Vitamin A Deficiency Reduces Insulin-Like Growth Factor (IGF)-I Gene Expression and Increases IGF-I Receptor and Insulin Receptor Gene Expression in Tissues of Japanese Quail (*Coturnix coturnix japonica*)[1]

Zhengwei Fu, Tadashi Noguchi and Hisanori Kato[2]

Laboratory of Nutritional Biochemistry, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, 113-8657, Japan

ABSTRACT The insulin-like growth factor (IGF) system is regulated by various stimuli, including hormones, growth factors and nutritional status. We examined the effects of vitamin A on components of the IGF system in Japanese quail. Male quail (1 d old) fed a vitamin A–deficient diet for 14 or 21 d developed vitamin A deficiency, as confirmed by a depletion of serum retinol and hepatic retinyl palmitate. Consuming the vitamin A–deficient diet for 14 d did not affect growth rate, but decreased the serum IGF-I concentrations by 22% compared with the control group. The decreased serum IGF-I levels were accompanied by 21–52% lower levels of IGF-I mRNA in the testis, lung, liver and heart, whereas IGF-I receptor (IGF-IR) and insulin receptor (IR) gene expressions were unaffected in these tissues. Continuous feeding of the vitamin A–deficient diet for 21 d retarded growth and further decreased the levels of serum IGF-I and tissue IGF-I mRNA. Serum IGF-I levels were reduced by ~50%; IGF-I mRNA levels were > 90% lower in the liver and lung and ~60% lower in the heart and testis. In contrast, levels of the IGF-IR and IR mRNAs were ~100% greater in some tissues examined. When vitamin A–deficient quail received a single injection of retinol or retinoic acid (0.1 mg/bird), tissue IGF-I, IGF-IR and IR gene expressions did not change after 4 h. These results suggest a possible physiologic role of the IGF system in mediating vitamin A–supported growth of Japanese quail. J. Nutr. 131: 1189–1194, 2001.

KEY WORDS: • *vitamin A* • *insulin-like growth factor system* • *gene expression* • *Japanese quail*

The insulin-like growth factors (IGF-I and IGF-II)[3] play critical roles in proliferation, differentiation and transformation in a variety of vertebrate tissues (1,2). The liver is the main source of serum IGF-I. It has been thought that IGF-I produced in the liver is secreted into the circulation and has an endocrine action on target tissues (3,4). In addition to its endocrine effects, IGF-I is also produced in most extrahepatic tissues and can function as an autocrine and/or paracrine growth stimulator (3,4). The biological actions of IGF-I are mediated mainly through the IGF-I receptor (IGF-IR), and partly through the insulin receptor (IR) (5). Furthermore, IGF-binding proteins (IGFBP) are important modulators of the biological actions of IGF (6).

The concentrations of IGF-I and insulin in plasma and the expression of genes encoding for IGF-I, IGF-IR and IR are regulated by many factors, including developmental stage, growth hormone, insulin and nutritional status. For example, food restriction or deprivation decreases serum IGF-I and reduces the abundance of IGF-I mRNA in many tissues of both mammals and birds (7–10). In contrast, the expression of IGF-IR and IR mRNAs, and the numbers of these two receptors in many tissues are upregulated by food deprivation in rats and chickens (7,11–15). These results suggest that nutrition is an important factor in the regulation of plasma IGF-I and insulin and of IGF-I, IGF-IR and IR gene expression in many tissues.

Vitamin A has been shown to be an essential micronutrient for normal growth in numerous nutritional studies. Vitamin A encompasses a class of compounds, including retinol, retinoic acid (RA) and other derivatives, all of which exhibit striking effects on cell proliferation and differentiation (16). These effects are exerted mainly by regulation of the expression of target genes through specific receptors, retinoic acid receptors (RAR) and retinoid X receptors (RXR) (17). Recent studies have demonstrated the important effects of RA on the expression of IGFBP in many cell lines (18–23), including up-regulation or down-regulation depending on either the cell line or the type of IGFBP. Furthermore, RA causes a transient increase in IGF-I and IGF-II mRNA levels after 6 h of RA treatment in osteoblast cell lines (24). In an in vivo study, vitamin A deficiency caused a decrease in the plasma IGF-I concentrations and a significant increase in the testicular IGF-I concentrations in rats (25). Taken together, these data suggest that vitamin A might also be an important regulator of

[1] Supported in part by a Grant-in-Aid for Fellows of the Japan Society for Promotion of Science (JSPS) (No. 98218) to Z.W.F. from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed.
E-mail: akatoq@mail.ecc.u-tokyo.ac.jp.

[3] Abbreviations used: IGF, insulin-like growth factor; IGFBP, IGF binding protein; IGF-IR, IGF-I receptor; IR, insulin receptor; PCR, polymerase chain reaction; RA, retinoic acid; RAR, retinoic acid receptor; RARE, retinoic acid responsive elements; RXR, retinoid X receptor; +VA diet, vitamin A–sufficient diet; −VA diet, vitamin A–deficient diet.

Manuscript received 2 October 2000. Initial review completed 3 November 2000. Revision accepted 22 December 2000.

TABLE 1

Composition of the vitamin A–deficient diet

Ingredient	Amount
	g/kg
Isolated soybean protein	315.0
Glucose monohydrate	524.9
Rapeseed oil	50.0
Cellulose	30.0
DL-Methionine	6.2
Choline chloride	3.0
Calcium carbonate	20.0
Potassium dihydrogenphosphate	19.0
Sodium dihydrogenphosphate dihydrate	6.8
Sodium chloride	2.8
Magnesium hydrogenphosphate trihydrate	5.3
Mineral mixture[1]	5.0
Vitamin mixture[2]	10.0
Cornstarch	2.0

[1] Mineral mixture contained the following (mg/kg diet): copper phosphate, 22; iron phosphate octahydrate, 360; manganese carbonate, 240; zinc sulfate heptahydrate, 280; potassium iodate, 1; sodium selenite, 0.5; disodium molybdate dihydrate, 12, which were mixed into the cornstarch.

