

Effect of saturated fatty acid on GnRH-induced gonadotropin secretion from anterior pituitary cells of pubescent ewe lambs

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Summary

The pathologically changed pattern of gonadotropin secretion, responsible for ovulation disorders in fatty ewes, may result from the prolonged increase in leptin concentration as well as from diminution of leptin receptors expression in anterior pituitary cells. Leptin acting peripherally reduces the secretion of insulin – the potent inhibitor of lipolysis. Consequently, an increment in plasma fatty acids level is observed. It was also found that in ewe lambs born to obese sheep carrying twins or triplets, a high plasma level of saturated fatty acids (SFA) is in positive correlation with the delay in puberty. However, the relationship between SFA and gonadotropin secretion from the ovine pituitary cells in pubescent ewe lambs is not clear. Therefore, the aim of the study was to establish the effect of SFA on GnRH-induced secretion of FSH and LH. Pituitary glands were isolated from 7 month old ewe lambs. Pituitary cells were cultured in McCoy 5A medium without GnRH and SFA (negative control), with GnRH only (positive control), with GnRH and 10^{-9} - 10^{-3} M/l of the butyric (C4:0), caprylic (C8:0), lauric (C12:0), palmitic (C16:0) or stearic acid (C18:0). After 2 or 6 h of exposure to SFA followed by 2, 6, 12, 18, 24 or 30 h incubation, the media for LH and FSH analysis were collected. It was found that all used SFA reduce GnRH-induced LH and FSH secretion from pituitary cells *in vitro*. The most significant ($P \leq 0.05$) suppressive effect was observed after 6 h exposure of cells to 10^{-3} M/l of caprylic acid, 10^{-4} M/l of palmitic acid and 10^{-4} M/l of stearic acid compared to the positive control.

Keywords: saturated fatty acids, gonadotropin, pituitary cells *in vitro*, ewe lambs

The pathologically changed pattern of gonadotropin secretion can be caused by the prolonged increase in plasma leptin concentration as well as by the diminution of leptin receptor expression in anterior pituitary cells (7). Leptin acting peripherally reduces the secretion of insulin – the potent inhibitor of lipolysis (2). Consequently, the increment in plasma fatty acids concentration is observed. Additionally, there was

found that in ewe lambs, especially born to obese sheep carrying twins or triplets and characterised by high daily body mass gains, the augmented plasma concentrations of leptin (10) as well as palmitic and stearic acid were in positive correlation with delay in puberty. There are some reports showing that fatty acids may be involved in modulation of pituitary hormones secretion. According to Garrel et al. (3) short-term treatment

with the monounsaturated oleate or the polyunsaturated linoleate significantly enhances LH release in both rat pituitary and mouse L β T2 cells (3). These authors noted also that oleate and linoleate increase LH β mRNA levels and LH release in rat primary cell cultures in a dose-dependent manner. Moreover, it is known that high plasma palmitate and stearate level is correlated with a delay in puberty in young ewes. However, according to the authors best knowledge, the relationship between saturated fatty acids (SFA) and gonadotropin secretion from the ovine pituitary cells isolated from pubescent ewe lambs is not established. Therefore, the aim of our study was to analyse the effect of SFA on LH and FSH secretion stimulated with GnRH *in vitro*.

Material and methods

Pituitary glands were obtained from seven-month old ewe lambs of meat-prolific SCP breed (37.5% Polish Lowland Sheep, 25% Suffolk, 25% Charolaise, 12.5% Romanov) ($n = 10$) immediately after slaughter. Due to collecting the tissue samples during a routine commercial slaughter of sheep, the study does not require the approval of an ethical committee in Poland. Isolation of cells was carried out through the digestion of adenohypophysis with 0.25% trypsin solution. Pituitary cells were finally cultured in McCoy 5A medium containing 2.5% fetal calf serum, 10% horse serum, mixture of amino acids and vitamins, 0.59% HEPES, gentamicin (20 μ g/ml), and adjusted to pH 7.4. 1 ml (in the case of LH and FSH secretion analysis) or 100 μ l (in the case of proliferation index (PI) determination) of dispersed cell suspension at concentration of 2.5×10^5 cells/ml was transferred to each culture dish of 24 or 96 well culture plates and incubated for 86 h at 37°C under the atmosphere of 5% CO₂.

