

IWC
PARIS
2008



5th International Whey Conference

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Application of antimicrobial proteins lactoferrin and lactoperoxidase for oral hygiene

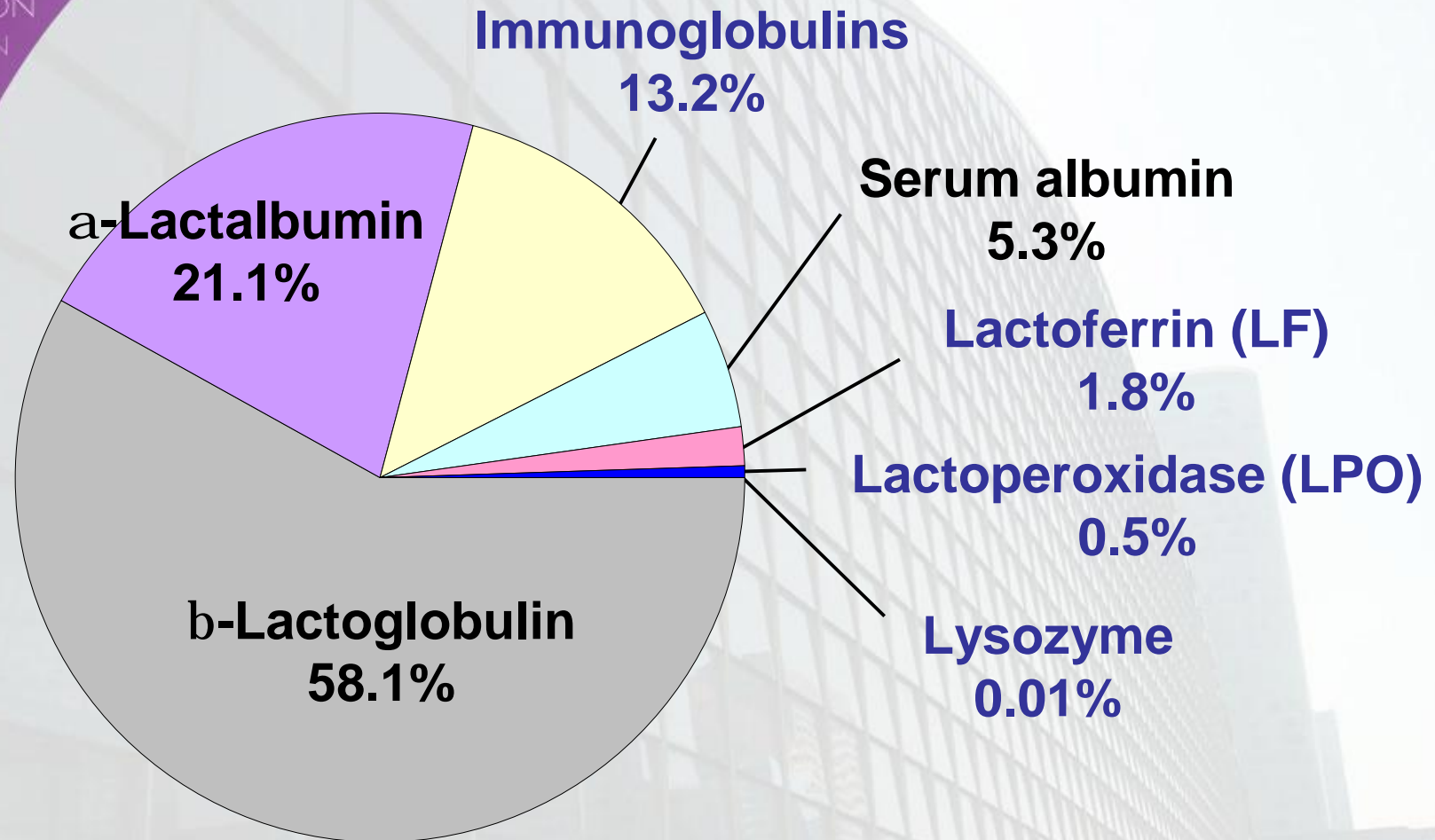
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Whey proteins in bovine milk





Distribution of antimicrobial components in milk and saliva

Protein	Concentration (mg/L)	
	Bovine milk	Human saliva
Immunoglobulins	500~1000	200
LF	100	9~24
LPO	30	2
Lysozyme	0.4	5~50



Oral hygiene

Practices to prevent oral problems such as:

- ~ Dental caries
- ~ **Periodontal disease**
- ~ **Oral malodor**

By the means of:

- ~ Tools; toothbrush, dental floss, tongue brush
- ~ Agents; toothpaste, mouthwash
- ~ Professional cleaning
- ~ **Functional foods**; sugar alcohols, fluoride, casein phosphopeptides



To study *in vitro* and *in vivo* effects of antimicrobial whey components:

- ~ **LF;**
Periodontal pathogens and disease
- ~ **A composition containing LPO;**
Oral bacteria and breath odor

