Supplementation of diet with lactic acid bacteria did not influence the body weight gain (6, 13). However, in the present study V-cell group showed tend to decrease in body weight gain and food intake more than other groups (Table 2). In our previous study, we observed that rats consumed viable cells of *L.* GG showed tend to increase the body weight gain and food intake (3). The influence of viable GG cells on body weight gain needs further investigation.

In conclusion, the results of this study demonstrated that viable and heat-sterilized cells of *L.* GG decreased the cholesterol concentrations in serum and liver in the presence of excess cholesterol in the diet by an increased excretion of cholesterol and bile acid; and this hypocholesterolemic action of viable GG cells was higher than that of heat-sterilized GG cells. In addition, it is suggested that viable GG cells might increase the fecal cholesterol excretion by binding cholesterol, and might increase the fecal bile acid excretion by binding bile acid and lowering intestinal pH. However, the binding of cholesterol and bile acid remains controversial *in vitro*, we want to measure the absorption of cholesterol and bile acid in rats fed GG cell in future experiment. Further work will be needed.

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Iron-fortified milk can improve iron status in young women with low iron stores

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A considerable proportion of the populations of developing and industrialised nations does not meet the recommended daily allowance for iron and are thus at risk of chronic iron-deficiency anaemia. In a placebo-controlled, double-blind study we investigated whether supplementation with iron-enriched milk can improve the iron status in young women with low iron stores. Sixty-two women aged 20–36 years with serum ferritin concentrations </= 22 µg/l were given 400 ml/d of commercial milk (reference milk; n=30) or milk enriched with 1.75 mg/100 ml micro-compartmented iron and 10 mg/100 ml vitamin C (Fe-milk; n=32) as part of their habitual diets for 8 weeks. The Fe-milk was found to increase ferritin concentrations from 13.3±6.9 µg/l (mean±SD) to 17.7±11.8 µg/l after 8 weeks, whereas the reference milk resulted in a decline from 12.6±6.8 µg/l to 10.6±8.1 µg/l (p=0.01). After 8 weeks haemoglobin was higher in women receiving Fe-milk (135.5±1.0) than in women receiving reference milk (131.4±1.5 g/l; p=0.03). Conclusion: Milk enriched with micro-compartmented iron and vitamin C can increase depleted iron stores in reproductive age women.

Verbesserung des Eisenstatus durch Supplementation mit Eisen angereicherter Milch bei jungen Frauen

Text Die Versorgung mit Eisen bei Frauen im reproduktionsfähigen Alter erreicht nur 75% der Empfehlung, wodurch die Gefahr eines chronischen Fe-Mangels/einer Anämie besteht. In einer Plazebo-kontrollierten Doppeltblindstudie wurde geprüft, ob durch den Verzehr einer mit Eisen angereicherten Milch die Eisenspeicher bei jungen Frauen mit geringem Eisenstatus erhöht werden können. Frauen (20-40 Jahre, mit Plasma-Ferritinspiegeln </= 22 µg/l) verzehrten zu ihrer gewohnten Kost über 8 Wochen täglich 2x200 ml Milch, die entweder keine (Referenzgruppe, n=30) oder eine Anreicherung mit 1,75 mg mikrokompartmentiertem Eisen plus 10 mg Vitamin C /100 mL (Fe-Milch, n=32) erfuhr. Der Verzehr von 400 ml mit Eisen angereicherter Milch, entsprechend 7 mg Fe/d, erhöhten die Plasmakonzentration des Ferritins von 13.3 ± 6.9 µg/l (Mittelwert ± SD) auf 17.7 ± 11.8 µg/l nach 8 Wochen, während die Werte in der Kontrollgruppe von 12.6 ± 6.8 µg/l auf 10.6 ± 8.1 µg/l sank (p = 0.01). Nach 8 Wochen hatten Frauen die Eisenmilch verzehrt hatten, signifikant höhere Hämoglobinwerte (135.5 ± 1.0) als Frauen der Referenzgruppe (131.4 ± 1.5 g/l; p=0.03). Schlussfolgerung: Milch, die mit mikrokompartmentiertem Eisen angereichert war, kann den Eisenstatus bei jungen Frauen mit geringen Eisenspeicherwerten erhöhen.

