versions 8e and 9.1 for windows®).

The amount of BA iron was estimated for breast milk: 34% [9], formula: 20% [19], meat: 15.5% [20], fruits: 10%, vegetables: 13.5% [21], cereals: 4% [22]; iron fortified infant cereals: 3% [23].

To examine differences between the study groups either the χ^2 test or the Wilcoxon two-sample test was used as appropriate. For the secondary analysis (BM group), differences of matched pairs (HM vs. LM, $n = 25$) were examined with Student paired t test.

Repeated measurement ANOVA was performed to investigate, whether changes in selected iron biomarkers (Hb, Fer, ZPP; dependent variables) over time (within-subject effects) were different between the study groups (LM vs. HM; independent variable) (between-subject effects). Subsequently, sex, duration of breast-feeding, interaction between study group and sex, measurements over time as potential confounders were incorporated into the model.

The statistical significance was set at $P < 0.05$. With regard to the biomarkers as outcome variables, only the P value related to the primary outcome (Hb) was considered as confirmatory, whereas all other P values were interpreted in a more descriptive exploratory sense, since there was no adjustment for multiple testing planned.

Ethical considerations

The study was approved by the Ethics Committee of the University of Muenster, Germany. Written informed consent of parents was obligatory for study participation.

Parents were informed about possible advantages and disadvantages of HM and LM. They were motivated for participation in the study by free consultation on optimal nutrition, free medical examinations of the infants by the pediatric clinic, and study food free of charge.

Results

LM versus HM

For final analysis 107 of the 132 enrolled participants completed the study and 97 (48 HM; 49 LM) were available (Fig. 1). Measurements were taken very close to the scheduled times [128 ± 5 days (mean ± SD) of life at 4 months; 220 ± 6 days at 7 months; 309 ± 5 days at 10 months]. Length and weight (Table 2) of the infants were close to reference values. No information on cord clamping time at birth was available.

Participants of the groups LM ($n = 49$) and HM ($n = 48$) did not differ in clinical, nutritional or socio-demographic characteristics including breast-feeding, except for a higher mean birth weight and a lower percentage of primipara in the LM group (Table 2).

Mean iron content of the study meals was significantly lower in the LM than in the HM varieties (Table 1). Mean daily consumption of the study meals as well as mean total daily energy intake did not differ between the study groups (Table 3). The HM group consumed more meat and had a higher intake of dietary iron and BA iron from the study meals. Total dietary iron intake did not differ between the groups. In total, a relatively small portion of total iron intake and BA iron derived from study food in contrast to high portions from fortification of formula (all products) and cereal-based meals (most products).

Regarding the total sample, there were no differences discernible in means (95% CI) of iron status biomarkers between infants of the LM and the HM group (Table 4). Hb