Periodontal disease

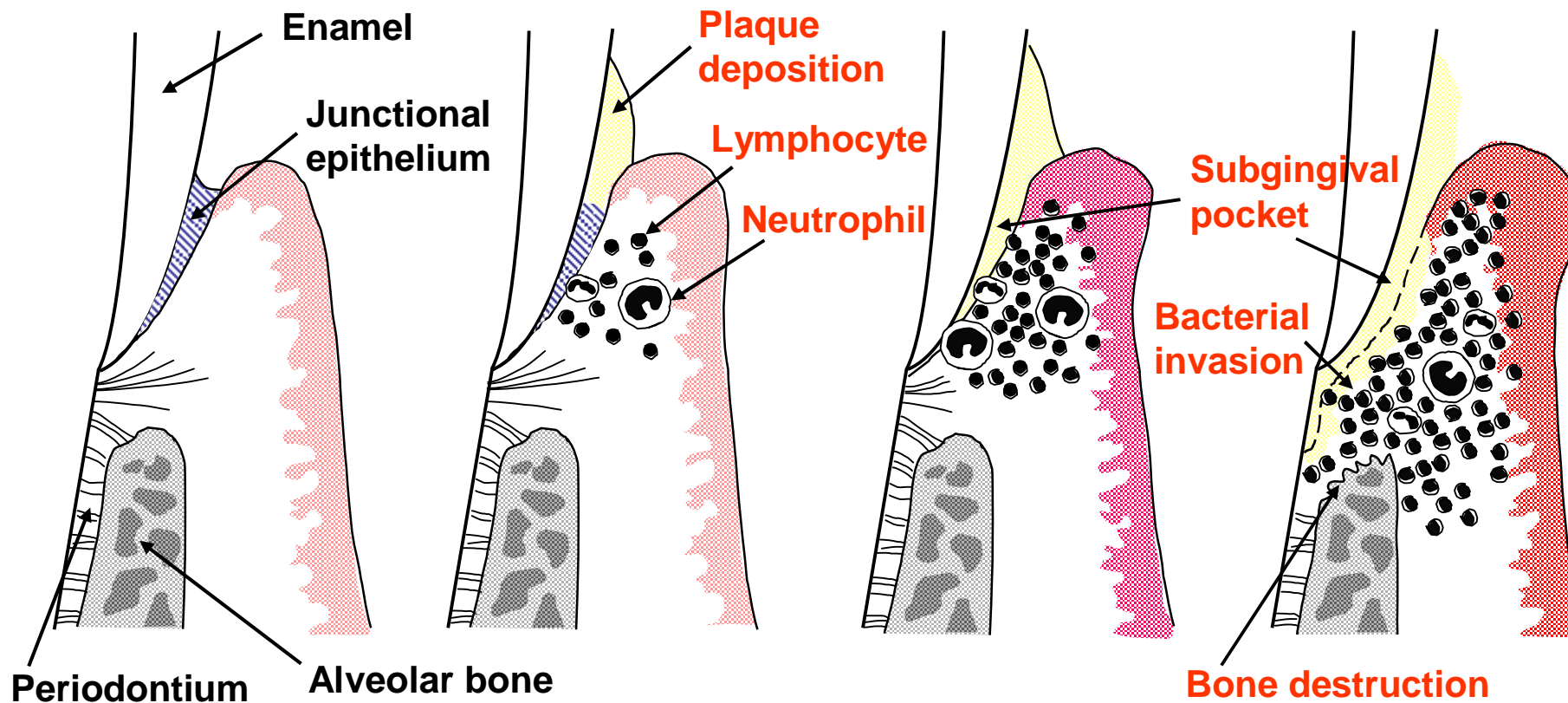
Normal
gingiva

Progression of Periodontitis

Weak

Mild

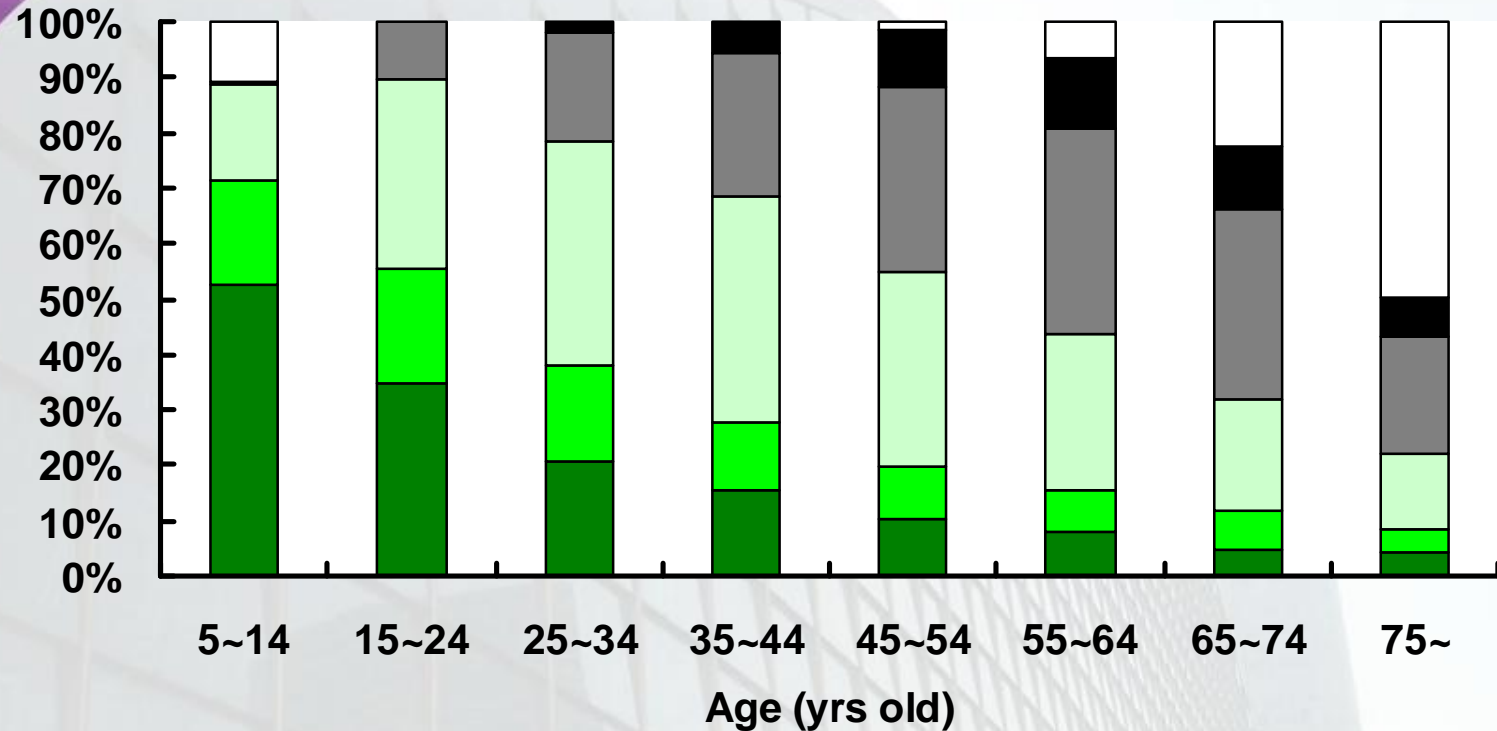
Severe





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Periodontal disease in Japan



- No tooth to diagnose
- Periodontal pockets ≥6 mm
- Periodontal pockets 4 to 5 mm
- Calculus deposition
- Gingival bleeding after probing
- No finding (healthy periodontal conditions)

