

# The Association between Free Glycogen in the Vaginal Fluid and Colonization by Lactobacilli



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## INTRODUCTION

- *L. crispatus* is the *Lactobacillus* most commonly isolated from both the vagina and rectum (1).
- Goh and Klaenhammer demonstrated in a mouse model that *L. acidophilus* strains having the capacity to produce intracellular glycogen had a competitive advantage for GI tract colonization (2).
- Mirmonsef et al. reported that women having a Nugent score of 0-6 had significantly higher levels of glycogen and lactate compared to women with BV (3). Further, free glycogen was associated with decreased vaginal pH (4).
- Lactobacilli can make both D-lactate and L-lactate but it differs by species (5):
  - *L. crispatus* produces both D- and L-lactate
  - *L. jensenii* produces D- but not L-lactate
  - *L. iners* produces only L-lactate

## OBJECTIVES

- Primary: To assess the association between free glycogen, D-lactate and L-lactate in cervicovaginal lavage (CVL) with vaginal microbiota (Nugent score, quantitative PCR and culture for lactobacilli).
- Secondary: To confirm the association between vaginal pH and free glycogen in the CVL.

## METHODS

**Study population:** Women (n=55) aged 18-45 without clinical BV, GC, CT or trichomoniasis

**Vaginal samples collected:**

- Dacron swab: pH
- Dacron swab: Nugent criteria
- Two Dacron swabs: Quantitative culture
- Flocked swab: qPCR for *L. crispatus* and *L. iners*.
- 10-mL CVL sample: Collected after all swabs.

**pH:** Swab is rolled onto pH strip having 3.6-6.1 range.

**Nugent:** Swab rolled on slide and Gram stained.

**Culture methods:**

- Columbia agar w/ 5% sheep blood and human blood bilayer Tween agar, 37°C, 5-6% CO<sub>2</sub>, 48 hrs.
- Rogosa SL agar, 37°C, anaerobic chamber, 72 hrs.
- *Lactobacillus* identification: Repetitive-sequence PCR DNA fingerprinting, 16S HpyCH4V restriction digest or sequencing.

**qPCR Methods:**

- Bacterial DNA extracted with QIAamp DNA Mini Kit (Qiagen), combined with species-specific primers and SYBR green binding dye for detection of gene copies per swab.

**Measurement of glycogen, lactate, and protein:**

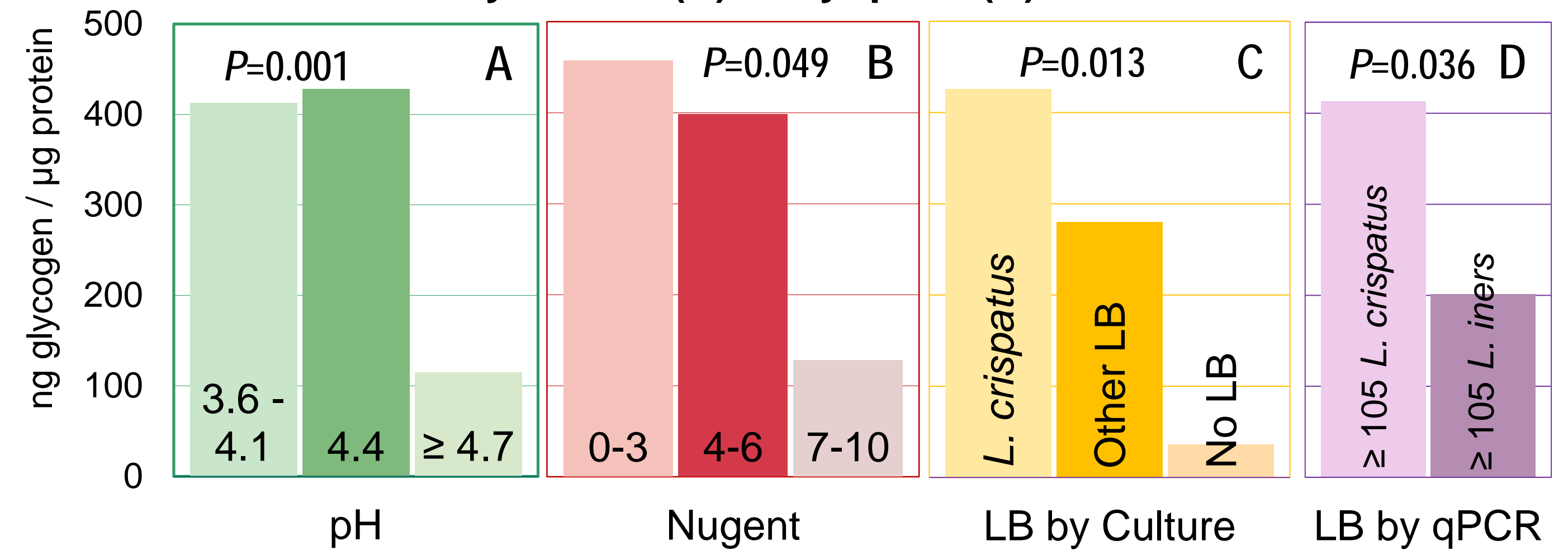
- Glycogen, D-lactate and L-lactate were measured using fluorometric assays (BioVision).
- All were standardized to protein levels in the CVL using Lowry assay.

**Statistical methods:** Differences in median levels of glycogen, D-lactate and L-lactate were evaluated using the Kruskal-Wallis test.