[2] Vitamin mixture contained the following (mg/kg diet): cholecalciferol, 0.05; vitamin E, 50; vitamin K-3, 2; calcium pantothenate, 22; thiamin hydrochloride, 5; riboflavin, 11; nicotinic acid, 80; pyridoxine hydrochloride, 6; D-biotin, 0.5; folic acid, 2; vitamin B-12, 0.02, which were mixed into the cornstarch.

IGF-I, IGF-IR, IR and IGFBP gene expression. However, there is no information regarding the effects of vitamin A on the gene expression of the IGF system in any in vivo system.

To elucidate the relationship between vitamin A and the IGF system, we examined the effects of vitamin A deficiency on serum IGF-I concentrations and on the expression of IGF-I, IGF-IR and IR mRNA in different tissues of Japanese quail (*Coturnix coturnix japonica*). We also examined the effects of vitamin A repletion on vitamin A–deficient quail in terms of the expression of these genes.

MATERIALS AND METHODS

Animals and experimental design. All-*trans*-retinol palmitate and all-*trans*-retinoic acid were purchased from Sigma Chemical (St. Louis, MO). Two different diets, a vitamin A–deficient diet (−VA) (**Table 1**) and a −VA diet supplemented with 4.2 mg/kg of all-*trans*-retinol palmitate (+VA), were prepared as described previously (26–27). In the −VA diet, nutrients other than retinol were sufficient to support normal growth. These two diets were prepared weekly and stored at −20°C until use.

In Experiment1, 1-d-old male Japanese quail were randomly divided into two groups (the control and vitamin A–deficient groups). They were housed in a brooder with continuous illumination for 3 d, and then were subjected to a light-dark cycle (14 h light:10 h dark). The vitamin A–deficient group was fed the −VA diet, whereas the control group was fed the +VA diet. Diets and water were available at all times throughout the experiment. Five birds from each group were randomly selected as samples at 14 and 21 d of age. Each bird was weighed, killed by decapitation and bled. Their tissues, including the brain, liver, heart, lung, kidney and testis, were quickly dissected and frozen in liquid nitrogen immediately, then stored at −80°C for analysis of the expression of IGF-I, IGF-IR and IR mRNA. In addition, serum was prepared and used for measuring the serum retinol and IGF-I concentrations.

In Experiment 2, 1-d-old quail were fed the +VA or the −VA diet for 21 d. Then 5 quail from each group were injected intramuscularly with 0.1 mg of all-*trans*-retinoic acid (Sigma), or 0.1 mg of all-*trans*-retinol palmitate (Sigma) or an equal amount of vehicle. All-*trans*-retinoic acid and all-*trans*-retinol palmitate were dissolved in ethanol (10 g/L), and were diluted 5 times with rapeseed oil before injection. The quail were killed 4 h after the treatment; different tissues were dissected and frozen in liquid nitrogen immediately and stored at −80°C until analysis.

All experiments were performed under the guidelines of the Animal Usage Committee of the Faculty of Agriculture, The University of Tokyo.

Assessment of vitamin A-depletion. To assess the degree of vitamin A depletion in the vitamin A–deficient group, serum retinol and hepatic retinyl palmitate levels were determined at 14 and 21 d of age by HPLC as described previously (26,28). Consumption of the −VA diet for 14 d reduced the serum retinol concentrations by ~80% and the hepatic retinyl palmitate concentrations by ~90%, findings that were in agreement with our previous reports (26,28). In 21-d-old quail, both the serum retinol and hepatic retinyl palmitate levels in the vitamin A–deficient group were below the detectable levels. The detection limits of serum retinol and hepatic palmitate were 3.4 μmol/L and 0.04 μmol/g, respectively.

IGF-I RIA. Serum concentrations of quail IGF-I were determined by a heterologous RIA. A commercial assay kit (I-AA27), which includes iodinated IGF-I, anti-human IGF-I and other necessary reagents, was purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA). The hydrochloric acid/ethanol extract was adopted, and the assay was carried out according to the kit's protocol. All serum samples were included in a single assay to avoid interassay variability and were assayed in duplicate, with a 5% CV.

Measurement of expression of genes encoding IGF-I, IGF-IR and IR

Preparation and radiolabeling of antisense RNA probes. A 260-bp polymerase chain reaction (PCR) fragment of quail IGF-I (GenBank accession no. AF260131), containing positions 374–634, was subcloned into the pGEM-T Easy vector (Promega, Madison, WI). A 666-bp PCR fragment of quail IGF-IR (GenBank accession no. AF260702) and a 430-bp PCR fragment of quail InsR (GenBank accession no. AF260703) were subcloned into PCR II vector (Invitrogen, Carlsbad, CA). To generate the antisense IGF-I and IR cRNA probes, IGF-I and IR plasmids were linearized with Nco I and Xba I restriction endonucleases, respectively, and transcribed by SP6 RNA polymerase in the presence of a ^{32}P-αUTP as described previously (14). For generating ^{32}P-αUTP–labeled antisense IGF-IR, IGF-IR plasmid was linearized with Hind III restriction endonuclease and transcribed by T7 polymerase.

