

Does Irisin Have an Effect on Female Reproductive Function? Initial in-Vitro Studies

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Abstract

Irisin is a recently discovered hormone secreted by muscle and adipose tissue which plays a role in fat and energy metabolism. Because energy metabolism is closely linked to reproduction, we hypothesized that irisin may have effects in the reproductive system. In this study we examined the effects of irisin on luteinizing hormone (LH) secretion in murine pituitary cells and on estradiol (E2) secretion in human granulosa cells.

Methods: The adult mouse pituitary cell lines (mPitA12 and mPitA19, CELLutions Biosystems Inc. and UCLA at San Diego, respectively) were incubated with or without increasing concentrations of irisin (0-250ng/mL) and GnRH (0-50nM).

Human granulosa cells from in-vitro fertilization were cultured with and without irisin (0-275ng/mL) and insulin (0-10,000ng/mL).

ELISA was used to measure LH or E2 concentrations in the conditioned culture medium. Two-way analysis of variance (ANOVA) was used to compare mean LH or E2 for various combinations of GnRH and irisin or insulin and irisin.

Results: In the pituitary cell experiments, GnRH alone (without irisin) stimulated LH secretion in a dose-dependent manner ($p < 0.0005$), thus validating the assay. Irisin alone (without GnRH) did not show a clear pattern of either stimulation or inhibition of LH. When irisin and GnRH were used together, there was significant GnRH/irisin interaction ($p < 0.0019$), with the pattern of LH still depending on GnRH concentration.

In the granulosa cell experiments, E2 concentration in the medium increased as insulin concentration increased ($p < 0.0494$), thus validating the assay. Irisin alone (without insulin) showed no clear trend for E2 as a function of irisin at any concentration. With both irisin and insulin in the system, there was significant irisin/insulin interaction ($p = 0.0049$) at insulin concentrations of 0 and 50 ng/mL. Additional experiments are in progress.

Conclusions: In these preliminary in-vitro experiments, irisin appears to interfere with the effects of GnRH in the pituitary cells and with the effects of insulin in granulosa cells. To understand the mechanisms of these interactions, if confirmed, will require further study.

Introduction

Irisin is a recently discovered hormone secreted by muscle and adipose tissue(1) responsible for “browning” of fat and therefore implicated to play a role in fat and energy metabolism, insulin resistance and metabolic syndrome (Mets). Irisin is cleaved from Fibronectin type III domain-containing protein 5 (FNDC-5)(2) and is induced by PGC1 α , a PPAR γ co-activator- a modulator of uncoupling protein-1 responsible for thermogenesis in brown adipose tissue (BAT). Circulating irisin concentrations correlate positively with BMI (3,4). Polycystic ovary syndrome (PCOS), the most common cause of infertility in females, often presents with obesity, insulin resistance and elevated luteinizing hormone (LH) levels (5). In this study we examine the effects of Irisin on LH secretion in murine pituitary cells and estradiol (E2) secretion in human granulosa cells.

