

Inhibitory Properties of Lactoferrin on Adhesion of Oral Bacteria to Hydroxyapatite

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ABSTRACT

Caries is one of the most common infectious diseases in the world, caused by an interplay between different variables in the oral cavity resulting in a demineralization of the teeth. Human milk is considered the “golden standard” as nutrient source for infants, but in some cases baby formula is used instead. Today formulas contain an unnecessarily high amount of iron, which could have a negative impact on health and development. A high iron content could be replaced with a low iron content together with lactoferrin.

This in vitro study examines the capability of *Streptococcus mutans* and *Lactobacillus gasseri* to bind saliva coated hydroxyapatite beads covered in formulas containing various amounts of lactoferrin and iron. The aim is to investigate the effect of baby formulas on adhesion of caries related bacteria. Saliva collected from children aged 0-5 years was pooled and added to wells on a round-bottom microtiter plate, together with radiolabeled *Streptococcus mutans* or *Lactobacillus gasseri*. The binding capacity of the bacteria was tested by using the hydroxyapatite assay (HA-assay). Excel was used for calculations. The results indicated that the levels of iron and lactoferrin in formula do affect the ability of bacteria to bind to the tooth surface. The formula assumed containing lactoferrin showed a significant difference to the formula assumed not containing lactoferrin. Lactoferrin containing formula was also significantly different from human milk and bovine milk for both bacteria tested. The results will be confirmed when the code is broken for the main study.

INTRODUCTION

One of the most common chronic diseases world wide caused by infection and metabolic events in the oral biofilm is dental caries (Aas *et al.*, 2008). Tooth surfaces covered with pellicle act as binding sites for caries related bacteria. More specific, some glycoproteins and proteins like Proline rich proteins (PRP:s) and Statherin secreted by human saliva promote binding to bacteria by absorption to dental surfaces, while others act as inhibitors e.g lysozyme and lactoferrin (Lf) (Wernersson *et al.*, 2006). Other functions of the dental pellicle are promotion of remineralization and protection of dental surfaces from erosion, caused by acids from foods and drinks. The positively charged enamel attracts proteins with negatively charged side-chains and forms a layer with a thickness of about 1µm (Fejerskov *et al.*, 2015).

Formation of the biofilm initiates with the contact between bacterial cells and the tooth surface covered by pellicle. More specific, adhesins expressed by bacteria interacts with receptors in the pellicle to form the first bacterial layer covering the tooth. Selective adherence appears between early colonizers and specific receptors in pellicle by the ability to recognize these by colonizing bacteria. Once the first bacteria layer is attached to the pellicle, other bacteria adhere to the already existing bacteria and the biofilm formation continues (Fejerskov *et al.*, 2015).

The biofilm ecology promoting dental caries is distinguished by a higher amount of acidogenic (acid producing) and aciduric (acid resistant) bacteria. Streptococci and Lactobacilli species are known to benefit from the low pH (Danielsson Niemi, 2010). Several bacteria have been recognized as initiators to the development of caries and *Streptococcus mutans* (*S. mutans*) is a well-known pathogen causing demineralization by production of lactic acid. Another bacteria, also related to caries, is different species of lactobacilli which contributes to demineralization by its ability to produce acid and tolerate acidic environments (Becker *et al.*, 2002; Vestman *et al.*, 2013). While some lactobacilli are related to disease, others are known to promote oral health. There are species of lactobacilli that are especially seen in children and particularly common among breastfed infants, eg. *Lactobacillus gasseri* (*L. gasseri*) (Vestman *et al.*, 2013). This specie has been proven to inhibit *S. mutans* binding to hydroxyapatite in vitro and also to depress the growth of *S. mutans*. One reason to this probiotic property is that both share the same host receptor, gp340. A specific phenotype of this receptor seems to favor the binding of *L. gasseri*, which will have an inhibiting effect on colonization of *S. mutans* on tooth surface. Since *Lactobacilli*

species are second invaders, their colonization of teeth are dependent on early colonizers and their ability to create a favorable niche (Vestman *et al.*, 2013; Caufield *et al.*, 2015).

