Aberrant hypothalamic-pituitary-ovarian axis in the Watanabe heritable hyperlipidemic rabbit

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Abstract The WHHL rabbit has a defective low density lipoprotein receptor and is a model for familial hypercholesterolemia. WHHL rabbits are less fecund than NZW rabbits, the strain into which the defect has been inbred. This lower fecundity could be related to impaired ovarian steroidogenesis due to reduced intracellular availability of cholesterol. Here we compare the WHHL and NZW rabbits with regard to oocyte morphology and fertilization rates after stimulation with equine chorionic gonadotropin. We also compare hypothalamic-pituitary-ovarian axis function by measuring baseline and gonadotropin releasing hormone-stimulated plasma estradiol, progesterone, and gonadotropin levels, both before and after simvastatin inhibition of de novo cholesterol synthesis. WHHL rabbit oocytes remained encased in cumulus and had a lowered fertilization rate (9/50 vs. 83/87, $P < 0.05$). WHHL rabbits had lower baseline estradiol levels (7.1 ± 0.72 vs. 10.2 ± 0.94, $P < 0.05$) and had higher baseline follicle stimulating hormone ($P < 0.05$) and luteinizing hormone ($P < 0.05$) levels. Simvastatin lowered luteal progesterone concentrations only in WHHL rabbits ($P < 0.05$). We conclude that the hypothalamic-pituitary-ovarian axis in WHHL rabbits is abnormal. The reduced availability of intracellular cholesterol for progesterone synthesis by inhibition of de novo cholesterol biosynthesis leads to a significant reduction in plasma progesterone concentrations in the WHHL. These findings have implications for women with familial hypercholesterolemia, particularly regarding treatment with inhibitors of de novo cholesterol synthesis.—**Robins, E. D., L. M. Nelson, and J. M. Hoeg.** Aberrant hypothalamic-pituitary-ovarian axis in the Watanabe heritable hyperlipidemic rabbit. *J. Lipid Res.* 1994. **35:** 52–59.

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The WHHL rabbit is a model for familial hypercholesterolemia. As in familial hypercholesterolemia, the pathophysiology in the WHHL rabbit stems from a defect in LDL receptor function (LDL receptor activity can be less than 1% of normal) (1, 2). Due to this LDL receptor defect, the WHHL rabbit has a 10-fold increase in LDL serum concentration and the sequelae of premature cardiovascular disease and cholesterol deposition (1).

One problem with the WHHL rabbit as a model, however, is its low fecundity. The WHHL rabbit has an average litter size of 2–4 kits (3) compared to a liter size of 8–10 kits in wild-type rabbits (4). Chai (5) showed that implantation was impaired in inbred females that had normal LDL-receptors. However, Shiomi, Ito, and Watanabe (3) established that closed-colony breeding does not cause the rapid fall of reproductive vigor seen in the inbreeding described by Chai (5). This reduced fecundity in the WHHL may be due to disorders of ovulation, implantation, and gestation (6). Because of poor fecundity, WHHL rabbits must be regularly outbred to normal rabbits in order to continue the colony. The WHHL rabbits here at the National Institutes of Health have been outbred with NZW rabbits since 1983 so that NZW rabbits serve as the control rabbits (personal communication with Nathan N. Jackson D.V.M., Chief of the Genetic Resources Section, Scientific Services Branch, Veterinary Resources Program, National Center For Research Resources, National Institutes of Health).

As cholesterol is the precursor for steroid hormones, the LDL receptor defect might contribute to this lowered fecundity by altering steroidogenesis. There are two main pathways that provide cholesterol to the cell for steroid production. Cholesterol is available from intracellular de novo synthesis where HMG-CoA reductase is the rate-limiting step, or cholesterol is imported from the serum in lipoprotein particles (7). The low density lipoprotein particle is the preferred source for ovarian steroid synthesis, particularly for the corpus luteum (8). Thus, defective LDL receptor function may play a role in the reduced fecundity.

Abbreviations: WHHL, Watanabe heritable hyperlipidemic; NZW, New Zealand white; LDL, low- density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; eCG, equine chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle stimulating hormone; RIA, radioimmunoassay; GnRH, gonadotropin releasing hormone.

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Although the LDL receptor defect impairs adrenal function in WHHL rabbits (9), it is not known whether the LDL receptor defect alters ovarian steroid production. In humans, patients without LDL in their serum have low serum concentrations of progesterone in the luteal phase (10) and during pregnancy (11). The corpus luteum is the sole source of progesterone in the pseudopregnant rabbit, and changes in progesterone production are responsible for the variations in peripheral progesterone concentrations in the rabbit (12).