After attachment to the dishes and obtaining the monolayer, the cells were washed with McCoy 5A medium without serum. Next, the cells were incubated with McCoy 5A medium without GnRH and SFA (negative control), with GnRH only (4×10^{-9} M/l) (positive control), with GnRH (4×10^{-9} M/l) and 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} M/l of butyric (C4:0), caprylic (C8:0), lauric (C12:0), palmitic (C16:0) or stearic (C18:0) acid, respectively. Each sample was performed in duplicate. After 2 or 6 h of exposure to SFA followed by 2, 6, 12, 18, 24 or 30 h incubation the media were collected to analyse LH and FSH in control and experimental cultures. Simultaneously, PI of control cells and those treated with SFA was estimated. Assessment of cell proliferation was based on the reduction of the tetrazolium salt (MTT) into a blue formazan. Control cultures and those incubated with SFA were

pulsed with 15 μ l of MTT (for 3 h at 37°C) and then solubilized with SDS overnight. The optical density (OD) of the formed blue formazan was measured by ELISA microplate reader (Biotek Elx800 Microplate Reader, Agilent, CA, USA) at the wavelength of 600 nm. The results were expressed as PI values and used to calculation of gonadotropin secretion. LH and FSH concentrations in the culture media were determined using LH [125I] IRMA KIT or FSH [125I] IRMA KIT (Orion Diagnostica, Spectria, Finland), respectively. Gonadotropin secretion was expressed as a concentration (mIU/ml) of given hormone which was released into the culture medium by about 2.5×10^5 cells during 2, 6, 12, 18, 24 and 30 h, respectively. Each sample was performed in duplicate.

Statistical analysis. The obtained results were calculated using Statistica 10.0 PL and expressed as a mean and standard deviation ($\bar{x} \pm SD$). Comparisons between the control and experimental cultures were performed using analysis of variance and the paired t-tests. Differences were considered as significant at $P \leq 0.05$.

Results and discussion

The effect of SFA on GnRH-induced FSH secretion from ovine pituitary cells *in vitro*. FSH secretion averaged 1.85 ± 0.52 mIU/ml/ 2.5×10^5 cells/30 h in negative control culture, whereas 2.98 ± 0.19 mIU/ml/ 2.5×10^5 cells/30 h in positive control. The exposure of the cells to butyric, caprylic and lauric acid for 2 hours did not affect FSH secretion significantly compared to the positive control. However, 2-hour exposure of the cells to palmitic and stearic acid resulted in a decrease in FSH secretion. The most significant effect ($P \leq 0.05$) was observed after the exposure of the

