The Interactive Effect of Dietary Protein and Vitamin Levels on the Depression of Gonadal Development in Growing Male Rats Kept under Disturbed Daily Rhythm

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(Received August 4, 2006)

Summary The purpose of this study was to clarify the effects of nutrients on the gonadal development of male rats kept under constant darkness as a model of disturbed daily rhythm. The present study examined protein and vitamins, and their interactions. This study was based on three-way ANOVA; the three factors were lighting conditions, dietary protein and dietary vitamins, respectively. The levels of dietary protein were low or normal: 9% casein or 20% casein. The levels of dietary vitamins were low, normal or high: 1/3.3 of normal (AIN-93G diet) content, normal content, or three times the normal content, respectively. Other compositions were the same as those of the AIN-93G diet, and six kinds of experimental diet were prepared. Four-week-old rats (Fischer 344 strain) were kept under constant darkness or normal lighting (12-h light/dark cycle) for 4 wk. After 4 wk, the gonadal weights and serum testosterone content were evaluated. In the constant darkness groups (D-groups), the low-protein diet induced reduction of gonadal organ weights and serum testosterone concentrations. This reduction of gonadal organ weights was exacerbated by progressively higher levels of dietary vitamins. In the case of a normal-protein diet, the depression of gonadal development was not accelerated by high-vitamin intake. In the normal lighting groups (N-groups), the low-protein and high-vitamin diet slightly depressed gonadal development. These results suggest that the metabolism of protein and vitamins is different in rats being kept under constant darkness, and that excess dietary vitamins have an adverse effect on gonadal development in rats fed a low-protein diet.

Key Words constant darkness, disturbed daily rhythm, gonad, protein, vitamin

The number of people who are living under disturbed daily rhythm has been increasing due to the globalization of business and social activities, and diversification of the forms of labor. Such irregularities in the daily rhythms adversely affect bio-regulatory mechanisms, resulting in an abnormal diurnal rhythm that can impede biological activities structurally and functionally. The process by which such disorders are induced is, in theory, dependent upon the nutritional condition of the individual. However, there are no basic data on the nutritional aspects of maintaining or promoting health under the condition of disturbed daily rhythms. The metabolic function changes under the condition of disturbed daily rhythms such as constant darkness, and it can be presumed that nutritional requirements in such an environment change, as well. The perspective behind this research is as follows: as basic data on the

relationship between nutritional status and gonad development in rats with disturbed daily rhythm accumulate, these data can be used for human research, and finally, dietary reference intakes can be compiled for persons living under disturbed daily rhythm.

Lighting is one of the key external factors for the formation of daily rhythm. It has been observed that rats kept under constant darkness develop disturbances in their feeding and motor-activity rhythms, and suffer from altered rhythms of hormone secretion and enzyme activity (1, 2).

Sakai (3, 4) and Esashi et al. (5, 6) have reported that rats kept in constant darkness showed a decrease of gonadal development. It has also been reported that the depression of gonadal development in these rats was accelerated by a low-protein diet (5, 6). Clearly, gonads have high sensitivity to constant darkness and nutrients. Hence, we have focused on gonadal development in our efforts to accumulate basic nutritional data on rats kept under constant darkness as a model of daily disturbed rhythm.

In previous papers, we have reported the effects of protein, methionine, vitamins, minerals and oil (7), various minerals (8), and various amino acids, an AIN-76 diet and an AIN-93G diet (9). The depression of

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gonadal development in rats kept under constant darkness was mitigated by normal levels of protein, the addition of methionine, low vitamin levels, low mineral levels and normal levels of oil, and was accelerated by low protein plus methionine, normal vitamins, normal minerals and a low oil diet. The purpose of the present study was to clarify the effects of protein and vitamins, and the interactive effect of protein and vitamins, on gonadal development. Esashi et al. first reported the effect of protein on gonadal development (5, 6). However, the interactive effect of dietary protein and vitamins on the gonads of rats kept under constant darkness has not been reported. Therefore, in this study, we attempted to clarify the effects of dietary protein levels combined with different vitamin levels.