To determine whether the LDL receptor defect in the WHHL rabbit has an effect on the hypothalamic-pituitary-ovarian axis, we compared plasma concentrations of FSH, LH, estradiol, and progesterone to concentrations of these hormones found in age-matched control NZW rabbits. We determined the impact of inhibition of de novo cholesterol synthesis to supply precursor for ovarian steroidogenesis in the WHHL rabbit by using the HMG-CoA reductase inhibitor simvastatin.

MATERIALS AND METHODS

Animals

Sexually mature NZW female rabbits, obtained from Hazelton Laboratory Products Inc. (Denver, PA), were age-matched to sexually mature female WHHL rabbits acquired from the Small Animal Section of the National Institutes of Health. These rabbits were caged separately at constant temperature (68°-72°F) on a 12-h light cycle. They were given 120 g of alfalfa-based pressed pellet daily and water ad libitum.

Agents

Buserelin, a GnRH agonist generously donated by Hoechst Roussel AG (Frankfurt, Germany) was administered by subcutaneous injection (0.6 μg/kg body weight). ECG was obtained from Sigma Chemical Company (St. Louis, MO) as pregnant mare serum gonadotropin and injected intramuscularly (28 IU/kg body weight). Bio-Serve Inc. (Frenchtown, NJ) incorporated simvastatin, obtained by generous donation from Merck, Sharp & Dohme (Rahway, NJ), into alfalfa pellets in doses of 2 mg per g of pellet.

Blood samples

Central venous catheters were place in the right atrium of each rabbit as previously described (13). Heparin flushes (1:10,000, 0.5 ml, 3 times weekly) kept the catheters patent. Blood samples for cholesterol measurements were obtained after animals had fasted 12 h. Samples for hormone assay were obtained from unrestrained rabbits at time 0, 1 h, 2 h, 4 h, 4 days, and 8 days after buserelin injection. All samples were collected in EDTA tubes and placed on ice. The plasma was separated by centrifugation at 4°C, then stored at −20°C until assayed.

Assays

We measured the total cholesterol plasma concentrations using the Abbott analyzer model VP Super System with Abbott reagents purchased from Abbott laboratories (Chicago, IL). Plasma progesterone was measured by an extraction, double antibody RIA technique as previously described (14). Estradiol concentration was measured by an extraction chromatography RIA technique as previously described (15) with modification of the chromatographic separation as described by Abraham et al. (16), and also using 4 ml rather than 2 ml of plasma for the initial extraction of estradiol. Plasma LH and FSH were measured utilizing radioimmunoassay kits kindly supplied by A. F. Parlow, at the Pituitary Hormones and Antisera Center (Torrance, CA). These kits included purified LH and FSH standard and respective antibodies raised in guinea pigs. The second antibody was a goat anti-guinea pig serum purchased from ICN Biomedicals (Costa Mesa, CA).

Experimental design

Experiment I: In transgenic experiments in another study with WHHL rabbits, oocytes were recovered from mated WHHL and NZW rabbits as previously described in mice (17) with the following exception: eCG was administered subcutaneously (28 IU/kg) to the rabbits instead of 5 IU administered to mice. Potentially sperm-penetrated oocytes were examined by phase contrast microscopy (100×). If the potentially sperm-penetrated oocytes were both free of cumulus and had pronucleus formation they were considered normal. These findings were noted and were the genesis for experiments II and III described below.

Experiment II: The endocrine status of the hypothalamic-pituitary-ovarian axis of 8 WHHL rabbits and 8 NZW rabbits was compared. We measured plasma estradiol, progesterone, and gonadotropin concentrations in blood samples obtained from non-anesthetized and unrestrained rabbits, and compared these hormone concentrations in the two rabbit strains. Then we measured concentrations of progesterone in plasma samples obtained from these same rabbits at 1 h, 2 h, 4 h, 4 days, and 8 days after injecting the GnRH agonist buserelin (0.06 μg/kg). Gonadotropin concentrations were also measured on the sample obtained 1 h post-buserelin injection. We compared the plasma progesterone and gonadotropin concentrations in the two rabbit strains to assess the response to buserelin.

