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Caloric restriction and IGF-I administration promote rabbit fecundity: Possible interrelationships and mechanisms of action

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ABSTRACT

The aim of these *in vivo* and *in vitro* studies was to examine the influence of caloric restriction (CR), and the administration of insulin-like growth factor (IGF-I), on rabbit fecundity and to understand the interrelationships between CR and IGF-I, as well as the endocrine and intracellular mechanisms of their effects. Female rabbits were subjected to 50% CR, injections of IGF-I (20 μg/animal/day) and a combination of the two for 10 d before and 2 d after ovulation induced by 25 IU PMSG and 0.25 IU hCG. On the day of ovulation blood samples were collected and analyzed IGF-I, leptin, progesterone (P_4) and estradiol (E_2) concentrations by RIA. Some animals from each group were killed in their periovulatory period and weighed, as were their ovaries. Granulosa cells isolated from ovaries of does subjected or not to CR were cultured for 2 d with and without IGF-I (100 ng/mL). Accumulation of markers of cell proliferation (PCNA and cyclin B1), apoptosis (bax), MAP/ERK1,2 kinase (MAPK), protein kinase A (PKA) and IGF-I were evaluated by immunocytochemistry. In addition, E_2 release by cells isolated from ovaries of animals subjected or not to CR and cultured with and without IGF-I (1, 10, 100, 1000 or 10000 ng/mL) was assessed by RIA. The remaining animals were kept until parturition, when the number of pups was recorded.

CR did not affect animal and ovarian weight, but significantly increased the number of pups per litter and plasma levels of IGF-I and decreased plasma leptin and P_4, but not E_2 concentration. Injections of IGF-I did not influence body and ovarian weights, but increased the number of pups per litter and plasma IGF-I and leptin concentration and reduced plasma E_2 but not P_4 level. IGF-I administration did not modify the main effects of CR, although it prevented the CR-induced decrease in plasma P_4 level.

CR reduced accumulation of PCNA, bax, promoted accumulation of cyclin B1 but not of MAPK, PKA or IGF-I within ovarian granulosa cells. Addition of IGF-I to culture medium reduced accumulation of bax, MAPK, and IGF-I and promoted PKA accumulation and E_2 release. CR promoted the stimulatory effect of IGF-I on E_2 output.

Thus, CR can increase rabbit fecundity, probably via changes in IGF-I, leptin and steroid hormones released, which in turn can affect ovarian cell cycle, apoptosis, and response to IGF-I. Furthermore, they demonstrate the stimulatory influence of IGF-I on rabbit fecundity, which was associated with changes in plasma leptin, E_2 and ovarian cell apoptosis, PKA, MAPK, IGF-I and E_2 release. The promotion of IGF-I output by CR and the ability of IGF-I to mimic/replace but not to modify CR effects on fecundity, plasma IGF-I, and ovarian cell apoptosis suggest that IGF can mediate the action of CR on these reproductive indexes. In contrast, differences in the action of CR and IGF-I on other hormones, ovarian cell proliferation, protein kinases and IGF-I suggest that CR action on these indexes is not mediated by IGF-I. We thus demonstrate that both CR and IGF-I administration can increase rabbit fecundity, and that their

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effects can be mediated by changes in reproductive hormones, ovarian cell proliferation, apoptosis, and the response of ovarian cells to IGF-I.

1. Introduction

It is widely accepted, that caloric restriction (CR) improves health, prevents aging and extends life span across multiple species [1–3], but it inhibits reproductive processes via changes in metabolic hormones and subsequent suppression of hypothalamo-hypophysial-gonadal axes and, as a result, puberty and fertility [1,4–6]. Some evidence suggests that rabbits can be excluded from this rule. Several studies [7–12] have demonstrated that CR can affect rabbit metabolic and reproductive hormones and suppress post-ovulatory embryo growth and viability but it does not affect the number of developing ovarian follicles, number of ovulations, oocytes, and kindling rate. Other experiments have shown that either intermittent or continuous [13–18] pre-mating CR can significantly promote the subsequent rabbit oocyte maturation, fertility and kindling rate. Therefore, examining the character of CR influence on fertility in this model species can be important for understanding the general interrelationship between reproduction and metabolism, for medical application of CR without jeopardizing reproductive processes, and also for biologically and economically efficient rabbit breeding.