23 Iron status (iron enrichment of milk), **Iron enrichment milk** (iron status women)

23 Eisenstatus (Eisenanreicherung von Milch) **Eisenanreicherung von Milch** (Eisenstatus Frauen)

1. Introduction

Iron is among the scarcest nutrients in human diets worldwide (5, 6), which is a contributing factor to the more than one billion people suffering from iron deficiency (27). The prevalence in developing nations ranges from 30 to 70%, while in industrialized countries it has declined to about 20% (25), due in part to the implementation of multifaceted strategies to ensure the widespread availability of affordable iron-fortified foods (30). Even in developed countries, however, certain groups remain at risk, including children, women of child-bearing age, vegetarians, and migrants (21), with consequent problems of fatigue and diminished capability. Most of these groups are characterised by iron intakes below recommended amounts. According to the German National Food Consumption Survey (24), only 30% of women in Germany aged 19 to 51 years achieved the recommended dietary allowance (RDA) of 15 mg/d proposed by the German, Austrian and Swiss Societies of Nutrition (D-A-CH), while 90% of men in the same age group met their RDA of 10 mg/d (7). Comparable trends have been reported for the Netherlands (3). Naturally occurring blood loss expose women to a gender-specific risk of iron-deficiency anaemia. To meet the RDA for iron, sufficient amounts must be provided in bioavailable form.

In the present study, we examined whether supplementing the diet of women of child-bearing age with low iron stores as indicated by low serum ferritin concentrations with iron-fortified milk (Fe-milk) can lead to an increase in their iron stores. The Fe-milk was a commercial milk fortified with a micro-compartmented form of iron to prevent impairments of taste and colour. Milk as a vehicle for iron supplementation has the drawback that the calcium abundantly present in milk may inhibit absorption of iron (12, 13). To overcome these possible inhibitory effects of calcium, the milk was fortified with vitamin C as well.

2. Materials and methods

2.1 Experimental design

A placebo-controlled, double-blind intervention study was performed in women aged 20 to 40 years with presumably low iron status but without anaemia at the Federal Research Centre for Nutrition and Foods (former Federal Dairy Research Centre) in Kiel, Germany. The women were recruited from written advertisement posted at different institutions in Kiel.

Approval was obtained from the University Medical Ethics Committee, and each volunteer gave her informed written consent. Sixty-seven women of child-bearing age were recruited from the 170 women who responded to the advertisements. Exclusion criteria as checked by anamnesis were: pregnancy, lactation, regular blood donation or at least one donation within the past two months, chronic gastrointestinal (GI) disease, macrobiotic or vegan life style, avoidance of milk, use of mineral or vitamin supplements, regular use of antibiotics, laxatives, diuretics or antacids, Hb < 110 g/l, and clinically relevant blood parameters (safety parameters) outside the normal range. Each candidate was screened for haemoglobin and ferritin concentrations – a well-accepted parameter for assessing iron deficiency in adult women (22). Women with low iron stores as indicated by ferritin levels </= 22 µg/l were enrolled. Sixty-two of the 67 women completed the study (drop out rate of 7.5%). To ensure similar baseline values, the reference milk group and Fe-milk group were matched for ferritin concentrations and body mass index. Each woman was asked to complete a questionnaire on GI well-being before and during each week of the intervention period. The questionnaire was based on COOK *et al.* (4) and FOWLIE *et al.* (9) and inquired about the intensity, duration, and frequency of abdominal pain as well as the frequency of bowel movement and any accompanying symptoms. Each questionnaire produced a score for GI symptoms between 1 (no symptoms) and 7 (most symptoms). The subjects were given instructions from a nutritionist before filling out a photo-illustrated food frequency questionnaire (FFQ) from the German Institute of Human Nutrition (Potsdam-Rehbrücke, Germany) (8,18) before and at the end of the study to assess their habitual intakes of calcium, iron, vitamin C, phosphorus, sodium chloride and protein. Calculation of the nutrients was based on the German Food Code and Nutrient Data Base BLS.II2 (8).

