SUPPLEMENTARY DATA

# Hypothalamic and striatal insulin action suppresses endogenous glucose production and may stimulate glucose uptake during hyperinsulinemia in lean but not in overweight men

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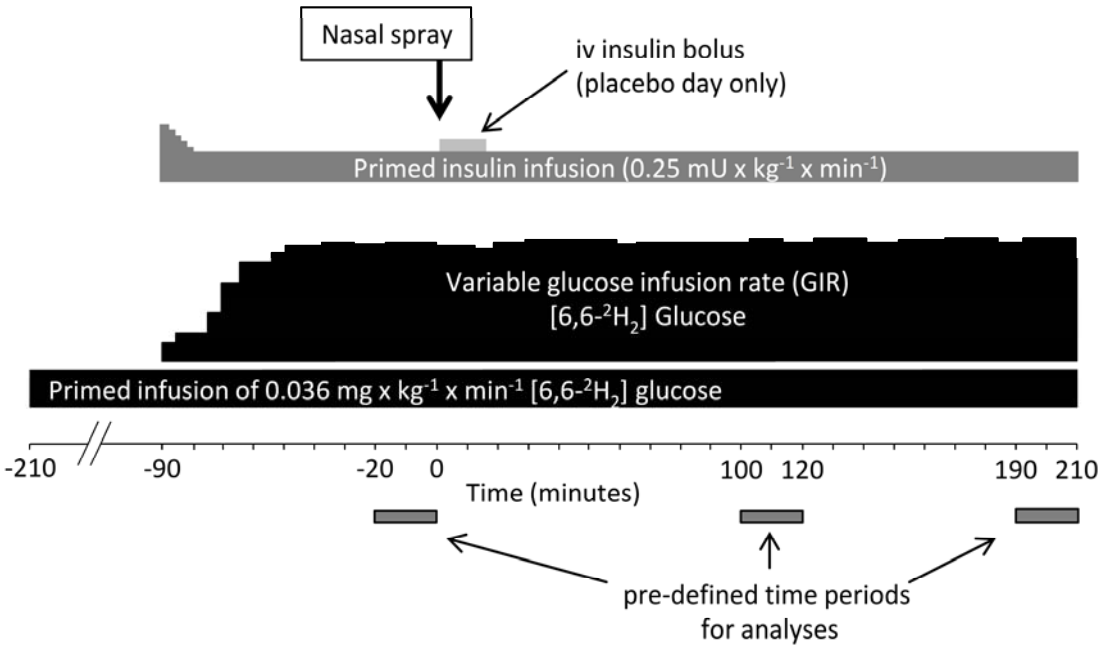
**Supplementary Figure 1. Outline of the hyperinsulinemic euglycemic clamp experiments.**
After the participants had fasted overnight, experiments commenced at t=-210 minutes with a primed continuous infusion of [6,6-$^2H_2$]-labeled glucose which was maintained throughout the experiment. At t=-90 minutes, primed insulin infusion was initiated and variable amounts of labeled glucose were infused to maintain plasma glucose at 5 mmol/l throughout the experiment. 160 U of intranasal insulin or placebo spray were administered at t=0 minutes in randomized order. An additional insulin bolus was infused intravenously over 15 minutes after placebo spray only. After spray administration, hyperinsulinemic euglycemic clamp was maintained for a further 210 minutes. The three predefined periods for the assessment of changes in GIR and tracer dilution (-20 to 0 minutes, 100-120 minutes, and 190-210 minutes) are indicated as gray boxes in the figure.



