Nangarhar University
International Journal of Biosciences

Received: 09 July 2023
Accepted after revision: 25 Sep 2023
Published Online: 30 Sep 2023

ORIGINAL ARTICLE Vol 2, Issue 3 (2023) e-ISSN: 2957-9988

Long-term Glucose Infusions had no Influence on Gonadotropins Hormone Secretion in Ovariectomized Goats

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ABSTRACT

Background: It is reported that high nutritional supplementation treatment for seven days stimulated pulsatile LH secretion attended by increased in the plasma glucose and insulin concentrations in goat. The present study tested the hypothesis that whether providing peripheral glucose to goats associated with digestion of high nutrition supplementation increase gonadotropin secretion in ovariectomized goats.

Materials and Methods: There was two experimental groups. The treatment animals from both experiences received glucose infusion. In the first experiment (constant glucose infusion; 50% glucose + saline at a rate of 7.5 ml/h for 7 days) and in the second experiment (progressive glucose infusion; 20% glucose + saline by increasing 5% glucose in 24hr intervals for 7 days at a rate of 15 ml/h for 7 days). The control animals from both experiences received saline infusion in the same manner.

Findings: In the first experiment, there was no significant diversity in the concentrations of glucose and insulin among the treatment period as compared with mean pre-infusion period or compared to control groups except for Day 2 ($p<0.05$). In the second experiment, concentrations of both the glucose and insulin started increasing significantly from Day 2 and remained with elevated levels until day 7 as compared with mean pre-infusion period or compared to control groups ($p<0.05$). The mean Follicle stimulating hormone concentration in treatment group was not affected by glucose infusion as compared with pre-infusion periods or control group. The mean LH (concentration and pulse frequency) was not affected by glucose or saline infusion in both experiments.

Conclusion: The present studies suggest that an increase in the glucose availability does not control the gonadotropin secretion as a stimulatory mediator in ovariectomized goats.

Keywords: Glucose infusion, Insulin, Luteinizing hormone, Follicle stimulating hormone, Ovariectomized goats

INTRODUCTION

In mammals, reproductive function is influenced by negative or positive energy balance at various stages of their reproductive cycle. A key question to be answered is to identify the metabolic factor(s) linking between the nutrition and reproduction. One candidate is the glucose availability that is mainly regulated by blood glucose

and/or insulin levels (Daniel et al., 2000; Archer et al., 2005). In lactating dairy cows, negative energy balance induces a reduction of glucose and insulin, and has certain straight inhibitory effects on both folliculogenesis and oocyte quality (Butler, 2000; Leroy et al., 2008a; Leroy et al., 2008b). The treatment of a long-acting insulin restored estrous cyclicity with increases in the numbers and diameters of follicles in acyclic goats (Sarath et al., 2008). Our previous study has also reported in goats that short-term food restriction suppresses both pulsatile luteinizing hormone (LH) secretion and plasma glucose and insulin concentrations (Tanaka et al., 2004). The regulatory relation between glucose accessibility and LH pulsatile secretion has been proved by the previous studies that physiological level of insulin supplementation is able to maintain the normal LH pulsatile secretion in diabetic sheep model (Tanaka et al., 2000), and that pharmacological blockade of glucose availability inhibits pulsatile LH frequency in ram (Bucholtz et al., 2000). Thus, glucose availability is a factor mediating reproductive dysfunction induced by negative energy balance in domestic animals.

The enormous publications reported that high-energy levels of nutritional supplementation has stimulatory affect on reproductive function such as ovarian activity (Wettemann and Bossis, 2000; Wettemann et al., 2003), folliculogenesis (Diskin et al., 2003), and ovulation rate. The glucose availability is also preoccupied in the effect of such positive energy balance on reproductive function. We have recently indicated that 7 days nutritional supplementation stimulated LH pulsatile secretion attend by increased the glucose and insulin plasma concentrations in cycling (Haruna et al., 2009) and ovariectomized (Zabuli et al., 2009) goats, suggesting that insulin and glucose as metabolic factors are associated with the stimulatory effects of nutritional supplementation on LH secretion. However, it seems to be beneficial to test induce of artificially-promoted glucose availability on pulsatile LH secretion by several reasons of methodological issues.