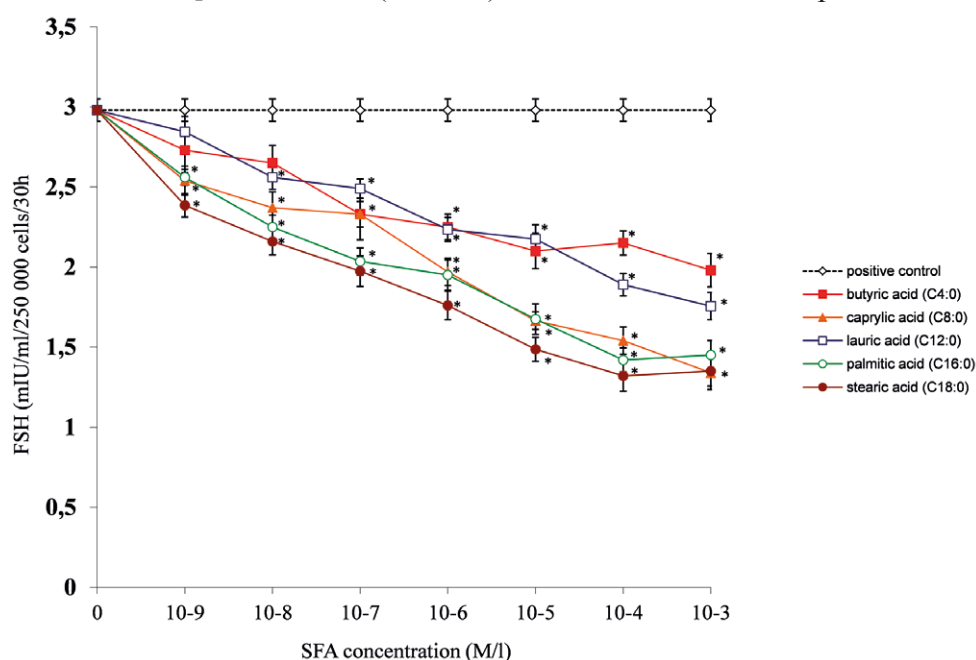


Fig. 1. The effect of 6-hour exposure of the ovine pituitary cells to saturated fatty acids (SFA) (butyric (C4:0), caprylic (C8:0), lauric (C12:0), palmitic (C16:0) and stearic acid (C18:0), each in concentration 10^{-9} – 10^{-3} M/l) on GnRH-induced secretion of FSH *in vitro*. The cells were isolated from 7-month old ewe lambs

Explanation: * – significant difference ($P \leq 0.05$) compared to the positive control (4×10^{-9} M/l GnRH)

cells to 10^{-4} and 10^{-3} M/l of stearic acid (2.48 ± 0.07 and 2.12 ± 0.10 mIU/ml/ 2.5×10^5 cells/30 h) as well as to 10^{-4} and 10^{-3} M/l of palmitic acid (2.48 ± 0.07 and 2.49 ± 0.10 mIU/ml/ 2.5×10^5 cells/30 h). After 6-hour exposure of cells to all used SFA we noted the marked reduction of GnRH-induced FSH secretion. The most significant ($P \leq 0.05$) suppressive effects were observed under the influence of 10^{-3} M/l of caprylic acid (FSH: 1.34 ± 0.10 mIU/ml/ 2.5×10^5 cells/30 h), 10^{-4} M/l of palmitic acid (FSH: 1.42 ± 0.14 mIU/ml/ 2.5×10^5 cells/30 h) and 10^{-4} M/l of stearic acid (FSH: 1.32 ± 0.21 mIU/ml/ 2.5×10^5 cells/30 h) in comparison to positive control (Fig. 1). Negative correlations between FSH secretion and butyric, caprylic, lauric, palmitic or stearic acid concentration in culture media ($r = -0.98$, $r = -1.00$, $r = -0.64$, $r = -0.98$, $r = -0.98$, respectively) were also found.

The effect of SFA on GnRH-induced LH secretion from ovine pituitary cells *in vitro*. LH secretion averaged 1.76 ± 0.67 mIU/ml/ 2.5×10^5 cells/30 h in negative control culture, whereas 2.76 ± 0.19 mIU/ml/ 2.5×10^5 cells/30 h in positive control. 2-hour treatment of the cells with butyric, caprylic, lauric and palmitic acids did not significantly change LH secretion compared to the positive control, whereas stearic acid (10^{-4} M/l) decreased LH release ($P \leq 0.05$) (1.67 ± 0.12 mIU/ml/ 2.5×10^5 cells/30 h). However, after 6 hours exposure of cells the significant ($P \leq 0.05$) inhibitory influence of SFA on LH secretion during the entire time of the experiment was observed. The most significant suppressive effects ($P \leq 0.05$) were found after exposure of the cells to 10^{-3} M/l of caprylic

acid (LH: 1.18 ± 0.12 mIU/ml/ 2.5×10^5 cells/30 h), 10^{-4} M/l of palmitic acid (LH: 1.27 ± 0.14 mIU/ml/ 2.5×10^5 cells/30 h) and 10^{-4} M/l of stearic acid (LH: 1.35 ± 0.17 mIU/ml/ 2.5×10^5 cells/30 h) in comparison to the positive control (LH: 2.76 ± 0.09 mIU/ml/ 2.5×10^5 cells/30 h) (Fig. 2). Therefore, there was negative correlation between LH and butyric, caprylic, lauric, palmitic, stearic acid ($r = -0.95$, $r = -0.98$, $r = -0.98$, $r = -0.98$, $r = -0.98$, respectively).