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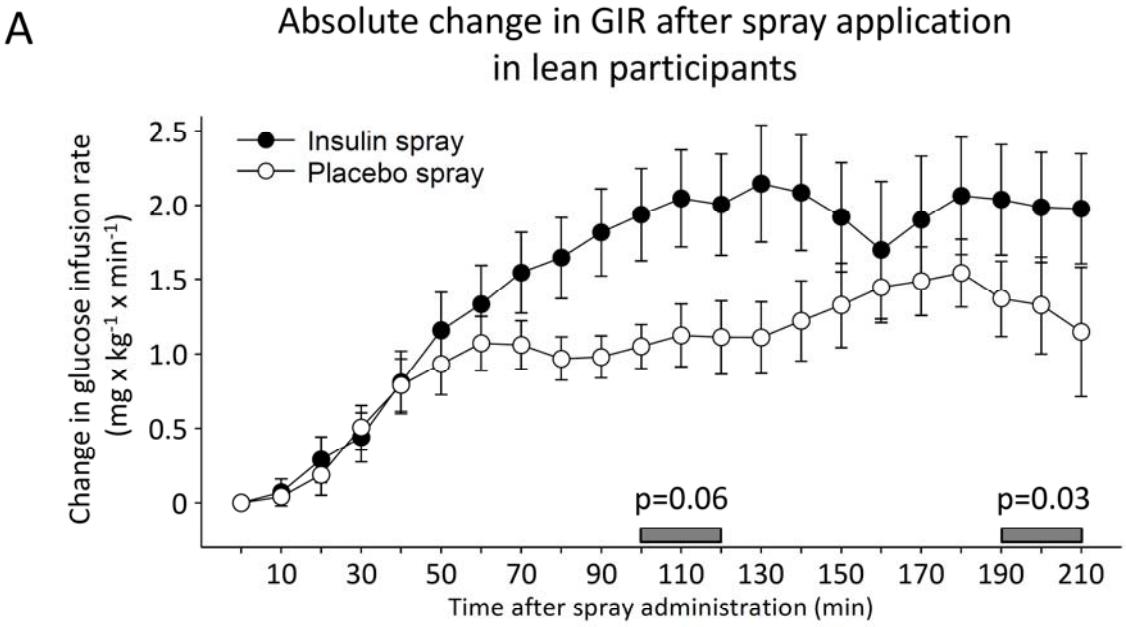
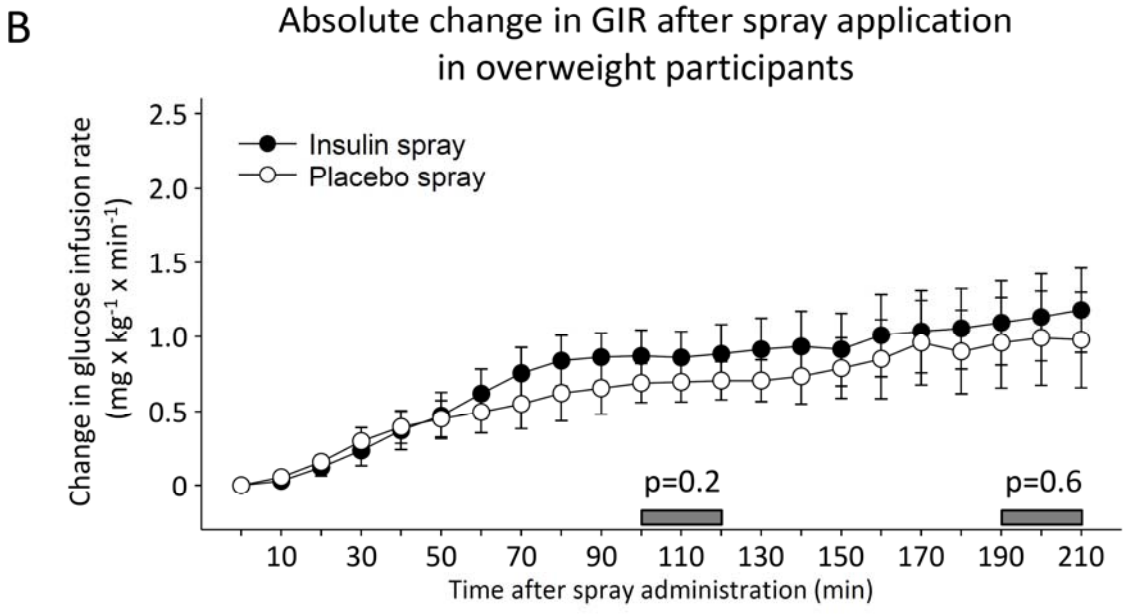
**Supplementary Figure 2. Absolute change in glucose infusion rate after spray administration.**
The absolute change in glucose infusion rate after spray administration at t=0 minutes is presented here for lean **(A)** and overweight participants **(B)**. Presented are means ± SEM. Differences in means between insulin and placebo spray during these designated periods were tested by pairwise two-tailed t-tests.



