Effects of Vitamin A and Inositol on Term Weights of H-2 Congenic Mice (43927)

MARVIN L. TYAN[1]
Veterans Affairs Medical Center, Los Angeles, California 90073

Abstract. **Pregnant H-2 congenic mice, C57BL/10, B10.A, B10.A(15R), and B10.A(18R) were fed Purina Laboratory Chow 5001 or the same diet supplemented with 400 IU vitamin A dissolved in corn oil or 0.4% (w/w) myo-inositol. On the 18th day of gestation, the dams were sacrificed, and the fetuses were weighed, sexed, and examined for developmental abnormalities. Term fetal weight was found to be significantly reduced in progeny of dams bearing d alleles distal to Ea in the H-2 complex when the diet was supplemented with vitamin A or myo-inositol (B10.A and B10.A [18R]). Fetuses from dams of all strains fed the diet supplemented with vitamin A had significantly increased frequencies of microphthalmia; the frequency of microphthalmia was moderately but not significantly increased in one of the two strains fed the diet supplemented with myo-inositol (B10.A[15R] but not B10.A[18R]).** [P.S.E.B.M. 1995, Vol 210]

Poor nutrition in the form of inadequate caloric intake, deficiencies in protein, vitamins, and specific minerals are factors known to contribute to the birth of underdeveloped infants (1). Vitamin A and inositol are dietary factors essential for normal growth and development of vertebrate embryos (2–6). In the absence of either of these factors in the diet of the pregnant animal, fetal growth is retarded, organogenesis impaired, and death may result.

Similarly, an excess of vitamin A during pregnancy can retard overall growth and induce malformations of the craniofacial region and the skeletal, respiratory, gastrointestinal, genitourinary, cardiovascular, and central nervous systems (2, 7–9). The effects of excess dietary inositol on fetal growth appear to be unknown.

Recent work from this laboratory has shown that 11-day-old mouse fetuses from mothers fed diets supplemented with (i) vitamin A, 200 IU/day; (ii) inositol, 0.1%; or (iii) corn oil (2%), a rich source of inositol and essential fatty acids, are approximately 25% larger and have significantly advanced development of the eyes and hind limbs when compared with fetuses from dams fed a standard mouse chow, Purina 5001 (10). At a higher dose of vitamin A, 500 IU/day, growth and eye and limb development are impaired.

Presented below are data on the effects of supplemental dietary vitamin A, 400 IU/day, or myo-inositol, 0.4%, on fetal growth and development measured at term in four H-2 congenic strains of mice.

Materials and Methods

The H-2 congenic strains C57BL/10, B10.A, B10.A(15R), and B10.A(18R) were maintained in this laboratory by brother × sister matings. The differences in H-2 haplotypes are shown in Table I (see also Ref. 11 and 12). In the experiments, one male and two virgin 10 to 12 week-old females were placed in each cage. The day a vaginal plug was detected was considered to be Day 0 of pregnancy.

On Day 0, the dams were fed Purina Mouse Chow 5001, the same diet supplemented with vitamin A (400 IU in 0.2 ml corn oil/5 g) or the same diet supplemented with myo-inositol, 0.4% (w/w).

The vitamin A palmitate and myo-inositol were obtained from Sigma Chemical Co. (St. Louis, MO), and were added to the 5001 biscuits as described previously (10). The average pregnant female mouse consumes approximately 5 g of food per day. The 5001 chow contains 15 IU vitamin A/g and corn oil less than 1 IU/ml. Therefore, the mice fed the control diet con-

[1] To whom requests for reprints should be addressed at West Los Angeles Veterans Affairs Medical Center 111M, Los Angeles, CA 90073.

Received January 30, 1995. [P.S.E.B.M. 1995, Vol 210]
Accepted May 5, 1995.

0037-9727/95/2101-0077$10.50/0

Table I. Alleles at H-2 Loci on Chromosome 17 in Four H-2 Congenic Mouse Strains

Congenic strain	Centromeric to K	K	Ea	C4	BAT 5	D
C57BL/10	b	b	b	b	b	b
B10.A(18R)	b	b	b	b	d	d
B10.A	k	k	k	d	d	d
B10.A(15R)	k	k	k	d	b	b

sumed approximately 75 IU vitamin A daily and those fed the diet supplemented wtih vitamin A, 475 IU.