RNase protection assay. Total RNA was extracted from the brain, liver, heart, lung, kidney and testis according to the method described previously using TRIzol reagent (GibcoBRL, Rockville, MD). Sample quality and quantity were assessed by measuring the optical density of each sample at 260 and 280 nm. Sample quality was also checked by ethidium bromide staining of denatured agarose gel. Equal amounts of total RNA (40 μg) from each sample were then used for determining the expression of IGF-I, IGF-IR and IR mRNAs by an RNase protection assay as described previously (29). Briefly, total RNA was hybridized with 4.726 kBq of each probe overnight, then digested with RNase A and RNase T1. RNase A/T1 were inactivated by proteinase K solution (RNA grade, GibcoBRL). Protected mRNA was directly precipitated by adding isopropanol at the same volume and 4 μL of tRNA (5 g/L). In this way, all processes from total RNA to electrophoresis were completed in the same tube. To compare the equivalent loading of RNA samples, quail β-actin was used as an internal control.

Data analysis. Hybridized blots were visualized and analyzed using BAS-2000 (FujiPhoto Film, Tokyo, Japan). All values for mRNA levels are given relative to controls within an experiment. All data including body weight, serum IGF-I concentrations, and the levels of IGF-I, IGF-IR and IR mRNAs are expressed as means and SEM. Repeated-measures ANOVA was performed to determine the effects of time and diet on the body weight and serum IGF-I concentrations. Post-hoc analysis was performed with the Fisher's Protected Least Significant Difference test (Statview J-4.51.1 for Macintosh,

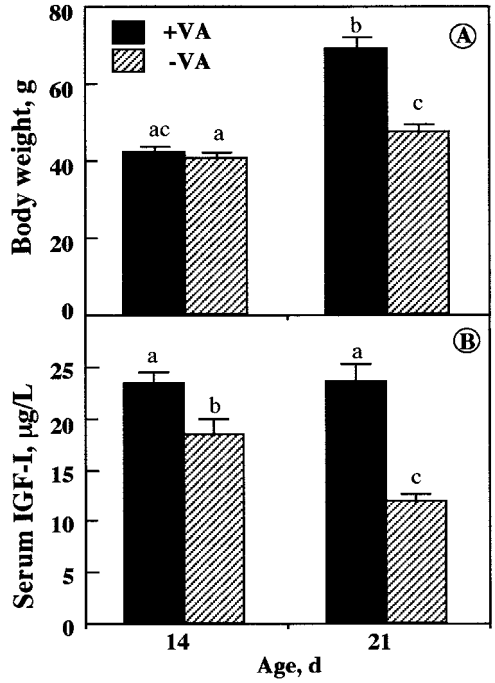

FIGURE 1 The influence of vitamin A deficiency on body weight (*A*) and serum insulin-like growth factor (IGF)-I concentrations (*B*) of 1-d-old male quail fed a vitamin A–sufficient (+VA) or vitamin A–deficient diet (−VA) for 14 or 21 d. The +VA group was fed the −VA diet supplemented with 4.2 mg/kg diet of all-*trans*-retinol palmitate. Values are means ± SEM, $n = 5$. Means not having a common letter differ significantly ($P < 0.05$).

Abacus Concepts, Berkeley, CA). One-way ANOVA was used to analyze the effects of diet on the gene expression of IGF-I, IGF-IR and IR. Differences with *P*-values < 0.05 were considered significant.

RESULTS

Effect of vitamin A deficiency on the level of serum IGF-I. Consuming the −VA diet for 14 d did not retard the growth of the quail, but significantly reduced their growth during the next 7 d (**Fig. 1***A*, $P < 0.05$). In contrast to the rapid body weight gain (+27 g) in the control group (+VA) from 14 to 21 d of age, only a slight increase in body weight (+7 g) was observed in the vitamin A–deficient group. Serum IGF-I concentrations in the control group were not different between 14 and 21 d of age. However, serum IGF-I concentrations were significantly reduced by vitamin A deficiency (Fig. 1*B*). Serum IGF-I concentrations at 14 and 21 d were 78% ($P < 0.05$) and 50% ($P < 0.01$), respectively, of the values for the control group. Moreover, serum IGF-I concentrations in the vitamin A–deficient quail continued to decrease from 14 to 21 d of age as the vitamin A deficiency progressed ($P < 0.05$).

Effect of vitamin A deficiency on the expression of the IGF-I gene. The decrease in serum IGF-I concentrations induced by vitamin A deficiency (Fig. 1*B*) was accompanied by a significant decrease in the IGF-I mRNA levels in many tissues. Consumption of the −VA diet for 14 d reduced the mRNA levels of IGF-I by 35, 52, 31 and 21% in the liver ($P < 0.05$), heart ($P < 0.01$), lung ($P < 0.05$) and testis ($P = 0.09$), respectively, compared with those in the control group (**Fig. 2**). Consumption of the −VA diet for another 7 d caused a further decrease ($P < 0.01$) in the expression of the IGF-I gene in the liver, heart, lung and testis (**Fig. 3**). IGF-I expressions in the vitamin A–deficient quail were 90, 60, 98

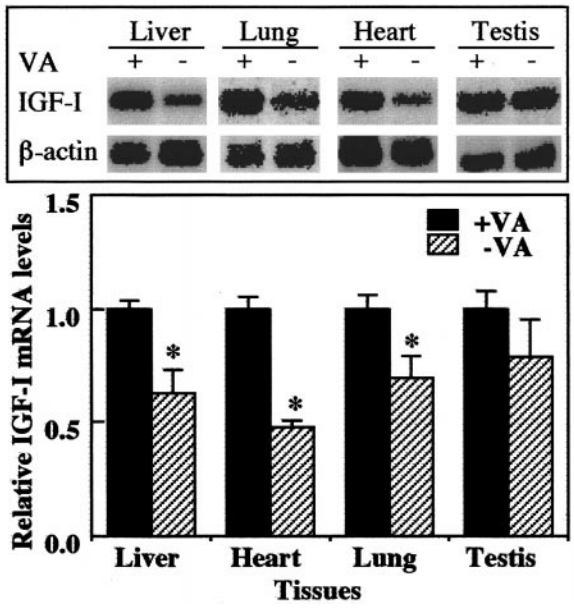

FIGURE 2 Effect of 14-d feeding of a vitamin A–deficient diet on insulin-like growth factor (IGF)-I mRNA levels in several tissues of Japanese quail. Male quail (1 d old) were fed as described in the legend of Figure 1. Representative results obtained by RNase protection assay from the selected tissues are shown in the *upper panel*. The quantitative representations of multiple results expressed as values relative to the abundance in the control are presented in the *lower panel*. Data represent means ± SEM, $n = 5$. *Different from +VA, $P < 0.05$.