Human milk affects the oral cavity for instance by contributing with *Lactobacillus*. Holgerson *et al.*, (2013) identified three species of *Lactobacillus* in breastfed infants, among those three, *L. gasseri* was the most prevalent. The study also showed that *Lactobacillus* collected from exclusively breast fed infants had an inhibitory effect on *S. mutans* whilst *Lactobacilli* from formula fed infants had no inhibitory effect. The biofilm in breastfed and formula fed infants was also compared, where the group that was exclusively breastfed had a higher prevalence of bacterial species associated with oral health in contrast to formula fed biofilms were species related to oral disease was detected (Holgerson *et al.*, 2013).

Human milk is known to be the main source of nutrients in the early months of life (Danielsson Niemi, 2010; Labbok *et al.*, 2004). There are several studies that show the advantages of breastfed compared to formula fed infants considering health and development. Among other nutrients, human milk contains Lf, which plays a crucial part in the early immune system of the child (Danielsson Niemi, 2010). In all cases human milk as nutrient source is not possible and a substitute, baby formula, normally based on cow's milk protein, can be used. It is of importance that the composition of the formula fulfills the same requirement as human milk so that the favorable health benefits are as comparable as possible (Clemens *et al.*, 2010).

Lf is a multifunctional iron-binding protein, synthesized by epithelial cells of the mammary glands but is also present in other exocrine fluids (Danielsson Niemi, 2010; Drago-Serrano *et al.*, 2017; Johnston *et al.*, 2015). Some of the characteristics of Lf are anti-inflammatory activity, involvement in cellular proliferation and differentiation, iron status maintenance and iron absorption in the small intestine as well as host defense against microbial infections. By its high affinity to bind iron, the protein is able to reduce the amount of free iron, which prevents the possibility for iron-requiring pathogens to use it. Another antimicrobial function of Lf is present when a domain of the protein is disengaged when digested and exposed to pepsin. It has therefore been suggested that the presence of Lf affects the composition and function of gut microbiota. Since Lf is also present in other exocrine fluids like saliva, as mentioned earlier, its antimicrobial properties are also shown in the oral cavity. Like other salivary proteins with antimicrobial functions both minor and major glands in the oral

mucosa, mainly by the serous cells, secrete Lf. In the oral environment the protective function of the protein comes from its high affinity to iron and the ability to keep iron unreachable for iron-requiring bacteria, making it bacteriostatic (Amerongen and Veerman, 2002; Fejerskov *et al.*, 2015). The human and bovine Lf proteins are structurally similar and have demonstrated comparable bioactivity, the proteins share ~70 % sequence homology. Although, the human Lf antimicrobial properties have been shown to be more effective. Concentration of Lf in bovine milk is lower than the concentration found in human milk (Johnston *et al.*, 2015; Lönnerdal, 2016). As seen in the literature the Lf content in bovine milk varies not only between different cow breeds but also between individual cows (Arnould *et al.*, 2009). According to Cheng *et al.*, (2008) concentration of Lf varied between 115.4 mg/L (± 67.4) in mature bovine milk whilst Arnould *et al.*, (2009) calculated the Lf mean to 137.8 mg/L (± 176.74). The content of Lf in human milk are very high and differs between women in the same way it varies in bovine milk. Lf in human milk range from 7000 mg/L in colostrum to 1000 mg/L in mature milk (Brock, 1980).

Iron, a mineral essential for development of the infant and necessary to carry oxygen in haemoglobin as well as vital for development and cell growth in the immune and neural systems. Iron is also a pro-oxidant and acts as a substrate for iron requiring pathogens and contributes to their survival and virulence. An iron deficiency can cause anemia and inhibit a normal infant growth, on the other hand a too high iron intake has been seen to increase growth of some iron required pathogens (WHO 2016). The amount of iron in human milk is 0.3 mg/L, much lower compared to the amount iron available in baby formulas, containing 8-14 mg/L (Ziegler *et al.*, 2009).

In a study (LIME, Laktoferrin I ModersmjölksErsättning) the effect of a baby low iron formula (2 mg/l) enriched, or not, with Lf, is compared with the “golden standard” human milk and a high iron formula (8 mg/l, no Lf) (PI Staffan Berglund, UmU). The study is a randomized, double blind intervention trial, with 4 arms and 72 children in each arm.