METHODS

1. Animals. Seventy-two Fischer strain (F344) male rats (purchased from Charles River Japan Inc., Kanagawa, Japan, at 3 wk of age) were preliminarily maintained for 1 wk on the AIN-93G purified diet (10), and then divided into experimental groups. There were a total of twelve groups with six rats in each group. No differences were found in mean body weights of rats from any of the twelve groups. The rats were kept under constant darkness (D-groups) or normal lighting (12-h light/dark cycle, N-groups) for 4 wk. Food intake and body weight were recorded every other day. The care of rats kept under constant darkness included lighting a red lamp for about 2 h; the lamp was for photographs and would not cause a phase variation of circadian rhythms. The rats were housed in individual, stainless steel, wire-mesh-bottomed cages at $22 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ humidity, in a room free from specific pathogens. Food and distilled water were provided to all rats ad libitum.

Animals were maintained in accordance with the Guideline for the Care and Use of Laboratory Animals.

2. Diets. Examined factors and their levels, and compositions of the experimental diets are shown in Tables 1 and 2, respectively. The dietary protein levels were either low or normal, 9% casein or 20% casein, respectively. Dietary vitamin levels were one of three levels, low, normal or high: specifically, 1/3.3 of normal content, normal content and three times the normal content. Other components were based on the AIN-93G diet (10). Six kinds of experimental diets were prepared.

3. Analysis. After 4 wk of treatment, the rats were decapitated, and the blood and gonadal organs (testes, epididymides, seminal vesicles and prostate) were collected. The gonadal organs were weighed. Blood was centrifuged (3,000 rpm, 15 min, 4°C), and the serum obtained was stored at -20°C until analysis, when serum testosterone concentration was measured by a radioimmunoassay kit (CIS Diagnostic Co., Tokyo, Japan).

4. Statistical analysis. The results of the experiment were analyzed by three-way ANOVA and subsequently by Fischer's exact test using Stat View/Version 5.0 (SAS Institute Inc., NC, USA). Differences among mean values were considered significant at $p < 0.05$.

Table 1. Estimated factors and their levels in three-way classification.

Factor	Level 1	Level 2	Level 3
A: Lighting condition	Normal lighting	Constant darkness	—
B: Protein (Casein)	Low*	Normal*	—
C: Vitamin mixture	Low**	Normal**	High**

*Casein level, low: 9% casein, Normal: 20% casein.

**Vitamin mixture level, Low: 0.3% ($\times 1/3.3$), Normal: 1%, High: 3% ($\times 3$).

Table 2. Composition of the diets.

(g/100 g diet)

Ingredients	Diet No.					
	1	2	3	4	5	6
Milk casein ¹	9	9	9	20	20	20
L-Cystine	0.135	0.135	0.135	0.3	0.3	0.3
α -Corn starch	10	10	10	10	10	10
Sucrose	64.99	64.11	61.61	53.82	52.95	50.45
Fiber	5	5	5	5	5	5
Soybean oil	7	7	7	7	7	7
Vitamin mix. ²	0.3	1.0	3.0	0.3	1.0	3.0
Choline bitartrate	0.075	0.25	0.75	0.075	0.25	0.75
Mineral mix. ³	—	—	—	3.5	3.5	3.5
Mineral mix. ⁴	3.5	3.5	3.5	—	—	—
tert-Butylhydroquinone	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014

¹ Vitamin-free milk casein.

² AIN-93 vitamin mixture.

³ AIN-93G mineral mixture.

⁴ AIN-93G mineral mixture containing 67.2 g/kg of phosphorus.

Table 3. Results of dietary protein and vitamin levels on body weight and total food intake.