Data from a survey by Ministry of Health, Labour and Welfare, 1999

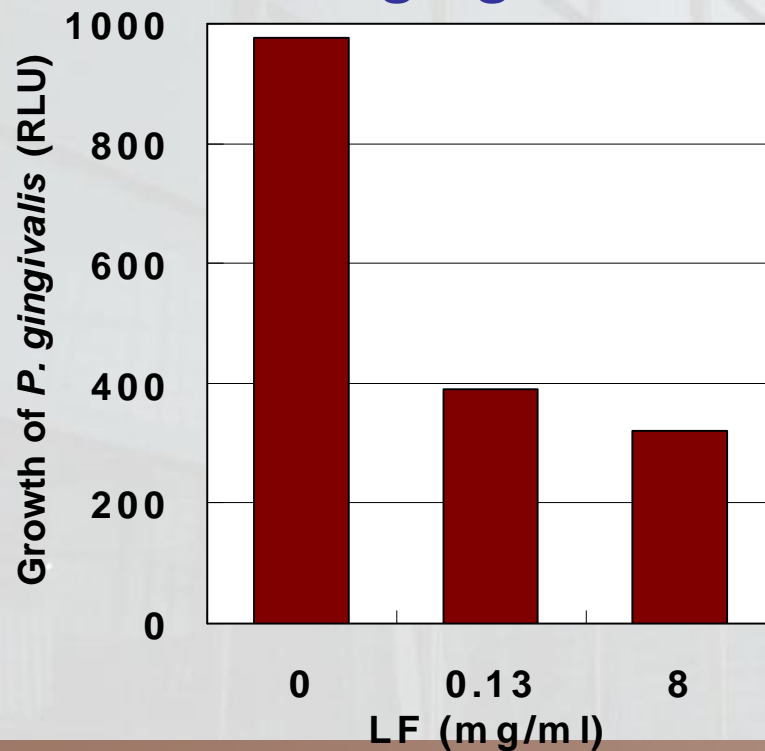


Antibacterial activity of LF

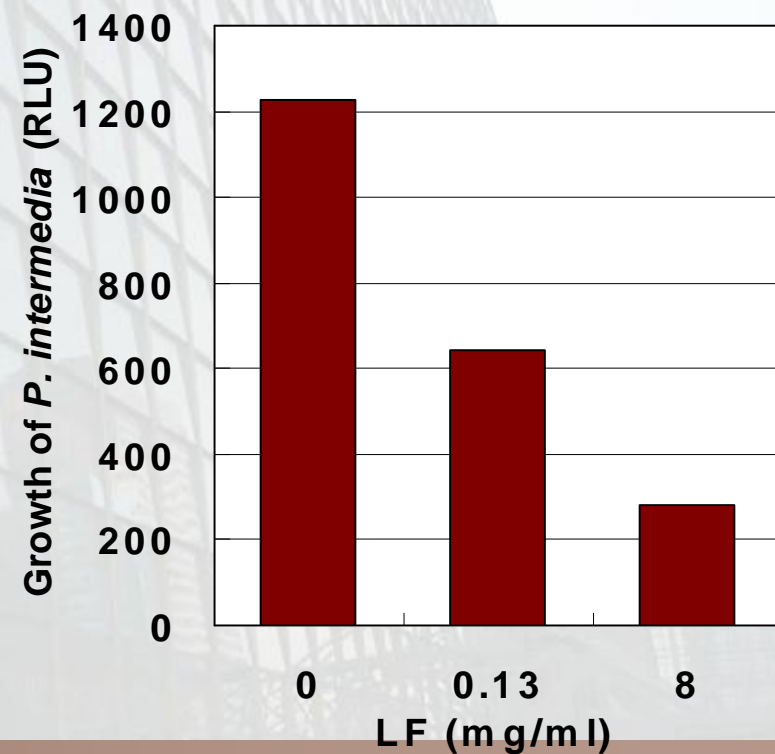
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- ~ Chelation of iron
- ~ Interaction with bacterial cell surface
- ~ Antimicrobial peptide (e.g. Lactoferricin®)
- ~ Synergistic action with secretory components

P. gingivalis



P. intermedia





Effects of LF on periodontitis

- ~ Randomized, double-blind, placebo-controlled trial at Niigata University
- ~ Subjects: patients with mild periodontitis (two teeth with pocket depth of 4-5 mm)
- ~ 6 tablets daily administered for 3 m
 - è LF group (LF 1.8 g/d), n=8
 - è Placebo group, n=10
- ~ Real-time PCR: total bacteria, *P. gingivalis*, and *P. intermedia* in subgingival plaque
- ~ ELISA: human and bovine LF in gingival crevicular fluid (GCF)
- ~ Limulus test: lipopolysaccharide (LPS) in GCF

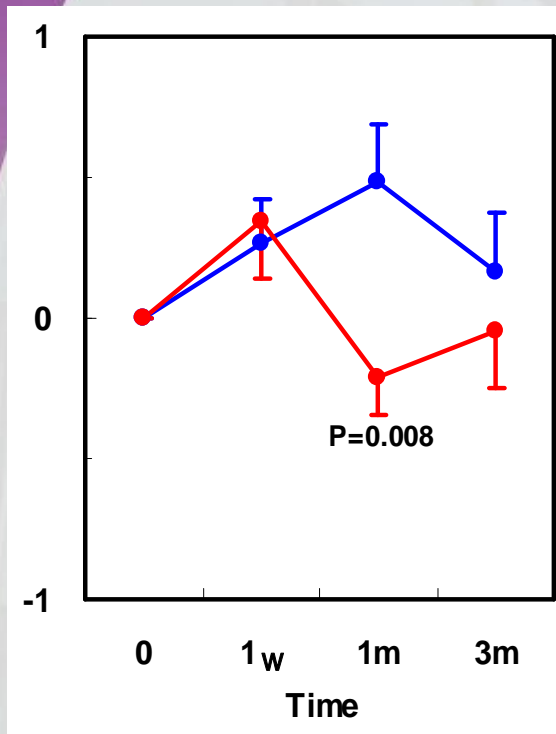


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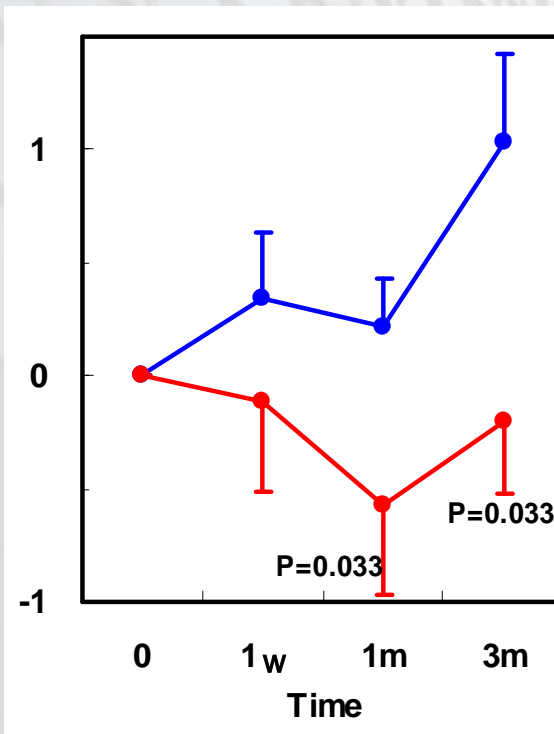
Bacterial number in subgingival plaque