2.2 Intervention

The milk for both groups was provided by Campina Melkunie, Woerden, Netherlands. It contained either no added nutrients (reference milk, n=30) or 1.75 mg microcompartmented iron (iron pyrophosphate microencapsulated with glycerol esters of fatty acids, Taiyo Kagaku, Japan) plus 10 mg of vitamin C/100 ml (BASF,

Ludwigshafen, Germany) referred to as "Fe-milk" in this study, n=32). The Fe-milk thus provided nearly 50% of the German RDA of iron for women. The women drank one cup (200 ml) with breakfast because the absorption ratio is highest after overnight fasting (26), and the second cup at any other time of the day. The volunteers picked the milk up from the institute twice a week. Blood samples were taken between 7:00 and 11:00 a.m. in the fasted state before intervention was started and after the third and 8h week of intervention. Compliance was high (>95%), as assessed by forms completed weekly, and did not differ between the groups.

2.3 Clinical analyses

On each day of blood collection, the following parameters were analysed in blood, serum and plasma: haemoglobin, ferritin, reticulocytes, transferrin, iron, iron-binding capacity, transferrin saturation, and mean corpuscular haemoglobin (MCH).

All analyses were performed using routine laboratory methods in the certified central laboratory of the Kiel Municipal Hospital.

2.4 Statistical analyses

Analysis of variance (ANOVA) was performed to test variances for significance, followed by a Newman-Keuls-test (two-tailed). Means and standard deviation are given throughout. Differences were considered significant if $p<0.05$. Comparison of means was performed on absolute values and on changes over time (Δ). A one-tailed t-test was also performed to test whether intervention with iron-enriched milk improved parameters of iron status.

3. Results

The two groups did not differ at the start and end of the study with regard to mean age, body weight, body mass index, habitual daily intake of food energy (kJ), and several nutrients (Table 1). Mean intragroup values did not change over time.

Initial serum ferritin concentrations were 12.6±6.78 µg/l for reference milk and 13.3±6.90 µg/l for Fe-milk, mean ±SD, ($p=0.69$). Ferritin levels tended to increase in the Fe-milk group, to decrease in the reference milk group.

After three weeks the difference between the Fe-milk group (16.80±12.30 µg/l) and reference milk group (11.80 ±8.45 µg/l) was significant in the one-tailed t-test ($p<0.05$). After eight weeks the difference (17.65±11.85 µg/l for Fe-milk, 10.60±8.13 µg/l for reference milk) was also significant in the two-tailed test ($p=0.01$) (Fig. 1a). The change in ferritin levels over time (Δ) differed significantly between the reference milk (–0.8±8.02 µg/l) and Fe-milk (3.51±9.30 µg/l) after 3 weeks in the one-tailed test ($p<0.05$), and after 8 weeks in the two-tailed test (reference milk –2.0±7.54 µg/l vs. Fe-milk 4.34±8.85 µg/l, $p<0.05$).

Haemoglobin concentrations in EDTA-blood did not differ between the 2 groups before (132.1±8.6 g/l for reference milk and 133.9±6.1 g/l for Fe-milk), and 3 weeks after start of intervention (130.7±9.2 g/l for reference milk and 131.5±6.1 g/l for Fe-milk). After 8 weeks haemoglobin was higher in women on Fe-milk (135.5± 5.6 g/l) than in women on reference milk (131.4±8.1 g/l) (Fig. 1b; $p=0.03$; two-tailed tests). The 2 groups differed significantly with regard to changes in haemoglobin levels over time (Δ) (–0.63±5.49 g/l for reference milk vs. 1.59±4.73 g/l for Fe-milk) after 8 weeks ($p<0.05$; one-tailed t-test).