This in vitro study, nested to the above mentioned RCT-study, aims to investigate the ability of *S. mutans* and *L. gasseri* to bind hydroxyapatite beads covered in saliva mixed with the different baby formulas which contain high or low amounts of iron with or without Lactoferrin. The hypothesis is that baby formula containing Lactoferrin is thought to decrease the capacity of *S. mutans* and *L. gasseri* binding the tooth surface.

MATERIALS & METHODS

Saliva sampling

Healthy children aged 0-5 years were recruited by asking their parents for permission to participate, the parents gave their informed consent (appendix 1). Unstimulated saliva were collected with a suction set modified from Noakes *et al.* (2007), specially designed to minimize discomfort for the participants. A suction is attached to the dental unit and the saliva is collected in a sterile tube. The modified suction set is validated and has been used in previous studies (Noakes *et al.*, 2007). Saliva from all participants was pooled and stored in a test tube at -80°C.

Bacteria radiolabeling

When culturing and radiolabeling the bacteria, the following methods were used. It should be noted that this step was not done within this study. *S. mutans* (CCUG 11877T) was refrigerated in skim milk, a sterile loop was used to take 1 µl bacterial rash to a blood agar plate. The plate was incubated at 37° CO₂ for two days; one colony was then transported to a new blood agar plate and incubated at 37° CO₂ for another day. *L. gasseri* (CCUG 31451) was refrigerated in skim milk, a sterile loop was then used to take 1 µl bacterial rash to an acid (pH 5.5) Rogosa plate (Merck Germany) and incubated anaerobically at 37° for two days. One colony was then transported to a new Rogosa plate and incubated anaerobically at 37° for one day.

The next day one loop (1 µl colonies) from each bacterial culture was placed in two different tubes containing 80 µl sterile M-dil solution (4.4 g NaCl, 0.42 g KCl, 1.0 g Na₂HPO₄ x 2H₂O, 1.0 g KH₂PO₄, 10.0 g C₃H₉Na₂O₇P x H₂O mixed with 0.1 g MgCl₂ x 6H₂O, 500 mL distilled water), and 20 µl S³⁵ (400 µCi) was added to each tube. The whole volume (100 µl) of the *S. mutans* solution was added to a preheated blood agar plate and the whole volume (100 µl) of the *L. gasseri* solution was added to a Rogosa plate (if the radioactivity had been reduced by half, the S³⁵ increases gradually, for example 60 µl M-dil + 40 µl S³⁵). A sterile loop was swept over the two different plates until it was all dry; the plates were then incubated anaerobically overnight at 37°. All bacteria were then swept of the plates using a cotton swab, by adding a 1-2 ml ADH-buffer. ADH was added to a total amount of 7 ml and rotated for 10 minutes at 5000 rpm. 1ml ADH-buffer was added to 6 ml ADH and rotated for 10 minutes. 1ml ADH-buffer from the solution was used to calculate abs (550 nm): 50 µl sample + 950 µl

ADH-buffer. The OD-rate was checked using a precalculated diagram and the concentration at a 20 times dilution was then calculated.

Bacteria binding HA-method

To test the bacteria binding the hydroxyapatite assay (Gibbons and Hay, 1988) was used (appendix 2). 5 mg (4.9-5.1 mg) hydroxyapatite beads were placed in 60 wells on a round-bottom microtitre plate (Nunc Surface, NUNC, Roskilde, Denmark), then 125 µl ADH-buffer was added to each well and the plate was left in a refrigerator overnight. The beads were then washed one time with ADH-buffer and 125 µl saliva were added to cover the beads. Additional to the collected child saliva a control saliva known to have a great binding capacity for caries related bacteria was used. The plate was then rotated for 1 hour. Thereafter the beads were washed three more times with ADH-buffer. Next, 125 µl blocking solution (5 % BSA with ADH) was added to the HA-beads and the plate was incubated at 37° C while rotated for 1 hour. The beads were washed three times with 125 µl ADH-buffer after removing the blocking solution. 63 µl ADH was added to the 18 control wells and 63 µl of each baby formula was added to six wells. 63 µl human milk was added to six wells and finally 63 µl bovine milk was added to six wells. 63 µl bacteria suspension of *S. mutans* was added to three out of six wells containing baby formula, human milk or bovine milk and 63 µl bacteria suspension of *L. gasseri* was added to the three remaining wells. Both bacteria suspensions were radiolabeled. The plate was rotated for 1 hour and washed three times with ADH. 6.3 µl of each bacteria suspension was added to three empty wells. 150 µl scintillation fluid was added to all wells and left overnight. The following day the radioactivity was measured in a scintillation vial and the counts per minute (CPM) for each well was measured. For 63 µl bacteria suspension the CPM value was calculated.