Methods

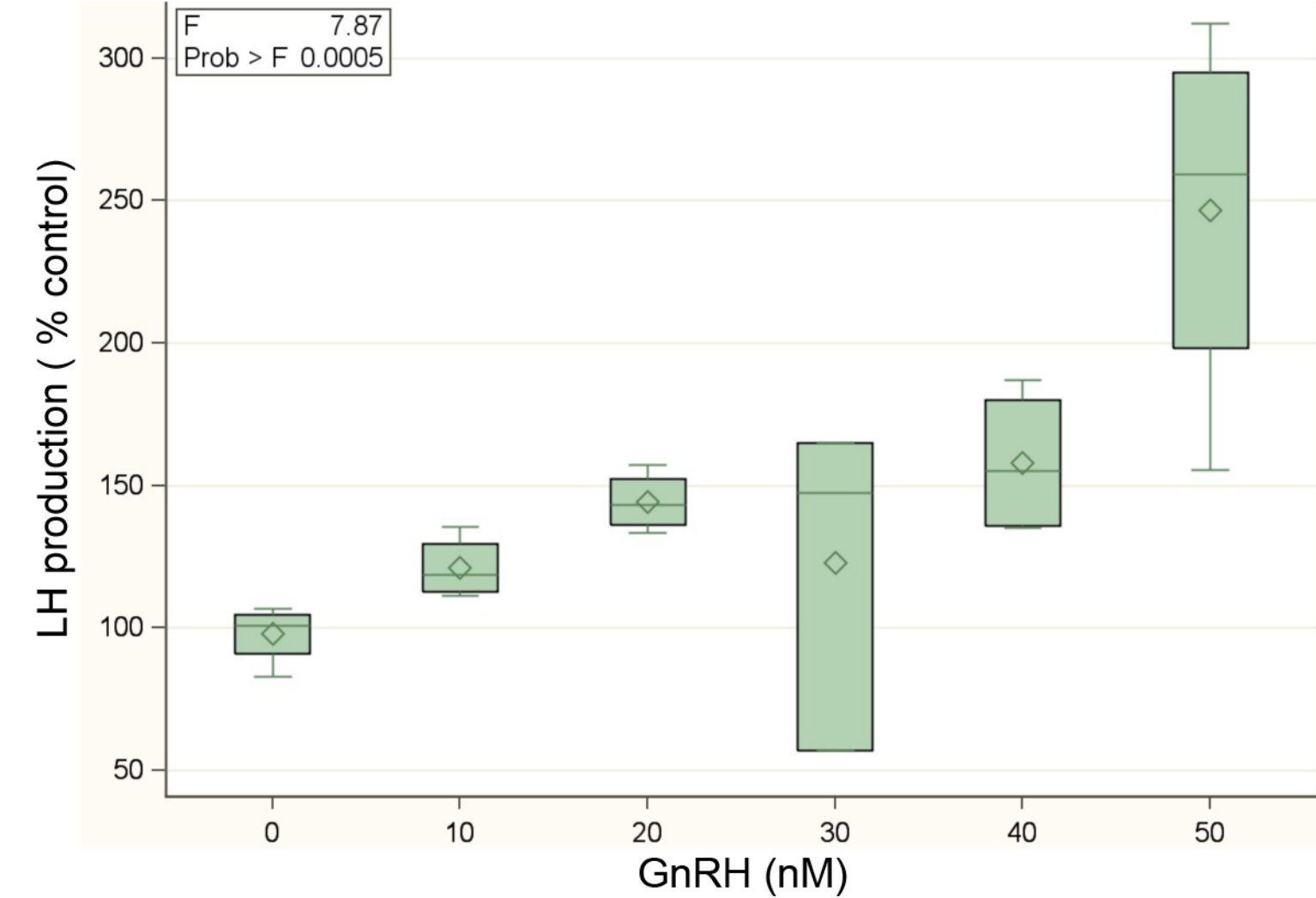
The adult mouse pituitary cell lines (mPitA12, mPitA19) were obtained from CELLutions Biosystems Inc. or Cedars Sinai Medical Center. Pituitary cells have been incubated in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 UI penicillin and 100 mg/mL streptomycin at 37°C with 5% CO₂. The cells were grown to 70-90% confluence, trypsinized and then incubated for 48 hours under the same conditions. The cells were further incubated for another 24 hours in 6-well plates with 2% fetal bovine serum (FBS), 100 UI penicillin and 100 mg/mL streptomycin at 37°C with 5% CO₂. One set of cells was incubated 19 hours with the same medium containing Irisin 0 ng/mL or 125 ng/mL and GnRH (0, 10, 20, 30, 40, 50 nM). The other set of cells was incubated with GnRH 0 nM or 25 nM and Irisin ranging from 0-125 ng/mL for the same duration. LH or FSH concentration was measured with ELISA assay (MyBioSource San Diego, CA).

Human granulosa cells were obtained from the IVF program at NY-Presbyterian Hospital-Weill Cornell. They were purified on the Percoll gradients (40% Percoll/Hank’s balanced salt solution) as previously described. Purified human granulosa cells were counted using hemocytometer, and 1mL of 0.5 $\times 10^5$ cells/mL suspension was placed in 24 well tissue culture plates (5). The cells were cultured for 48 hours at 37°C, 5% CO₂, 90% humidity in M199 medium supplemented with 10% FBS, 10 μ g/mL gentamicin, and 250 ng/mL amphotericin B. After 48 hours of incubation, the medium supplemented with 10% FBS was replaced by a medium with 2% FBS, in which the cells were incubated for additional 24h before 10 μ M testosterone was added to the same medium. One set of cells was incubated with insulin 0 ng/mL or 50 ng/mL and irisin 0-275 ng/mL (0,25,50,100,125,150,200,250) . The other set was incubated with irisin 0 ng/mL or 125 ng/mL and insulin 0-10,000 ng/mL (0,10,50,100,1000,10,000). E2 concentration was measured by estradiol ELISA assay (ALPCO Salem, NH).

Statistics: Two-way analysis of variance (ANOVA) was used to compare mean LH or E2 for various combinations of GnRH and irisin or insulin and irisin.