The main goal of the present study was to examine the hypothesis that an increase in glucose availability stimulates gonadotropins hormone secretion in ovariectomized goats. The present approach was to test the effect on LH pulsatile secretion of continuous glucose infusion that can maintain blood glucose and insulin levels observed on the day when nutritional supplementation stimulates LH pulsatile secretion in intact goat.

MATERIALS AND METHODS

Animals

The ovariectomized (OVX) Shiba goats from experimental farm of the Tokyo University of Agriculture and Technology (TUAT) were used. Alfalfa hay cube at the maintenance diets (basically 700g /head/day given twice daily) provided according to previous report (Haruna et al., 2009). Mineralized salt licks and clean water were available ad libitum. All research processes were approved by the TUAT Committee for the Use and Care of Animals.

Experimental 1 (constant glucose infusion)

All OVX goat were implanted subcutaneously with a silicone capsule containing oestradiol (Kim et al., 2003; Zabuli et al., 2009) on 8 days before the start of infusion treatment, and then divided into two groups, control (n=4) and treatment (n=4) group. They were moved to indoor cages and were attached with jugular catheters bilaterally; one side was for glucose infusion and the other side was for taking blood samples frequently. The treatment group was continuously infused with a constant dose of glucose (50%) dissolved in saline at a rate of 7.5 ml/h with a peristaltic pump (SJ-1211 Atto, Tokyo, Japan) for 7 days (Day 1 = day of the start of treatment). The concentration of glucose and infusion rate was based on the previous (Zabuli et al.,

2009) and our preliminary studies. The control group received saline in the same way. In both groups, blood samples were taken for 6 h at 10 min intervals on Days 0, 3, and Day 7 for analysis of LH pulsatile patterns and daily from Day –7 to Day 7 to check the plasma glucose and insulin levels.

Experimental 2 (progressive glucose infusion)

The additional experiment was conducted because plasma levels of glucose and insulin were increased only on the several days after the start of glucose infusion in experiment 1 (see results section). The experimental procedure was same as experiment 1 except for the manner of a glucose treatment. The concentration of glucose solution for infusion was gradually increased during the infusion period; it was 20% on Day 1 and then increased at a rate of 5% every 24 hrs. The concentration of glucose solution was finally 50% on Day 7. The infusion rate was 15 ml/h in both groups and saline was used instead of glucose in the control group. In this experiment, plasma FSH concentrations were also analyzed.

Hormone assays

The LH and FSH plasma concentrations were measured by a double-antibody radioimmunoassay (RIA) according to previous report (Suganuma et al., 2007). The sensitivity of assays for FSH and LH were 3.75 ng/ml and 0.19 ng/ml, respectively. The intra- and inter-assay coefficients of variation were 2.97 % and 0.18 % for FSH and 2.7 % and 1.3 % for LH, respectively. Insulin concentrations were determined by an RIA kit (EIKEN CHEMICAL CO., LTD. Tokyo, Japan) as reported previously (Zabuli et al., 2009). Assay sensitivity averaged 0.19 μU/ml, and intra- and inter-assay coefficients of variation were 22.4 % and 4.36 % respectively. Glucose was quantified by the glucose oxidize method (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as reported previously (Zabuli et al., 2009).

Statistical Analysis

All research data are described as mean ± SD. Two-way (between group, within time) or one-way ANOVA for repeated measures followed by Tukey test (Treatment vs. Controls) or Dunnett test (before vs. after the treatment in each group) was accepted to figure out statistical differences between the mean values. $P<0.05$ was reflected to be statistically significant. According to previous report (Zabuli et al., 2009) the Cluster Analysis program was used to identification of LH pulses in the samples collected at 10 min intervals. The nadir and peak clusters for the detection of LH pulses were 2/1 points, and the t–statistics for significant increases and decreases were 2.0/2.0.

