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Effects of Dietary Caloric Restriction and Aging on Thyroid Hormones of Rhesus Monkeys

Abstract

Plasma levels of thyroid hormones – triiodothyronine (T_3), thyroxin (T_4), and thyroid-stimulating hormone (TSH) were measured in male and female rhesus monkeys *(Macaca mulatta)* fed either *ad libitum* or a 30% calorie-restricted (CR) diet (males for 11 years; females for 6 years). The same hormones were measured in another group of young male rhesus monkeys during adaptation to the 30% CR regimen. Both long- and shorter-term CR diet lowered total T_3 in plasma of the monkeys. The effect appeared to be greater in younger monkeys than in older counterparts. No effects of CR diet were detected for either free or total T_4, although unlike T_3, levels of this hormone decreased with age. TSH levels also decreased with age, and were increased by long-term CR diet in older monkeys only. No consistent effects of shorter-term CR diet were observed for TSH. In the light of the effects of the thyroid axis on overall metabolism, these results suggest a possible mechanism by which CR diets may elicit their well-known beneficial 'anti-aging' effects in mammals.

Key words
Primate · Thyroxin · Triiodothyronine · Dietary Restriction

Introduction

Dietary caloric restriction (CR) is the most robust intervention for retarding aging in mammals [1,2]. Recent studies suggest that, in addition to rodents and lower animals, CR may exert its beneficial anti-disease and anti-aging effects in primates [2,3], and may even benefit humans, at least in theory [3]. However, difficulties associated with maintaining a 30–40% reduced caloric intake for the bulk of the life span will probably preclude any wide adoption of this intervention by humans, even with its promise of an extended life span and health span [1–3]. Consequently, a more practical strategy would be to elucidate the biological mechanism(s) by which CR exerts its effects and attempt to mimic them by other means [4].

A number of possible mechanisms for CR have been proposed, including reduction in damage through oxygen free radicals [5], decreased glycation of macromolecules [6], stress protection [7], and metabolic adaptations [8]. Evidence suggests that all may contribute, at least in part, but the latter mechanism might have more global consequences since metabolic adaptations may provide a coordinated set of physiological readjustments to mediate all others [9]. One key metabolic manifestation of the CR state in mammals is a reduced body temperature. A recent study from our laboratory demonstrated that rhesus monkeys on CR also exhibit a reduction in body temperature [8]. In addition, a transient reduction in metabolic rate is observed when rodents and primates are subjected to CR [8,10].

It is therefore very important to determine the mechanisms by which such metabolic adaptations occur. One class of hormones that serves to regulate metabolism is produced by the thyroid gland [11]. There is substantial information suggesting that CR may affect thyroid hormones in various species relatively soon after its introduction [12–14]. In particular, triiodothyronine (T_3) levels in the blood have been reported to decrease in CR rats [14], while concentrations of thyroxin (T_4), from which T_3 is formed in the periphery, are generally left unaltered. Since T_4 is the predominant species directly secreted by the thyroid, it is possible that CR affects the conversion of T_4 to T_3. However, one

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Bibliography
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study on rhesus monkeys at the University of Maryland (UMd) reported exactly the opposite – namely, a CR-induced decrease in T_4, but not T_3 [15]. The dietary regimen and body composition of monkeys in the UMd study were somewhat different from the present investigation (such as weight stabilization in the UMd study as opposed to a direct 30% caloric reduction and a higher body fat content in both control and CR animals in our study). Control monkeys in the present investigation are relatively lean, with only 14 – 16 percent body fat on average. The findings in the UMd study suggested that monkeys might respond to CR differently than rodents, at least in terms of thyroid regulation. The possibility of species differences in thyroid hormone responses to CR would be important to determine to define possible biological mechanisms of this metabolic intervention.

For this reason, it became critical to reassess the effects of CR in non-obese monkeys under defined intake restriction conditions. The results presented here provide the first such documentation of thyroid hormone responses to such dietary intervention in non-obese rhesus monkeys, and suggest a possible biological mechanism for the metabolic effects of CR.