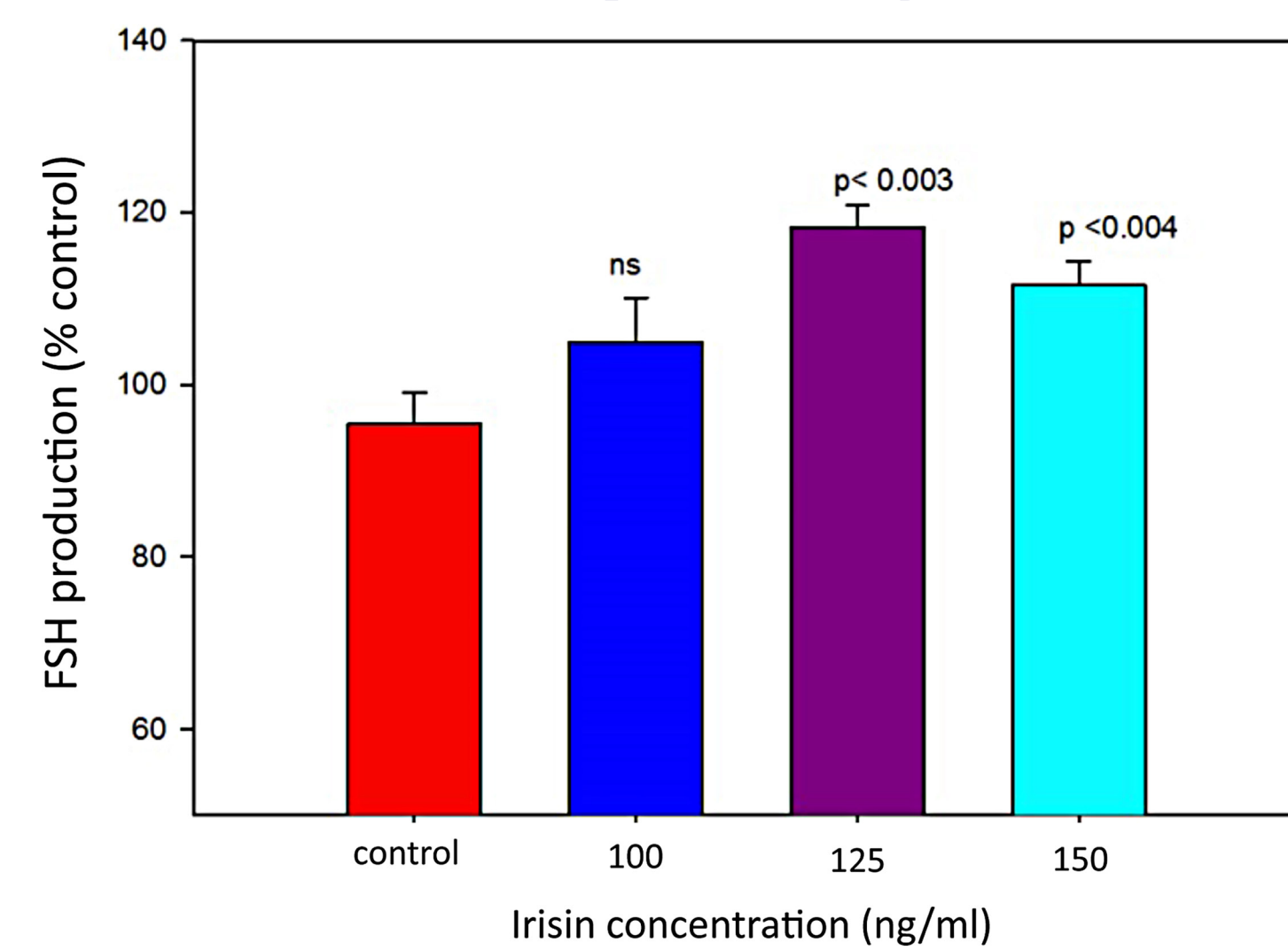
Results

Figure 1. GnRH effect on LH production in mouse pituitary cells



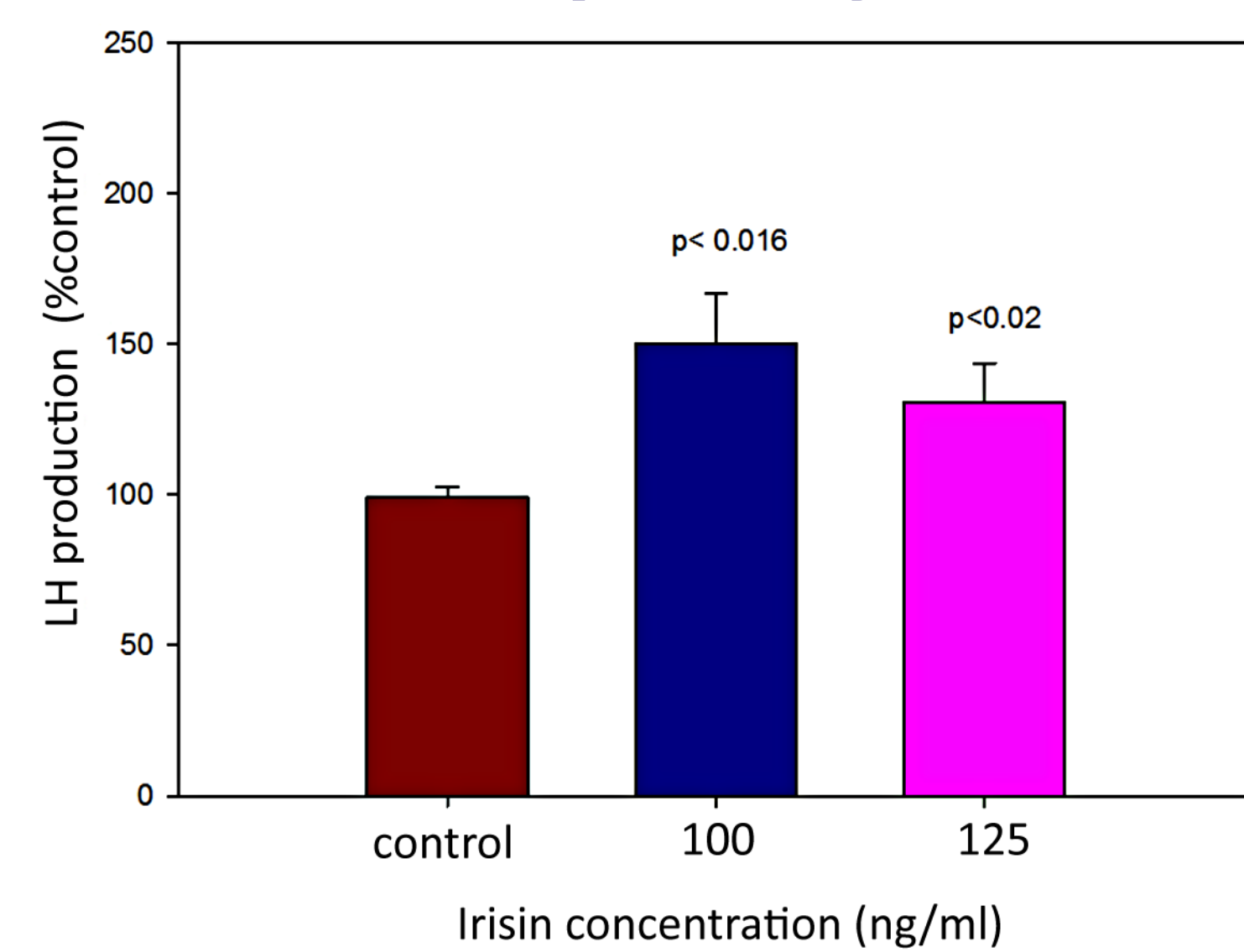
LH is stimulated by GnRH in a dose dependent manner. LH production is expressed as percent of control.