Experiment III: We next assessed simvastatin's effect on the preovulatory and pseudopregnant plasma progesterone concentration. We controlled for the elevated plasma

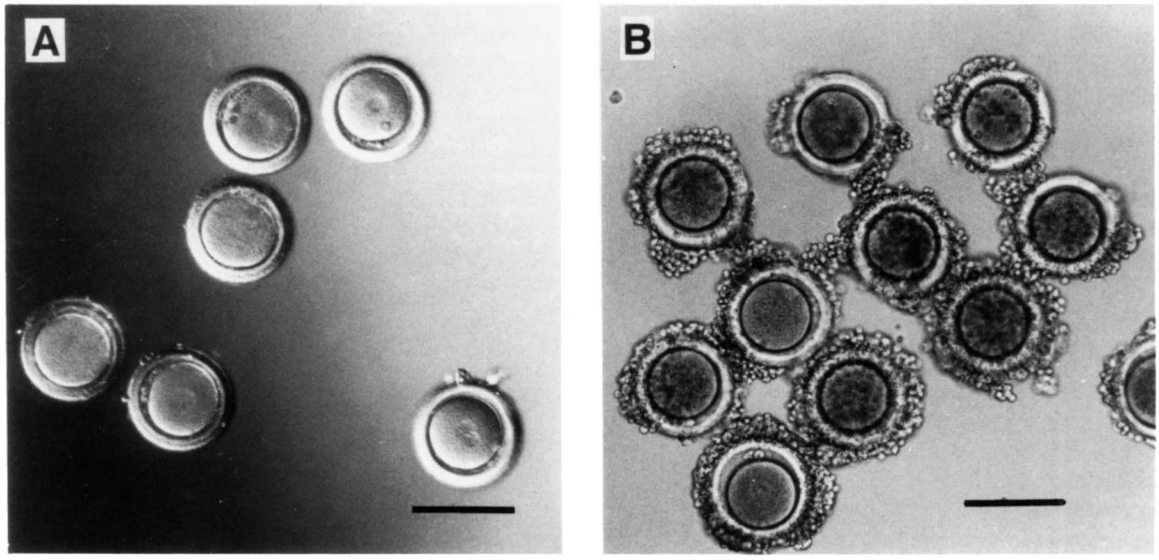

Fig. 1. Oocytes retrieved from NZW and WHHL rabbits. Oocytes retrieved 20 h after ovulation induction in NZW (A) and WHHL (B) as seen by phase contrast microscopy (100×). The polar bodies as well as double pronuclei can be seen just inside the zona pellucida in all the NZW oocytes indicating fertilization. The WHHL oocytes are coated with the cumulus oophorus and few are fertilized. The solid bar indicates 150 μm.

FSH concentration in the WHHL rabbit by injecting eCG (28 IU/kg) 48 h prior to buserelin injection and obtained serial blood samples after buserelin as in experiment II (6 WHHL rabbits and 6 NZW rabbits). Then we supplied the simvastatin-incorporated feed in the diet to the same 12 rabbits so that they would receive 20 mg per kg of body weight of simvastatin each day for 4 weeks. During the 4th week of simvastatin therapy the blood samples were again obtained on the same schedule. Twelve-hour fasting bloods were obtained for plasma cholesterol concentration before and after simvastatin treatment.

Statistics

Chi square was used to test the significance of the difference between the frequency of fertilized oocytes between the two rabbit strains. Differences among multigroup means were compared by two-way analysis of variance with repeated measures. Two-tailed Student's *t* tests were used to determine significance between means. Because of lack of normality of the data, the Wilcoxon rank sum test was used to determine the significance between the means of the baseline WHHL and NZW rabbit plasma estradiol and progesterone concentrations. All *P* values < 0.05 were considered statistically significant.

RESULTS

The NZW potentially sperm-penetrated oocytes were free from surrounding cells and mucin and the majority of the cells were fertilized with clearly visualized polar bodies and pronucleus (**Fig. 1**). By contrast, the WHHL rabbit potentially sperm-penetrated ova were encased in cumulus and mucin. Occasional polar bodies were seen but few WHHL rabbit potentially sperm-penetrated oocytes contained pronucleuses. Eighty-three of 87 NZW potentially sperm-penetrated oocytes were fertilized and free of cumulus while 9 of 50 potentially sperm-penetrated oocytes recovered in WHHL rabbits were both free of cumulus and fertilized ($P < 0.05$).

The mean plasma estradiol concentration was 30% lower in the WHHL rabbits than in NZW rabbits ($P < 0.05$) (**Table 1**). The mean plasma concentration of baseline FSH was 87% higher in the WHHL rabbits than in the NZW rabbits ($P < 0.05$). Similarly, the baseline mean plasma concentration of LH was 61% higher in the WHHL rabbits ($P < 0.05$) (**Fig. 2**). One hour after buserelin administration the, LH plasma concentration was 79% higher in the WHHL rabbits ($P < 0.05$).

Although baseline progesterone concentrations were not significantly different, post-LH surge plasma progesterone concentrations were significantly higher in WHHL compared to NZW rabbits ($P < 0.05$). The peak progesterone concentration in the WHHL rabbits was 76% higher than in the NZW rabbits at hour 2 (**Fig. 3**).