On the 18th day postcoitus (pc) the pregnant mice were sacrificed. The fetuses were dissected free of maternal tissues and membranes, weighed, and examined for external abnormalities. Sex was determined by visual inspection of the gonads. The carcass of the dam was weighed.

The sampling unit in these studies is the litter. Mean weights were compared using the unpaired *t* test and the Mann-Whitney two sample test. Frequencies were compared using the Fisher exact test. No corrections are made for multiple determinations.

Results

In the first study pregnant C57BL/10, B10.A, B10.A(15R), and B10.A(18R) mice were fed the control diet, Purina 5001, or the same diet supplemented with vitamin A, 400 IU/day (Table II). It was found that 18-day pc fetuses from B10.A and B10.A(18R) dams fed the diet supplemented with vitamin A weighed significantly less than did those from dams fed the control diet; no differences in weight were noted among C57BL/10 and B10.A(15R) fetuses fed the control and supplemented diets. Maternal weights on the 18th day pc were virtually identical in the groups fed the control and vitamin A supplemented diets, suggesting that the added vitamin A had neither a positive nor a negative effect on caloric intake (see Table IV). As previously reported (13) fetuses from dams fed the vitamin A supplemented diet had increased frequencies of microphthalmia.

In the next series of experiments, B10.A(15R) and B10.A(18R) dams were fed the control diet or the same diet supplemented with 0.4% myo-inositol. Eighteen-day pc fetuses from B10.A(15R) dams fed the diet supplemented with 0.4% inositol were found not to differ significantly in weight from those in the control groups as was the case with those from dams fed the vitamin A supplemented diet (Table III); however, fetuses from B10.A(18R) dams fed the diet supplemented with myo-inositol, again as was the case with vitamin A, were approximately 10% smaller than their controls. The frequency of microphthalmia was moderately but

Table II. Effects of Supplemental Dietary Vitamin A on 18-day pc Fetal Weight and the Frequency of Microphthalmia in Four H-2 Congenic Strains of Mice

Congenic strain	Diet: Purina 5001				
	Litters	Male		Female	
		Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)	Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)
C57BL/10	12	1048 ± 103[a] (37)	0/232[b] (0.0)	986 ± 114[a] (37)	11/263[b] (4.2)
B10.A(18R)	24	1043 ± 94[a] (86)	5/539[b] (0.9)	981 ± 103[a] (75)	22/547[b] (4.0)
B10.A	12	1039 ± 84[a] (48)	5/375[b] (1.3)	934 ± 96[a] (31)	27/364[b] (7.4)
B10.A(15R)	7	1020 ± 61 (22)	2/140[b] (1.4)	956 ± 92 (34)	9/171[b] (5.3)
Congenic strain	Diet: Purina 5001 plus vitamin A, 400 IU/day				
	Litters	Male		Female	
		Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)	Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)
C57BL/10	13	1029 ± 103 (39)	1/45[b] (2.2)	934 ± 103 (35)	5/41[b,c] (12.2)
B10.A(18R)	26	948 ± 96[d] (80)	1/30[b] (3.3)	919 ± 113[d] (80)	6/33[b,d] (18.2)
B10.A	15	949 ± 122[d] (55)	0/55[b] (0.0)	887 ± 95[c] (52)	7/44[b,c] (15.9)
B10.A(15R)	5	1024 ± 61 (10)	2/101[b] (1.9)	961 ± 102 (22)	26/128[b,d] (20.3)

[a] Data reported previously (14).
[b] Data reported previously (13).
[c] Comparison with control diet: $P < 0.05$.
[d] Comparison with control diet: $P < 0.01$.

Table III. Effects of Vitamin A and Inositol on the Frequency of Microphthalmia and on 18-Day pc Weights of Fetuses from Two H-2 Congenic Mouse Strains