RESULTS

Insulin and Glucose profiles

The concentrations changes of the insulin and glucose in experiment 1 and experiment 2 are shown in Fig. 1. In the treatment group in experiment 1 (Fig. 1a), significant increases in the concentrations of glucose and insulin were observed only on Day 2 (140.7 ± 35.2) and (47.4 ± 21.1) as compared with mean pre-infusion period (59.6 ± 17.7) and (8.0 ± 4.0) respectively. There was no significant difference in the concentrations of glucose and insulin between the treatment and control groups ($p>0.05$) except for Day 2 (140.7 ± 35.2 vs. 55.3 ± 4.1) and (47.4 ± 21.1 vs.± 8.6 ± 2.6) respectively ($p<0.05$). In the treatment group in experiment 2, the concentrations of glucose and insulin (Fig. 1b) started increasing significantly ($p<0.05$) from Day 2 and remained with elevated levels at the ranges of (72.9 ± 10.4 mg/dl vs. 53.5 ± 6.1 mg/dl) for glucose and of (48.3

± 23.2 µU/ml vs. 10.0 ± 6.0 µU/ml) for insulin during the treatment as compared with pre-infusions period. The plasma glucose and insulin were significantly ($p<0.05$) higher from Day 2 to Day 7 than those of the control group for glucose (52.9 ± 5.4 mg/dl) and insulin (11.3 ± 6.0 µU/ml) respectively.

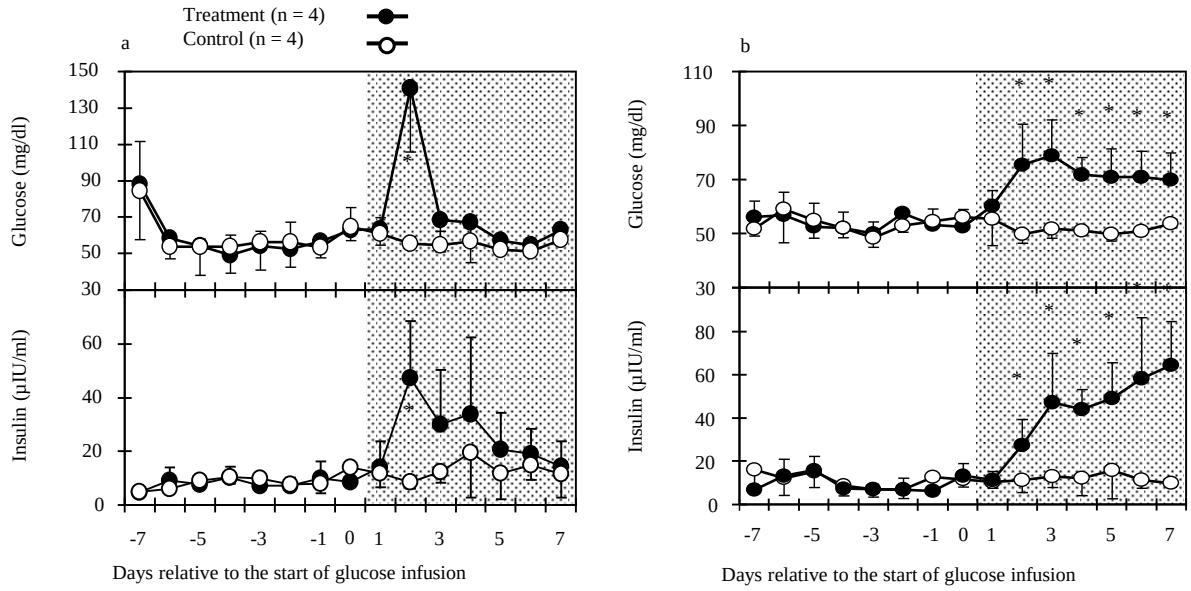

Figure 1. Shows the daily profiles (mean ± SD) of glucose (upper tow panels) and insulin (bottom tow panels) from both, the experiment 1 (left two panels) and experiment 2 (right two panels) in the control (open circle, n=4) and treatment (closed circle, n= 4) groups. The shaded region indicates the duration of treatments and the stars shows the significant point vs. pre-treatment and vs. controls.