	Protein levels	Vitamin levels	Body weight (g)	Total food intake (g)	Food efficiency (g)
Normal lighting	20% Casein	Low ¹	170.9±7.2 ^{4a}	296.7±11.2 ^{aa}	0.356±0.018Aab
		Normal ²	178.4±8.9A ^{6a7b8)}	306.7±13.5a	0.385±0.044Aaa
		High ³	180.3±7.1Aab	322.2±21.0Ab	0.357±0.013Aab
	9% Casein	Low	154.6±7.2A	338.4±16.5Ab	0.285±0.004b
		Normal	156.9±9.7Ab	333.5±23.1Ab	0.296±0.011b
		High	152.6±7.3Ab	323.0±12.5A	0.292±0.008b
Constant darkness	20% Casein	Low	162.0±8.3a	301.1±18.3	0.321±0.014Ba
		Normal	162.0±11.1Ba	299.4±13.2	0.322±0.019Ba
		High	163.5±12.4Ba	299.9±15.5B	0.327±0.018Ba
	9% Casein	Low	143.3±4.5Bb	319.0±8.8Ba	0.267±0.005b
		Normal	143.3±9.5Bb	306.7±18.2B	0.277±0.013b
		High	138.1±9.8Bb	292.0±18.4Bb	0.273±0.014b
Three-way ANOVA	Lighting conditions ⁵ (A)		** ⁹	**	**
	Protein (B)		**	**	**
	Vitamin (C)				
	A×B			*	**
	A×C				
	B×C			**	
	A×B×C				

¹Low level (×1/3.3), ²Normal level, ³High level (×3).

⁴Mean ± SD (n=6).

⁵Lighting conditions, Normal lighting or Constant darkness.

⁶Having different capital letters within a column means a significant difference of at least 95% probability for lighting conditions.

⁷Having different lower-case letters within a column means a significant difference of at least 95% probability for protein levels.

⁸Having different italic letters within a column means a significant difference of at least 95% probability for vitamin levels.

⁹*p<0.05, **p<0.01.

RESULTS

1. Final body weight, total food intake and food efficiency

Final body weights, total food intake and food efficiency for each of the twelve groups are shown in Table 3.

Lighting conditions and protein level were observed to have an effect on the final body weight. Lighting conditions and protein, and interactions between protein and both lighting conditions and vitamin levels were observed to have an effect on total food intake. Lighting conditions and protein, and the interaction between lighting conditions and protein were observed to have an effect on food efficiency.

The final body weights of the D-groups were lower than that of N-groups, and the 9% casein (low protein) diet reduced the final body weight compared with the 20% casein (normal protein) diet. In the D-groups, there was a tendency for the final body weight of rats given the 9% casein and high-vitamin diet to be reduced. But there was no significant difference among the three groups of 9% casein diet rats kept in constant darkness.

The total food intake of the D-groups given the 9%

casein diet was low compared with that of the N-groups. In the D-groups, the total food intake of rats fed the 9% casein and high-vitamin diet was lower than that of those fed the 9% casein and low-vitamin diet.

The food efficiency of the D-groups given the 20% casein diet was low compared with that of the N-groups. In the D-groups, no effect of vitamins on food efficiency was observed.

2. Gonadal organ weights

Measured weights of the testes, epididymides, seminal vesicles and prostate are shown in Table 4.

Effects of lighting conditions and protein, and interactive effects of protein and vitamins were observed in the gonadal organ weights of almost all groups. The gonadal organ weights of D-groups given the 9% casein diet were low compared with those of N-groups given the 9% casein diet. Among the D-groups, the gonadal organ weights were especially reduced by the 9% casein and high-vitamin diet. In the N-groups, there was no effect of vitamin levels on the gonadal organ weights of rats fed the 9% casein diet. The testes and epididymides weights of rats in the D-groups given the 20% casein diet were no different from those of the N-groups and were not impacted by dietary vitamin levels.

Table 4. Results of dietary protein and vitamin levels on gonadal organ weights.