As the baseline and stimulated LH concentrations were significantly higher in the WHHL, the rabbits did not receive a uniform stimulus for luteinization. To standardize the progesterone concentration in response to gonadotropin stimulation we evaluated ΔP4/ΔLH, where ΔP4 is the plasma progesterone difference between hour-1 and hour-0 and ΔLH is the plasma LH concentration difference between hour-1 and hour-0. The mean ΔP4 in

TABLE 1. Plasma estradiol concentration in WHHL and NZW adult female rabbits

Rabbit	Estradiol
	pg/ml
WHHL	
W327-3	5.6
W330-1	6.52
W330-3	6.9
W331-1	5.5
W335-1	10.2
W325-1	7.93
Mean ± SEM	7.1 ± 0.72[a]
NZW	
N19396	13.4
N25111	12.4
N58093	9.16
N63335	9.63
N70669	9.65
N78504	7.11
Mean ± SEM	10.2 ± 0.94

[a]Differs from NZW ($P < 0.05$ by the Wilcoxon rank sum test).

the WHHL rabbits was over 100% higher than in the NZW rabbits ($P < 0.05$) and the mean ΔLH was 79% higher ($P < 0.05$); however, the mean ΔP4/ΔLH was not significantly different between the two strains ($P = 0.4$) (**Table 2**). Therefore, the mean concentration change in plasma progesterone per unit plasma concentration change of LH in the first hour was not significantly different between WHHL and NZW rabbits.

The mean plasma cholesterol concentration in the WHHL rabbits was more than 10-fold higher than in the NZW rabbits ($P < 0.05$). Simvastatin (20 mg/kg per day) effectively lowered cholesterol levels in both WHHL rabbits and NZW rabbits (**Table 3**). The simvastatin treatment decreased fasting plasma cholesterol concentrations by approximately 60% in both strains ($P < 0.05$). The

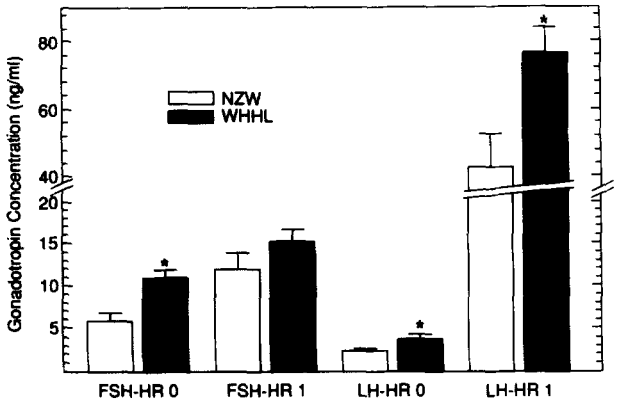

Fig. 2. Plasma gonadotropin concentrations in NZW and WHHL female rabbits. Mean plasma FSH and LH concentrations (ng/ml) in NZW (white bars) and WHHL (black bars) before and 1 h after buserelin injection. The error bars represent standard error of the mean. *Differs from NZW ($P < 0.05$).

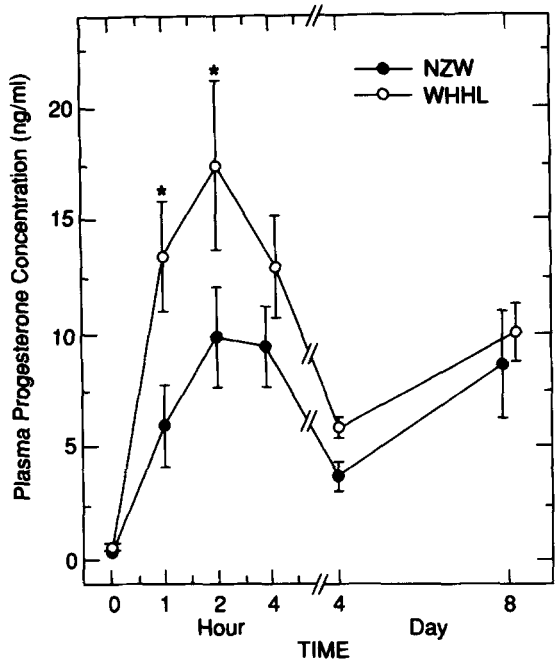

Fig. 3. Plasma progesterone concentrations after buserelin infusion in NZW and WHHL female rabbits. Mean plasma progesterone concentrations (ng/ml) in NZW (●) and WHHL (○) before and after buserelin (0.6 mg/kg). The error bars represent standard error of the mean. *Differs from NZW.

cholesterol-lowering effect of the HMG-CoA reductase inhibitor was evident in each individual rabbit of both strains (**Fig. 4**).