Figure 2. Irisin effect on FSH production in mouse pituitary cells



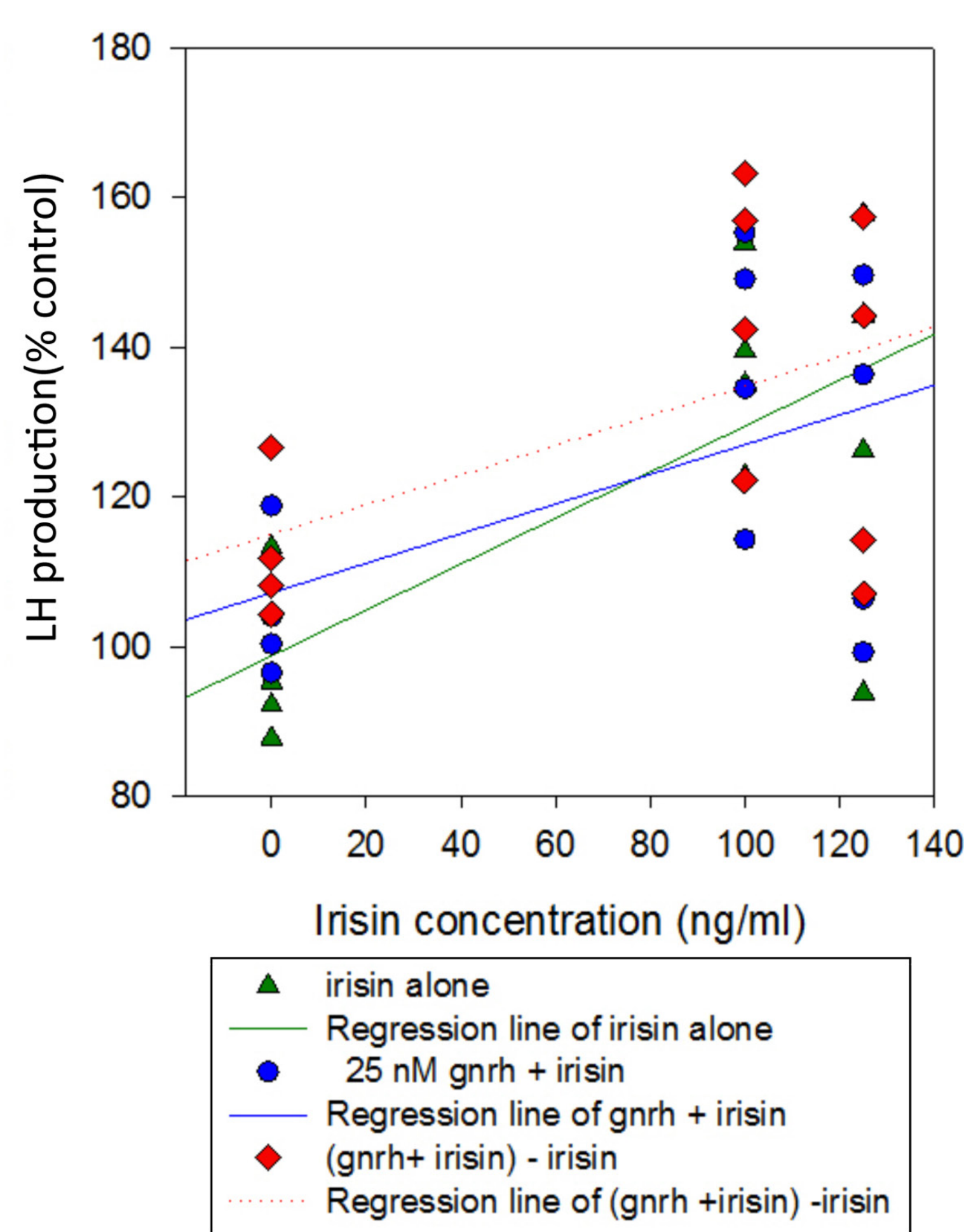
Irisin stimulates FSH production at 125 and 150ng/mL in mouse pituitary cells. Stimulation at 100 ng/mL is not significant.