	Protein levels	Vitamin levels		Testes Wt		Epididymides Wt		Seminal vesicle Wt		Prostate Wt	
				(g)	(g/100 gBW)	(mg)	(mg/100 gBW)	(mg)	(mg/100 gBW)	(mg)	(mg/100 gBW)
Normal lighting	20% Casein	Low ¹		2.11±0.30 ⁴	1.23±0.14	217±49 ^a	127±25	100±30 ^{Aa}	58±16	88±17 ^{Aa}	51±8 ^{Aa}
		Normal ²		2.32±0.12	1.30±0.03	265±53	148±26	129±20 ^A	72±8 ^A	92±16 ^{Aa}	51±7 ^a
		High ³		2.30±0.20 ^{a7}	1.27±0.07	283±55 ^{ab}	156±27 ^a	130±23 ^{Aab}	72±11 ^A	111±15 ^{Ab}	62±7 ^{Ab}
	9% Casein	Low		2.00±0.24	1.29±0.13	221±57	142±34	107±23 ^A	69±14 ^A	88±9 ^A	57±6 ^A
		Normal		2.04±0.26 ^{A6}	1.30±0.11	221±55	140±28	119±28 ^A	75±14 ^A	92±12 ^A	59±5 ^A
		High		1.82±0.18 ^{Ab}	1.19±0.08 ^A	184±28 ^{Ab}	120±14 ^{Ab}	95±21 ^{Ab}	62±12 ^A	89±13 ^A	58±7 ^A
Constant darkness	20% Casein	Low		2.03±0.36	1.25±0.20	233±71	143±41	69±30 ^B	42±19	63±8 ^B	39±4 ^{Ba}
		Normal		2.09±0.40 ^a	1.29±0.26	203±74	124±39	67±34 ^B	41±18 ^B	72±17 ^{Ba}	44±8
		High		2.08±0.43 ^a	1.27±0.20 ^a	226±79 ^a	137±42 ^a	75±35 ^{Ba}	45±20 ^{Ba}	71±18 ^{Ba}	43±9 ^B
	9% Casein	Low		1.81±0.28 ^{a8}	1.27±0.19 ^a	192±42 ^a	134±29 ^a	63±21 ^B	44±14 ^B	69±10 ^{Ba}	49±7 ^{Ba}
		Normal		1.70±0.18 ^{Bba}	1.19±0.09 ^a	163±30	113±18	42±10 ^B	29±6 ^{Ba}	55±5 ^{Bb}	38±3 ^{Bb}
		High		1.27±0.31 ^{Bbb}	0.91±0.17 ^{Bbb}	119±27 ^{Bbb}	85±15 ^{Bbb}	34±15 ^{Bb}	24±9 ^{Bbb}	51±8 ^{Bbb}	37±4 ^{Bbb}
Three-way ANOVA	Lighting conditions ⁵ (A)		** ⁹			**	*	**	**	**	**
	Protein (B)		**		*	**	*	**	**	**	**
	Vitamin (C)				*						
	A×B										
	A×C										*
	B×C		*		*	*	*	*	*	**	**
	A×B×C										

¹ Low level (×1/3.3), ² Normal level, ³ High level (×3) .⁴ Mean±SD (n=6).⁵ Lighting conditions, Normal lighting or Constant darkness.⁶ Having different capital letters within a column means a significant difference of at least 95% probability for lighting conditions.⁷ Having different lower-case letters within a column means a significant difference of at least 95% probability for protein levels.⁸ Having different italic letters within a column means a significant difference of at least 95% probability for vitamin levels.⁹ *p<0.05, **p<0.01.

Table 5. Results of dietary protein and vitamin levels on serum testosterone concentration.

	Protein levels	Vitamin levels	Testosterone (ng/mL)
Normal lighting	20% Casein	Low ¹	2.700±1.984 ^{4a} ^{6,7}
		Normal ²	2.051±0.708
		High ³	1.567±1.179 ^b
	9% Casein	Low	1.127±1.116 ^b
		Normal	1.590±1.010
		High	1.189±1.142
Constant darkness	20% Casein	Low	1.625±0.169
		Normal	1.557±0.451
		High	1.543±0.561
	9% Casein	Low	0.857±0.742
		Normal	0.860±0.486
		High	0.885±0.201
Three-way ANOVA	Lighting condition ⁵ (A)		* ⁸
	Protein (B)		**
	Vitamin (C)		
	A×B		
	A×C		
	B×C		
	A×B×C		

¹ Low level (×1/3.3), ² Normal level, ³ High level (×3).