Fig. 1 Participant flow

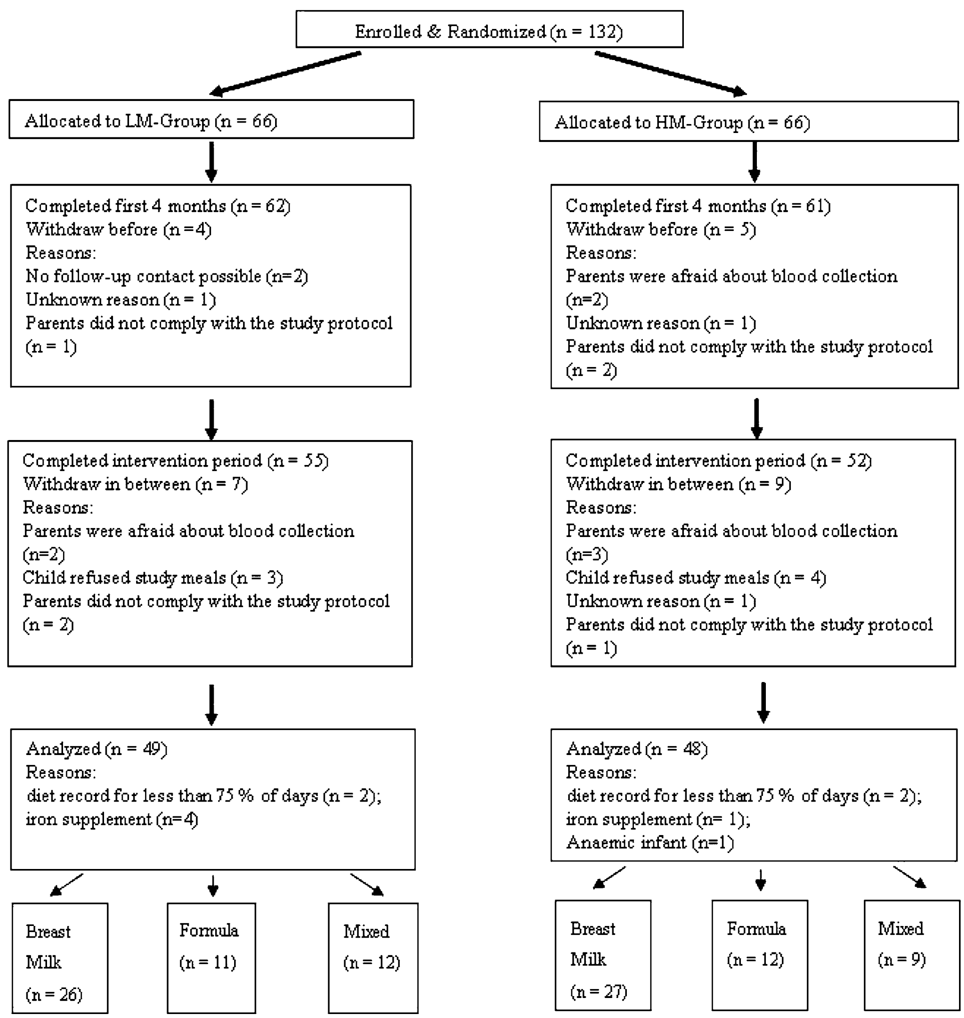

Table 2 Characteristics of the total sample ($n = 97$) and the study groups low meat (LM, $n = 49$) and high meat (HM, $n = 48$)

	Low meat ($n = 49$)	High meat ($n = 48$)
Child		
Boys/girls (n)	25/24	24/24
Gestational age (week)[a,b]	39.8 (1.2)	39.5 (1.3)
Birth weight (kg)[a,b]	3.5 (0.4)*	3.4 (0.4)*
Bodyweight at 4 months (kg)[a,b]	7.0 (0.8)	6.9 (0.7)
Bodyweight at 7 months (kg)[a,b]	8.3 (0.8)	8.2 (0.8)
Bodyweight at 10 months (kg)[a,b]	9.2 (1.0)	9.0 (0.9)
Fully breast-fed ≥17 weeks (%)[c]	53	56
Fully breast-fed ≥26 weeks (%)[c]	16	17
Partially breast-fed at 10 months (%)[c]	23	23
Start of complementary feeding (week)[a,b]	22 (3.7)	21 (3.6)
Mother		
Age (years)[a,b]	33 (4.7)	32 (4.1)
Primipara (%)[c]	49*	71*
University entrance diploma (%)[c]	71	65
Smoking during pregnancy (%)[c]	10	12
Vegetarian diet during pregnancy (%)[c]	0	2
Iron supplement during pregnancy (%)[c]	57	52

[a] Wilcoxon two-sample test

[b] Mean (SD)

[c] Chi^2

* $P < 0.05$

values tended to increase with age, while Fer, representing the iron stores, tended to decrease pointing to a preferred formation of Hb (besides iron needed for lean tissue synthesis). At the same time, values of ZPP increased, pointing to a higher need for alimentary iron. Trends for changes of Hb and Fer during the intervention period were observed on a slightly less favorable level in the LM than in the HM group. The Repeated measurement analysis supported these results.

From the 11 participants with Hb values between 10.0 and 11.0 g/dl at 4 (7 or 10, respectively), months, 5 (7 or 5, respectively), infants were found in the HM group and 6 (4 or 7, resp.) in the LM group. However, from the 6 infants with marginal Hb values below 10.5 g/dl, 4 infants were in the LM group, 2 in the HM group. All 6 of them were in the BM group.

Breast-fed subgroups

In the secondary analysis, 53 infants were allocated to the BM group (27 HM; 26 LM) (Fig. 1). Similar to the total sample, regarding basic characteristics, birth weight alone (LM 3.63/HM 3.41 kg) and percentage of primipara (LM 39%/HM 78%) were significantly different. Mean age of introducing complementary food was 23 weeks of life in both groups. Also, percentage of infants fully breast-fed at 6 months (LM 19%/HM 7%) and percentage of infants partially breast-fed at 10 months (LM 39%/HM 41%) were not significantly different (data not shown). Compared to the total sample, complementary food was introduced later by 1 week on average and proportions of long breast-fed infants (both, fully and partially) were higher in the BM subgroup.

This retrospective analysis did not reveal a sound evidence of a lower mean Hb concentration in the LM compared to the HM group, as the difference between the groups did not reach significance at the age of 10 months (Table 5). In detail, at 10 months 4 out of the 53 breast-fed infants presented with marginal Hb values <10.5, indicating development towards IDA. Those 2 out of 3 infants presenting with Hb <10.5 at 7 months and recovering until 10 months belonged to the HM group.