Having established that the hypothalamic-pituitary-ovarian axis in the WHHL rabbit is aberrant, but progesterone concentrations were not significantly less in the WHHL rabbit, we tested the effect of simvastatin on the WHHL and normal rabbits. HMG-CoA reductase inhibition by simvastatin in the WHHL rabbits significantly reduced plasma progesterone concentrations in response to GnRH stimulation ($P < 0.05$). In contrast, simvastatin did not have this effect in NZW rabbits (**Fig. 5**). The trend of the mean plasma progesterone in the simvastatin-treated NZW rabbits did appear to drop below the untreated NZW rabbits mean plasma progesterone concentration at day 8, but the difference was not significant ($P = 0.2$).

DISCUSSION

We have noted that potentially sperm-penetrated oocytes obtained from WHHL rabbits after eCG stimulation are less likely to be fertilized than those obtained from NZW rabbits under the same conditions. The WHHL potentially sperm-penetrated oocytes flushed from the fallopian tube have both a tenacious mucous covering and an extensive cumulus that we did not ob-

TABLE 2. Progesterone and LH responses to the GnRH agonist buserelin in WHHL and NZW female rabbits

Rabbit	ΔP4[a]	ΔLH[b]	ΔP4/ΔLH
NZW1	1.39	9.95	0.14
NZW2	3.68	60	0.061
NZW3	16.6	48.35	0.34
NZW4	5.2	64.1	0.08
NZW5	1.99	13.95	0.14
NZW6	5.64	62.9	0.09
NZW7	7.74	34.35	0.23
NZW8	11.1	30.65	0.36
Mean ± SEM	6.67 ± 1.8	40.53 ± 7.6	0.18 ± 0.04[c]
WHHL1	11.2	62.45	0.18
WHHL2	10.9	93.3	0.12
WHHL3	13.7	128.05	0.11
WHHL4	8.62	38.75	0.22
WHHL5	29.1	38.15	0.76
WHHL6	15.5	64.05	0.24
WHHL7	6.75	76.35	0.09
WHHL8	19.9	61.25	0.32
Mean ± SEM	14.4 ± 2.53[d]	70.29 ± 0.4[c]	0.255 ± 0.08[c]

[a] ΔP4 is hour-1 progesterone concentration − hour-0 progesterone concentration.
[b] ΔLH is hour-1 LH concentration − hour-0 LH concentration.
[c] No significant difference ($P = 0.4$).
[d] Differs from NZW mean ΔP4 ($P = 0.012$).
[e] Differs from NZW mean ΔLH ($P = 0.019$).

serve in oocytes from NZW rabbits. This difference probably does not reflect the normal cumulus expansion base on oocyte maturation noted in human oocytes obtained by ovarian follicle aspiration for assisted reproductive techniques as the rabbit oocytes are 20 h post-ovulatory, subject to rabbit fallopian tube processing, and potentially sperm-penetrated. How these differences between the ova of the two rabbit strains arise cannot be said with certainty. Steroid hormones have been shown to have an effect on the interaction of the oocyte and fallopian tube. The secretion of tubal fluid is under hormonal control, estrogen increases secretion rate while progesterone decreases the secretion rate, and estrogen decreases mucin deposition while progesterone increases it (18). Furthermore, components of tubal fluid may play a role in removing the cumulus oophorus (19). Low concentration of estradiol and high concentration of progesterone that we have observed in the WHHL rabbit might be the mechanism affecting oocyte quality and thus another route by which the LDL defect lowers fecundity.

TABLE 3. Fasting plasma cholesterol concentration in NZW and WHHL female rabbits before and after 21 days treatment with simvastatin (20 mg/kg per day)

Rabbit	Baseline	Simvastatin-Treated
NZW	55 ± 6.2[a]	20.4 ± 1.3[b]
WHHL	705.3 ± 34.2[c]	292.4 ± 35.7[d]

[a] Mean plasma cholesterol concentration, mg/dl ± SEM.
[b] Differs from untreated NZW ($P < 0.001$).
[c] Differs from untreated NZW ($P < 0.001$).
[d] Differs from untreated WHHL ($P < 0.001$).