Diet of Purina 5001 plus:	B10.A(15R)				
	Litters	Male		Female	
		Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)	Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)
Nothing added	7	1020 ± 61[a] (22)	2/140[b] (1.4)	956 ± 92[a] (34)	9/171[b] (5.3)
Vitamin A, 400 IU in corn oil	5	1024 ± 61 (10)	2/101[b] (1.9)	961 ± 102 (22)	26/128[b,c] (20.3)
Inositol, 0.4%	9	1056 ± 160 (39)	0/39 (0.0)	968 ± 155 (30)	4/30[g] (13.3)
Diet of Purina 5001 plus:	B10.A(18R)				
	Litters	Male		Female	
		Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)	Weight ± SD (mg) (*n*)	Microphthalmia no./total (%)
Nothing added	24	1043 ± 94[a] (86)	5/539[b] (0.9)	981 ± 103[a] (75)	22/547[b] (4.0)
Vitamin A, 400 IU in corn oil	26	948 ± 96[d] (80)	1/30[b] (3.3)	919 ± 113[a,e] (80)	6/33[b,f] (18.2)
Inositol, 0.4%	10	935 ± 104[d] (34)	0/34 (0.0)	888 ± 105[d] (28)	0/28 (0.0)

[a] Data reported previously (14). All treatments were run concurrently over a 2-year period.
[b] Data reported previously (13).
[c] Comparison with control group: [c] $P = 0.005$; [d] $P < 0.01$; [e] $P < 0.05$; [f] $P = 0.006$; [g] $P = 0.11$.

not significantly increased among the B10.A(15R) fetuses from dams fed the myo-inositol-supplemented diet; the same diet clearly did not increase the incidence of microphthalmia in B10.A(18R) fetuses. The addition of myo-inositol to the diet had no significant effect on 18-day pc maternal weights (Table IV).

Discussion

It has been shown here that moderate excesses of dietary vitamin A and myo-inositol can induce significant reductions in term weights of fetuses from B10.A and B10.A(18R) but not C57BL/10 or B10.A(15R) dams. In theory, these H-2 congenic strains differ only in the region of the major histocompatibility complex. The two strains in which birth weight was affected share d alleles distal to Ea (Table I), suggesting that a locus in this region may be involved in fetal growth: the data suggest that the activity of this locus can be modulated to some degree by vitamin A and myo-inositol or one of its derivatives. Furthermore, the finding of an increased incidence of microphthalmia in fetuses from B10.A(15R) dams fed the diet supplemented with myo-inositol suggests the possibility that like vitamin A this agent may be teratogenic at high dose levels.

Table IV. Maternal 18-Day pc Weights Minus Conceptus of Two Mouse Strains Fed Purina 5001 or the Same Diet Supplemented with Vitamin A or Inositol

Diet of Purina 5001 plus:	B10.A(15R)		B10.A(18R)	
	Dams	Maternal weight (g ± SD)	Dams	Maternal weight (g ± SD)
Nothing added	7	25.8 ± 2.1	24	26.1 ± 1.8
Vitamin A, 400 IU/day	5	26.3 ± 2.5	26	26.2 ± 1.6
Inositol, 0.4%	9	26.5 ± 1.9	9	25.8 ± 2.4

Water-soluble vitamin A (200 IU/day) and myo-inositol (0.1%) added separately to the diets of pregnant mice have previously been reported to increase the weight of 11-day gestations by approximately 25% and enhance development of the eyes by the equivalent of 0.5–1.0 day (10); at a dose of 500 IU vitamin A/day fetal growth and eye developed was found to be impaired in a subset of fetuses. Similarly, growth and eye development have been found to be reduced in 11-day pc fetuses from dams fed a diet supplemented with 0.4% myo-inositol (unpublished data).

Taken together, these observations show that over the dose ranges tested dietary vitamin A and myo-inositol induce similar changes in growth and development. This suggests that myo-inositol of dietary origin or a derivative may act within the inositol second messenger system mimicking at least certain actions of vitamin A. One possible mechanism may be through regulation of insulin-like growth factor-I (IGF-I). Using weight as an index of development, fetal growth

has been found to correlate with cord blood levels of IGF-1 (15, 16). After birth, growth is mediated by IGFs under the control of pituitary growth hormone (GH): *in vitro* the release of growth hormone from pituitary cells can be modulated by retinoic acid (17). However, GH secreted by the fetal hypophysis does not increase the synthesis or release of IGF-I in fetal liver because of the immaturity of GH receptors (18). Fetal blood levels of IGF-I correlate well with those of placental lactogen (PL) which has 90% homology with GH, suggesting that PL may be involved in the regulation of IGF-I (19, 20). It is not known what effect, if any, retinoids have on the production or release of PL; however, it has been shown that myo-inositol can modulate the release of PL from trophoblastic giant cells of the placenta (21, 22) and that it can reverse the growth inhibitory effects of hyperglycemia in early rat embryos (23).