and 57% lower in the liver, heart, lung and testis, respectively. However, the expression of the IGF-I gene in brain was unaffected by vitamin A deficiency (Fig. 3). In addition, IGF-I

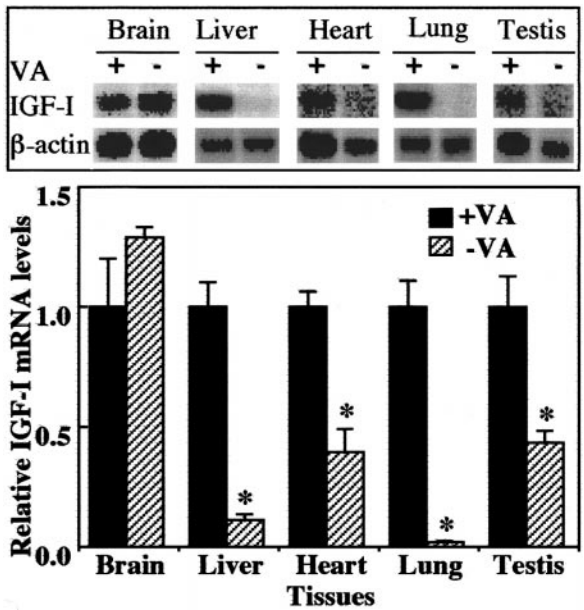

FIGURE 3 Effect of 21-d feeding of the vitamin A–deficient diet on insulin-like growth factor (IGF)-I mRNA levels in several tissues of Japanese quail. Quail were depleted of vitamin A by feeding them a vitamin A–free diet for 3 wk. After this treatment, neither serum retinol nor hepatic retinyl palmitate could be detected. Representative results of RNase protection assay are shown in the *upper panel*. The quantitative representations of multiple results expressed as values relative to the abundance in the control are presented in the *lower panel*. Values are means ± SEM, $n = 5$. *Different from (+VA) $P < 0.01$.

mRNA was not detected in the kidney of either the control or the vitamin A–deficient quail at the age of 21 d (data not shown).

Effect of vitamin A deficiency on the expression of IGF-IR and IR genes. The expression of genes encoding IGF-IR and IR in the liver, heart, lung and testis of quail fed the −VA diet for 14 d did not differ from those in the control quail (data not shown). However, a further depletion of vitamin A (21 d of consuming the −VA diet) altered the expression of both IGF-IR and IR genes in a tissue-specific manner (**Fig. 4**). Vitamin A deficiency caused an ~100% increase ($P < 0.05$) in the mRNA levels of IGF-IR in the heart, kidney and testis, whereas the expression of this gene in the brain, liver and lung was unaffected (Fig. 4*B*). Vitamin A deficiency also significantly ($P < 0.05$) increased IR mRNA levels by ~40–65% in the brain, lung and testis, and by >100% in the heart and kidney (Fig. 4*C*). In contrast, the expression of the hepatic IR gene was unaffected by vitamin A deficiency.

Effect of repletion of vitamin A on the expression of IGF-I, IGF-IR and IR genes. We examined whether the decrease in the mRNA levels of IGF-I and the increase in those of IGF-IR and IR that had been caused by vitamin A deficiency could be restored by repletion of vitamin A. **Figure 5** shows IGF-IR mRNA in the testis of vitamin A–sufficient and –deficient quail

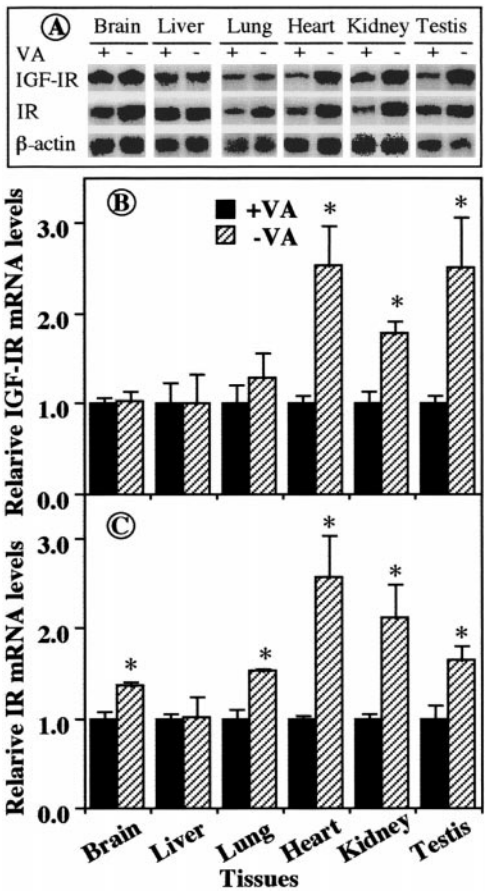

FIGURE 4 A 21-d deficiency in vitamin A increases the mRNA levels of both insulin-like growth factor (IGF)-I receptor (IGF-IR) and insulin receptor (IR) and IR genes in a tissue-specific manner in Japanese quail. Tissues used in this experiment were the same as those described in the legend of Figure 3. Representative results obtained by RNase protection assay are shown in (*A*). The quantitative expression of both IGF-IR (*B*) and IR (*C*) mRNAs was presented as a value relative to the abundance in each respective tissue of the control quail. Data represent means ± SEM, $n = 5$. *Different from +VA, $P < 0.05$.