Materials and Methods

Animals and husbandry

A total of 51 male and 45 female rhesus monkeys (*Macaca mulatta*) in our longitudinal study of aging and calorie restriction (CR) in non-human primates were used in the present study. Both groups were split into adult (female 11.79 ± 0.56 years; male 13.86 ± 0.21 years) and old age groups (female 24.61 ± 0.90 years; male 28.23 ± 0.6 years). Half of the monkeys in each group were fed approximately *ad libitum*, whereas the other half were fed 30% less calories compared to control animals of a similar age and body weight. Experimental monkeys were maintained on 30% restriction for 6 and 11 years for females and males, respectively. Additionally, 24 male rhesus monkeys (n = 12, 4 years at start of study; n = 12, 20 years at start of study) were used to examine changes during the initial adaptation to CR. Each group was divided into six control animals (fed *ad libitum*) and six experimental animals (fed *ad libitum* for one month after which caloric intake was reduced 10% per month to a final restriction level of 30%).

Diet composition was identical for all monkeys and has been described previously [16,17]. A vitamin and mineral premix was added to the diet during manufacturing to insure normal levels of vitamins, minerals, and trace elements in the diet of CR monkeys. Monkeys were fed twice daily (7 h and 14 h) and had free access to water.

All monkeys had been housed continuously at the NIH Animal Center, VRP, ORS, Poolesville, MD. The protocol was approved by the Gerontology Research Center Animal Care and Use Committee, and NIH was accredited by the Association for Accreditation of Laboratory Animal Care International.

Experimental measures

Total triiodothyronine (T_3), total and free thyroxin (T_4) and thyroid stimulating hormone (TSH) were assayed from a plasma sample obtained from animals on the longitudinal study of CR in males and females at 11 and 6 years on CR, respectively. To examine the changes during the initial adaptation to CR, plasma samples were obtained and assayed at each intake level (*ad libitum*, 10%, 20%, and 30%) as well as 6 months at 30% and one year at 30%. All samples were collected *via* venipuncture of the femoral vein between the hours of 7 and 10 a.m. in anesthetized animals (Telazol 3 – 4 mg/kg IM) following an overnight fast. Whole blood was centrifuged and the resultant plasma stored at – 80 °C. Frozen samples were sent to Yerkes Regional Primate Research Center for analysis.

Statistical analysis

Simple linear regression was performed to assess age effects in control monkeys. Individual 2 × 2 (age × diet) ANOVA tests were utilized to determine sex and CR effects in each hormonal parameter.

Results

Effects of aging on thyroid hormones

Since CR has been shown to retard age-related changes in many endocrine parameters, it was first important to characterize the effects of aging on total T_3, total T_4, and TSH in plasma from the control monkeys. Fig. **1** shows that females generally have higher total T_3 levels than males ($p < 0.004$); however, there is no significant effect of age on either sex separately or when combined. In contrast, total T_4 levels decline significantly by approximately 35% over the adult life span ($p < 0.04$). A similar effect occurs for free T_4 (data not shown). Sexes were combined since values did not differ significantly between them. Plasma TSH also decreased significantly ($p < 0.01$) by more than 50% with age. Again, values of males and females did not differ significantly.