Figure 3A. Irisin effect on LH production in mouse pituitary cells



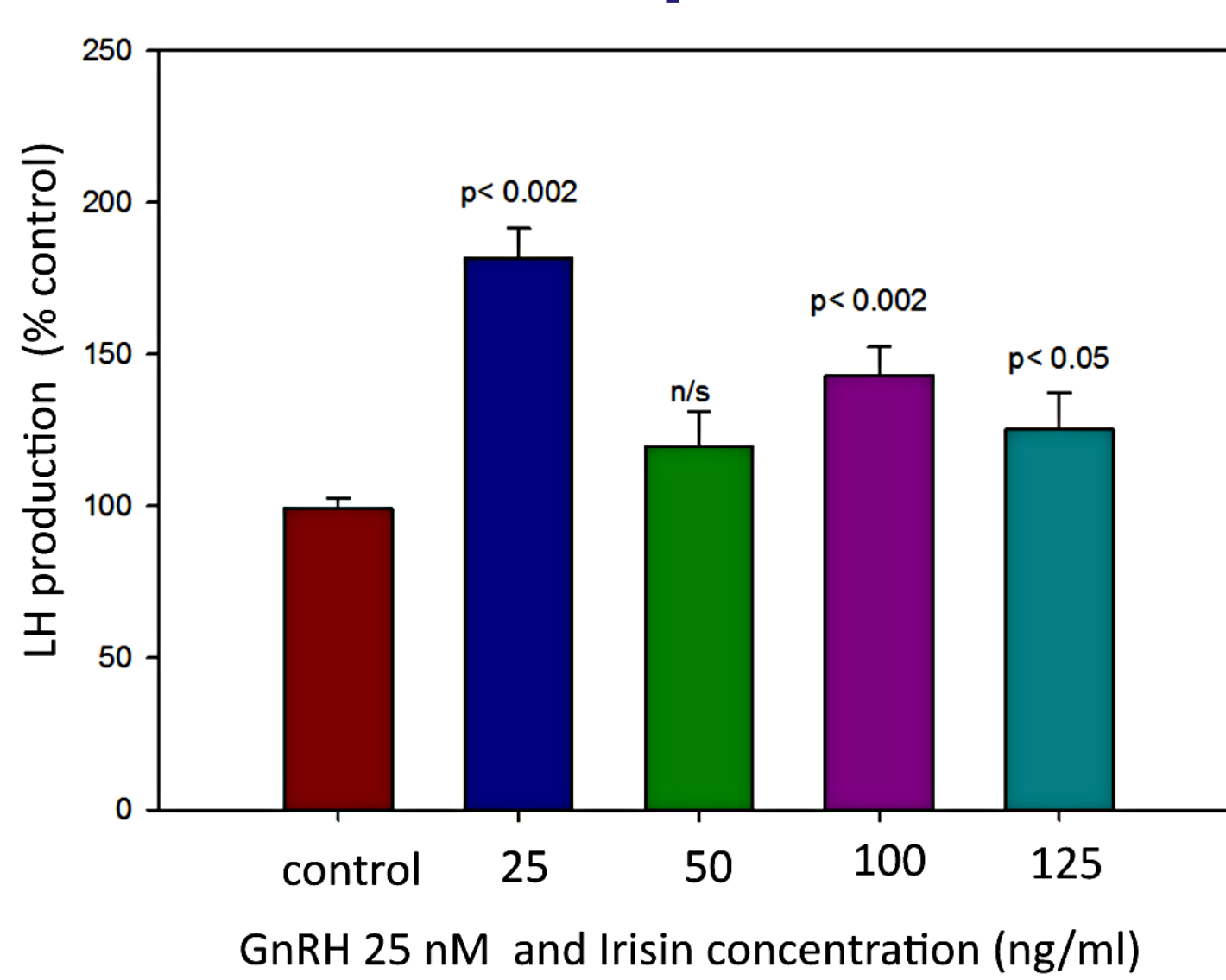
Irisin at 100ng/mL and 125ng/mL stimulates LH production.