Table 3 Dietary characteristics of the study groups low meat (LM; $n = 49$) and high meat (HM; $n = 48$) during the intervention periods

	5–7 months		8–10 months	
	LM	HM	LM	HM
Energy (kcal/day)	667 (642; 689)	662 (638; 687)	734 (691; 776)	723 (690; 754)
Total Iron (mg/day)	3.72 (2.96; 4.48)	3.86 (3.00; 4.72)	5.74 (5.15; 6.34)	5.84 (5.25; 6.44)
Iron from breast milk (mg/day)	0.29 (0.22; 0.36)	0.29 (0.22; 0.36)	0.12 (0.07; 0.17)	0.13 (0.08; 0.18)
Iron from formula (mg/day)	2.51 (1.75; 2.71)	2.44 (1.62; 3.64)	2.29 (1.75; 2.83)	2.39 (1.79; 2.99)
Iron from CF other than study food (mg/day)	0.15 (0.11; 0.20)	0.18 (0.13; 0.22)	0.70* (0.58; 0.82)	0.91* (0.77; 1.05)
Iron from study food (mg/day)	0.25* (0.20; 0.29)	0.35* (0.29; 0.41)	0.59** (0.54; 0.63)	0.77** (0.71; 0.82)
Iron from meat (mg/day)	0.09 (0.08; 0.11)	0.15 (0.12; 0.17)	0.22 (0.20; 0.24)	0.37 (0.34; 0.40)
Total BA iron (mg/day)	0.66 (0.53; 0.79)	0.66 (0.51; 0.81)	0.70 (0.61; 0.79)	0.74 (0.64; 0.85)

BA estimated amount of bioavailable iron

* $P < 0.05$ LM versus HM (Wilcoxon two-sample test); ** $P < 0.001$ LM versus HM (Wilcoxon two-sample test)

Table 4 Means (95% CI) of clinical and functional iron biomarkers at the ages of 7 and 10 months (before and after the intervention period) in the intervention groups low meat (LM, $n = 49$) and high meat (HM, $n = 48$)

	Clinical parameters							
	Hemoglobin (g/dl)		Hematocrit (%)		MCV (fl)		MCH (pg)	
	LM	HM	LM	HM	LM	HM	LM	HM
7 months	11.6 (11.4; 11.8)	11.8 (11.5; 12.0)	35.6 (35.0; 36.1)	35.8 (35.5; 36.6)	78.7 (77.7; 79.6)	81.1 (77.3; 79.8)	25.6 (25.3; 26.0)	26.8 (25.2; 26.1)
10 months	11.9 (11.7; 12.1)	12.1 (11.9; 12.4)	36.3 (35.5; 36.8)	37.1 (36.4; 37.8)	78.4 (77.5; 79.2)	78.7 (77.6; 79.8)	25.7 (25.3, 26.1)	25.6 (25.2; 26.0)
	Functional parameters							
	Ferritin (ng/ml)		Iron (µg/dl)		ZPP (µmol/mol heme)		TfR (mg/l)	
	LM	HM	LM	HM	LM	HM	LM	HM
7 months	35.5 (27.7; 43.2)	33.3 (26.5; 40.0)	58.2 (50.7; 65.8)	56.5 (50.3; 62.8)	39.2 (33.2; 45.3)	39.9 (33.6; 46.3)	1.7 (1.6; 1.8)	1.8 (1.7; 1.9)
10 months	25.5 (21.4; 29.6)	28.8 (23.8; 33.8)	70.2 (56.8; 83.5)	54.1 (47.5; 60.6)	45.0 (39.2; 50.8)	48.7 (41.8; 55.7)	1.7 (1.6; 1.8)	1.8 (1.6; 1.9)

Table 5 Means (95% CI) of clinical and functional iron biomarkers at the ages of 7 and 10 months (before and after main intervention period) in (4 months fully, and at least 6 months partially) breastfed infants (BM, $n = 53$) of the study groups low meat (LM, $n = 26$) and high meat (HM, $n = 27$)

	Hemoglobin (g/dl)		*P*
	LM	HM	
7 months	11.3 (11.1; 11.5)	11.6 (11.3; 11.8)	ns
10 months	11.7 (11.3; 12.0)	12.0 (11.7; 12.3)	0.0563*

* Student's *t* test on differences of matched pairs ($n = 25$)

Discussion

In a group of healthy infants consuming either breast milk or iron fortified formula along with complementary feeding according to dietary guidelines, mean values for biomarkers of iron status were adequate before (4 months), during (7 months), and after (10 months) intervention with different levels of meat intake. No significant biomarker differences between the HM and the LM group were found, as might have been expected because of the small difference in dietary iron intake. However, in the subgroup of infants who were fully breast-fed for at least 4 months as generally recommended and thus had a relatively low intake of iron throughout the first half of infancy, there was weak evidence of an increased risk to develop a marginal iron status during the second half of infancy.