Products

The products used for estimating the ability of caries related bacteria to bind hydroxyapatite was skimmed bovine milk, fat free human milk and baby formula. Five different cans of baby formula with three different content, with or without Lf, were used. Which of the cans of baby formula that contains high or low content of iron with or without Lf is not announced until the LIME study is complete and the code was planned to be revealed in the summer 2018. Lf in the baby formula was derived from bovine milk.

Statistical analyses

Radiation was registered and CPM was calculated in percent. All data from the study was collected and processed in Excel, where the mean of the total amount bacteria, the percent of the mean and standard deviation was calculated. The statistical significance for the binding to the two bacteria tested was calculated with student's t-test in Excel. Tables and diagrams were also modeled in Excel.

Literature search

The PubMed database was used for locating most of the articles. The results were found using Mesh terms in the free text search were sufficient. By using mesh database, the search result would have been too restrictive and many articles would have been missed; more recent articles and other articles lack the search terms in their abstract but still have a relevant main focus. The keywords mainly used were child, dental caries, *S. mutans*, *Lactobacillus*, Lactoferrin, baby formula, human milk and bovine milk. Other sources of information were found in textbooks and dissertations related to caries and oral bacteria.

Ethical considerations

The Ethics Committee at Institution of Odontology, Umeå University approved the implementation of the study. All participants were given both verbal and written information. Since the participants included in this study were too young to make a decision on their own whether to participate or not, this decision was transferred to their legal guardians or parents. The participation was though completely voluntary and could be cancelled at any time. The participants could potentially perceive saliva sampling as unpleasant because of their young age and their general inexperience of being in a dental clinic, but the method used ensured that the collection was made carefully and secure. The situation could possibly be experienced as offensive by the children, due to the parents great impact on the decision whether to participate or not. With this in mind, all contact and meetings with the children were performed with respect to their own autonomy.

RESULTS

The two different bacteria *S. mutans* and *L. gasseri* showed a resembling binding capacity to the five different baby formulas, containing a high or low amount of iron with or without Lf. HA-beads covered in saliva and baby formula nr. 1 resulted in the highest amount of binding *L. gasseri* and *S. mutans* whilst baby formula nr. 5 had the least amount of binding bacteria. As shown in table 1 the mean number, in percent, of bacteria binding baby formula nr. 1 for *L. gasseri* was 2.6 and for *S. mutans* was 7.8. For baby formula nr. 5 the binding capacity for *L. gasseri* was 1.2 and for *S. mutans* 4.0.

The formula that had the highest binding capacity for *S. mutans*, formula 1, showed significant difference to formula 4 and formula 5 (p-value < 0.001). For *L. gasseri* there was significant differences for all formulas (p-value < 0.05 and p-value ≤ 0.001) (table 2). The results for human milk and bovine milk was significant different from formula 1 for both bacteria tested (p-value < 0.05) (figure 1a and 1b).

Table 1 shows that the percentage of binding bacteria to saliva coated HA-beads varied between 1.5-2.0 for *L. gasseri* and between 5.9-6.4 for *S. mutans* when covered with baby formula nr. 2, 3 and 4. The percentage of binding bacteria for baby formula nr. 2, 3 and 4 thus lies between the percentage binding bacteria for baby formula nr. 1 and 5, regarding both *L. gasseri* and *S. mutans* (figure 2). The capacity of binding the HA-beads differed between the two bacteria tested (figure 2). *S. mutans* attached to a greater amount of the hydroxyapatite surface compared to *L. gasseri* in wells containing baby formula. This was also the case in the wells containing only saliva.