The corpus luteum is the sole source of progesterone in the pregnant rabbit (12). Donnelly et al. (6) found lower serum concentrations of progesterone from the 10th to the 25th day of pregnancy when they compared the WHHL rabbit to the NZW rabbit. However, we found that the WHHL rabbit does not have decreased plasma progesterone concentrations at baseline or in the first 8 days of

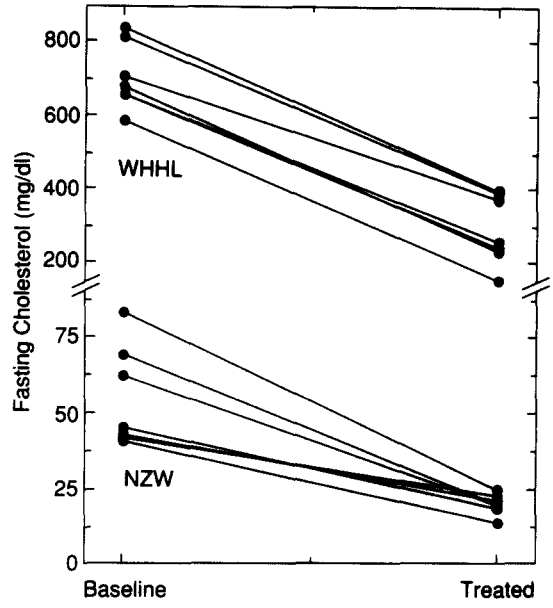

Fig. 4. Fasting plasma cholesterol concentrations in WHHL and NZW female rabbits before and after simvastatin treatment. The symbols and lines represent individual animals with the points representing the fasting cholesterol (mg/dl) in the animal before and after 4 weeks treatment with simvastatin (20 mg/kg per day).

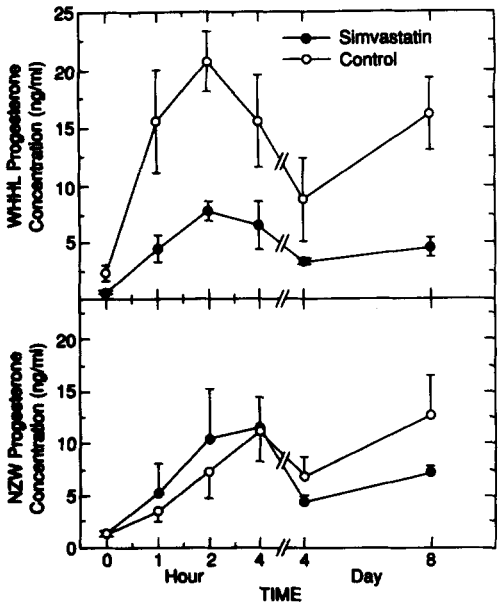

Fig. 5. Plasma progesterone concentrations after buserelin injection in simvastatin-treated and untreated NZW and WHHL female rabbits. Mean plasma progesterone concentrations (ng/ml) before and after buserelin (0.6 mg/kg) in simvastatin-treated (○) and untreated (●) WHHL and NZW rabbits. The error bars represent standard error of the mean.

pseudopregnancy compared to the NZW rabbit. It appears that there is a compensatory source of cholesterol for progesterone steroidogenesis during the preovulatory period and early pseudopregnancy. The source of cholesterol for progesterone production in response to LH stimulation (20) in the preovulatory rabbit comes from intracellular pools of cholesteryl esters (21). With the robust LH surge of the WHHL rabbit in response to buserelin injection, large amounts of progesterone can be produced. This transient pre-ovulatory progesterone surge may be a cause of low fecundity in a manner similar to low-dose progesterone birth control in humans. Our findings suggest that the LDL receptor is less important in the early corpus luteum function but the LDL receptor may become more important to rabbit corpus luteum function in mid to late pregnancy.

In the rabbit ovary, HMG-CoA reductase and the LDL receptor both play a role in supplying cholesterol for steroidogenesis (22). The rabbit is highly estrogen-dependent for luteal progesterone production. The decreased estrogen levels in the WHHL rabbit may be a cause of reduced fecundity. Additionally, during the transition of luteal progestin production to becoming estrogen-dependent, there is a fall in HMG-CoA reductase activity and an increased dependence on LDL-derived cholesterol occurring after the first week of corpus luteum function (12). Our data suggest that, without normal LDL receptor activity, ovarian function is impaired as indicated by reduced baseline plasma estradiol concentrations and elevated plasma gonadotropin concentrations. Presumably, the WHHL rabbit is able to maintain baseline and preovulatory plasma progesterone concentrations by compensating through de novo cholesterol synthesis. This is supported by our findings in simvastatin-treated animals. Simvastatin therapy significantly lowers plasma cholesterol concentrations in WHHL rabbits as has been shown with the other HMG-CoA reductase inhibitors lovastatin and pravastatin (23–25). After simvastatin treatment the plasma progesterone concentrations in the preovulatory and early pseudopregnant WHHL rabbits were reduced, but simvastatin treatment did not significantly affect the plasma progesterone concentrations in the preovulatory and pseudopregnant NZW rabbits. It appears that the WHHL rabbit relies on de novo synthesis of cholesterol for preovulatory and early pseudopregnancy progesterone production. It is possible, however, that an alternative pathway independent of the LDL receptor exists for transport of extracellular cholesterol (26, 27), and that this pathway was impaired by the lower plasma concentrations of cholesterol due to simvastatin treatment. This is unlikely, however, as the mean plasma total cholesterol concentration in the simvastatin-treated WHHL rabbit is still nearly 300 mg/dl, about 5-fold the concentration of the mean plasma concentration of total cholesterol in the untreated NZW rabbit.