So far only a few studies have gathered information on the development of iron metabolism and on the impact of iron supplementation via complementary food in this life stage, which is characterized by a rapid increase of iron requirements. Besides Hb as primary outcome here, TfR and ZPP are sensitive markers for iron deficiency during infancy, TfR primarily indicating cellular and tissue needs for iron [24, 25]. Both parameters increase in case of elevated iron needs and they may help to discriminate between various diagnoses underlying iron deficiency [26, 27]. The observed changes of parameters of iron status by age at an iron intake just covering the BA requirements found in this trial might add to the scarce information on iron requirements and relationship between specific complementary foods and iron status [10].

Strength of this study is the detailed dietary data throughout the complementary feeding period, with the possibility to estimate the amount of BA iron. Hereby, it was possible to distinguish the small impact of the study food on total iron intake in contrast to the common iron fortification of formula and cereal-based meals. The diet of the study sample may reflect the dietary habits in western populations with similar breast-feeding rates.

The small difference in meat content between HM and LM study foods is a limitation of the study, as the resulting small difference in dietary iron intake reduced the probability to find significant differences of iron biomarkers in the primary analysis. Regarding the secondary analysis, this small meat difference together with the small sample size of the subgroup of breast-fed infants made it difficult to show definitive results; moreover, this analysis has not been the primary outcome variable. Maybe some trends that were already indicated in the total sample would have reached significance with a larger sample of infants fully breast-fed for 4–6 months such as calculated basically for the intervention.

Nevertheless, the trends found in our secondary analysis are consistent with a recent reevaluation [28] of current recommendations [29] for the optimal duration of exclusive breast-feeding. The authors concluded that there is a persuasive scientific evidence for exclusive breast-feeding for 6 months rather than 4–6 months for infants in developing countries, but is weaker for infants in developed countries.

Our findings show that among healthy well-nourished infants in a developed country some infants fully breast-fed for 4–6 months are not immune from a marginal iron status (in the lowest range of reference ranges describing sufficiency) during the second half of infancy. Mean start of complementary food in the group of infants with Hb <10.5 g/dl at 7 or 10 months was 24 weeks of life (data not shown), thus 2 weeks later than in the total sample and 1 week later than in the total BM group.

According to European and US pediatric recommendations, complementary food should be introduced between 4 and 6 months of age, providing readily BA iron [10, 30]. Meat could be an option; however, there is no consensus on the optimal meat content of complementary food (in terms of a general public health advice). EU law allows a meat content as low as 8% by weight, whereas the German pediatric guidelines recommend 12%. Our study meals were designed just to examine a possible difference between these two levels of meat content.

Meat, in particular beef, is a source of only not highly BA heme iron, but also zinc and arachidonic acid. In our study, we also included poultry because in Germany it is more convenient for baby food. Meat per se increases the absorption of non-heme iron from a meal, also proven in infants [31]. Moreover, iron absorption increases in case of high iron requirements [32, 33].

Infants, fully breast-fed for 4–6 months and thus with the lowest habitual iron intake during the first half of infancy in our sample, tended to have profited from higher meat content of the study food, and probably from development to an efficient iron absorption and distribution in the body.

A similarly designed Danish intervention study with an intervention period of 2 months and akin differences in meat consumption (10 vs. 27 g/day), found that a higher meat proportion at the same total dietary iron intake favored Hb levels in partially breast-fed infants in late infancy [34]. Two recent studies [35, 36] reported favorable effects on the psychomotor development of infants related to early introduction of meat as a complementary food.

Although we used best available data on iron bioavailability, there are some weaknesses in our calculations. Bioavailability has mostly been determined in adults and may be different in infants. It depends on the presence and the amount of promoters and inhibitors of absorption and on the iron status of the individual [32, 37]. However, our estimation of BA iron just meeting the requirements without any safety margin quite nicely fits to the tendency of lower Hb values in the subgroup of breast-fed infants with lowest estimated amount of BA iron intake.

Conclusion

In this double-blinded RCT in healthy, well-nourished term infants, the primary analysis could not discern sound evidence for an impaired iron status in infants receiving complementary food with meat content just at the lowest level allowed by EU law. A secondary analysis found that such low meat content possibly increases the risk of developing a marginal iron status during the second half of infancy in the subgroup of infants who were fully breast-fed 4–6 months as recommended and thus are characterized by a habitually low iron intake during the first half of infancy. A further trial may be indicated to study this question.

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Conflict of interest statement All authors have no conflict of interest.

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