Initial plasma iron levels were 0.71±0.32 mg/l for reference milk vs. 0.80±0.37 mg/l for Fe-milk, ns). After 8 weeks, the difference between the 2 groups was significant (0.75±0.40 mg/l for reference milk vs. 0.95± 0.48 mg/l for Fe-milk; $p<0.05$; one-tailed test; Fig. 1c).

Reticulocytes were significantly higher in the Fe-milk group before intervention but the difference lost significance over time (Table 2). Changes in reticulocyte counts over time (values not shown) fell short of significance ($p=0.07$).

Table 1: Age, height, body weight and effect of iron-fortified milk on body mass index (BMI), and habitual nutrient intake in young women

		Reference milk Mean n=30	SD	Fe-milk Mean n=32	SD
Age (Y)	Before	25.2	4.2	24.2	2.7
Height (cm)	Before	170.7	10.6	171.5	9.6
Body weight (kg)	Before	65.0	5.8	65.7	5.4
BMI (kg/m^2)	Before	22.3	3.2	22.3	2.7
	After 3 weeks	22.1	3.3	22.4	2.7
	After 8 weeks	22.4	3.3	22.3	2.7
Habitual daily intake					
Energy (kJ)	Before	8323	2301	8852	2894
	After 8 weeks	8201	2221	8200	2574
Protein (g)	Before	67.10	20.59	69.89	27.99
	After 8 weeks	64.03	19.09	66.37	24.45
Calcium (mg)	Before	1052	319	1215	724
	After 8 weeks	1044	318	1104	553
Phosphorus (mg)	Before	1955	501	2116	967
	After 8 weeks	1884	520	1897	819
NaCl (mg)	Before	5621	348	5507	318
	After 8 weeks	5250	1725	4926	1620
Iron (mg)	Before	14.37	3.76	14.69	5.70
	After 8 weeks	13.98	4.63	12.85	4.56
Vitamin C (mg)	Before	103.25	41.21	128.89	74.73
	After 8 weeks	102.43	45.14	116.06	61.79

Means and SD from ANOVA followed by Newman-Keuls-test. No significant differences between the 2 groups for absolute and Δ values.

Table 2: Effect of iron-fortified milk on haematological parameters

		Reference milk Mean n=30	SD	Fe-milk Mean n=32	SD
Reticulocytes (‰)	Before	9.40	3.07	11.56	3.67$^{\&}$
	After 3	10.00	3.55	11.84	3.90§
	After 8 weeks	10.40	2.80	11.59	3.40
Transferrin (g/l)	Before	3.40	0.75	3.19	0.50
	After 3	3.24	0.59	2.97	0.45§
	After 8 weeks	3.05	0.59	2.83	0.39§
Transferrin saturation (%)	Before	15.13	6.30	19.12	10.97
	After 3	17.30	7.32	20.28	12.92
	After 8 weeks	18.58	11.42	24.74	14.53§
Fe-binding capacity (µmol/l)	Before	85.74	19.12	80.45	13.16
	After 3	81.79	15.62	74.82	11.97§
	After 8 weeks	77.00	14.92	71.39	9.69§
MCH (pg)	Before	28.80	2.15	29.83	1.95
	After 3	28.80	1.94	29.95	1.89$^{\#,\&}$
	After 8 weeks	28.74	1.94	29.95	1.78$^{\#,\&}$

Means and SD from ANOVA. Values of women on Fe-milk differed significantly from those on reference milk in the one-tailed test as follows: $^{\S}p<0.05$, $^{\#}p<0.01$; in the two-tailed test as follows: $^{\&}p<0.05$, $^{\$}p<0.01$. Mean corpuscular haemoglobin (MCH).

Although there were significant differences between the dietary groups for transferrin, transferrin saturation, plasma iron-binding capacity, and changes in MCH, these significances were lost when means were corrected for initial values (Δ-values, not shown).

The GI symptoms score indicated a slight reduction in frequency, intensity and duration of abdominal pain in both groups, which attained significance in the reference milk group. There was no appreciable effect on frequency of bowel movement (not shown).