Change of number in subgingival plaque
(log₁₀ copies/paper point)

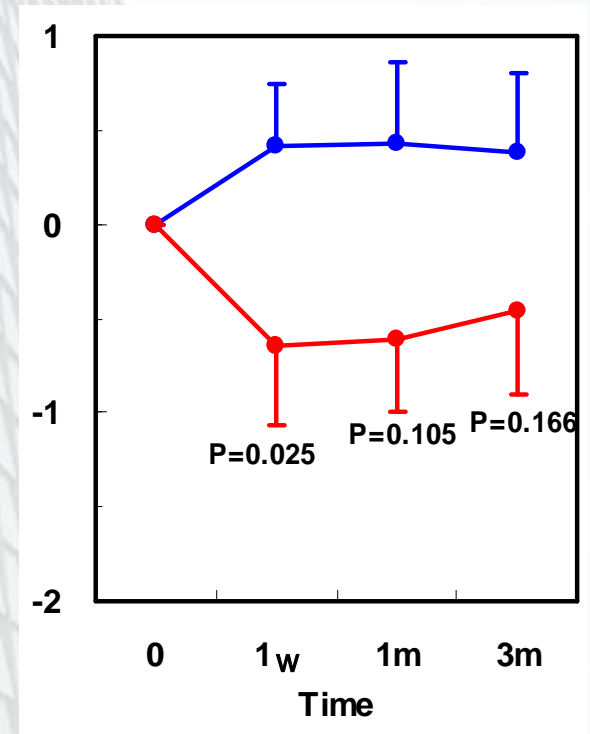
Total bacteria



P. gingivalis



P. intermedia



● Placebo ● LF



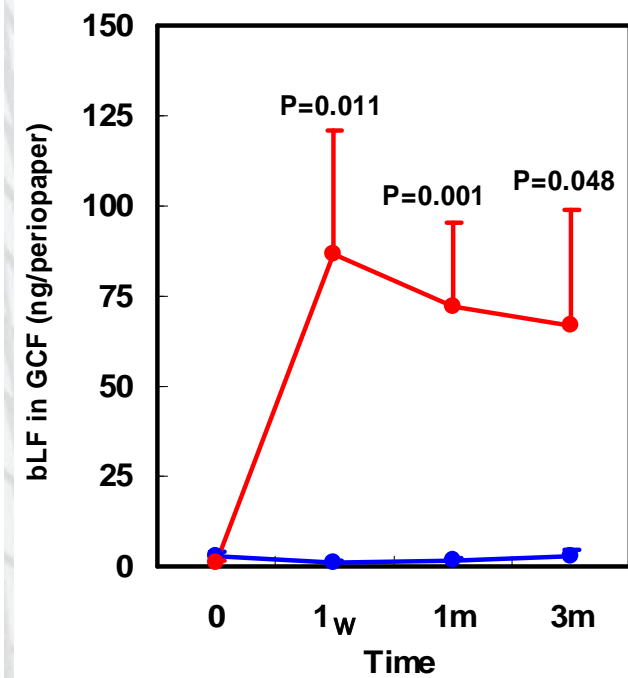
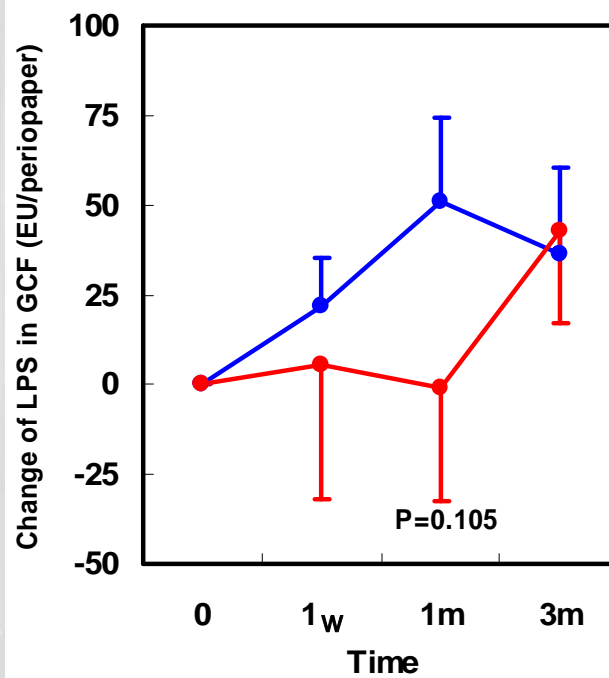
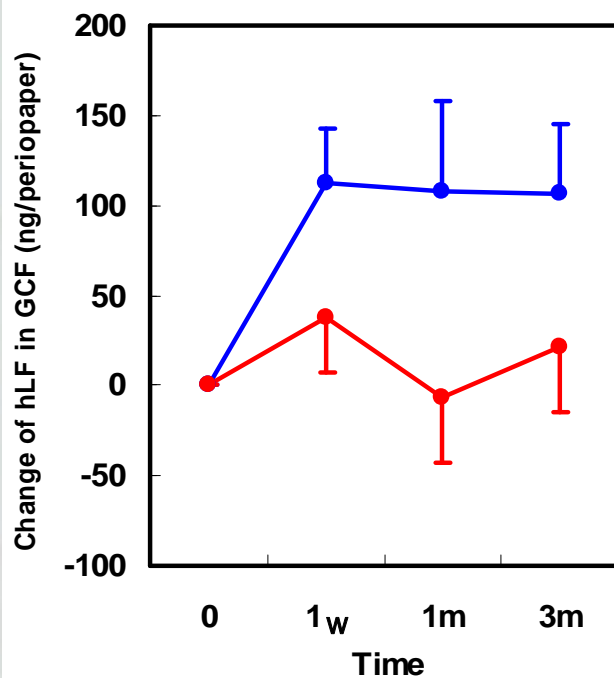
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Levels of LF and LPS in gingival crevicular fluid (GCF)