HA-beads covered by bovine milk and HA-beads covered by human milk showed a similar percentage of binding bacteria, regarding both *S. mutans* and *L. gasseri*, as shown in table 1. The quantity of binding bacteria was higher in both strains of bacteria when added to HA-beads covered by exclusively saliva compared to the ones covered by both types of milk.

L. gasseri in wells containing saliva and the five different formulas showed a mean percentage of binding bacteria to HA-beads varying from 1.2 to 2.6. The wells containing *L. gasseri* and human milk had a mean of 4.7 and the well containing bovine milk 4.5. *S. mutans* had a mean percentage of binding bacteria varying from 4.0 to 7.8 in the wells with saliva and

the different formulas. In the wells containing human milk the mean percentage was 4.8 and in the wells containing bovine milk it was 4.5.

DISCUSSION

The present study showed that the baby formulas used, containing a high or low amount of iron, with or without Lf, had varying capability in inhibiting binding between caries related bacteria and a tooth like surface, in the case of this study HA-beads was used for that purpose. Formula 5, which according to our results most likely contains Lf, had the greatest ability to inhibit *L. gasseri* and *S. mutans* from binding HA-beads, probably partly due to the protective properties of Lf. It should be noted that other protective substances in the baby formulas could affect the result. Likewise the result visualized the lesser capability of formula 1, which according to our results most likely does not contain Lf, to inhibit the same bacteria from attaching.

The significant difference in binding bacteria regarding both *S. mutans* and *L. gasseri* indicates that there is evidence for the assumption (hypothesis) that presence of Lf and different amounts of iron affect the binding capacity of *S. mutans* and *L. gasseri* to a tooth like surface. This study therefore suggests that presence of Lf in baby formula reduces the capability of caries related bacteria to interact with the tooth surface. Baby formula nr. 2, 3 and 4 showed the most comparable results regarding percent binding bacteria among all baby formulas. It is therefore tempting to assume that the amount of iron is equal to, or close to equal in these formulas and thereby would have similar properties in an oral environment.

Human milk and bovine milk, naturally containing Lf, had almost the same ability to prevent the two tested bacterias capacity of binding the tooth like surface. As the result showed the number of binding bacteria was reduced with $\frac{1}{3}$ - $\frac{1}{2}$ in the wells covered with milk compared to the wells containing only saliva, regarding *S. mutans*. There is a significant difference between baby formula nr. 1 and human milk as well as bovine milk. This indicates that, when taking only the risk of caries development in to account, human milk or bovine milk are a more preferable source of nutrients than baby formula nr 1.

The ability to inhibit the binding of *S. mutans* was greater in human milk and bovine milk compared to the five different baby formulas. Since human milk and bovine milk contains a certain amount of *L. gasseri* and knowing that *S. mutans* are inhibited by *L. gasseri* (Holgerson *et al.*, 2013) this could probably be the reason that a lesser amount of *S. mutans* were capable of binding the HA-beads containing human milk and bovine milk than to the

HA-beads containing only child saliva and the HA-beads containing child saliva and different baby formulas. In the present study this can be seen in all baby formulas except from nr 5.

Regarding *L. gasseri*, the five baby formulas had a greater capability to inhibit binding bacteria compared to both human milk and bovine milk, which might be due to *L. gasseri* being a secondary invader and the possibility that binding to the tooth surface would require a specific niche created by early colonizers (Caufield *et al.*, 2015). There is a possibility that the baby formulas lack substances needed for *L. gasseri* to bind the hydroxylapatite surface, which is not taken into account in this study. The reason for the greater amount of *L. gasseri* in the wells containing human milk and bovine milk might be on account of the naturally existing amount of *L. gasseri* in the two milks. Different substances in human milk, bovine milk and in the baby formulas, not examined in this study, might affect the binding capacity of the bacterias to the hydroxylapatite like surface and thereby influence the outcome of this study (Martín *et al.*, 2007).

Based on the result in this study it is only possible to assume and from our side yet only speculate that baby formula nr 5 contain Lf, while the other four tested baby formulas probably do not. It should also be noted that measurement bias may occur. This can be confirmed when the content of the baby formulas are released.