Even more important could be understanding the mechanisms mediating the influence of metabolic state on reproduction so that these mediators can be used for characterization, prediction, and control of metabolism and reproduction. It is proposed, that such mediators could be metabolic hormones including IGF-I and leptin because (1) CR change their production and reception and (2) because their up- and down-regulation can affect reproduction and change the effect of CR on reproductive systems [1,4,6]. The action of CR on rabbit IGF-I, leptin and ovarian steroid hormones [10,17–20], and the effect of these molecules on rabbit ovarian cell functions [21–25], are documented. These data could indicate that IGF-I and other molecules could potentially mediate the effect of CR on rabbit reproduction. Nevertheless, the direct evidence for it could be the ability of such potential mediators to mimic and replace effects of CR. To our knowledge, such studies have not yet been performed. Hormones can affect ovarian functions via intracellular messengers—protein kinases, regulators of the cell cycle, and apoptosis [24]. These regulatory molecules are present in rabbit ovaries, they can affect rabbit reproductive functions and mediate the effect of hormones on these functions [21,25]. The interrelationships between CR and regulators/markers of ovarian cell proliferation, apoptosis, and protein kinases, and the role of these molecules in the mediation of CR action, remain unknown. Moreover, the influence of IGF-I on rabbit reproductive functions *in vivo* has not been previously studied.

The aim of our *in vivo* and *in vitro* studies was to examine the influence of both CR and IGF-I on rabbit fecundity and to understand their interrelationships and the endocrine and intracellular mechanisms of their influence, including the role of some hormones, protein kinases, regulators/markers of cell cycle, apoptosis and the response of ovarian cells to IGF-I.

2. Materials and methods

The effects of CR and IGF-I were investigated in both *in vivo* and *in vitro* systems in one series of experiments, as follows: (1) rabbits were subjected to CR or IGF-I administration and a combination of the above, and investigated for reproductive parameters and plasma concentrations of hormones; (2) granulosa cells were isolated from ovaries of control and CR-treated animals and evaluated for accumulation of indices of cell proliferation and apoptosis; some protein kinases and IGF-I (3) these cells were cultured with and without IGF-I and evaluated for the secretion of estradiol.

2.1. In vivo experiments

Female nulliparous non-cycling New Zealand White rabbits, line M91, 6 months of age, weight 4.0–4.9 kg were bred and kept in individual cages under standard conditions (photoperiod 16 h:8 h temperature 17 ± 3 °C, air humidity 75+ 10%) at the local rabbit farm of Research Institute of Animal Production. All the animals were fed individually 1 times per day with previously weighted standard granulated mixture KK (PD Cataj, Cataj, Slovakia) containing 18% NL, 12% fibers, 2.6% fat, ME = 10.8 MJ. The composition and nutrient content of this mixture is indicated in Table 1. .From days 1–12, animals were randomly divided into 4 groups (15 animals per group): (1) control (received full food dose), (2) subjected to 50% CR (received 50% of food dose), (3) injected with recombinant IGF-I (GroPep, Adelaide, Australia, 20 μg/mL PBS/day) and (4) subjected to a combination of both CR + IGF-I (as mentioned above). The animals not subjected to IGF-I administration were injected with PBS only. Aliquots of freshly prepared stock solution of IGF-I (1 mg/mL PBS) were frozen at −17 °C and thawed immediately before injections. All females were injected on the 6th day of the experiment with PMSG (Sergon, Bioveta, Ivanovice Ivanovice na Hané, Czech Republic; 25 IU/animal in 1 mL PBS), and on the 9th day with LH-RH (Supergstran, Ferring-Leciva, Pohori-Chotoun, Czech Republic; 0.25 IU/animal in 1 mL of PBS). On day 10, all animals were weighted, artificially inseminated and blood was

Table 1
The composition and nutrient content of feed mixture for rabbits used in the experiments Components Percentage (%).

Lucerne forages	36
Oat	13
Barley	8
Wheat bran	9
Corn-DDGS	5
Rapeseed (sunflower) extracted meal	11
Malt sprouts	15
Ground limestone	1
Vitamin-mineral premix	1.7
NaCl	0.3
Crude protein	min.16.5
Crude fiber	16
Fat	3
Starch	14
Lysine	0.75–1.0
Methionine and Cystine	0.4–0.6
Arginine	0.6–0.9
Ca	0.8–1.2
P	0.3–0.7
K	0.7–0.9
E672 (Vitamin A)	6000 m.j./kg
E671 (Vitamin D 3)	1000 m.j./kg
Vitamin E (Alphatocopherol)	50 mg/kg