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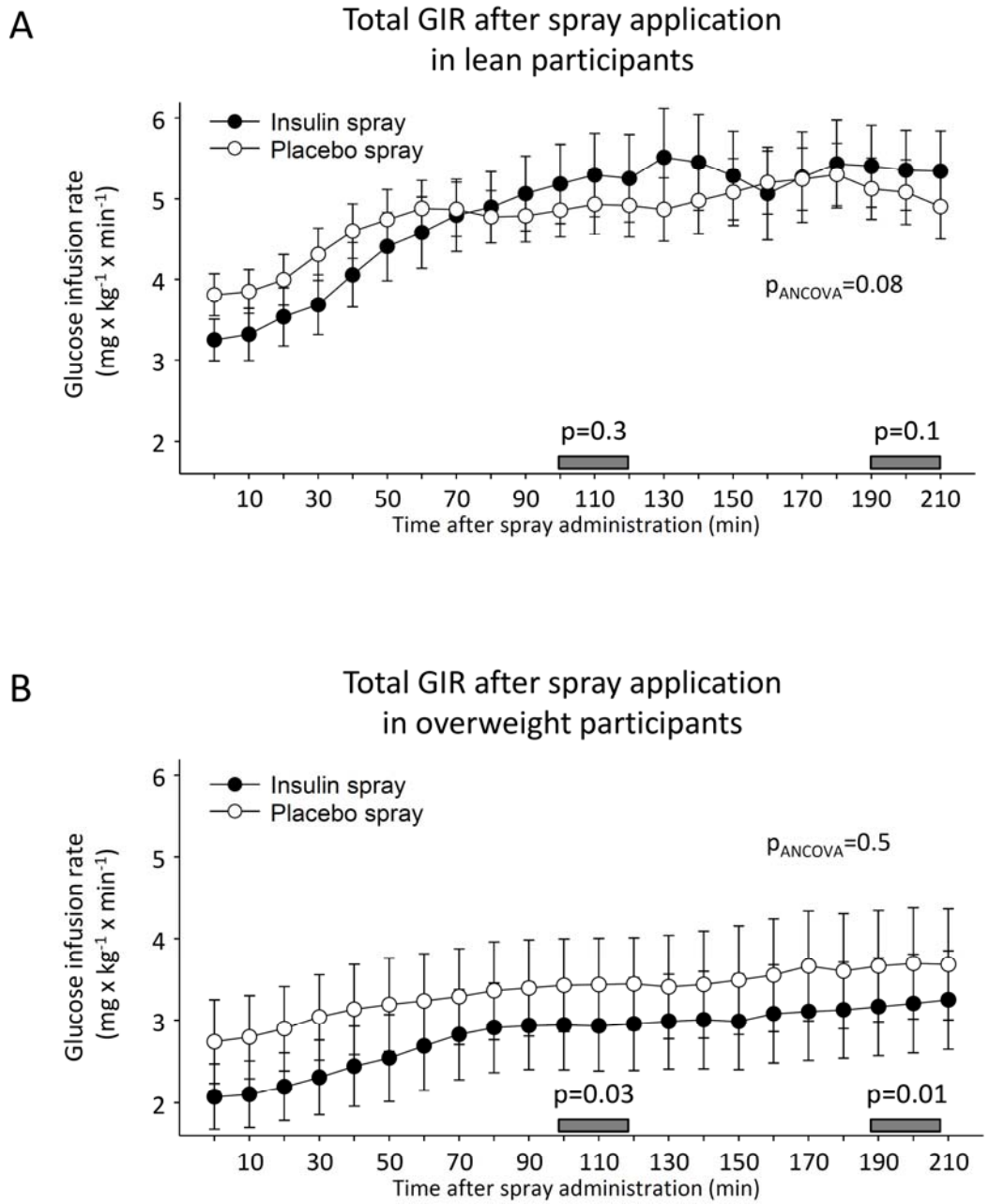
**Supplementary Figure 3. Total glucose infusion rates after spray administration.**
Total glucose infusion rates after spray administration at t=0 minutes is presented here for lean **(A)** and overweight participants **(B)**. Presented are means ± SEM. Differences in means between insulin and placebo spray during these designated periods were tested by pairwise two-tailed t-tests. ANCOVA was used for interactions between condition (insulin versus placebo spray) and time.



