

Production of c9,t11- and t10,c12-conjugated Linoleic Acids in Humans by *Lactobacillus rhamnosus* PL60

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***Lactobacillus rhamnosus* PL60 was tested for whether it can produce c9,t11- and t10,c12-conjugated linoleic acids (CLAs) in human. After consumption of *L. rhamnosus* PL60, *L. rhamnosus* was detected in feces 1 week after the start of intake. Analysis by gas chromatography showed that concentrations of c9,t11- and t10,c12-CLAs in serum had increased and concentrations of serum leptin had significantly decreased. Results showed that *L. rhamnosus* PL60 can survive in human intestines and produce CLAs in humans. This is the first report that bacteria can produce CLAs in humans.**

Keywords: Conjugated linoleic acid, *Lactobacillus rhamnosus*, anti-obesity

Conjugated linoleic acid (CLA) is the generic name of a mixture of linoleic acid isomers that have conjugated double bonds. Many animal studies have reported various effects of CLAs, such as anticarcinogenic and antiatherogenic activities, reduction of the catabolic effects of immune stimulation, and reduction of body fat [6, 10]. Among the isomers of CLA, c9,t11-CLA shows anticancer activity [1] and t10,c12-CLA exerts anti-obesity effects [4, 7]. Although many researchers have doubted the latter, several reports support a role for the isomer in weight control, including a recent meta-analysis showing that a dose of 3.2 g CLA/d modestly reduces fat mass in humans [11]. CLAs in animal milk and meat are believed to be formed by rumen microbiota that incompletely biohydrogenate the unsaturated fatty acid, linoleic acid. Since the discovery that *Butyrivibrio fibrisolvens* produces CLA [2], various bacteria, mainly lactic acid-producing bacteria (LAB), have been reported to produce CLAs [5]. If CLA-producing LAB survive and colonize the human intestinal mucosa,

they can continuously produce CLAs that exert various beneficial effects on human health.

Recently, we showed that *Lactobacillus rhamnosus* PL60 (PL60) produced c9,t11- and t10,c12-CLAs *in vitro* as well as *in vivo* and decreased the leptin levels, body weight, and white adipose tissue content of mice [3]. In the current study, we tested whether PL60 could survive in human intestines long enough to produce CLA isomers and decrease leptin levels as observed *in vivo* experiment with mice.

Four volunteers consumed 1 g of freeze-dried PL60 (10^{12} colony forming units per gram) once daily for 21 days. Feces and blood were obtained on days 0 (baseline), 7, 14, and 21 of intake, and 7 days after discontinuation. Serum was prepared as described previously [3].

Feces were inoculated on MRS medium (Difco, Detroit, MI, U.S.A.) containing 0.002% bromophenol blue and 30 µg/ml vancomycin to exclude *Enterococcus*. Small white colonies with the characteristics of PL60 appeared on the media. They were collected and identified by multiplex polymerase chain reaction (PCR) performed using group- and species-specific primers (Lu-5, 5'-CTAGCGGGTGCGA CTTTG-3'; Rha II, 5'-GCGATGCGAATTTCTATTATT-3') designed by other investigators [9]. The PCR comprised 30 cycles of denaturation at 95°C for 20 sec, annealing at 62°C for 2 min, and polymerization at 74°C for 2 min. CLAs were prepared as described previously [3]. Gas chromatography (GC) was carried out by Lab-Frontier (Anyang, Kyunggi, Korea) on a Agilent 6890 GC/FID (Kyoto, Japan) instrument equipped with a DB-FFAP capillary column (30 m×0.25 i.d.; film thickness, 0.25 µm). CLAs were identified by comparing retention times with those of c9,t11- and t10,c12-CLA (controls). Its identification was confirmed again by mixing the sample and CLA controls in 1:1 (v/v), and measuring the increase in area of c9,t11- and t10,c12-CLA peaks. Leptin was assayed with a sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, U.S.A.).

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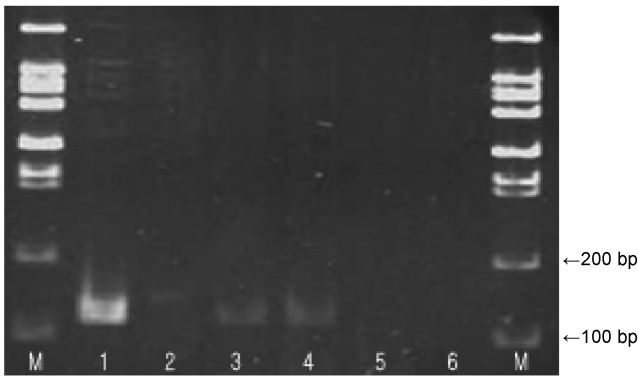


Fig. 1. Multiplex PCR assay. M, 100-bp marker; 1, *Lactobacillus rhamnosus* PL60; 2, 0th week; 3, 1st week; 4, 3rd week; 5, 1 week after stop; 6, negative control.