The pasteurization of the bovine milk might affect the substances as well as the composition and thereby possibly affect their protective properties against caries associated bacteria. When producing baby formula the processing of the raw product (milk) could result in changes in the composition, both structurally and regarding the actual content, and thereby its properties may change in relation to caries (Peila *et al.*, 2016). Additional, in further studies on *S. mutans* and *L. gasseri* an interplay between the two bacteria should be considered, an interplay which might increase the protective effect of *L. gasseri* on dental caries. The result in this pilot study are notable, although it's suggested to repeat the study employing a larger group of participants.

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Table 1.

Number of adhering *Streptococcus mutans* and *Lactobacillus gasseri* to hydroxyapatite beads in percent, testing the inhibition capacity in different formulas, human and bovine milk.

	<i>L. gasseri</i> Mean cpm	<i>L. gasseri</i> Mean % (\pm STD)	<i>S. mutans</i> Mean cpm	<i>S. mutans</i> Mean % (\pm STD)
CPM 63 μ l bak susp	46 030		18 843	
Control ADH	1 868	4.1(\pm 0,2)	552	2.9(\pm 0,1)
Control parotis saliva	3 015	6.6(\pm 0,2)	1 743	9.2(\pm 0,7)
Child saliva	3 256	7.1(\pm 0,8)	1 633	8.7(\pm 1,2)
Child saliva + Formula 1	1 174	2.6(\pm 0,1)	1 463	7.8(\pm 0,1)
Child saliva + Formula 2	917	2.0(\pm 0,2)	1 199	6.4(\pm 0,9)
Child saliva + Formula 3	866	1.9(\pm 0,1)	1 285	6.8(\pm 0,9)
Child saliva + Formula 4	685	1.5(\pm 0,1)	1 115	5.9(\pm 0,1)
Child saliva + Formula 5	569	1.2(\pm 0,0)	761	4.0(\pm 0,2)
Human milk	2 183	4.7(\pm 0,2)	898	4.8(\pm 0,2)
Bovine milk	2 079	4.5(\pm 0,6)	851	4.5(\pm 0,8)

Table 2.

Significant values for baby formulas to inhibit binding of *Streptococcus mutans* and *Lactobacillus gasseri* to hydroxyapatite beads. Formula 1 with the highest number of binding bacteria was compared to formula 2-5, human and bovine milk.

	<i>L. gasseri</i> p-värde	<i>S. mutans</i> p-värde
Formula 1 + Formula 2	0.01625	0.11575
Formula 1 + Formula 3	0.00304	0.20345
Formula 1 + Formula 4	0.00037	0.00004
Formula 1 + Formula 5	0.00116	0.00024
Formula 1 + Human milk	0.00039	0.00031
Formula 1 + Bovine milk	0.02302	0.01615