⁴ Mean±SD (n=6)

⁵ Lighting Conditions, Normal lighting or Constant darkness.

⁶ Having different lower-case letters within a column means a significant difference of at least 95% probability for protein levels.

⁷ Having different italic letters within a column means a significant of difference of at least 95% probability for vitamin levels.

⁸ **p*<0.05, ***p*<0.01.

The combined effects of dietary protein and vitamin levels on gonadal organ weights of the D-groups were in stark contrast to their effects on body weight. The testes weight of rats fed a low-protein diet was reduced by a high-vitamin diet, but the body weight was not influenced by vitamin level.

3. Serum testosterone concentration

Serum testosterone concentrations are shown in Table 5.

Lighting conditions and protein were observed to have an effect on serum concentration. The serum testosterone concentrations of D-groups were low compared with those of N-groups. The dietary vitamin levels didn't influence serum testosterone concentration in the D-groups.

DISCUSSION

As shown in the results, the body weights of the D-groups given a low protein diet were low compared with those of the N-groups. This was due to the decreased total food intake, because there was no difference in the

food efficiency. The reason for the decreased food intake of the D-groups given a low-protein diet is not clear. The decrease in food intake of rats kept under constant darkness compared with that of rats kept under the normal lighting conditions began only after 2 wk (data not shown). In general, the rats have a feeding rhythm of eating mostly in the nighttime and only a small amount in the daytime (11). But in the case of rats kept under constant darkness, the feeding rhythm is disturbed; they eat both night and day (12). However, the present data are insufficient to clarify or explain the relation between the disappearance of feeding rhythm and decrease of food intake.

In the N-groups, the total food intake of rats fed the low-protein diet increased compared with that of rats fed the normal protein diet. This reflects the usual nutritional adaptability of rats, which take in as much as they require of each nutrient (13). However, in the D-groups, the total food intake of rats fed the low-protein diet didn't increase. It is thought that their ability to regulate their intake was kept from being set in motion by their disturbed daily rhythm. The maturation of the mechanism of regulating their intake works closely with the biological rhythm adjustment mechanism in the growing period of rats (14). Therefore, there is a probability that the mechanism regulating the D-groups' intake was kept from maturing by their disturbed circadian rhythm. It is also thought that the nutritional requirements of the growing period differ for rats kept under constant darkness compared to those kept under normal lighting. But this hypothesis is not clear. Other systematic research on the ability of rats to regulate their nutritional intake is needed.

Leatham has reported that vitamins (V) A, V.E, V.B₁, V.B₂, and V.B₆, as well as panthotenic acid and biotin, influence gonad growth and function, and that deficiencies in these vitamins induce sperm malformation and atrophy of testes (15). It had also been reported that V.D deficiency induces reduction of testes function (16,17). Moreover, Huang and Marshall reported that a V.A-deficient diet induces failure of spermatid release in rats (18). These results were obtained by changing dietary V.D or V.A levels only; dietary protein levels remained the same.

As shown from the results of the interaction of dietary protein and vitamins, the influences of vitamins in the D-groups strongly depended on the dietary protein level. Namely, adverse effects on gonadal development were observed from excessive vitamins in D-groups given the low-protein diet. However, there were no significant effects of dietary vitamins on body weight. Therefore, it is thought that this is a specific effect on the gonads of rats kept under constant darkness and served a low-protein diet. By contrast, in the N-groups, there were no adverse effects of excess vitamins on gonadal development. These results suggest that the metabolisms of protein and vitamins were changed by keeping the rats under constant darkness, and that the requirements of vitamins for gonadal development came to differ between the N-groups and

the D-groups. Besides, it is thought that there is a possibility of interaction between protein or amino acids and individual vitamins. There have been no reports about changes in protein or amino acid metabolism under disturbed daily rhythm, such as constant darkness. But we have reported different requirements of amino acids for gonadal development in rats kept under constant darkness compared to normal-lighting controls (9). Specifically, we found that the methionine, cysteine and valine requirements for gonadal development were different between N-groups and D-groups. V.B₁ works as a co-enzyme for the valine metabolic enzyme (19). The valine requirement for gonadal development increased in rats kept under constant darkness (9). There is a possibility that the metabolism of valine and the physiologic function of V.B₁ were changed by the disturbed daily rhythm, but that cannot be confirmed at present.