This work was supported in part by the Department of Veterans Affairs.

1. Bergner L, Susser MB. Low birth weight and prenatal nutrition: An interpretive review. Pediatrics **46**:946–965, 1970.
2. Underwood RA. Vitamin A in animal and human nutrition. In: Sporn MB, Roberts AB, Goodman DS, Eds. The Retinoids. New York: Academic Press, pp298–302, 1984.
3. O'Toole BA, Frodkin R, Warkany J. Vitamin A deficiency and reproduction in rhesus monkeys. J Nutr **104**:1513–1524, 1974.
4. Morris HP. Review of the nutritive requirements of normal mice. J Natl Cancer Inst **5**:115–142, 1944.
5. Woolley DW. Synthesis of inositol in mice. J Exp Med **75**:277–284, 1942.
6. Holub BJ. Metabolism and function of myo-inositol and inositol phospholipids. Can J Physiol Pharm **62**:1–8, 1984.
7. Hale F. Pigs born without eyeballs. J Hered **24**:105–106, 1933.
8. Kalter H. Congenital malformations of the central nervous system. Am J Clin Nutr **12**:264–272, 1963.
9. Geeclen JAG. Hypervitaminosis A induced teratogenesis. CRC Crit Rev Toxicol **10**:351–375, 1979.
10. Tyan ML. Murine fetal development enhanced by dietary vitamin A, corn oil and inositol. Proc Soc Exp Biol Med **203**:485–489, 1993.
11. Vincek V, Sertic J, Zaleska-Rutezyuska Z, Figueroa F, Klein J. Characterization of H-2 congenic strains using DNA markers. Immunogenetics **31**:45–51, 1990.
12. Silver LM, Artzt K, Barlow D, Fisher-Lindahl K, Lyon M, Klein J, Snyder L. Mouse Chromosome 17. Mammalian Genome **3**:(Suppl):S241–S260, 1992.
13. Tyan ML. Effects of H-2 and vitamin A on eye defects in congenic mice. Proc Soc Exp Biol Med **199**:123–127, 1992.
14. Tyan ML. Fetal weight at term influenced by H-2 associated loci. Proc Soc Exp Biol Med **205**:85–88, 1994.
15. Lassare C, Hardouin S. Serum insulin-like growth factors in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res **29**:219–225, 1991.
16. Milner RDG, Hill DJ. Interaction between endocrine and paracrine peptides in prenatal growth control. Eur J Pediatr **146**:113–122, 1987.
17. Bedo G, Santisteban B, Aranda A. Retinoic acid regulates growth hormone expression. Nature **339**:231–234, 1989.
18. Gluckman PD, Grumbach MM, Kaplan SL. The neuroendocrine regulation and function of growth hormone and prolactin in the mammalian fetus. Endocr Rev **2**:363–395, 1981.
19. Freemark M, Comer M. Nutritional regulation of placental lactogen receptor in fetal liver: Implications for fetal metabolism and growth. Endocrinol **125**:1504–1512, 1989.
20. Fisher D. The unique endocrine milieu of the fetus. J Clin Invest **78**:603–611, 1986.
21. Petit A, Guillon G, Tence M, Jard S, Gallo-Payet N, Bellabarba D, Lehoux J-G, Belisle S. Angiotensin II stimulated both inositol phosphate production and human placental lactogen release from human trophoblastic cells. J Clin Endocrinol Metab **69**:280–286, 1989.
22. Petit A, Guillon G, Pantaloni C, Tence M, Gallo-Payet N, Bellabarba D, Lehoux J-G, Belisle S. An islet-activating protein-sensitive G-protein is involved in dopamine inhibition of both angiotensin-stimulated inositol phosphate production and human placental lactogen release in human trophoblastic cells. J Clin Endocrinol Metab **71**:1573–1580, 1990.
23. Hasimoto S, Akazawa S, Akazawa M, Akashi M, Yamamoto H, Maeda Y, Yamaguchi Y, Yamasaki H, Nakanishi T, Nagataki S. Effects of hyperglycemia on sorbitol and myo-inositol contents of cultured embryos. Diabetologia **38**:597–602, 1990.