Figure 4. Comparing irisin alone to GnRH + irisin



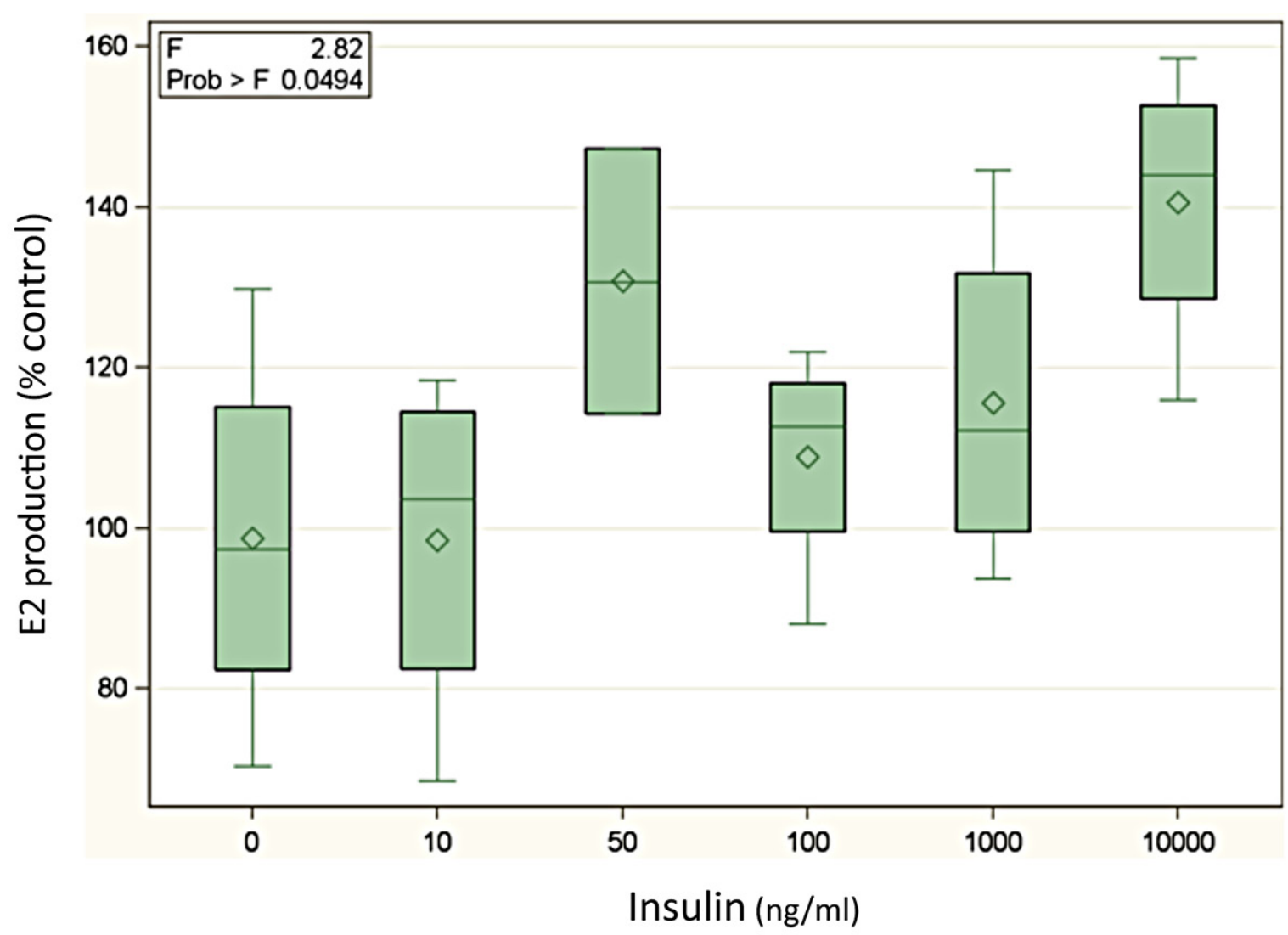
A comparison between the effect of irisin alone to the cumulative effect of irisin and GnRH in pituitary cells shows stimulation by irisin alone and no synergistic effect with GnRH.