It has been reported that a high dose of V.B₆, 500 or 1,000 mg/kg/d, induced testicular damage in rats (20). That experiment was done under a normal protein diet and normal lighting conditions. More over the V.B₆ dose of that study was about 3 to 14 times higher than that in the present study. Thus, that result and our result cannot be directly compared, but it can be considered that V.B₆ is a candidate vitamin for inducing adverse effects on gonads in rats kept under constant darkness.

In previous papers, we reported that serum testosterone concentrations were decreased by keeping rats under constant darkness (7–9) and also by feeding them a low-protein diet (7); the reduction of gonadal organ weight appeared to be caused by the decreased serum testosterone concentration. The results of present study supported these previous results.

Gonadal development depends on the androgen, testosterone. Furthermore, there are many molecular biological steps for functional expression of testosterone in each gonad (21). In the present study, the gonadal organ weight of the D-group given a 9% casein and high-vitamin diet was depressed compared with the D-groups fed the 9% casein and normal-vitamin diet and the 9% casein and low-vitamin diet. But the dietary vitamin level in the D-groups had no observed effect on serum testosterone concentration. Namely, the high-vitamin diet influenced gonadal development but didn't influence serum testosterone concentration in the D-groups given the 9% casein diet. These results show that the functional expression of testosterone in the gonads is inhibited by the low-protein and high-vitamin diet in the D-groups. It is also thought that dietary protein participates in gonadal development through the biosynthesis of testosterone, and that dietary vitamins are involved in gonadal development through a mechanism that has no relation to the biosynthesis of testosterone. But the exact mechanisms concerned could not be clarified in the present study.

The present study showed that a low-protein and high-vitamin diet accelerates the depression of gonadal development in rats kept under constant darkness as a model of disturbed daily rhythm; these effects were not shown in rats kept under normal lighting conditions.

These results suggest that the metabolism of protein and vitamins is different in rats kept under constant darkness, and that excess dietary vitamins have an adverse effect on gonadal development in rats fed a low-protein diet.

These days, there are numerous multivitamin supplements on the market, suggesting that excess consumption of vitamins is not unusual. Our result showed that people with insufficient protein intake and a disturbed daily rhythm must be careful in taking vitamin supplements.

The vitamin mixture used in the present study includes twelve kinds of vitamins. Further studies are needed to explain exactly which vitamin influenced the gonadal development of rats kept under constant darkness as a model of disturbed daily rhythm.