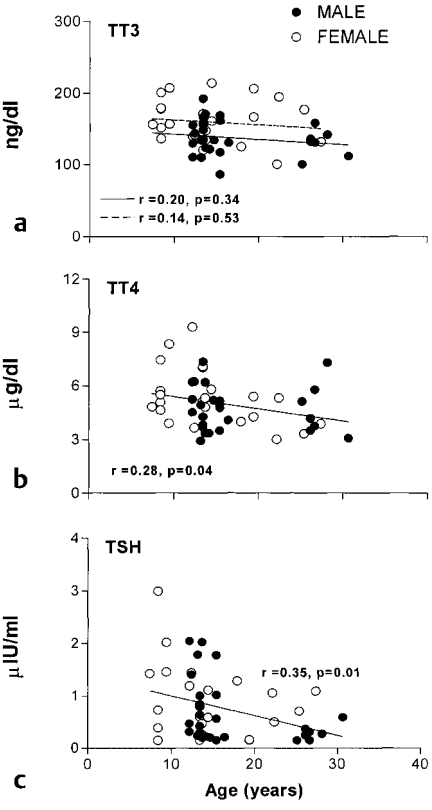

Fig. **1** Effects of aging on thyroid hormones. Total T_3 (TT_3), total T_4 (TT_4), and TSH levels were determined in plasma of control (approximately *ad libitum* fed) monkeys of various ages as described under **Materials and Methods**. TT_3 values for males and females are plotted on separate regression lines (males – solid lines; females – dashed lines), due to sex differences.

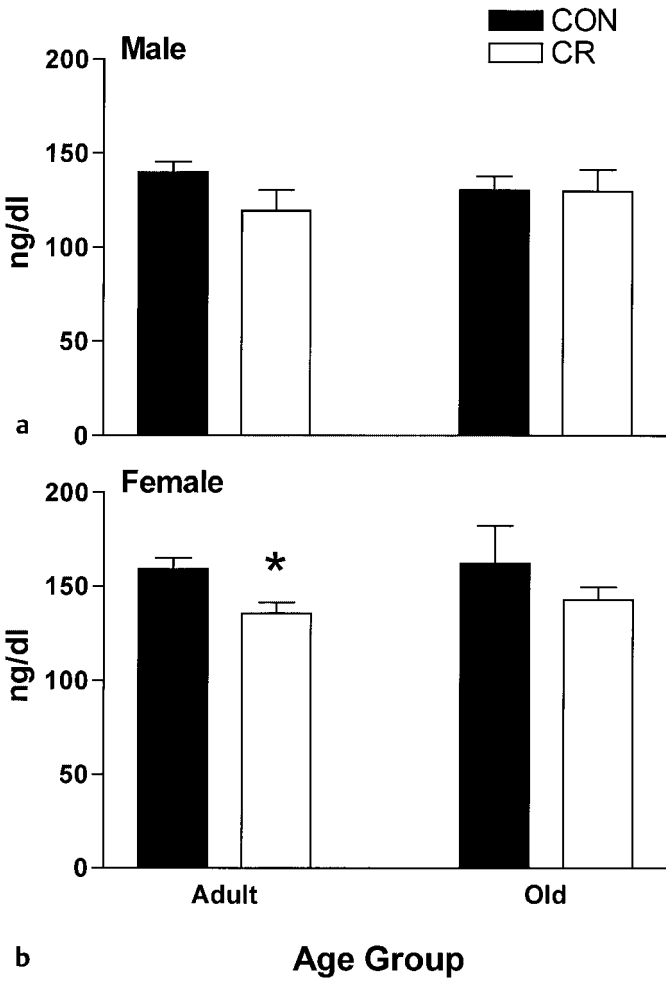

Fig. **2** Effects of CR on total T_3. Total T_3 levels were determined for control (CON) and CR monkeys as described under **Materials and Methods**. Values represent the mean ± standard error. *Adult female controls differ from adult female CR ($p < 0.007$).