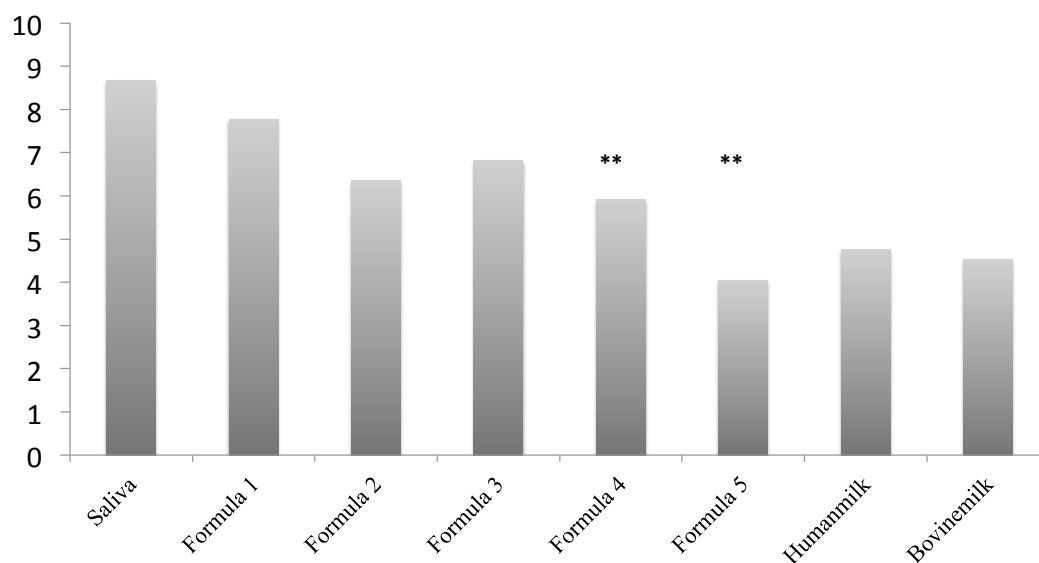


Figure 1a. Binding capacity for *S.mutans* after addition of baby formula containing different amounts of lactoferrin and iron, showing a significant difference between the formula 1 – with highest binding capacity and three of four of the other formulas (t-test, $p < 0.001$).

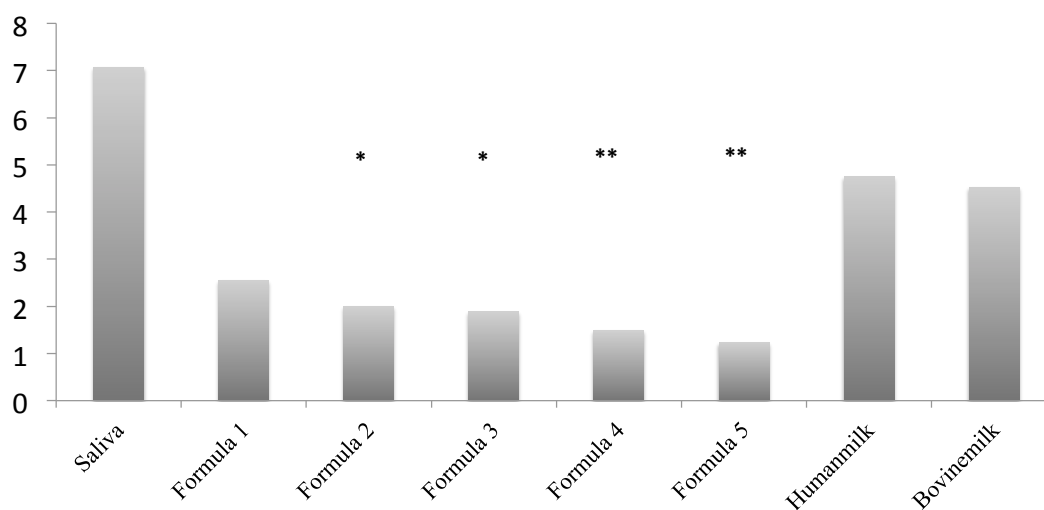


Figure 1b. Binding capacity for *L.gasseri* after addition of baby formula containing different amounts of lactoferrin and iron, showing a significant difference between the formula 1 – with highest binding capacity and all other formulas (t-test, $p < 0.05$ and $p \leq 0.001$)