## RESULTS

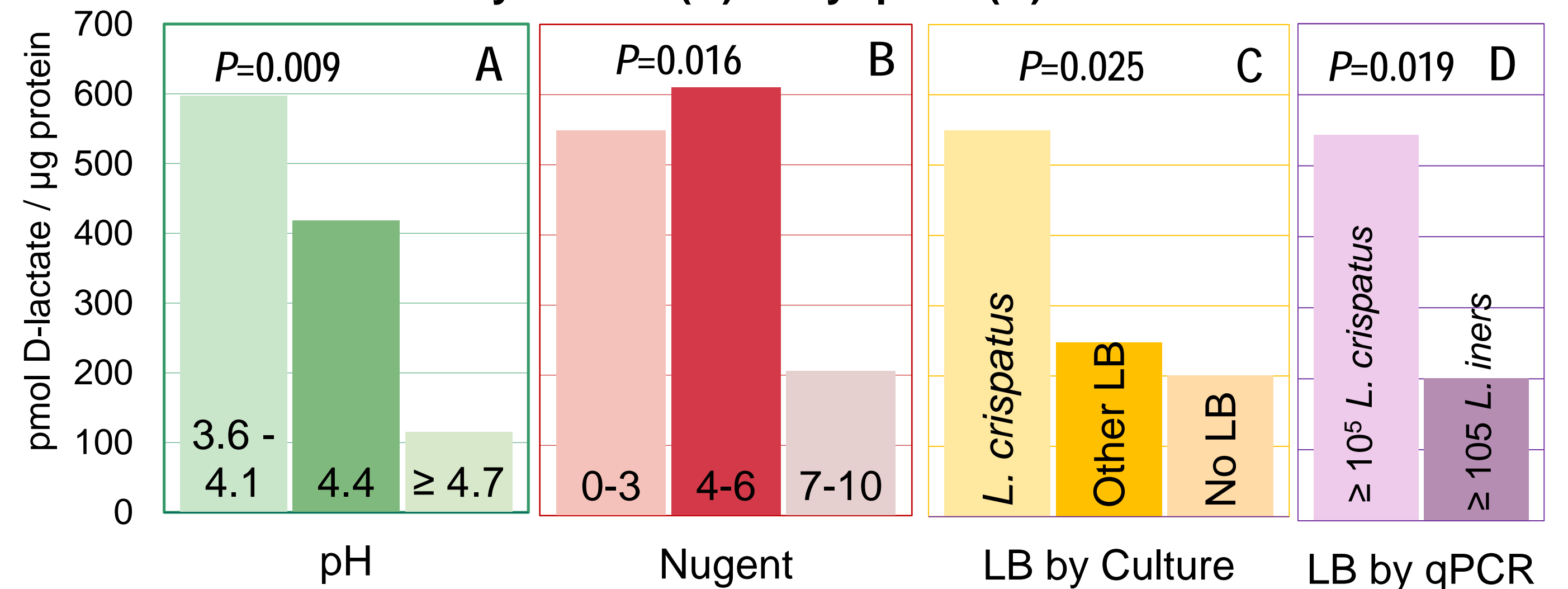
- **Study population (n=55):** Young (median age of 25.0), 54.5% white, 52.7% unmarried, and 49.1% currently sexually active.

**Figure 1. Association of free glycogen with pH (A), Nugent Score (B), and *Lactobacillus* detected by culture (C) or by qPCR (D). Median levels are shown.**



- Free glycogen in CVL was positively associated with lower pH, lower Nugent score, and *L. crispatus* detected by culture or qPCR (Fig 1).

**Figure 2. Association of D-lactate with pH (A), Nugent Score (B), and *Lactobacillus* detected by culture (C) or by qPCR (D). Median levels are shown.**



- D-Lactate in CVL was positively associated with lower pH, lower Nugent score, and *L. crispatus* detected by culture or by qPCR (Fig 2).
- L-Lactate was not associated with lower pH ( $P=0.70$ ), lower Nugent score ( $P=0.32$ ) or *L. crispatus* by qPCR ( $P=0.23$ ). L-Lactate was positively associated with colonization by other *Lactobacillus* species ( $P=0.04$ ).
- Table 1 presents the concentration of D- and L-lactate among the 39 women having a predominant *Lactobacillus* species by culture ( $\geq 1$  log greater than other species).

**Table 1. Association of D- and L-lactate median levels with *Lactobacillus* spp.**

Predominant <i>Lactobacillus</i> species	D-Lactate (pmol per μg protein)	L-Lactate (pmol per μg protein)
<i>L. crispatus</i> (n=21)	607	541
<i>L. jensenii</i> (n=8)	399	679
<i>L. iners</i> (n=6)	177	994
No or Other LB (n=4)	203	276
<b>P-value</b>	<b>0.028</b>	<b>0.06</b>

## CONCLUSIONS

- Although *L. crispatus* and *L. jensenii* have been previously reported to be associated with high levels of free glycogen (4), we found that *L. crispatus* dominant flora is also associated with the highest concentration of D-lactate.
- D-Lactate is associated with more markers of vaginal health than L-lactate.
- This work confirms previous publications about the association of free glycogen with lower pH and Nugent score.
- It is unknown whether glycogen enhances *L. crispatus* colonization, or whether *L. crispatus* synthesizes glycogen, increasing the glycogen content.

## ACKNOWLEDGEMENTS

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