LH and FSH profiles

The representative psychograph of LH pulsatile secretion from both experiments was shown in Fig. 2 and the pulsatile LH frequency and mean concentrations of LH for both experiments was summarized in Table 1. The mean pulsatile LH frequency and mean concentration of LH was not affected by glucose or saline infusion in both experiments. In the both experiments, there was no significantly difference in the mean pulsatile LH frequency and mean LH concentrations between treatment group and control group on Days 0, 3, and 7.

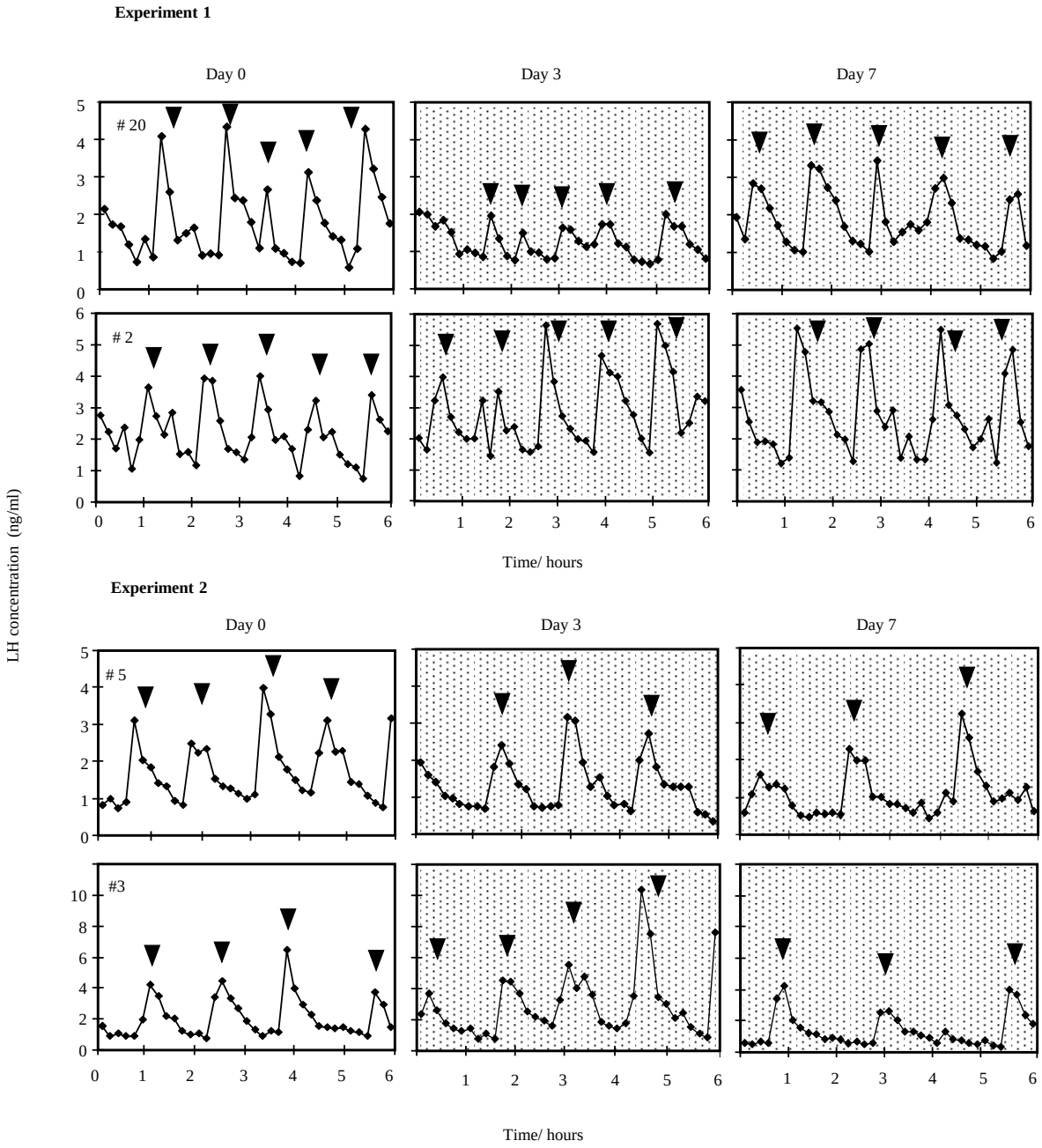

Figure 2. Shows the patterns of LH pulsatile secretion in the two OVX goat as a representative animals from the experiment 1 (upper 3 panels, #20 control and #2 treatment) and the two OVX goat for experiment 2 (upper 3 panels # 5 control and #3 treatment) on the day just before the start of infusion (Day 0, left) and the third (Day 3, middle) and seventh days (Day 7, right) of treatment. The shaded region point out the duration of infusion. Arrowheads point out the LH pulse recognized by Cluster Analysis.

Table 1. Effect of glucose infusion on LH secretion

Animal groups	LH profile	Experiment 1 (n = 8)			Experiment 2 (n = 8)		
		Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
Treatment	LH pulse/6h	5.3 ± 0.5	3.5 ± 1.9	4.0 ± 2.8	5.2 ± 1.0	5.0 ± 0.8	4.0 ± 0.8
	LH concentration (ng/ml)	2.3 ± 0.2	2.1 ± 0.6	2.1 ± 1.1	3.2 ± 2.0	3.0 ± 1.8	2.6 ± 1.8
Control	LH pulse/6h	6.3 ± 1.9	5.5 ± 1.7	6.0 ± 2.0	6.0 ± 1.4	5.7 ± 2.0	4.7 ± 1.7