We have demonstrated that the WHHL rabbit has an aberrant hypothalamic-pituitary-ovarian axis. Baseline plasma estradiol concentrations are low while baseline and stimulated plasma gonadotropin concentrations are high in comparison to control rabbits. Ovarian steroids exert negative feedback on the pituitary in the rabbit (28–30) as they do in other species. Thus, the elevated levels of gonadotropins in the WHHL rabbit may reflect impeded steroidogenesis consequent to altered transport of cholesterol substrate into steroid-producing cells expressing a defective LDL receptor. This suggests that LDL may be an important source of cholesterol for steroid production by the developing ovarian follicle in the rabbit. This raises the possibility that women with familial hypercholesterolemia might have an altered hypothalamic-pituitary-ovarian axis by the same mechanism. Certainly women with impaired estradiol synthesis have elevated gonadotropins, as seen, for example, in women with 17–20 lyase deficiency (31). We have also shown that HMG-CoA reductase inhibition decreases progesterone concentration in the GnRH-treated WHHL rabbit but not in the normal rabbit. This suggests that the WHHL corpus luteum is more dependent on de novo cholesterol synthesis to maintain progesterone concentrations. This raises the possibility that women with familial hypercholesterolemia might have impaired corpus luteum function while taking HMG-CoA reductase inhibitors. We are currently investigating these possibilities in patients with familial hypercholesterolemia and other LDL receptor pathway defects.