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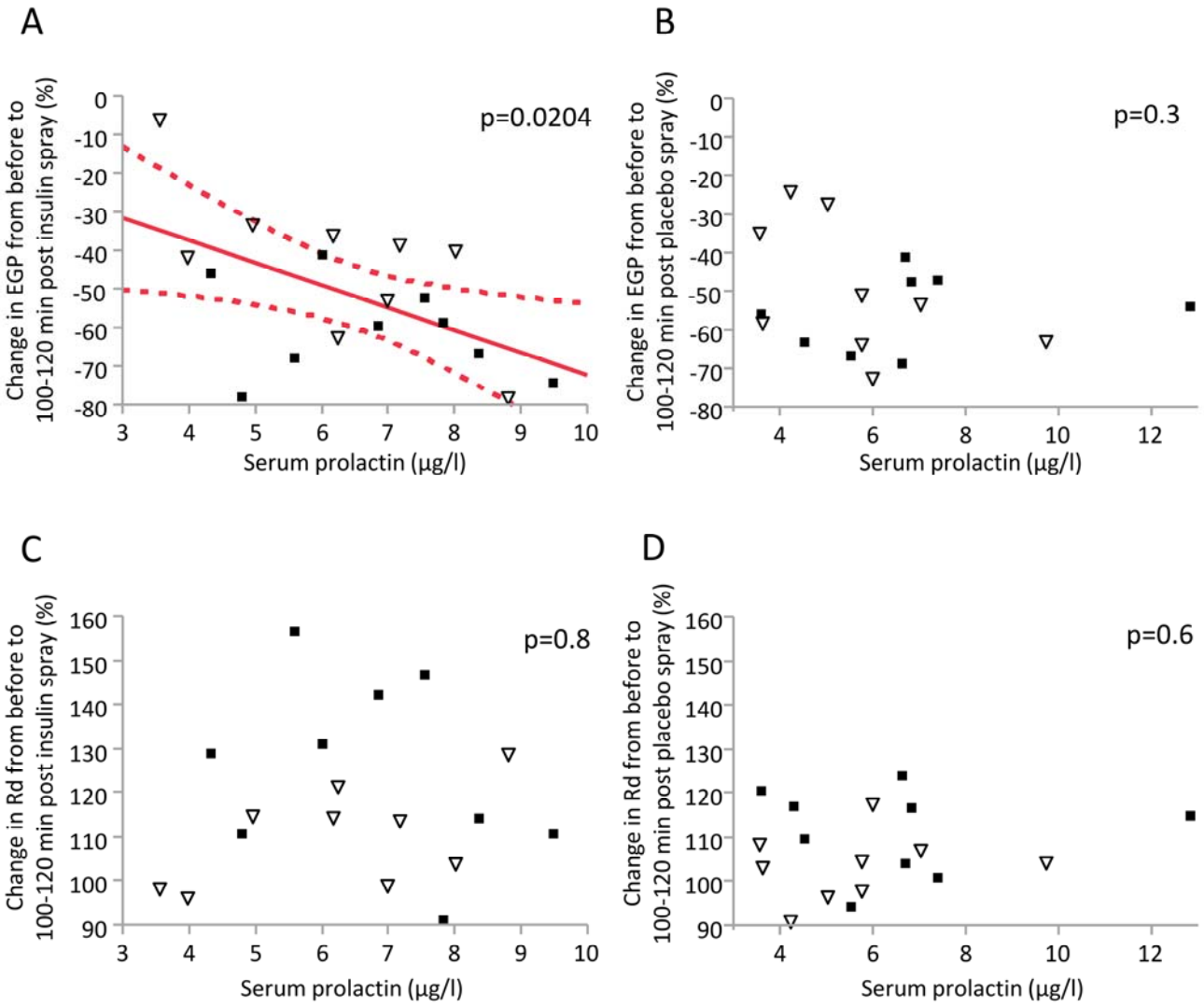
**Supplementary Figure 4. Association of serum prolactin levels with change in endogenous glucose production (EGP) and glucose disappearance rate (Rd) after insulin or placebo spray application.**
Changes from the 20 minutes before spray application to the first pre-defined time period after spray, i.e. 100-120 minutes post spray after insulin spray (left two panels, **A** and **C**) or placebo spray (right two panels, **B** and **D**) are given. **A** and **B** show changes in endogenous glucose production (EGP); **C** and **D** are changes in glucose disappearance rate (Rd). Filled squares represent lean participants, open triangles are overweight participants. Line represents fit line ± Confidence Interval from an unadjusted model.



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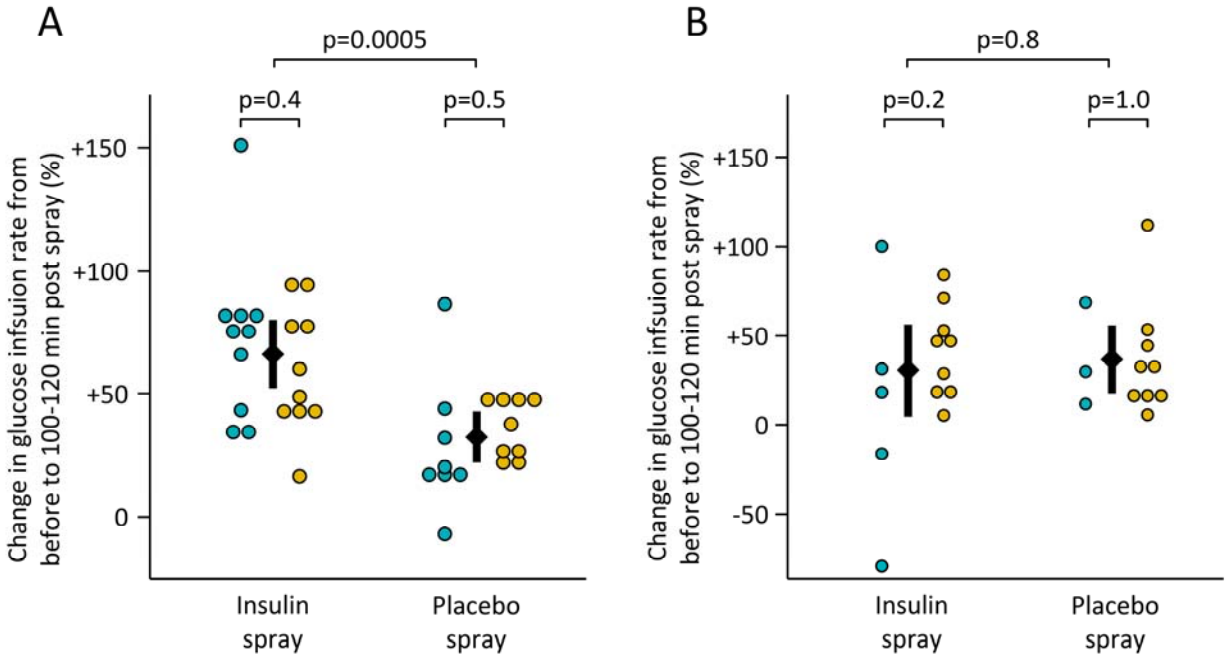
**Supplementary Figure 5. Effect of nasal insulin spray versus placebo on glucose infusion rates in participants of the current study and from a previous experiment.** Presented are individual changes in glucose infusion rate from before to 100-120 minutes post spray in participants of the current study (**yellow**) and our previous experiment (**blue**; Heni et al., Diabetes 2014). In lean participants **(A)**, glucose infusion rates had to be increased significantly more after nasal insulin than after nasal placebo spray. In overweight volunteers **(B)**, change in glucose infusion rate was not significantly different between the two spray conditions. Neither in lean nor in obese were the responses different between the current and the previous study. Comparisons of conditions (insulin versus placebo spray) were adjusted for study by multivariate linear regression analyses. Boxes indicate means, error bars represent SEM.