REFERENCES

- 1) Takahashi Y. 1991. Mammals. Endocrinology mechanism. In: Chronobiology Handbook (Chiba Y, Takahashi K, eds), p 112–129. Asakura Syoten Co Ltd, Tokyo (in Japanese).
- 2) Depres-Brummer P, Bourin P, Pages N, Metzger G, Levi F. 1997. Persistent T lymphocyte rhythms despite suppressed circadian clock outputs in rats. *Am J Physiol* **273**: R1891–R1899.
- 3) Sakai T. 1963. Activities of hypophysis, testis and pineal body in rats housed in continuous darkness. *Nippon Seirigaku Zasshi* **25**:140–145 (in Japanese).
- 4) Sakai T. 1963. Reproductive organs in rats housed in continuous darkness. *Nippon Seirigaku Zasshi* **25**:133–139 (in Japanese).
- 5) Esashi T, Nakamura K, Tsuchiya K, Tamura Y, Shimoto-mai A, Yokota F, Hayami H. 1973. The effect of illumination and darkness on the birth rate of rat. *Eiyogaku Zasshi (Jpn J Nutr Diet)* **31**: 195–198.
- 6) Esashi T. 1994. Light deficit and nutrition. *Eiyogaku Zasshi (Jpn J Nutr Diet)* **52**: 211–222 (in Japanese).
- 7) Hanai M, Kubo K, Esashi T. 2003. The effects of nutrients in improving the depression of gonadal development in growing male rats kept under disturbed daily rhythm—Investigations based on the L8(2⁷)-type orthogonal array. *J Nutr Sci Vitaminol* **49**: 388–396.
- 8) Hanai M, Esashi T. 2006. The effects of calcium, phosphorus, magnesium, sodium and zinc in improving the depression of gonadal development in growing male rats kept under disturbed daily rhythm—Investigations based on the L8(2⁷) type orthogonal array. *J Nutr Sci Vitaminol*: 368–375.
- 9) Hanai M, Esashi T. 2003. The effects of essential amino acids in improving the depression of gonadal development in growing male rats kept under constant darkness. *Rep Res Committee of Essential Amino Acids (Japan)* **167**: 61–72 (in Japanese).
- 10) Reeves PG, Nielsen FH, Fahey GC. 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **123**:1939–1951.
- 11) Zucker I. 1971. Light-dark rhythms in rat eating and drinking behavior. *Physiol Behav* **6**:115–126.
- 12) Esashi T, Suzuki K, Takahashi S, Suzue R, Nakamura A, Innami S. 1977. Effects of dietary casein content, L-tryptophan supplementation, melatonin injection and

- continuous darkness on the diurnal food intake rhythm of male rat. *Eiyogaku Zasshi (Jpn J Nutr Diet)* **35**:183–192 (in Japanese).
- 13) Hartstook EW, Nee JC. 1976. Effects in the rat of environmental temperature, diet dilution and treadmill running on voluntary food intake, body composition and endocrine organ mass: a multiple regression analysis. *J Nutr* **106**:1314–1325.
 - 14) Kubo K. 1984. The central mechanism for adjustment of feeding behavior circadian rhythm *In: Expression Mechanism of Biological Rhythm* (Kawakami M, Takasaka M, eds), p 68–95. Rikogakusya, Tokyo (in Japanese).
 - 15) Leatham JH. 1970. Nutrition. *In: The Testis 3* (Johnson AD, Gomes WR, Vandemark NL, eds), p 169–205. Academic Press, New York.
 - 16) Sood S, Reghunandanan R, Reghunandanan V, Marya RK, Singh PI. 1995. Effect of vitamin D repletion on testicular function in vitamin D-deficient rats. *Ann Nutr Metab* **39**: 95–98.
 - 17) Sood S, Marya RK, Reghunandanan R, Singh GP, Jaswal TS, Gopinathan K. 1992. Effect of vitamin D deficiency on testicular function in the rats. *Ann Nutr Metab* **36**: 203–208.
 - 18) Huang HF, Marshall GR. 1983. Failure of spermatid release under various vitamin A states—an indication of delayed spermiation. *Biol Reprod* **28**: 1163–1172.
 - 19) Itokawa Y. 1997. Vitamin B₁. *In: Vitamin Dictionary*. (Japanese Society of Vitamin, ed), p 150–167. Asakura Publishing Co. Ltd. Tokyo (in Japanese).
 - 20) Kaido M, Mori K, Ide Y, Inoue N, Koide O. 1991. Testicular damage by high vitamin B6 (pyridoxine) in rats: a light and electron microscopical study. *Exp Mol Pathol* **55**: 63–82.
 - 21) Sakuma Y. 2000. Reproductive grand. Development and function of reproduction. *In: Review of Medical Physiology* (Hoshi T, Okada Y, Kawahara K, Sugano T, Kumada M, Kurosawa M, Sakuma Y, Sato T, Suzuki Y, Takuwa Y, Nakamura Y, Fukuda K, tr), 19th ed, p 442–452. Maruzen Co Ltd, Tokyo (in Japanese).