Human LF

LPS

Bovine LF

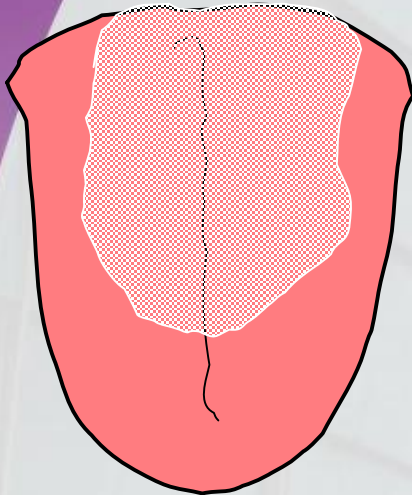


● Placebo ● LF



Mechanism of odor formation in the mouth

Tongue coating



Tongue dorsum

Oral epithelial cells

Protein degradation

Cysteine

Methionine

Hydrogen sulfide
(H_2S)

Methyl mercaptan
(CH_3SH)

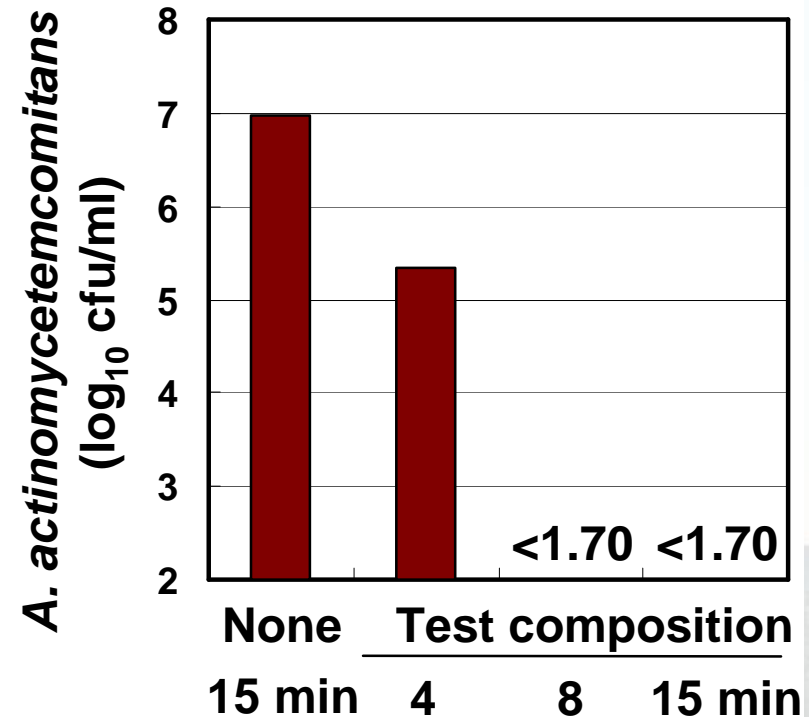
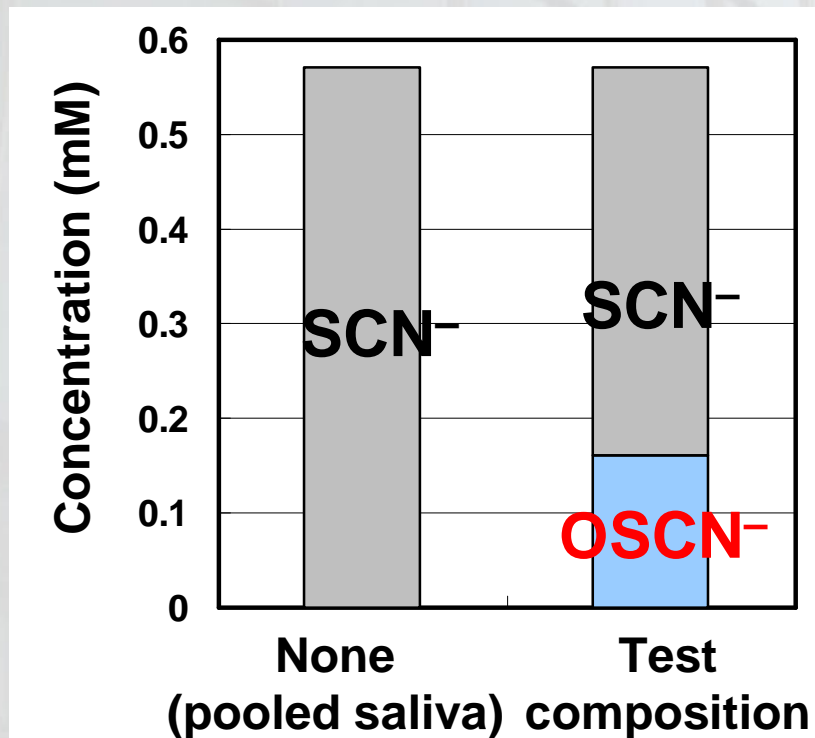
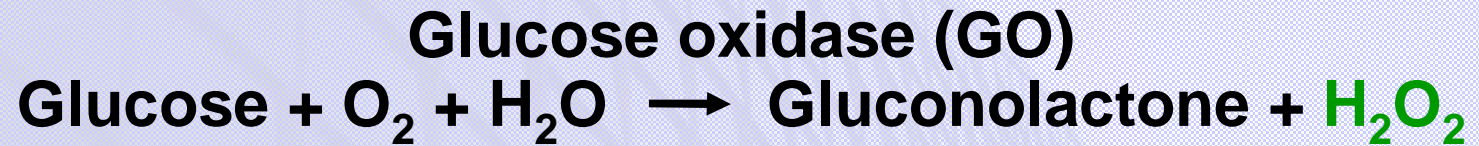
Volatile sulfur compounds (VSCs)