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REFERENCES

1. Tanzawa, K., Y. Shimad, M. Kuroda, Y. Tsujuita, M. Arai, and Y. Watanabe. 1980. WHHL rabbit: a low density lipoprotein receptor-deficient animal model for familial hypercholesterolemia. *FEBS Lett.* **118:** 81–84.
2. Kita, T., M. S. Brown, Y. Watanabe, and J. L. Goldstein. 1981. Deficiency of low density lipoprotein receptors in liver and adrenal gland of the WHHL rabbit, an animal model of familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **78:** 2268–2272.
3. Shiomi, M., T. Ito, and Y. Watanabe. 1987. Effects of hyperlipidemia on nursing ability of WHHL rabbits. *Lab. Anim. Sci.* **37:** 84–88.
4. Hagen, K. W. 1974. Colony husbandry. *In* The Biology of the Laboratory Rabbit. S. H. Weisbroth, R. E. Flatt, and A. L. Kraus. editors. Academic Press, New York. 23–45.
5. Chai, C. K. 1969. Effects of inbreeding in rabbits. *J. Hered.* **60:** 64–70.
6. Donnelly, T. M., S. F. Kelsey, D. M. Levine, and T. S. Parker. 1991. Control of variance in experimental studies of hyperlipidemia using the WHHL rabbit. *J. Lipid Res.* **32:** 1089–1098.
7. Holt, J. A., W. Wurfel, and M. W. Beckmann. 1991. Control and trafficking of cholesterol and other lipids within the ovary. *Semin. Reprod. Endocrinol.* **9:** 303–312.
8. Gwynne, J. T., and J. F. Strauss III. 1982. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. *Endocr. Rev.* **3:** 299–329.
9. Hoeg, J. M., L. Loriaux, R. E. Gregg, W. R. Green, and H. B. Brewer, Jr. 1985. Impaired adrenal reserve in the Watanabe heritable hyperlipidemic rabbit: implication for LDL-receptor function in steroidogenesis. *Metabolism.* **34:** 194–197.
10. Illingworth, D. R., D. K. Corbin, E. D. Kemp, and E. J. Keenan. 1982. Hormone changes during the menstrual cycle in abetalipoproteinemia: reduced luteal phase progesterone in a patient with homozygous hypobetalipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **79:** 6685–6689.
11. Parker, C. R., Jr., D. R. Illingworth, J. Bissonnette, and B. R. Carr. 1986. Endocrine changes during pregnancy in a patient with homozygous familial hypobetalipoproteinemia. *N. Engl. J. Med.* **314:** 557–560.
12. Holt, J. A. 1989. Regulation of progesterone production in the rabbit corpus luteum. *Biol. Reprod.* **40:** 201–208.
13. Walsh, T. J., J. Bacher, and P. A. Pizzo. 1988. Chronic silastic central venous catherization for induction maintenance and support of persistent granulocytopenia in rabbits. *Lab. Anim. Sci.* **38:** 467–471.
14. DeVilla, G. O., Jr., K. Roberts, W. G. Wiest, G. Mikhail, and G. A. Flickinger. 1972. Specific radioimmunoassay of plasma progesterone. *J. Clin. Endocrinol. Metab.* **35:** 458–460.
15. Jiang, N., and R. J. Ryan. 1969. Radioimmunoassay for estrogens: a preliminary communication. *Mayo Clin. Prod.* **44:** 461–464.
16. Abraham, C. E., J. D. Buster, L. A. Lucas, P. C. Corrales, and R. C. Teller. 1972. Chromatographic separation of steroid hormones for use in radioimmunoassay. *Anal. Lett.* **5:** 509–517.
17. Hogan, B. 1986. Recovery, culture and transfer of embryos. *In* Manipulating the Mouse Embryo. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 24–64.
18. Mastroianni, L., Jr., F. Beer, U. Shah, and T. H. Clewe. 1961. Endocrine regulation of oviduct secretions in the rabbit. *Endocrinology.* **68:** 92–100.
19. Zamboni, L., C. Hongsanand, and L. Mastroianni, Jr. 1965. Influence of tubal secretions on rabbit tubal ova. *Fertil. Steril.* **16:** 177–184.
20. YoungLai, E. V. 1977. Steroid production by isolated rabbit ovarian follicles: effects of luteinizing hormone from mating to implantation. *J. Endocrinol.* **73:** 59–66.
21. Solod, E. A., D. T. Armstrong, and R. O. Greep. 1966. Action of luteinizing hormone on conversion of ovarian cholesterol stores to steroids secreted in vivo and synthesized in vitro by the pseudopregnant rabbit ovary. *Steroids.* **7:** 607–620.
22. Kovanen, P. T., J. L. Goldstein, and M. S. Brown. 1978. High levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol synthesis in the ovary of the pregnant rabbit. *J. Biol. Chem.* **253:** 5126–5132.
23. Shiomi, M., T. Ito, Y. Watanabe, Y. Tsujita, M. Kuroda, M. Arai, M. Fukami, J. Fukushige, and A. Tamura. 1990. Suppression of established atherosclerosis and xanthomas in mature WHHL rabbits by keeping their serum cholesterol levels extremely low. *Atherosclerosis.* **83:** 69–80.
24. Watanabe, Y., T. Ito, M. Shiomi, Y. Tsujita, M. Kuroda, M. Ariai, M. Fukami, and A. Tamura. 1988. Preventive effect of pravastatin sodium, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, on coronary atherosclerosis and xanthoma in WHHL rabbits. *Biochim. Biophys. Acta.* **960:** 294–302.
25. Carew, T. E., D. C. Schwenke, and D. Steinberg. 1987. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Proc. Natl. Acad. Sci. USA.* **84:** 7725–7729.
26. Edge, S. B., J. M. Hoeg, T. Triche, P. D. Schneider, and H. B. Brewer, Jr. 1986. Cultured human hepatocytes. Evidence for metabolism of low density lipoproteins by a pathway independent of the classical low density lipoprotein receptor. *J. Biol. Chem.* **261:** 3800–3806.
27. Willnow, T. E., J. L. Goldstein, K. Orth, M. S. Brown, and J. Herz. 1992. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J. Biol. Chem.* **267:** 26172–26180.
28. Pau, K. Y. F., K. M. Orstead, D. L. Hess, and H. G. Spies. 1986. Feed-back effects of ovarian steroids on the

hypothalamic-hypophyseal-axis in the rabbit. *Biol. Reprod.* **35:** 1009–1023.
29. Scaramuzzi, R. J., C. A. Blake, H. Papkoff, J. Hilliard, and C. H. Sawyer. 1971. Radioimmunoassay of rabbit luteinizing hormone: serum levels during various reproductive states. *Endocrinology.* **90:** 1285–1291.
30. Osteen, K. G., and T. M. Mills. 1979. Serum LH and FSH levels in the pregnant rabbit (40703). *Proc. Soc. Exp. Biol. Med.* **162:** 454–457.
31. Larrea, F., R. Lisker, R. Bañuelos, J. A. Bermúdez, J. Herrera, V. N. Rasilla, and G. Pérez-Palacios. 1983. Hypergonadotropic hypogonadism in an XX female subject due to 17,20 steroid desmolase deficiency. *Acta Endocrinol.* **103:** 400–405.