Small white colonies appeared 1 week after consumption of PL60 and disappeared 1 week after discontinuation. PCR with species-specific primers for *L. rhamnosus* was used to detect 113-bp DNA fragments extracted from the colonies (Fig. 1). Consumption of PL60 increased the

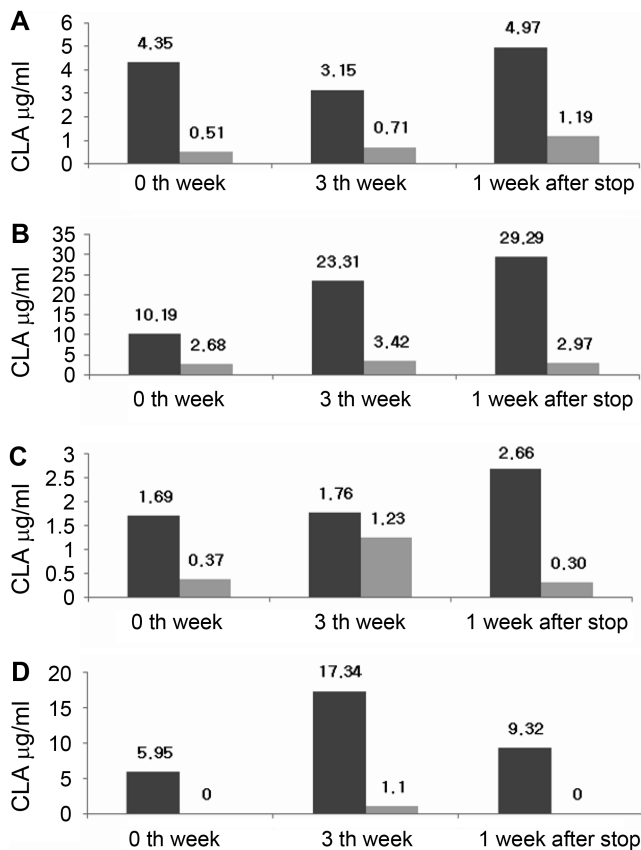


Fig. 2. Conjugated linoleic acid (CLA) in blood after consumption of PL60. **A.** A 51-year-old female with body mass index (BMI)=22; **B.** A 50-year-old female with BMI=23; **C.** A 37-year-old female with BMI=20; **D.** A 26-year-old male with BMI=23.4. ■, c9,t11-CLA; ■, t10,c12-CLA.

levels of both c9,t11- and t10,c12-CLAs over baseline in the blood of all four volunteers (Fig. 2). Concentrations of c9,t11-CLA were 1.69 to 10.19 µg/ml on day 0 (baseline) and 1.76 to 23.31 µg/ml on day 21. Concentrations of c10,t12-CLA were 0 to 2.68 µg/ml at on day 0 (baseline) and 0.71 to 3.42 µg/ml on day 21. Even 1 week after discontinuing consumption, the concentrations of both CLAs were higher than those measured at baseline. The difference in the CLA concentrations in each people must be due to the survival/attachment rate of PL60 and the efficiency of CLA production in each person.

The concentration of leptin significantly decreased in all four volunteers (Fig. 3). Leptin concentrations after 3 weeks of PL60 consumption were 5.8%, 26.8%, 24.9%, and 19.8% in volunteers A, B, C, and D, respectively. Concentrations dropped even further at 1 week after discontinuation: 6.4%, 30.7%, 33%, and 19.7% in volunteers A, B, C, and D, respectively. In this study, the individuals

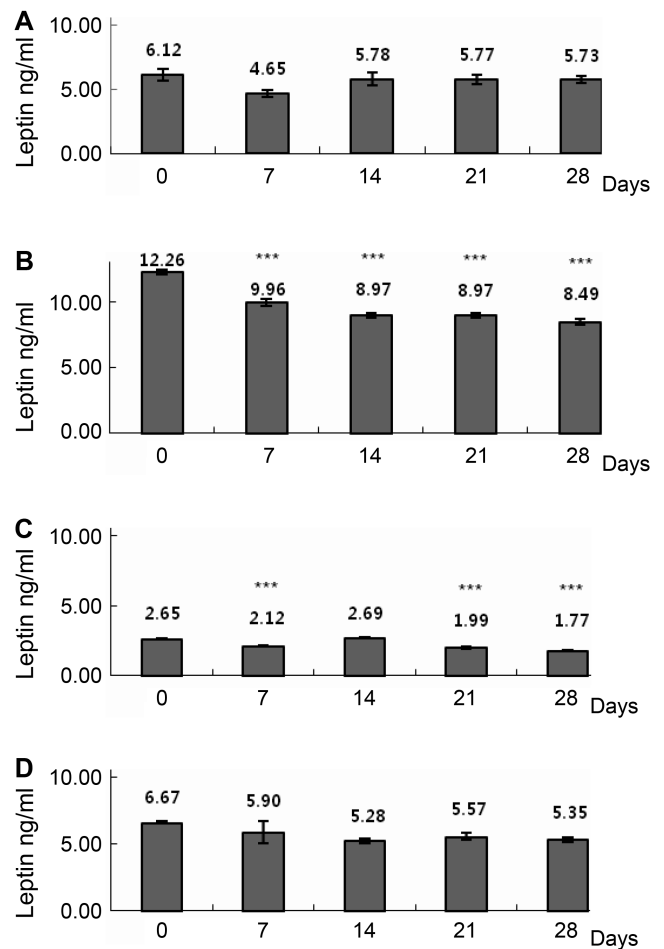


Fig. 3. Leptin concentrations in blood after consumption of PL60. **A.** A 51-year-old female with body mass index (BMI)=22; **B.** A 50-year-old female with BMI=23; **C.** A 37-year-old female with BMI=20; **D.** A 26-year-old male with BMI=23.4.

with increased CLA showed decreased leptin concentration whereas those with no change in CLA production showed very little change in leptin concentration. This result showed that an increase in CLA is related with the decreased leptin concentration. This observation is the same as that of another report that the CLA concentration is reversely related with leptin concentration [8].

This is the first study in humans to show that externally supplied LAB can survive in the intestine and produce detectable levels of CLAs that are similar to those achieved after consumption of the acids themselves. These results might explain the health and longevity of people who have a diet rich in LABs. Moreover, this study holds promise for the suppression of obesity and cancer by oral consumption of CLA-producing LAB.

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