Bacterial proteases

Bacterial metabolisms



Antibacterial activity of test composition in saliva





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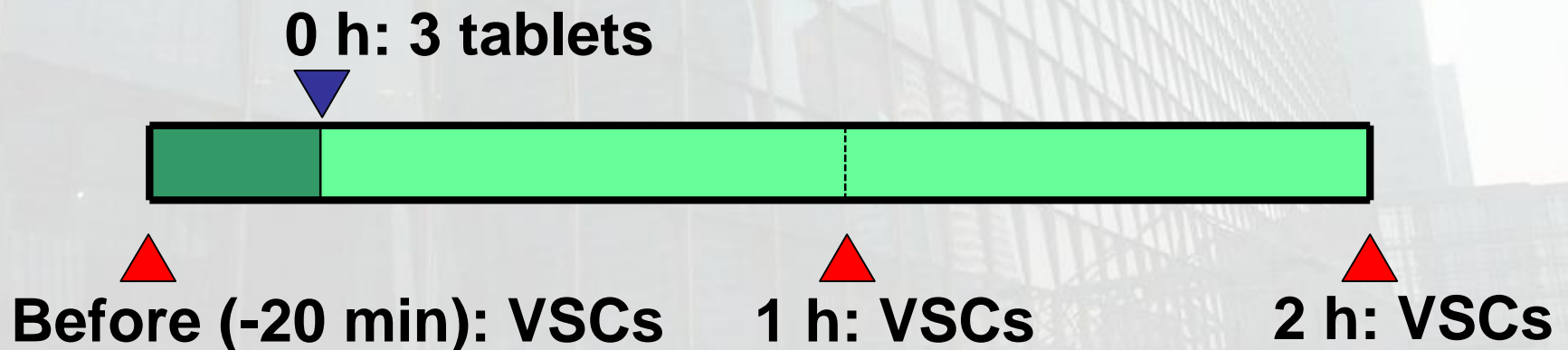
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Effects of test composition on breath odor

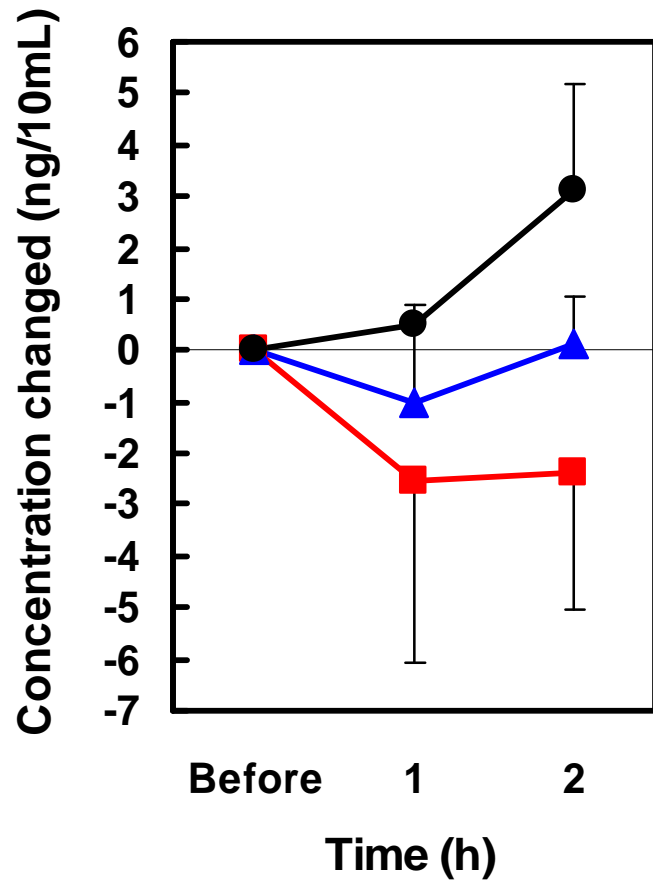
- ~ Subjects: 3 healthy adults refrained from oral cleaning, eating and drinking in the morning
- ~ Test tablet: 0.6 g of test composition containing LPO, GO, glucose and buffer salts
- ~ Control tablet: without LPO and GO
- ~ Compact gas chromatography: VSCs in oral air