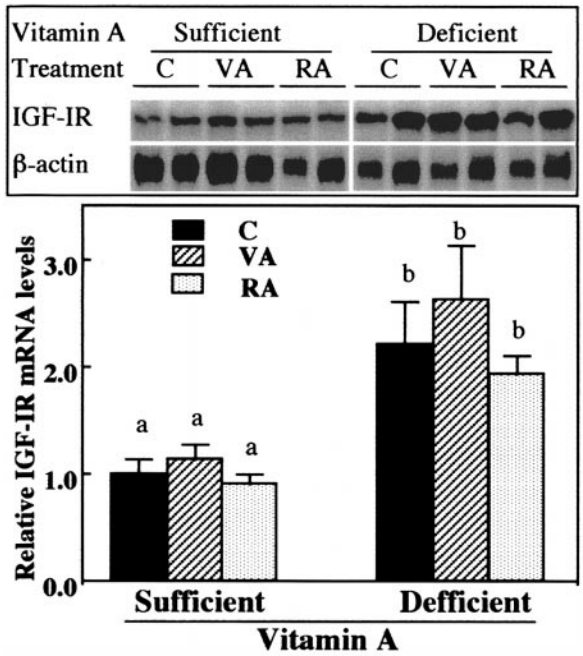

FIGURE 5 An injection of retinol or retinoic acid to the vitamin A–sufficient (+VA) or vitamin A–deficient (−VA) quail does not alter the gene expression of insulin-like growth factor (IGF)-I receptor (IGF-IR) in testis after 4 h of administration. Quail were fed the +VA or −VA diet for 3 wk. They were killed 4 h after treatment with 0.1 mg/bird of all-*trans*-retinol palmitate (VA) or all-*trans*-retinoic acid (RA). Typical results of IGF-IR mRNA expressed in the testis of both +VA or −VA quail are shown in the *upper panels*, and the quantitative representation of multiple results expressed as values relative to the abundance in the vehicle treatment (*C*) of the +VA quail are presented in the *lower panels*. Data represent means ± SEM, $n = 5$. Means not having a common letter differ significantly ($P < 0.05$) between or within the +VA and −VA groups.

4 h after intramuscular injection of retinol or RA. The significant increase in the mRNA levels of IGF-IR caused by vitamin A deficiency was confirmed. However, IGF-IR mRNA levels in both groups were unaffected by treatment with either retinol or RA. Similar results were also observed in other tissues (data not shown). In addition, IGF-I and IR gene expressions in the tissues of vitamin A–deficient quail were not affected by the injection of either retinol or RA (data not shown).

DISCUSSION

In this study, we used vitamin A–deficient quail to investigate the regulation of IGF-I, IGF-IR and IR gene expression by vitamin A in various tissues. This model system has been useful in the past for studying the involvement of vitamin A in many situations and has shown that vitamin A plays essential roles in a number of biological processes such as the maintenance of normal fetal development, the regulation of growth and the differentiation of various tissues (16,30–32).

Serum IGF-I concentrations are dependent on nutritional status. For example, starvation and food restriction markedly reduce serum IGF-I levels in comparison with the levels in well-fed controls in a number of species (7,33,34). The results presented in this study clearly indicate that dietary vitamin A levels also modulate the serum IGF-I concentrations in Japanese quail. Consuming the vitamin A–deficient diet for 14 d after hatching significantly decreased the serum IGF-I concentrations in the quail, and an additional 7-d feeding caused a

further reduction in the serum IGF-I levels. Consistent with our data, Bartlett et al. (25) reported that circulating IGF-I values decreased in vitamin A–deficient rats. As a result of the decrease in the serum IGF-I concentrations, growth in body weight gain was retarded, a finding that is consistent with our previous report (35). It is worth noting that the decrease in body weight was preceded by the decrease in serum IGF-I levels (Fig. 1), thus supporting the concept that IGF-I is an important factor for normal growth.

The liver is the major source of circulating IGF-I. It has been reported that the decrease in serum IGF-I that is caused by food deprivation or other nutritional changes is accompanied by a decrease in the levels of hepatic IGF-I mRNA in rats (36–38) and chickens (9,10). These results suggest that the decrease in the circulating IGF-I levels that results from the nutritional alterations may be caused at least in part by the decrease in the levels of hepatic IGF-I mRNA. No information regarding the effects of the vitamin A deficiency on IGF-I gene expression has been reported. In this study, we reported that the levels of IGF-I mRNA in several tissues were very sensitive to changes in the vitamin A nutritional status. Vitamin A deficiency reduced IGF-I mRNA levels in both the liver and extrahepatic tissues, including the heart, lung and testis. IGF-I gene expression in the brain, however, was unaffected by vitamin A deficiency, implying tissue-specific regulatory mechanisms of this gene.

The biological actions of IGF-I are mediated mainly through its specific cell-surface receptor, IGF-I receptor (IGF-IR), and in part through the insulin receptor (IR). Thus, it is worth examining how vitamin A deficiency affects the expression of both IGF-IR and IR mRNAs in various tissues. Compared with IGF-I gene expression, the expression of both IGF-IR and IR genes was less sensitive to changes in vitamin A nutritional status. A 14-d feeding of the vitamin A–deficient diet did not affect the expression of either IGF-IR or IR genes in any of the tissues examined, although it greatly reduced the levels of IGF-I mRNA in many tissues. However, further depletion of vitamin A by feeding the quail the deficient diet for 21 d altered the expression of IGF-IR and IR genes in a tissue-specific manner. IGF-IR mRNA increased in the heart, kidney and testis, and remained unchanged in the liver, brain and lung, whereas IR mRNA increased in all tissues examined except the liver. These results clearly indicate that the expression of IGF-IR and IR mRNAs was generally up-regulated by the vitamin A deficiency, whereas the serum IGF-I concentrations and tissue IGF-I mRNA were down-regulated.