Figure 3B. GnRH (25 nM) + Irisin effect on LH production



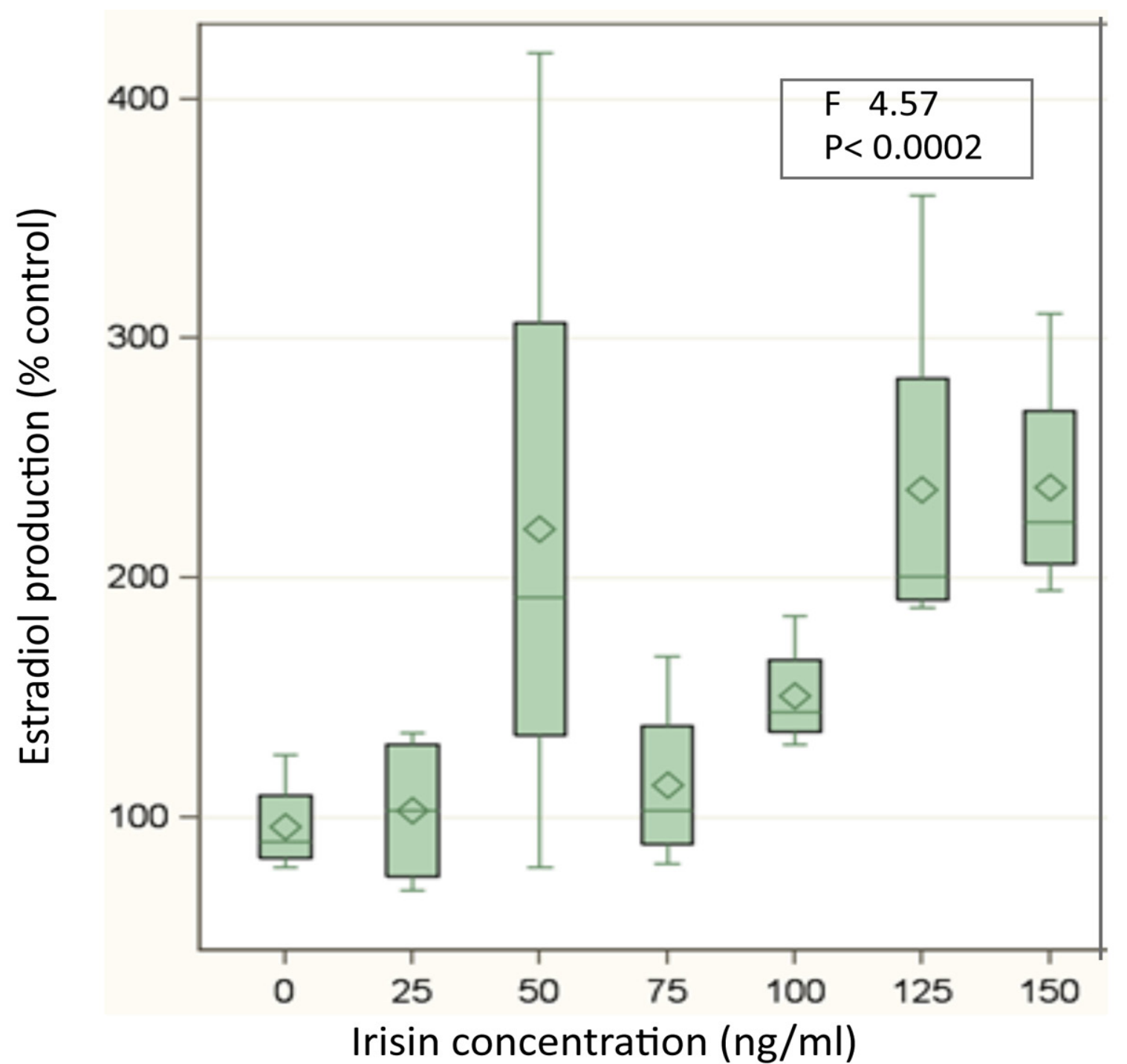
GnRH (25nM), when added to irisin, shows no additional effect on LH production.