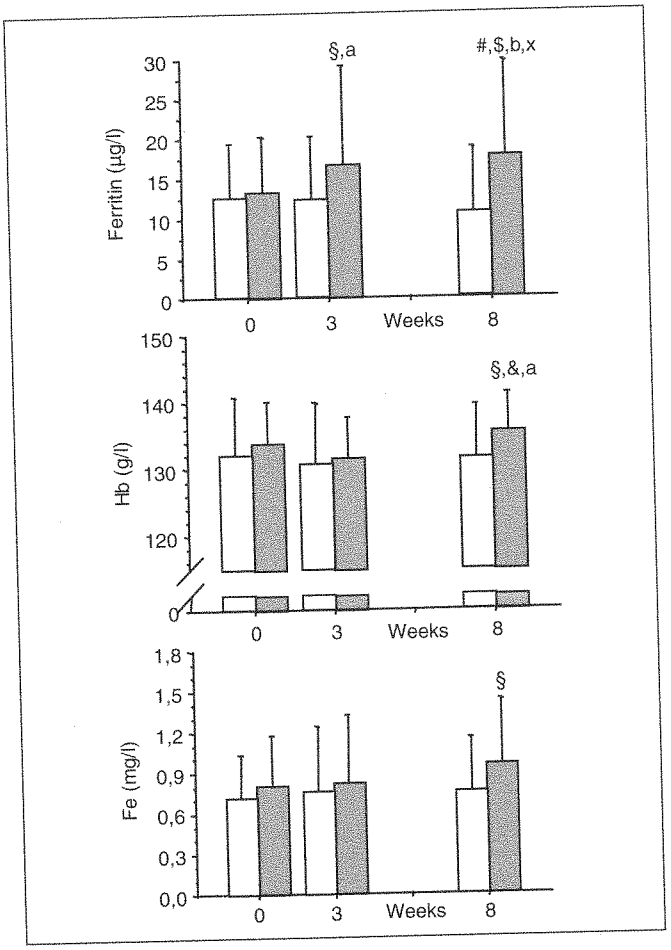

Fig. 1: Effect of iron-fortified milk on serum ferritin (top), haemoglobin (middle), and plasma iron (bottom) concentrations (mean and SD). Values (absolute) of women consuming Fe-milk differed significantly from those on reference milk in the one-tailed test: §: $p<0.05$, #: $p<0.01$; in the two-tailed test: &: $p<0.05$, $: $P<0.01$. Values (changes, Δ) of women consuming Fe-milk differed significantly from those on reference milk in the one-tailed test: a: $p<0.05$, b: $p<0.01$; in the two-tailed test as follows: x: $p<0.05$. ☐ Ref. milk, ▒ Fe-milk.

4. Discussion and conclusions

Forty-five percent of the women enrolled in our study had depleted iron stores (ferritin concentrations </=22 µg/l), a higher proportion than the 20% recently reported for populations of industrialised countries (25), or the 23% for premenopausal Danish women (14), and even higher than the 38% for women volunteers in South Africa (20). This high prevalence might have been because the information provided on the recruitment notices led to a greater response rate from women who – for whatever reason – considered themselves at risk of iron deficiency.

Increased ferritin concentrations 3 weeks after the start of intervention and a further increase after 8 weeks was associated with significantly higher haemoglobin levels after 8 weeks. The amount of iron absorbed from the diet depends largely on the individual's iron status, the ratio of haem to non-haem iron and on enhancers or inhibitors of iron absorption in the diet (10). A valid measure of bioavailability of non-haem iron is the ratio of its absorption in subjects with borderline iron deficiency (10), like the women in our study.