The activity of anterior pituitary cells is regulated by numerous factors, including signals related to nutritional status. It is known that hypothalamic GnRH is the main stimulatory signal eliciting gonadotropin synthesis and release. There are also some reports showing that fatty acids may be involved in modulation of pituitary hormones secretion. Most studies on the influence of fatty acids on the gonadotropin secretion report the role of unsaturated fatty acids in the endocrine regulation of reproductive functions in rodents. Garell et al. (3), using a rat model of central lipid overload combined with *in vitro* studies, demonstrated that unsaturated fatty acids such as linoleic acid directly stimulate LH β expression and interfere with basal and GnRH-stimulated LH secretion. These authors also reported that unsaturated fatty acids oppositely regulate the expression LH and FSH β -subunit genes. Moreover, they show that fatty acids disrupt cellular Smad signaling, providing a novel signalling cascade by which fatty acids can target gene expression (4). Moreover, Moriyama et al. (9) have shown that central exposure to linoleic acid increases LH β mRNA expression levels in rats. Another study conducted by Barb et al.

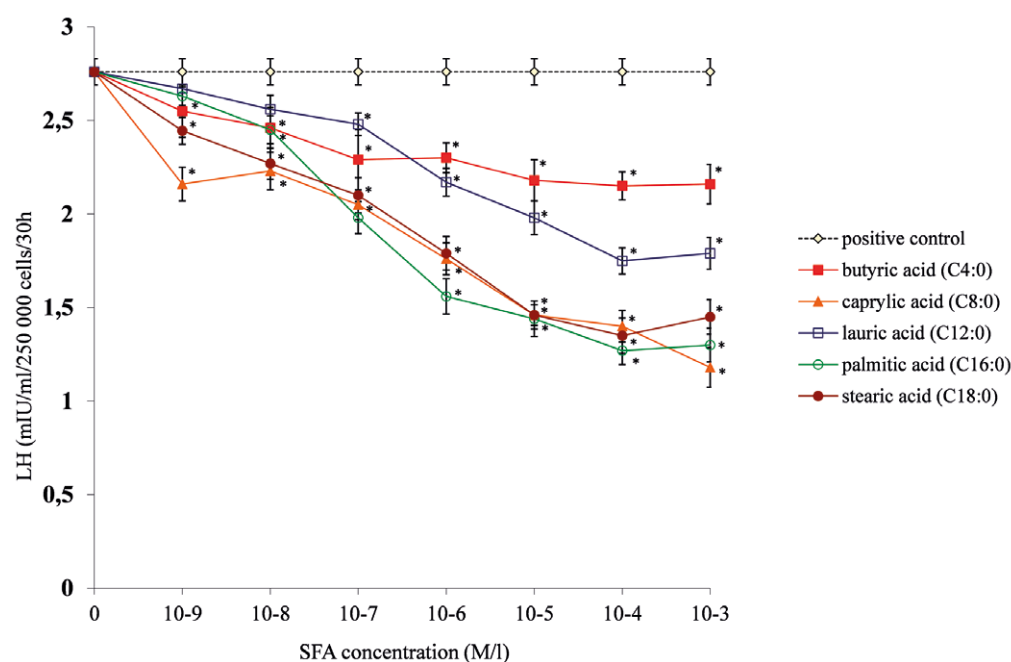


Fig. 2. The effect of 6-hour exposure of the ovine pituitary cells to saturated fatty acids (SFA) (butyric (C4:0), caprylic (C8:0), lauric (C12:0), palmitic (C16:0) and stearic acid (C18:0), each in concentration 10^{-9} – 10^{-3} M/l) on GnRH-induced secretion of LH *in vitro*. The cells were isolated from 7-month old ewe lambs