Figure 5 Effect of insulin on E2 production in human granulosa cells



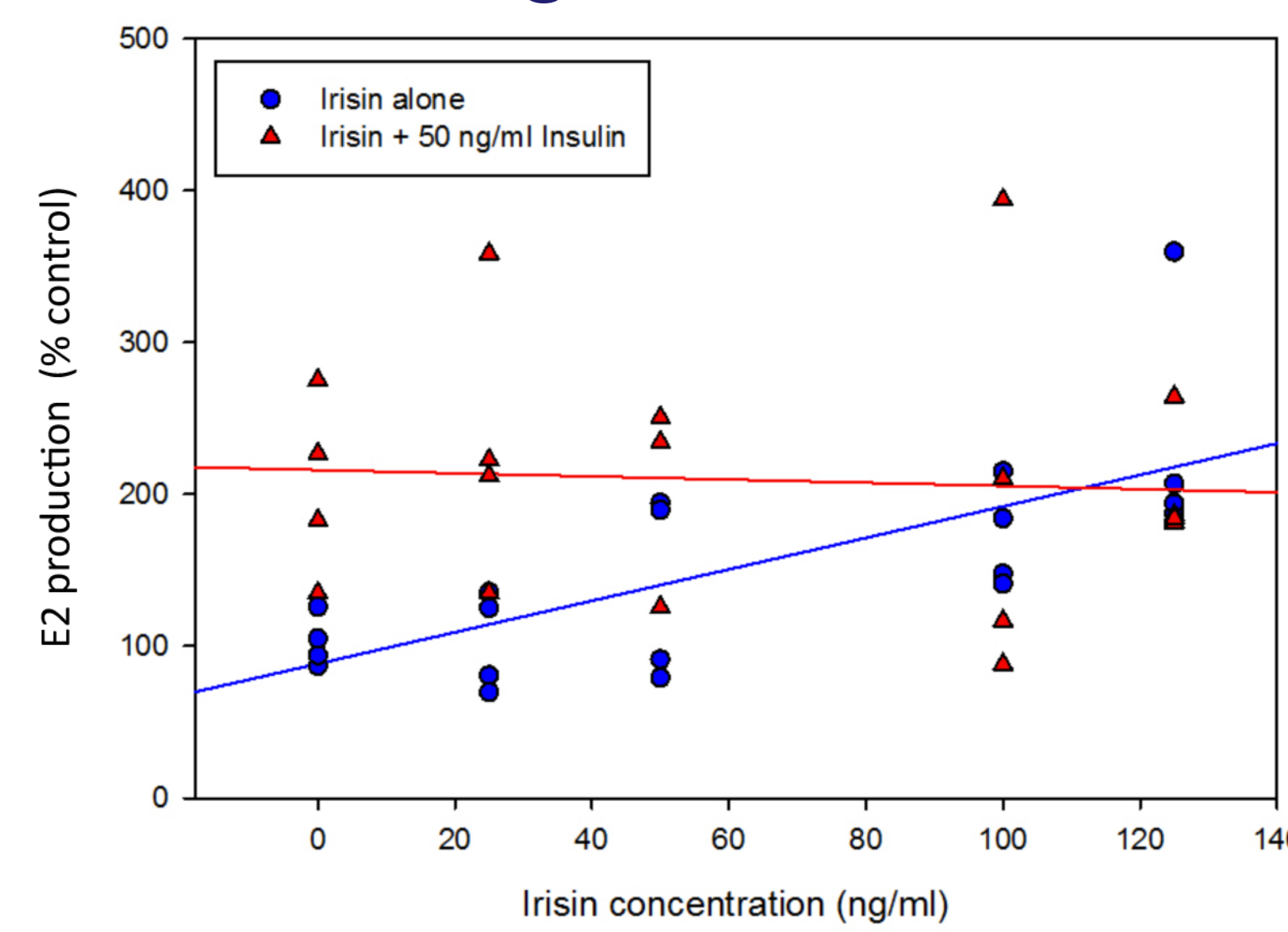
Insulin stimulates production of E2 in dose-dependent manner compared to control.

Figure 6. Irisin effect on E2 production in human granulosa cells



Irisin alone stimulates E2 production in dose-dependent manner.

Figure 7. Comparision of irisin alone to irisin + insulin effect on E2 production in human granulosa cells



When insulin(50 ng/mL) is added to irisin the effect of irisin on E2 production is lost.

In the pituitary cell experiments, GnRH alone stimulated LH secretion in a dose-dependent manner ($p < 0.0005$), thus validating the assay. Irisin alone stimulates LH ($p < 0.016$, $p < 0.02$) and FSH ($p < 0.003$, $p < 0.004$) production at concentrations of 100ng/mL to 150ng/mL. When irisin was combined with GnRH, no added effect of GnRH was observed.

In granulosa cells, E2 concentration in the medium increased as insulin concentration increased ($p < 0.0494$), thus validating the assay. Irisin alone stimulated production of E2 in a dose-dependent manner. In the presence of insulin (50 ng/mL) however, an enhanced effect on E2 production was not observed. This may be due to the fact that the cells may already be stimulated to its full capacity by insulin, rendering irisin ineffective.

Conclusion

In these preliminary in-vitro studies, irisin has been found to stimulate LH and FSH production in mouse pituitary cells. In the presence of GnRH, however this effect was blunted.

In human granulosa cells, irisin alone increased the concentration of E2 in the medium but, when insulin was present, this effect of irisin is lost.

Irisin appears to have no synergy with GnRH in the pituitary cells or with insulin in granulosa cells. To understand the mechanisms of these interactions will require further study.

References

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