IGF-I has been shown to down-regulate endogenous IGF-IR mRNA levels in several cell lines (39,40), a finding that has led to the hypothesis that both circulating and locally produced IGF-I are responsible for the regulation of IGF-IR mRNAs under several physiologic and pathologic conditions. This hypothesis may explain the changes in serum IGF-I concentrations, and in IGF-I and IGF-IR mRNA levels in the heart and testis of vitamin A–deficient animals. However, the hypothesis does not explain the situation in the lung and liver, in which vitamin A deficiency caused a dramatic decrease in the IGF-I mRNA levels with little or no change in the IGF-IR mRNA levels. In addition, both the IGF-I and IGF-IR mRNA levels in the brain did not change in response to vitamin A deficiency. Thus, these data demonstrate that the effect of vitamin A deficiency on IGF-I and IGF-IR is quantitatively different among tissues and suggest that the regulation of the gene expression of IGF-I and IGF-IR by vitamin A deficiency is discoordinate.

It has been reported that starvation-induced changes in plasma IGF-I concentrations, hepatic IGF-I mRNA levels, and tissue IGF-IR and IR mRNA levels can be partly or completely restored to control levels by a short-term refeeding. For instance, Knott et al. (41) reported that the increased insulin receptor mRNA levels in the skeletal muscle and brown adipose tissues declined rapidly to control levels after 4 h of refeeding after starvation in rats, and Kita et al. (10) reported that the plasma IGF-I concentration and hepatic IGF-I gene expression were fully restored within 2 h of refeeding after food deprivation in chickens. Thus, in the present study, a single dose of retinol or RA was given to the vitamin A–deficient quail to examine the short-term effects of retinol or RA on the gene expression of IGF-I, IGF-IR and IR in many different tissues. However, no restoration in the mRNA levels of IGF-I, IGF-IR or IR was observed. In this short-term study of vitamin A-replenishment, we used a RA-responsive gene, RARβ, as a positive control, and found a rapid increase in the expression of RARβ mRNA after 4 h of RA injection (Z.W., Fu, T., Kubo, K., Sugahara, T., Noguchi & H., Kato, unpublished observations). This finding confirms that our replenishment model worked satisfactorily. In addition, we also found that a single injection of RA can significantly induce the expression of quail IGFBP-5 mRNA in the same model (Z.W, Fu, T., Noguchi & H., Kato, unpublished observations). The ability of RA to alter gene expression of IGFBP has also been confirmed in several mammalian cell lines (18–23). From these results, it is not reasonable to postulate that the regulation of the genes for IGF-I, IGF-IR and IR by retinoid occurs directly by the binding of retinoid receptors to RA responsive elements (RARE). It is still not known whether RARE exist in the promoter regions of these genes. However, it has recently been reported that RA can cause a transient increase in IGF-I and IGF-II mRNA levels after 6 h of RA treatment in osteoblast cell lines (24). Therefore, further studies, such as an extension of the treatment period with retinol or RA, are required to elucidate the exact effects of vitamin A on the IGF system in vivo.

As discussed above, the IGF system is responsive to nutritional status such as food deprivation and protein malnutrition in mammals and birds (7,14,41–43). In addition, the short-term vitamin A repletion applied in the present study failed to correct the vitamin A deficiency–induced changes in gene expression of the IGF system. Thus, the possibility that the changes in the IGF system observed in the present study are due only to the overall impaired nutritional state must be considered. In fact, the average daily food intake per quail in the −VA group was less than that of the control group from d 15 to 21 of age (6.0 vs. 8.4 g). However, apparent differences existed between the many changes of the IGF system in response to food restriction and the response to vitamin A deficiency. For example, IGF-IR mRNA levels in testis increased >100% in quail fed the vitamin A–deficient diet for 21 d, but did not change in rats starved for 2 d (7). In the case of IR, the IR mRNA levels did not change in the brain of chickens starved for 5 d (14) and increased more than twofold in the liver of chickens starved for 2 d (15). In contrast, the IR mRNA levels showed a reverse response to vitamin A deficiency in these two tissues of Japanese quail reported here. We also found that IGFBP-2 mRNA levels, which are increased by food restriction in mammals (44), did not change in the vitamin A–deficient tissues used in the present study (Z.W, Fu, T., Noguchi & H., Kato, unpublished observations). In addition, different degrees of food restriction, ranging from 0.5 to 3.5 g/100 g body weight, did not affect IGF-I mRNA levels in the liver of *Sparus aurata* (45). Furthermore, the average daily food intake per quail in the −VA group until d 14 of age was not different from that of the +VA group, whereas the serum vitamin A and IGF-I levels and the expression of IGF-I

mRNA in many tissues of the −VA quail decreased significantly during the same period. Recently, we found that the reduced plasma IGF-I concentration caused by the vitamin A deficiency in rats could be restored by RA after 8 h of administration (unpublished data). Taken together, these results suggest that vitamin A indeed has its specific effect on the IGF system in the birds, although we cannot completely rule out the partial involvement of the food-intake factor at present.

In conclusion, the results presented here clearly indicate that dietary vitamin A levels modulate serum IGF-I concentrations and the gene expression of IGF-I, IGF-IR and IR in many different tissues of Japanese quail. Serum IGF-I concentrations and tissue IGF-I mRNA were down-regulated by vitamin A deficiency, whereas the expression of both IGF-IR and IR mRNAs was generally up-regulated. The drastic reduction in the levels of IGF-I mRNA and the resulting decrease in serum IGF-I concentrations may well explain the growth retardation in vitamin A–deficient animals. To our knowledge, this is the first in vivo report on the effects of vitamin A on IGF system components. Further analysis is warranted to elucidate the mechanisms by which vitamin A influences the expression of these genes.