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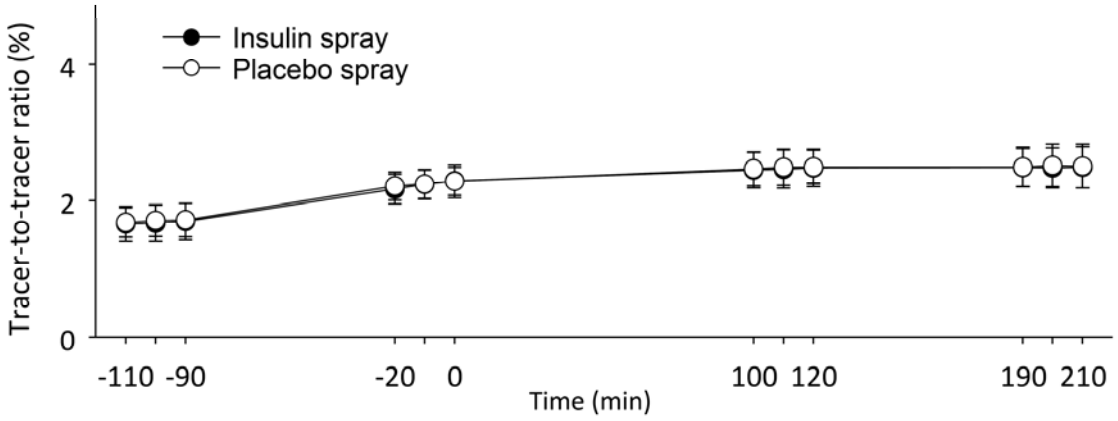
**Supplementary Figure 6 . Glucose tracer enrichment.**
Tracer enrichment is shown as incremental tracer-to-tracer ratio over time. Presented are means ± SD for the entire group (lean and overweight).



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**Supplementary Table 1.**

| | Lean | | | Overweight | | |
|---|---|---|---|---|---|---|
| | Insulin spray | Placebo spray | p | Insulin spray | Placebo spray | p |
| Fasting glucose (mmol/l) | 4.7 ± 0.4 | 4.9 ± 0.2 | 0.0006 | 5.2 ± 0.5 | 5.0 ± 0.4 | 0.0324 |
| Glucose before spray application (mmol/l) | 5.1 ± 0.4 | 5.0 ± 0.2 | 0.5 | 5.0 ± 0.3 | 5.0 ± 0.2 | 0.5 |
| Fasting plasma insulin (pmol/l) | 58 ± 21 | 52 ± 19 | 0.8 | 77 ± 34 | 74 ± 42 | 0.5 |
| Plasma insulin before spray application (pmol/l) | 178 ± 37 | 190 ± 41 | 0.6 | 206 ± 49 | 214 ± 65 | 0.9 |
| Fasting C-peptide (pmol/l) | 356 ± 56 | 355 ± 62 | 0.6 | 481 ± 141 | 417 ± 136 | 0.2 |
| C-peptide before spray application (pmol/l) | 403 ± 169 | 338 ± 100 | 0.4 | 352 ± 146 | 357 ± 172 | 0.8 |
| Fasting glucagon (pmol/l) | 21.8 ± 4.6 | 21.3 ± 3.0 | 0.7 | 23.0 ± 5.6 | 22.9 ± 5.2 | 0.9 |
| Glucagon before spray application (pmol/l) | 16.4 ± 3.9 | 18.3 ± 3.3 | 0.8 | 19.6 ± 6.1 | 18.9 ± 6.8 | 0.5 |
| Glucose infusion rate before spray application (mg x $kg^{-1}$ x $min^{-1}$) | 3.2 ± 0.8 | 3.8 ± 0.8 | 0.6 | 2.2 ± 1.2 | 2.7 ± 1.5 | 0.0335 |
| Fasting free fatty acids (µmol/l) | 247 ± 322 | 414 ± 589 | 0.6 | 338 ± 230 | 492 ± 249 | 0.2 |
| Free fatty acids before spray application (µmol/l) | 76 ± 80 | 45 ± 14 | 0.2 | 72 ± 39 | 115 ± 110 | 0.2 |