	LH concentration (ng/ml)	2.3 ± 0.6	2.0 ± 0.6	1.8 ± 0.5	3.1 ± 1.9	3.1 ± 2.3	2.6 ± 1.9

Mean ± SD. Day 1 is the day of start of infusion
P>0.05 compared with day 0 and control group

The mean FSH concentrations profiles of experiments two from daily blood polling was displayed (Fig. 3). The mean FSH concentrations was not affected by glucose infusion in treatment group as compared with pre-infusion periods, there was no significant difference between treatment and control group.

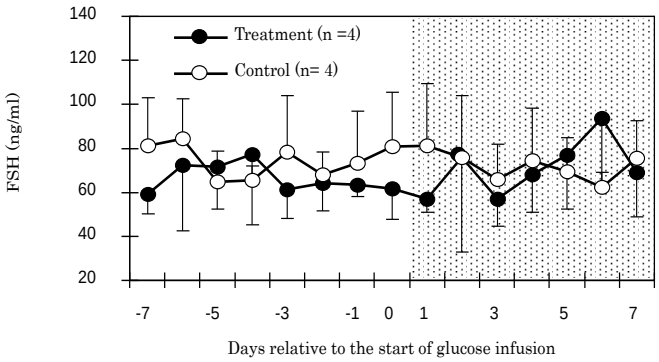

Figure 3. Shows the daily profiles (mean ± SD) of FSH from experiment 2 in the treatment (closed circle, n= 4) and control (open circle, n=4) groups. The shaded region point out the duration of infusion.

DISCUSSION

We have recently proved that the nutritional stimulus for short-term promotes LH pulsatile secretion go along with increased the insulin and glucose in the plasma concentrations in cycling (Haruna et al., 2009) and ovariectomized (Zabuli et al., 2009) goats. Peak levels of plasma insulin and glucose after the treatment in the present two experiments were similar to those of our previous studies. The present finding indicates that peripheral levels of glucose availability was stimulated by the glucose treatment up to a level that were observed in goats received short-term nutritional stimulus. On the other hand, patterns of the changes in the insulin and glucose concentrations were different between the constant and progressive infusion of glucose.