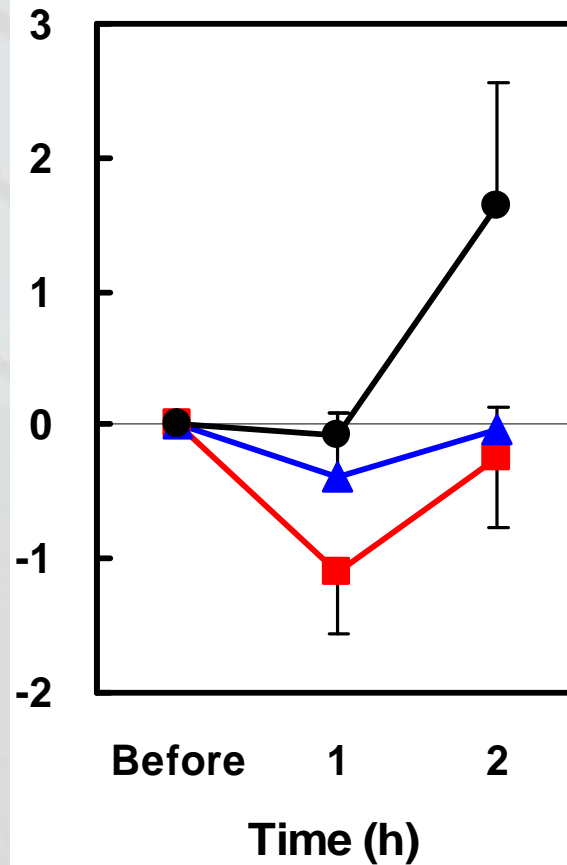


Levels of breath odor

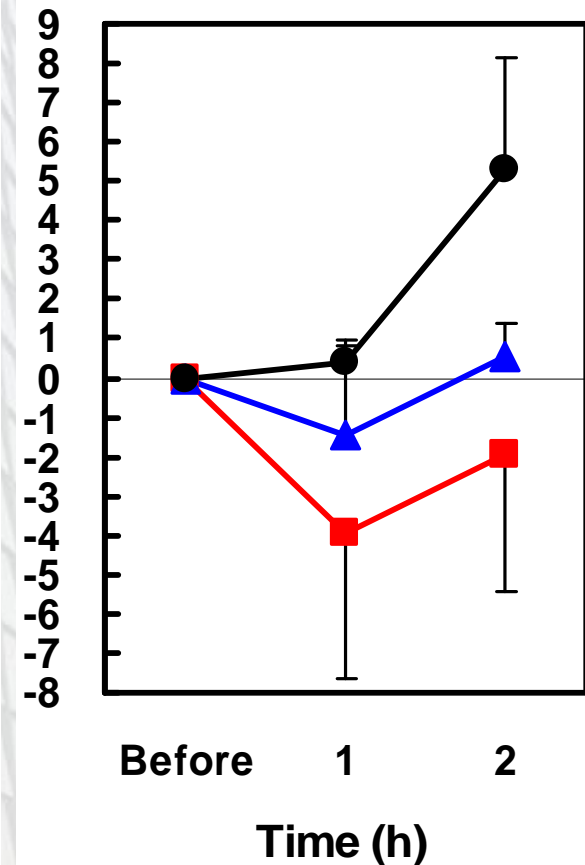
Hydrogen sulfide: H_2S



Methyl mercaptan: CH_3SH



Total VSCs: $H_2S+CH_3SH+(CH_3)_2S$



● Non-treatment

■ Test tablet

▲ Control tablet



Summary and conclusion

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- ~ **LF showed antimicrobial activity against periodontal pathogens *in vitro*.**
- ~ **In a clinical study, LF suppressed periodontal pathogens *in vivo*.**
- ~ **A composition containing LPO catalyzed the formation of OSCN– in saliva and showed bactericidal activity *in vitro*.**
- ~ **In a preliminary *in vivo* study, the composition reduced breath odor.**

These observations indicate the possibilities of LF and LPO as food ingredients for oral hygiene.