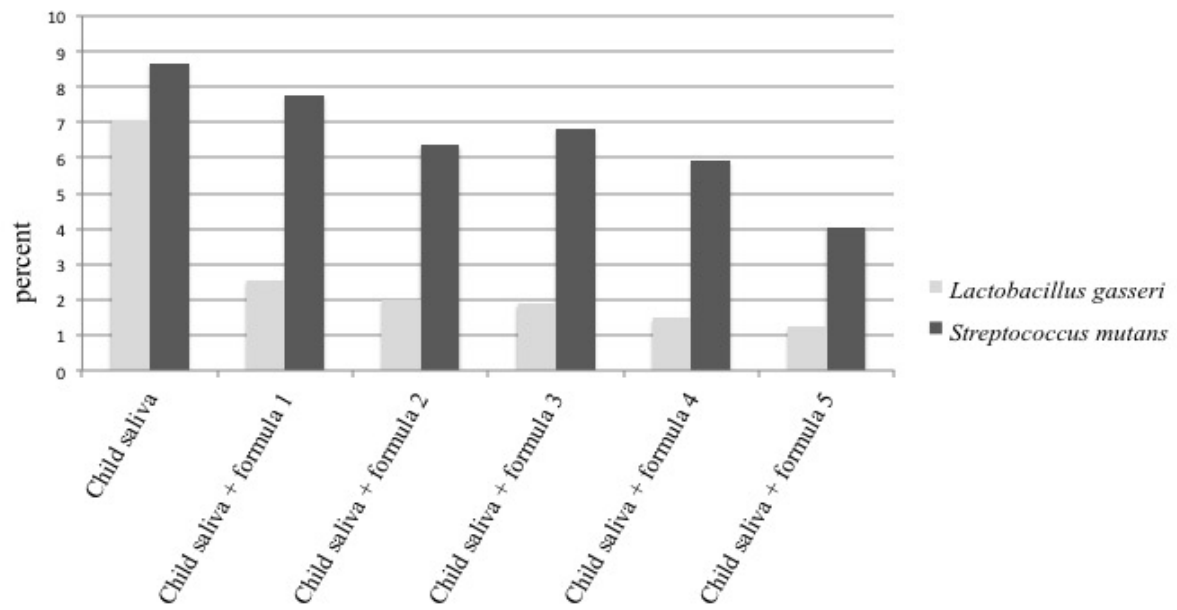


Figure 2. In percent, the number of adhering *Streptococcus mutans* and *Lactobacillus gasseri* to saliva coated hydroxyapatite beads covered in five different baby formulas.

Appendix 1

Informationsbrev till deltagare

Projekttitel: *Effect of baby formula with iron and lactoferrin on adhesion of oral bacteria to saliva coated hydroxyapatite.*

Hej, vi heter Elsa och Emma och är två studenter som läser termin 8 på tandläkarprogrammet vid Umeå universitet. Den här terminen kommer vi att påbörja vårt examensarbete som är en del i vår utbildning.

Arbetet genomförs som ett laborativt projekt. För att utföra projektet behövs saliv från barn i åldrarna 0-3 år. Insamlad saliv från alla deltagare blandas samman och analyseras med ett tandliknande material samt mjölkersättning. Målet är att kunna studera olika mjölkersättnings påverkan på några av de bakterier vi vet medverkar till att det blir hål i tänderna (karies).

Saliven samlas in med en specialutformad salivsug som lindrigt och smärtfritt samlar upp saliven. Saliven från ditt barn blandas med saliv från andra barn och kommer inte kunna härledas tillbaka till barnet. Insamlade data kommer att hanteras enligt personuppgiftslagen och biobankslagen. Resultatet kommer att presenteras muntligt samt skriftligt i ett examensarbete. Salivinsamlingen kommer ske i slutet av våren samt i början av hösten 2017.

Studieansvariga studenter vid institutionen för odontologi:

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HA-ASSAY # DATUM _____

	CCUG 31451			CCUG11877			CCUG31451			CCUG11877		
	1	2	3	4	5	6	7	8	9	10	11	12
A	ADH	ADH	ADH	ADH	ADH	ADH	Fettfri bröstmjök	Fettfri bröstmjök	Fettfri bröstmjök	Fettfri bröstmjök	Fettfri bröstmjök	Fettfri bröstmjök
B	JH parotis 1:1	JH parotis 1:1	JH parotis 1:1	JH parotis 1:1	JH parotis 1:1	JH parotis 1:1	Lätt mjök	Lätt mjök	Lätt mjök	Lätt mjök	Lätt mjök	Lätt mjök
C	Barnsaliv 1:1	Barnsaliv 1:1	Barnsaliv 1:1	Barnsaliv 1:1	Barnsaliv 1:1	Barnsaliv 1:1	Caug 31451	Caug 31451	Caug 31451	Caug 11877	Caug 11877	Caug 11877
D	Välling 1 8663-075	Välling 1 8663-075	Välling 1 8663-075	Välling 1 8663-075	Välling 1 8663-075	Välling 1 8663-075						
E	Välling2 8663-367	Välling2 8663-367	Välling2 8663-367	Välling2 8663-367	Välling2 8663-367	Välling2 8663-367						
F	Välling3 8663-458	Välling3 8663-458	Välling3 8663-458	Välling3 8663-458	Välling3 8663-458	Välling3 8663-458						
G	Välling4 8663-506	Välling4 8663-506	Välling4 8663-506	Välling4 8663-506	Välling4 8663-506	Välling4 8663-506						
H	Välling5 8663-700	Välling5 8663-700	Välling5 8663-700	Välling5 8663-700	Välling5 8663-700	Välling5 8663-700						