



Lovastatin Lactone Inhibits Methane Production in Human Stool Homogenates



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BACKGROUND

Research has linked an altered gut microbiome to symptoms of irritable bowel syndrome (IBS). Evidence suggests that methane and colonization with *Methanobrevibacter smithii* (*M. smithii*) may be important in the pathogenesis of constipation and constipation-predominant irritable bowel syndrome (C-IBS). The degree of constipation has been shown to be proportional to breath methane levels and is significantly improved with antibiotic elimination of methane. Recent data suggests certain HMG-CoA reductase inhibitors (statins) may reduce methane production by inhibiting methanogenesis

AIM

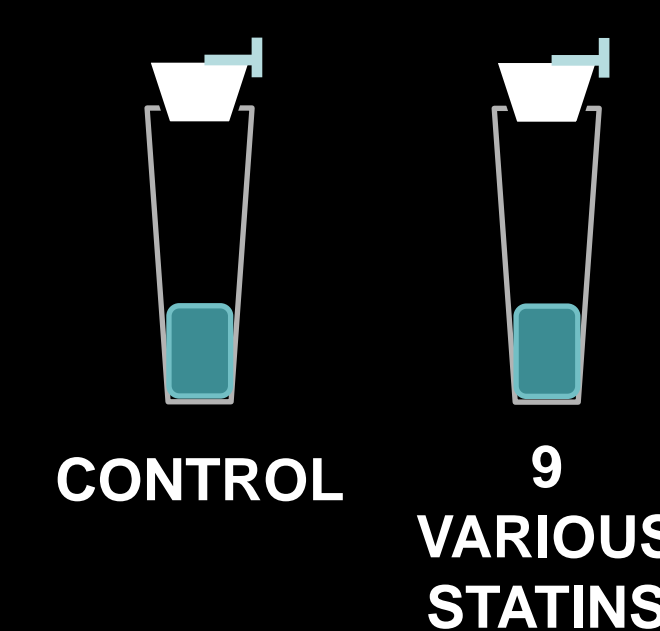
This study examines the effects of various statins on methane production in fresh human stool homogenates.

METHODS

- I. Five female subjects recruited based on high breath methane production (AVG = 69.6 ppm) provided a total of 8 fresh stool samples each.
- II. Samples were homogenized under anaerobic conditions @ 37° C in 1XPBS (3ml PBS/2g stool) and divided into stoppered flasks.
- III. **Assessment 1:** Head gas withdrawn through a stopcock was analyzed on a Quintron Model SC gas chromatograph for methane levels; at baseline, then every 30min for 270min
- IV. Nine statins were initially assessed for methane inhibition @ 5mg/g stool; lovastatin lactone & hydroxyacid, pravastatin lactone & hydroxyacid, atorvastatin lactone & hydroxyacid, simvastatin lactone, mevastatin lactone, and rosuvastatin lactone.
- V. **Assessment 2:** Upon determining lovastatin lactone as the ideal statin, concentrations of 0.04, 0.12, 0.48, 1, 5, and 10 mg/g stool were assessed; at baseline, then every 90min up to 720min.
- VI. **Assessment 3:** Final assessment was performed comparing three forms of lovastatin; lactone, diol, and hydroxyacid.

Study Design:

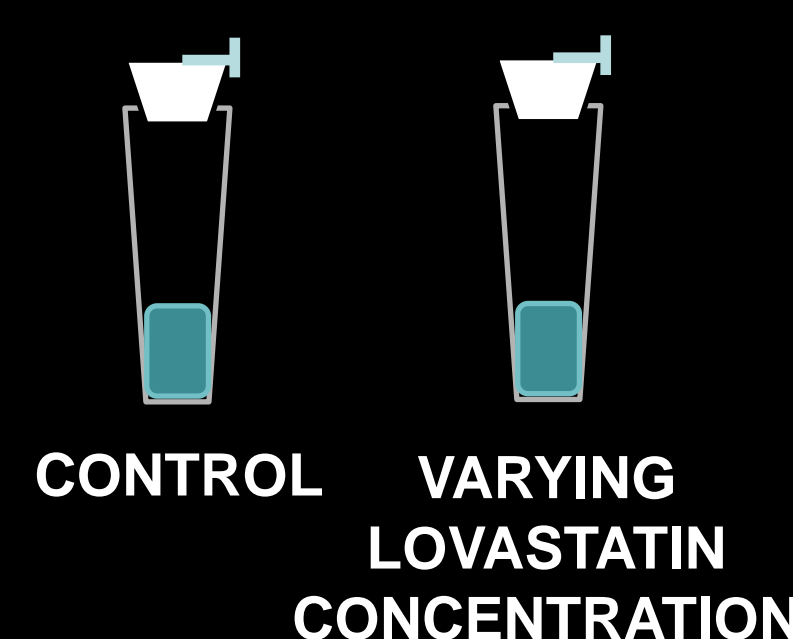
Assessment 1:



TEST PARAMETERS:

- Anaerobic
- 37° C
- 300 mL flasks
- Closed stopcocks
- 3ml 1XPBS/2g stool
- Statin: 5mg/g stool
- Readings : Baseline, 90min., 180, 270, Overnight

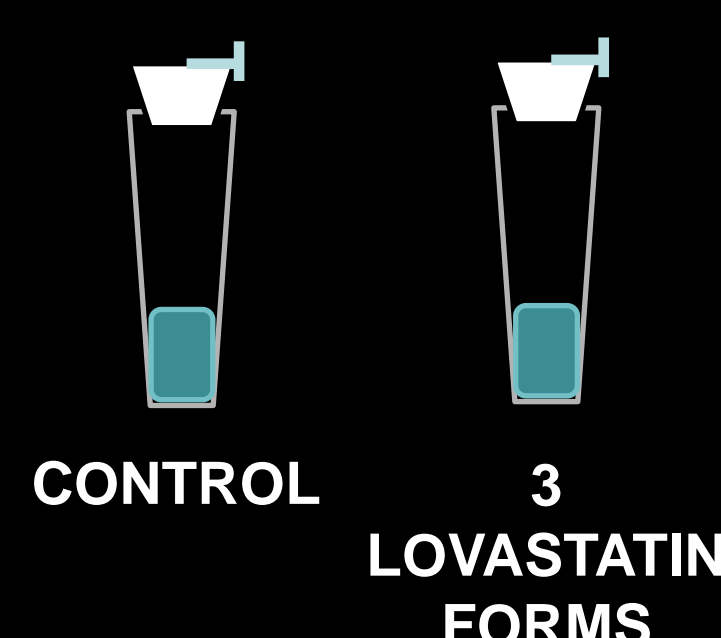
Assessment 2:



TEST PARAMETERS:

- Anaerobic
- 37° C
- 300 mL flasks
- Closed stopcocks
- 3ml 1XPBS/2g stool
- Lovastatin lactone: 0.04, 0.12, 0.48, 1, 5, 10 mg/g stool
- Readings : Baseline, 90min., 180, 270, Overnight

Assessment 3:



TEST PARAMETERS:

- Anaerobic
- 37° C
- 300 mL flasks
- Closed stopcocks
- 3ml 1XPBS/2g stool
- Lovastatin 5 mg/g stool; lactone, diol & hydroxyacid
- Readings : Baseline, 90min., 180, 270, Overnight

RESULTS

Lovastatin lactone was identified as the only effective methane inhibitor, significantly inhibiting methane levels by -65% of the control stool (Figure 1). Lovastatin lactone at 5mg/g produced the maximum inhibiting effect, resulting in an average methane level of 3% of the control over time (Figure 2). In a final validation comparison of 3 forms of lovastatin (5mg/g), both the lactone and diol forms proved to be effective (Figure 3). In all assessments, hydroxyacid forms were least able to inhibit methane production.