| Serum prolactin (µg/l) | 6.8 ± 1.7 | 6.5 ± 2.7 | 0.8 | 6.2 ± 1.8 | 5.6 ± 1.9 | 0.5 |
| Endogenous glucose production before clamp (mg x $kg^{-1}$ x $min^{-1}$) | 2.2 ± 0.2 | 2.1 ± 0.2 | 0.4 | 1.8 ± 0.3 | 1.9 ± 0.2 | 0.7 |
| Endogenous glucose production before spray application (mg x $kg^{-1}$ x $min^{-1}$) | 1.2 ± 0.3 | 1.1 ± 0.2 | 0.7 | 1.2 ± 0.3 | 1.2 ± 0.3 | 0.8 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Rate of glucose disappearance before spray application (mg x $kg^{-1}$ x $min^{-1}$) | 4.7 ± 0.7 | 5.0 ± 0.8 | 1.0 | 3.4 ± 1.1 | 4.1 ± 1.7 | 0.06 |
| Mean free fatty acids 100-120 minutes post spray application (µmol/l) | 43.3 ± 29.6 | 35.2 ± 11.8 | 0.3 | 65.9 ± 37.7 | 71.6 ± 54.7 | 0.2 |
| Mean free fatty acids 190-210 minutes post spray application (µmol/l) | 79.8 ± 141.6 | 50.5 ± 33.8 | 0.5 | 55.4 ± 22.8 | 73.2 ± 60.3 | 0.6 |
| Mean glucose 100-120 min after spray application (mmol/l) | 4.9 ± 0.2 | 4.8 ± 0.3 | 0.5 | 4.9 ± 0.3 | 4.8 ± 0.3 | 0.6 |
| Mean glucose 190-210 min after spray application (mmol/l) | 5.3 ± 0.3 | 5.0 ± 0.4 | 0.06 | 4.9 ± 0.2 | 4.9 ± 0.2 | 0.5 |
| Mean insulin 100-120 min after spray application (pmol/l) | 183 ± 27 | 172 ± 36 | 0.5 | 203 ± 41 | 216 ± 59 | 0.8 |
| Mean insulin 190-210 min after spray application (pmol/l) | 186 ± 48 | 174 ± 38 | 0.4 | 184 ± 26 | 207 ± 45 | 0.4 |
| Mean glucagon 100-120 min after spray application (pmol/l) | 13.9 ± 3.8 | 15.5 ± 4.4 | 0.8 | 18.5 ± 7.1 | 18.1 ± 5.9 | 0.7 |
| Mean glucagon 190-210 min after spray application (pmol/l) | 15.1 ± 3.4 | 15.3 ± 2.5 | 0.2 | 17.3 ± 7.7 | 17.1 ± 7.1 | 0.8 |

Given are unadjusted means ± standard deviation. P-values are from pairwise two-tailed t-tests.