In our previous studies, plasma insulin and glucose concentrations were transiently stimulated by high-levels of nutritional supplementation and then gradually decreased to the baseline levels while nutritional supplementation treatment continued for 7 days (Zabuli et al., 2009; Haruna et al., 2009). Regarding the profiles of the glucose and insulin concentrations during the infusion, it is likely that constant infusion of glucose could mimic the changes in the insulin and glucose during the continuous stimulation by high energy level of

nutritional supplementation, and that progressive infusion of glucose could maintain the peak levels after nutritional supplementation throughout the infusion. Such physiological responses of blood glucose and insulin after glucose infusion did not influence the pulsatile LH secretion, suggesting that a rise of LH pulse frequency in goats received short-term high-energy feeding is independent upon the changes in the glucose availability.

The failure of our hypothesis that an increase in glucose availability stimulates pulsatile LH secretion supports the previous observations when glucose was infused for 5 days (Downing and Scaramuzzi, 1991). It was reported that the pharmacological blockage of glucose availability inhibit LH pulses in goats (Ohkura et al., 2004). Moreover, several studies have indicated that insulin-dependent glucose availability in the central nervous system play a role in maintaining the normal pulsatile LH secretion in food restricted ewes (Daniel et al., 2000) and diabetic sheep model (Tanaka et al., 2000), suggesting that an inhibition of LH pulsatility under the condition of negative energy balance is partly regulated by a reduction of glucose availability. Besides that, the present investigate suggests that glucose availability does not work as a nutritional stimulator under the condition of positive energy balance. Taken together, it is likely that physiological role of glucose availability in the regulation of the LH pulsatile secretion is different between negative and positive energy balance conditions. In our recent study supporting this hypothesis, levels of body energy status to modulate the nutritional treatment effect on LH pulsatile secretion is different between negative and positive nutritional manipulation in goats (Tanaka et al., 2004; Tanaka et al., 2002).

The previous (Zabuli et al., 2009) and present study indicate that FSH secretion is not stimulated by a rise of glucose availability in ovariectomized goats. The evidence from intact female animals reported that the glucose infusion increased the plasma concentrations of insulin and glucose, and also stimulated folliculogenesis without alteration of the FSH secretion (Scaramuzzi et al., 2006). Other researchers reported that the five days of glucose infusion does not only increase blood glucose and insulin levels, but also stimulates folliculogenesis in sheep (Munoz-Gutierrez et al., 2002). The supplement of lupin grain feeding or the glucose infusion suppresses estradiol secretion during the follicular phase of the estrous cycle in ewes, suggesting that the outcome of these direct actions on the follicle are decreased on hypothalamic-pituitary pathway, negative feedback action and leads to stimulation of folliculogenesis in the presence of either unchanged or slightly increased FSH concentrations (Scaramuzzi et al., 2006). In previous report, the combined infusion (glucose and insulin) into the ovarian artery reduced the ovarian oestradiol secretion rate and androstenedione, which are suppressive to FSH secretion by negative feedback system (Downing et al., 1999). Although (Haruna et al., 2009) recently demonstrated that nutritional supplementation at a certain stage of the estrous cycle (from the late luteal phase to the follicular phase) promulgate the incidence of a new FSH wave and decreased the interpeak interval of wave-like FSH secretion with an ascent of insulin and glucose levels in cycling goats, it is possible that the glucose availability mediates FSH secretion and ovarian folliculogenesis as a metabolic factor via direct action to ovary.

CONCLUSION

The present studies suggest that an increase in the glucose availability does not play a role in the regulation of gonadotropin secretion in ovariectomized goats.

Acknowledgment: The authors express their grateful thanks to the kind people of Japan, especially TUAT University, for being there and doing research there. A special thanks to Prof. Dr. Gen Watanabe, Ms. Satoko Haruna, Mr. Jun Rinno and Ms. Kayoko Sawa for technical support.

Conflict of interest: The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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