Figure 1: Change in methane gas levels in different statin drugs compared to a control from 0-270 min

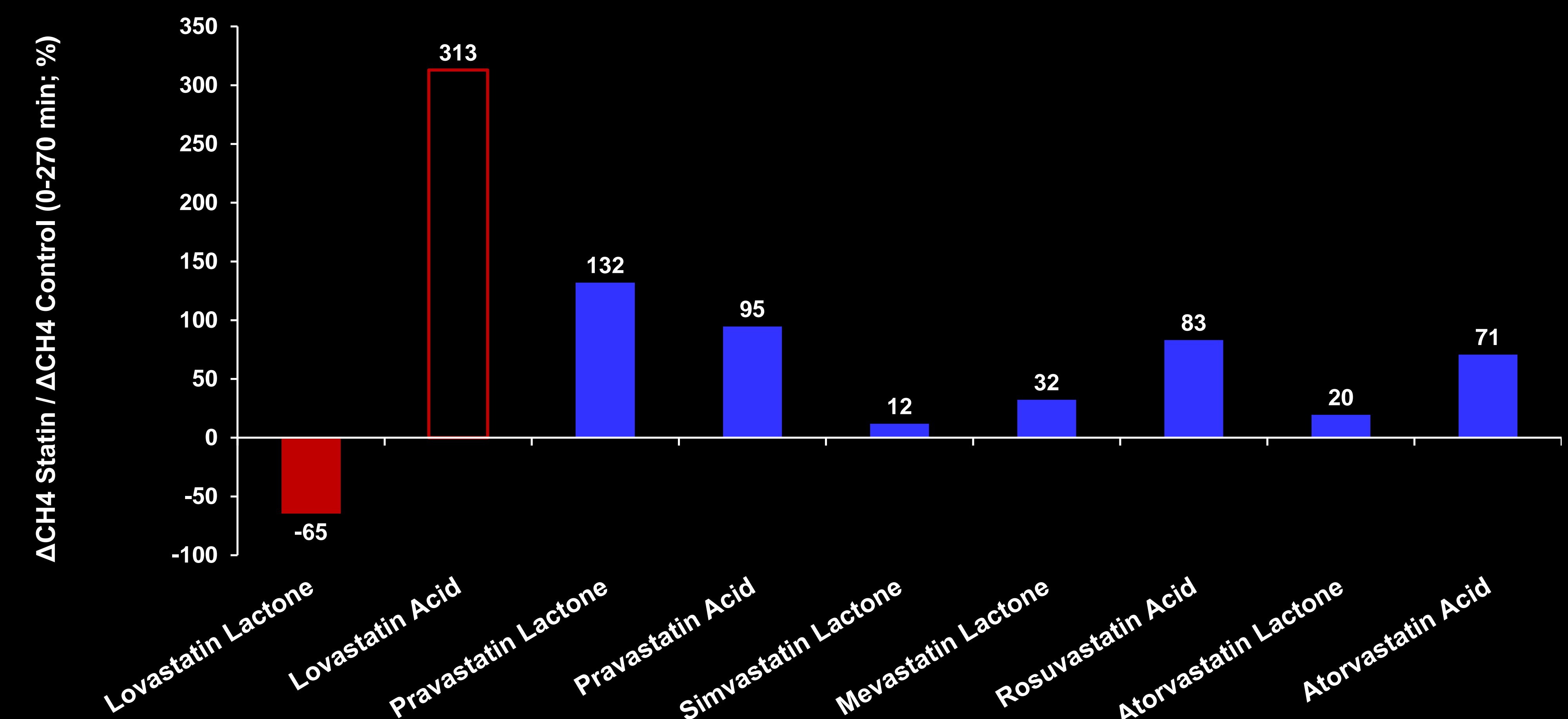


Figure 2: Lovastatin lactone dosage effect on methane levels over time