LITERATURE CITED

1. Stewart, C. E. & Rotwein, P. (1996) Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. Physiol. Rev. 76: 1005–1026.

2. Werner, H. & LeRoith, D. (1996) The role of the insulin-like growth factor system in human cancer. Adv. Cancer Res. 68: 183–223.

3. D'Ercole, A. J., Stiles, A. D. & Underwood, L. E. (1984) Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc. Natl. Acad. Sci. U.S.A. 81: 935–939.

4. Daughaday, W. H. (1989) Somatomedins: a new look at old questions. In: Molecular and Celllular Biology of Insulin-Like Growth Factors and Their Receptors (LeRoith, D. & Raizada, M. K., eds.), pp. 1–4. Plenum Press, New York, NY.

5. Nissley, P. & Lopaczynski, W. (1991) Insulin-like growth factor receptors. Growth Factors 5: 29–43.

6. Clemmons, D. R. (1991) Insulin-like growth factor-binding proteins: roles in regulating IGF physiology. J. Dev. Physiol. 15: 105–110.

7. Lowe, W. L., Jr., Adamo, M., Werner, H., Roberts, C. T., Jr. & LeRoith, D. (1989) Regulation by fasting of rat insulin-like growth factor I and its receptor. Effects on gene expression and binding. J. Clin. Investig. 84: 619–626.

8. Thissen, J. P., Ketelslegers, J. M. & Underwood, L. E. (1994) Nutritional regulation of the insulin-like growth factors. Endocr. Rev. 15: 80–101.

9. Kita, K., Tomas, F. M., Owens, P. C., Knowles, S. E., Forbes, B. E., Upton, Z., Hughes, R. & Ballard, F. J. (1996) Influence of nutrition on hepatic IGF-I mRNA levels and plasma concentrations of IGF-I and IGF-II in meat-type chickens. J. Endocrinol. 149: 181–190.

10. Kita, K., Hangsanet, K., Shibata, T., Conlon, M. A., Sasaki, T., Saito, N. & Okumura, J. (1998) Refeeding increases hepatic insulin-like growth factor-I (IGF-I) gene expression and plasma IGF-I concentration in fasted chicks. Br. Poult. Sci. 39: 679–682.

11. Freidenberg, G. R., Klein, H. H., Cordera, R. & Olefsky, J. M. (1985) Insulin receptor kinase activity in rat liver. Regulation by fasting and high carbohydrate feeding. J. Biol. Chem. 260: 12444–12453.

12. Saad, M. J., Araki, E., Miralpeix, M., Rothenberg, P. L., White, M. F. & Kahn, C. R. (1992) Regulation of insulin receptor substrate-1 in liver and muscle of animal models of insulin resistance. J. Clin. Investig. 90: 1839–1849.

13. Bisbis, S., Derouet, M. & Simon, J. (1994) Characterization of insulin receptors in chicken kidneys: effect of nutritional status. Gen. Comp. Endocrinol. 96: 37–49.

14. Matsumura, Y., Domeki, M., Sugahara, K., Kubo, T., Roberts, C. T., Jr., LeRoith, D. & Kato, H. (1996) Nutritional regulation of insulin-like growth factor-I receptor mRNA levels in growing chickens. Biosci. Biotechnol. Biochem. 60: 979–982.

15. Dupont, J., Derouet, M., Simon, J., & Taouis, M. (1998) Nutritional state regulates insulin receptor and IRS-1 phosphorylation and expression in chicken. Am. J. Physiol. 274: E309–E316.

16. Hofmann, C. & Eichele, G. (1994) Retinoids in development. In: The Retinoids: Biology, Chemistry, and Medicine (Sporn, M. B., Roberts, A. B. & Goodman, D. S., eds.), 2nd ed., pp. 387–441. Raven Press, New York, NY.

17. Mangelsdorf, D. J., Umesono, K. & Evans, R. M. (1994) The retinoid receptors. In: The Retinoids: Biology, Chemistry, and Medicine (Sporn, M. B., Roberts, A. B. & Goodman, D. S., eds.), 2nd ed., pp. 319–349. Raven Press, New York, NY.

18. Dong, Y. & Canalis, E. (1995) Insulin-like growth factor (IGF) I and retinoic acid induce the synthesis of IGF-binding protein 5 in rat osteoblastic cells. Endocrinology 136: 2000–2006.

19. Gabbitas, B. & Canalis, E. (1996) Retinoic acid stimulates the transcription of insulin-like growth factor binding protein-6 in skeletal cells. J. Cell. Physiol. 169: 15–22.

20. Glantschnig, H., Varga, F. & Klaushofer, K. (1996) Thyroid hormone and retinoic acid induce the synthesis of insulin-like growth factor-binding protein-4 in mouse osteoblastic cells. Endocrinology 137: 281–286.

21. Zhou, Y., Mohan, S., Linkhart, T. A., Baylink, D. J. & Strong, D. D. (1996) Retinoic acid regulates insulin-like growth factor-binding protein expression in human osteoblast cells. Endocrinology 137: 975–983.

22. Chambery, D., de Galle, B. & Babajko, S. (1998) Retinoic acid stimulates IGF binding protein (IGFBP)-6 and depresses IGFBP-2 and IGFBP-4 in SK-N-SH human neuroblastoma cells. J. Endocrinol. 159: 227–232.

23. Shang, Y., Baumrucker, C. R. & Green, M. H. (1999) Signal relay by retinoic acid receptors alpha and beta in the retinoic acid-induced expression of insulin-like growth factor-binding protein-3 in breast cancer cells. J. Biol. Chem. 274: 18005–18010.