Explanation: * – significant difference ($P \leq 0.05$) compared to the positive control (4×10^{-9} M/l GnRH)

(1) on porcine pituitary cells *in vitro* have demonstrated that oleic acid enhances basal LH release and suppressed the LH response to GnRH. Unfortunately, according to our knowledge, data on the effect of fatty acids on the reproduction of ruminants, including sheep, mainly concern the effect of polyunsaturated fatty acids on prostaglandin production and metabolism *in vivo* and *in vitro* (within reproductive cells), synthesis of progesterone and oestradiol, follicle development, oocyte maturation, onset of oestrus, ovulation rate, embryo survival and pregnancy rate (5). Among others, Mattos et al. (8) highlighted that dietary fatty acids of the n-3 family (polyunsaturated fatty acids such as linoleic, linolenic,

eicosapentaenoic and docosahexaenoic) reduce ovarian and endometrial synthesis of prostaglandin F2 α , and delay parturition in sheep. Also Soydan et al. (11), examined the effects of fatty acids on reproductive performance. These authors, taking into account that fatty acids are the precursors of progesterone and prostaglandins, have shown that they can influence follicular development, ovulation and embryonic implantation. What is more, they investigated that dairy cows fed diets high in eicosapentaenoic and docosahexaenoic acids or α -linolenic acid during early pregnancy had reduced prostaglandin PGF2 α production and increased pregnancy rates. They observed that feeding diets high in α -linolenic acid during the dry period may increase the incidence of placental retention. They suggested that the above mentioned fatty acids can improve fertility in dairy cows during the postpartum period (11).

However, there are no studies on the direct effect of SFA on gonadotropin secretion at the level of the pituitary gland in ruminants. According to our results, the saturated fatty acids reduce GnRH-induced LH and FSH secretion from pituitary cells *in vitro*. The most significant ($P \leq 0.05$) suppressive effect was observed after 6 h exposure of cells to 10^{-3} M/l of caprylic acid, 10^{-4} M/l of palmitic acid and 10^{-4} M/l of stearic acid compared to the positive control. In contrast, Garell et al. (3), using rodent L β T2 cells, have shown that saturated palmitate was ineffective in its impact on gonadotropin secretion. The difference in the results obtained may be due to the fact that the studies were conducted using a different species and different types of cell culture (primary and continuous cell cultures, respectively). Unfortunately, it may be difficult to compare our results more broadly and interpret them based on the work of other researchers due to the lack of other reports on the direct effect of saturated fatty acids on the secretion of gonadotropins by pituitary cells isolated from pubescent ewes. There are, however, some similar data relating to the influence of SFA on the secretion of adrenocorticotropin (ACTH) by rat pituitary cells *in vitro*. Katoh et al. (6) have demonstrated that saturated fatty acids (butyrate, caprylate, laurate, palmitate and stearate) significantly reduce ACTH release from rat anterior pituitary cells induced by CRH. Despite being based on another species and different tropic hormone this research is consistent with our studies, which indicates that saturated fatty acids reduced secretion of pituitary hormones (6).

To sum up, our study demonstrates that SFA reduce GnRH-induced LH and FSH secretion from pituitary cells isolated from pubescent ewes. Perhaps, analogously to the results obtained by Garell et al. (3), indicating that linoleic acid directly stimulates LH β expression, saturated fatty acids may also affect the expression of gonadotropin structural subunits, but reducing it rather than increasing. Additionally, taken into account that high palmitic and stearic acid are in positive correlation with delay in sexual maturation,

the observed decrease in gonadotropin secretion from pituitary cells under the influence of SFA may be involved in the mechanism of puberty delay in young ewes. To confirm this hypothesis, further research in this field is necessary.

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