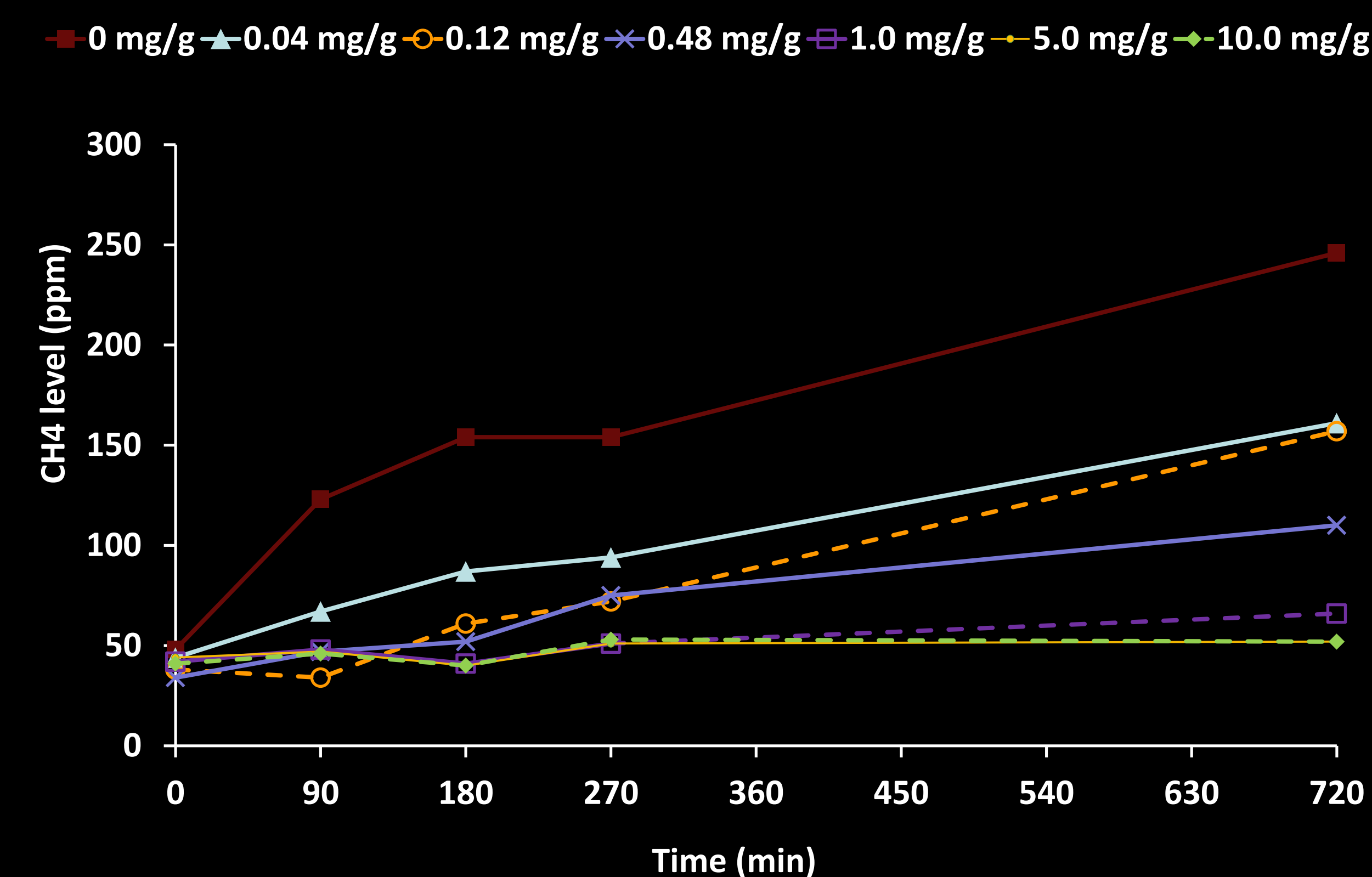
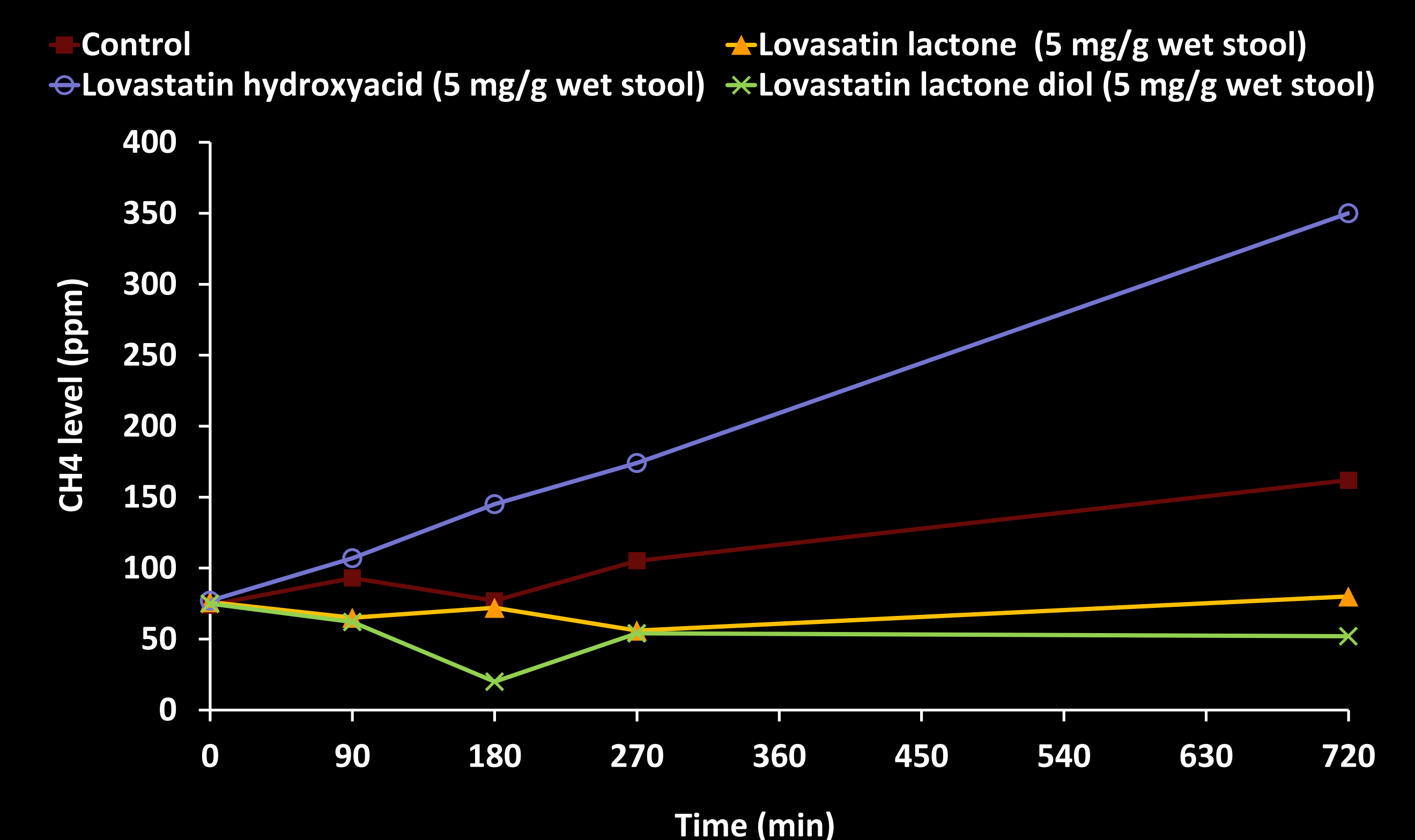


Figure 3: Comparison of lovastatin forms on methane levels over time



CONCLUSIONS

Lovastatin lactone and lovastatin diol at concentrations of 5mg/g stool significantly reduce methane production in human stool homogenates. Clinical studies are ongoing to assess the effect of this statin on methane production and IBS-C symptoms in humans.

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