24. Gabbitas, B. & Canalis, E. (1997) Retinoic acid regulates the expression of insulin-like growth factors I and II in osteoblasts. J. Cell. Physiol. 172: 253–264.

25. Bartlett, J. M., Spiteri-Grech, J. & Nieschlag, E. (1990) Regulation of insulin-like growth factor I and stage-specific levels of epidermal growth factor in stage synchronized rat testes. Endocrinology 127: 747–758.

26. Fu, Z. W., Kato, H., Kotera, N., Sugahara, K. & Kubo, T. (1999) Regulation of the expression of serotonin *N*-acetyltransferase gene in Japanese quail (*Coturnix japonica*): II. Effect of vitamin A deficiency. J. Pineal Res. 27: 34–41.

27. Fu, Z. W., Kato, H., Sugahara, K. & Kubo, T. (2000) Retinoic acid accelerates the development of reproductive organs and egg production in Japanese quail (*Coturnix coturnix japonica*). Biol. Reprod. 63: 1805–1810.

28. Fu, Z. W., Kato, H., Sugahara, K. & Kubo, T. (1998) Vitamin A deficiency reduces the responsiveness of pineal gland to light in Japanese quail (*Coturnix japonica*). Comp. Biochem. Physiol. 119A: 593–598.

29. Fu, Z. W., Kubo, T., Sugahara, K., Noguchi, T. & Kato, H. (2001) Cloning of complementary deoxyribonucleic acids encoding quail (*Coturnix coturnix japonica*) retinoic acid receptor β isoforms and changes in their gene expression during gonadotropic growth. Biol. Reprod. 64: 231–241.

30. Thompson, J. N., Howell, J. M. & Pitt, G.A.J. (1964) Vitamin A and reproduction in rats. Proc. R. Soc. Biol. 159: 510–535.

31. Thompson, J. N., Howell, J. M., Pitt, G.A.J. & McLaughlin, C. I. (1969) The biological activities of retinoic acid in the domestic fowl and effects of vitamin A deficiency on the chick embryo. Br. J. Nutr. 23: 471–490.

32. Zile, M. H. (1998) Vitamin A and embryonic development: an overview. J. Nutr. 128: 455S–458S.

33. Morishita, D., Sasaki, K., Wakita, M. & Hoshino, S. (1993) Effect of fasting on serum insulin-like growth factor-I (IGF-I) levels and IGF-I-binding activity in cockerels. J. Endocrinol. 139: 363–370.

34. Planas, J. V., Mendez, E., Banos, N., Capilla, E., Navarro, I. & Gutierrez, J. (2000) Insulin and IGF-I receptors in trout adipose tissue are physiologically regulated by circulating hormone levels. J. Exp. Biol. 203: 1153–1159.

35. Fu, Z. W., Ohara, S., Kato, H., Sugahara, K. & Kubo, T. (1997) Retinoic acid accelerates the testicular maturation in Japanese quail. Anim. Sci. Technol. (Japan) 68: 420–422.

36. Miura, Y., Kato, H. & Noguchi, T. (1992) Effect of dietary proteins on insulin-like growth factor-1 (IGF-1) messenger ribonucleic acid content in rat liver. Br. J. Nutr. 67: 257–265.

37. Straus, D. S. (1994) Nutritional regulation of hormones and growth factors that control mammalian growth. FASEB J. 8: 6–12.

38. Zhang, J., Chrysis, D. & Underwood, L. E. (1998) Reduction of hepatic insulin-like growth factor I (IGF-I) messenger ribonucleic acid (mRNA) during fasting is associated with diminished splicing of IGF-I pre-mRNA and decreased stability of cytoplasmic IGF-I mRNA. Endocrinology 139: 4523–4530.

39. Rosenthal, S. M. & Brown, E. J. (1994) Mechanisms of insulin-like growth factor (IGF)-II-induced IGF-I receptor down-regulation in BC3H-1 muscle cells. J. Endocrinol. 141: 69–74.

40. Hernandez-Sanchez, C., Werner, H., Roberts, C. T., Jr., Woo, E. J., Hum, D. W., Rosenthal, S. M. & LeRoith, D. (1997) Differential regulation of insulin-like growth factor-I (IGF-I) receptor gene expression by IGF-I and basic fibroblastic growth factor. J. Biol. Chem. 272: 4663–4670.

41. Knott, R. M., Trayhurn, P. & Hesketh, J. E. (1992) Changes in insulin-receptor mRNA levels in skeletal muscle and brown adipose tissue of weanling rats during fasting and refeeding. Br. J. Nutr. 68: 583–592.

42. Takenaka, A., Takahashi, S. & Noguchi, T. (1996) Effect of protein nutrition on insulin-like growth factor-I (IGF-I) receptor in various tissues of rats. J. Nutr. Sci. Vitaminol. 42: 347–357.

43. Chrysis, D., Moats-Staats, B. M. & Underwood, L. E. (1998) Effect of fasting on insulin receptor-related receptor messenger ribonucleic acid in rat kidney. J. Endocrinol. 159: R9–R12.

44. Ooi, G. T., Orlowski, C. C, Brown, A. L., Becker, R. E., Unterman, T. G. & Rechler, M. M. (1990) Different tissue distribution and hormonal regulation of messenger RNAs encoding rat insulin-like growth factor-binding proteins-1 and -2. Mol. Endocrinol. 4: 321–328.

45. Meton, I., Caseras, A., Canto, E., Fernandez, F. & Baanate, I. V. (2000) Liver insulin-like growth factor-I mRNA is not affected by diet composition or ration size but shows diurnal variations in regularly-fed Gilthead sea bream (*Sparus aurara*). J. Nutr. 130: 757–760.