The reports of HALLBERG *et al.* (12, 13) show that iron absorption is inhibited by simultaneous ingestion of calcium, thus impeding the benefit of iron supplementation via milk. We demonstrate that iron was effectively absorbed even in the presence of a food rich in calcium, as indicated by increased ferritin concentrations. There are several possible explanations for the discrepancy between HALLBERG'S findings and our own. HALLBERG *et al.* performed single meal experiments, with iron absorption as the measure of outcome, not parameters of iron stores and not over a longer period of time. Our subjects all had depleted iron stores – factors that enhance iron bioavailability (10). It is known that beyond a threshold calcium content of ≈300 mg per meal no further inhibition of iron absorption occurs (12, 13). Thus the initial calcium content of a meal determines whether or not calcium supplementation will influence iron absorption. In our study, each woman in the Fe-milk group consumed iron-enriched milk as part of her habitual diet containing more than 1000 mg/d of calcium before addition of the 480 mg/d of calcium contained in the iron-supplemented milk (Table 1). Thus, our finding of a low inhibitory effect of calcium on iron availability is not surprising. It agrees with those of other authors showing that calcium supplementation (1200 mg/d) had no long-term side effects on iron status in iron-replete adults (23) or in children accustomed to a diet high in calcium versus those accustomed to low calcium (1). Thus children could benefit from higher calcium intakes with respect to bone health without adversely affecting iron metabolism (1).

In free-living populations the correlation between the dietary calcium content and iron stores in young women is lower than one might have expected from single meal studies. VAN DE VIJVER and colleagues report a regression coefficient of –1.37 µg/l ferritin per 100 mg of calcium for women with transferrin saturation and iron levels similar to those of our subjects and whose habitual calcium intake ranged from 680 to 1227 mg/d (29). Extrapolated to the present study, our women on reference milk (+480 mg Ca) were at risk of reducing their ferritin concentrations from 12.6 to 6.0 µg/l. In fact their ferritin was 10.6 µg/l after 8 weeks of milk supplementation. It appears that the body adapts its iron absorption capacity to the presence of high levels of calcium (19).

Supplementation with highly bioavailable iron is important since it is not only the iron intake itself, but iron's low availability in the diet that is chiefly responsible for iron deficiency (2). The low iron status of our women volunteers despite their intake of the recommended allowance of iron as assessed by the FFQ indicates their habitual diets possessed iron sources of rather low bioavailability.

Absorption of iron is enhanced when vitamin C is taken (15). Inhibition of iron absorption can thus be reduced if iron supplements are given as part of a balanced diet. Vitamin C-rich fruit and vegetables help over-

come the effect of iron-absorption inhibitors in the same diet (28). The Fe-milk used in this study contained iron in the presence of calcium (potential inhibitor) plus vitamin C and was able to increase iron, ferritin, and haemoglobin. Vitamin C is, however, less effective at increasing parameters of iron stores, even at high dosages (16). Daily doses of 1500 mg vitamin C increased ferritin non-significantly from 10.7 to 11.9 µg /l (16), whereas our Fe-milk enriched with iron, but not more than 40 mg vitamin C, led to 7 µg/l higher ferritin concentrations than compared with the reference milk. An increase of 5 µg/l in ferritin is considered to be biologically significant (11). HUNT *et al.* (16) concluded that supplemental vitamin C has less effect on iron bioavailability in everyday balanced diets than was predicted from single meal studies. This inference was supported by a study on iron-deficient Mexican women showing that 50 mg/d supplemental ascorbic acid for 8 months resulted in no improvement in their iron status (11). The authors (11) attributed this to the low ratio of ascorbic acid to iron of 6:1 (wt/wt), which was comparable to the ratios in the present study of 7.3:1 in the reference milk group and 7.8:1 in the Fe-milk group. A ratio of 12:1 in the diet may be needed to improve absorption of iron with low bioavailability (17). The present results therefore indicate that the efficacy of the Fe-milk at improving iron stores was due mainly to the iron component.

Both the reference milk and the Fe-milk was tolerated well and did not induce weight gain, probably because it was consumed in place of other foods of equivalent energy content.

Conclusion: Parameters of iron stores can be increased in young women with depleted iron stores by consumption of 400 ml/d of milk enriched with microcompartmented iron plus vitamin C.

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