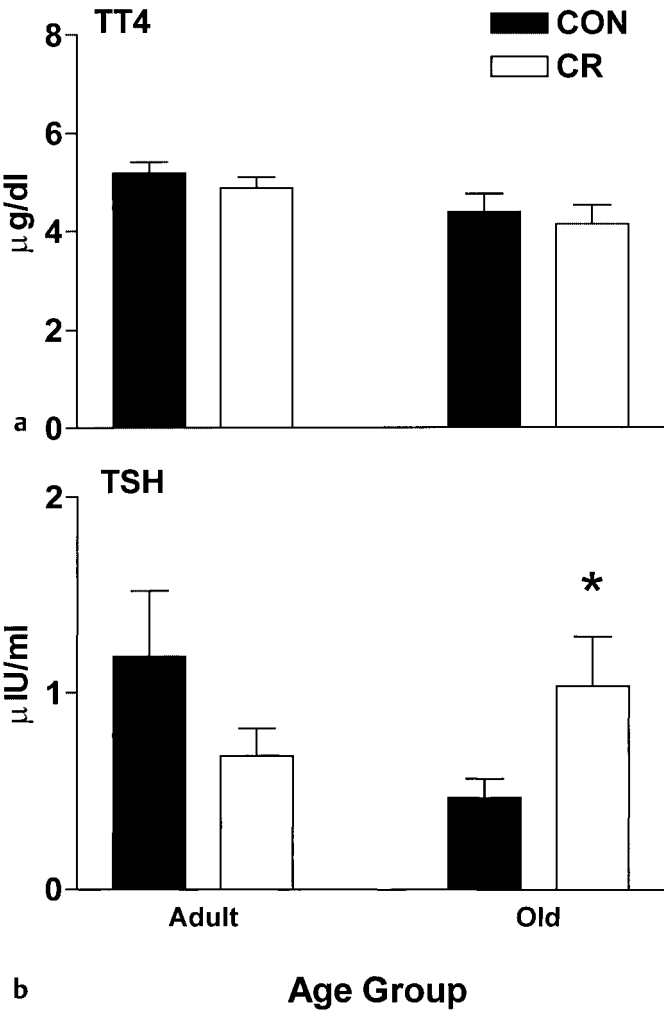

Fig. **3** Effects of CR on total T_4. (TT_4) and TSH. Total T_4 and TSH levels were determined for control (CON) and CR monkeys as described under **Materials and Methods**. Males and females were combined since there were no gender differences. Values represent the mean ± standard error. *Old female CR differs from old controls ($p < 0.02$).

Effects of CR on thyroid hormones

Since most studies on the effects of CR on the thyroid axis such as [14] have observed reductions in the levels of circulating T_3, this hormone was the first to be examined comprehensively. Fig. **2** compares the effects of age and diet on total T_3 concentrations in plasma of male and female rhesus monkeys. In this case, the animals were divided into two operational age groups as defined under **Materials and Methods** for simpler analysis of diet effects. Consistent with data in Fig. **1**, there is no significant effect of age on T_3, but monkeys on CR had lower T_3 levels than controls ($p < 0.04$, combined sex data not shown). Results are similar when each sex is analyzed independently, although males had been in the study for 11 years and females only 6 years at the time of sampling in 1998. It should be noted that when divided into separate age/sex groups with smaller numbers of monkeys, only the CR effect in adult females remained statistically significant ($p < 0.007$, although a trend toward lower levels remained in adult, but not old males). Some preliminary attempts to obtain T_3 values had been undertaken as part of another study in 1988, 6 to 18 months after respective cohorts of male monkeys entered the colony. At that time, there appeared to be a trend toward lower hormone levels in the CR groups, but this was not yet significant (unpublished data).

Since one other study in rhesus monkeys reported that CR lowered total T_4 levels [15], this hormone was examined next. Fig. **3** (upper panel) shows a tendency for an age-related decrease as illustrated above in Fig. **1**, but there was no significant effect of CR (and no effect on free T_4, data not shown). Thus, since CR reduces plasma levels of T_3 but not T_4 in the monkeys under study the effect of CR on the thyroid axis would appear to be at the level of conversion rather than secretion, as has been previously suggested for rodents [14]. Nevertheless, it was important to assess concentrations of TSH to determine whether or not stimulation of T_4 release from the pituitary could potentially be affected by diet. Also, since younger female monkeys (and possibly also males, although the trend was not statistically significant) appear to reduce their levels of T_3 more than their older counterparts do during CR (Fig. **2**) despite no significant effects on T_4, the possibility remained that differences in time course or hormone conversion kinetics might somehow compensate for age differences in TSH stimulation of T_4 secretion. Fig. **3** (lower panel) illustrates that there is a trend towards lower TSH levels in older monkeys ($p = 0.2$) as observed in Fig. **1**, and that CR tends to lower TSH in younger monkeys, but actually elevates or maintains more youthful levels of this hormone in older animals (age × diet effect, $p < 0.02$).

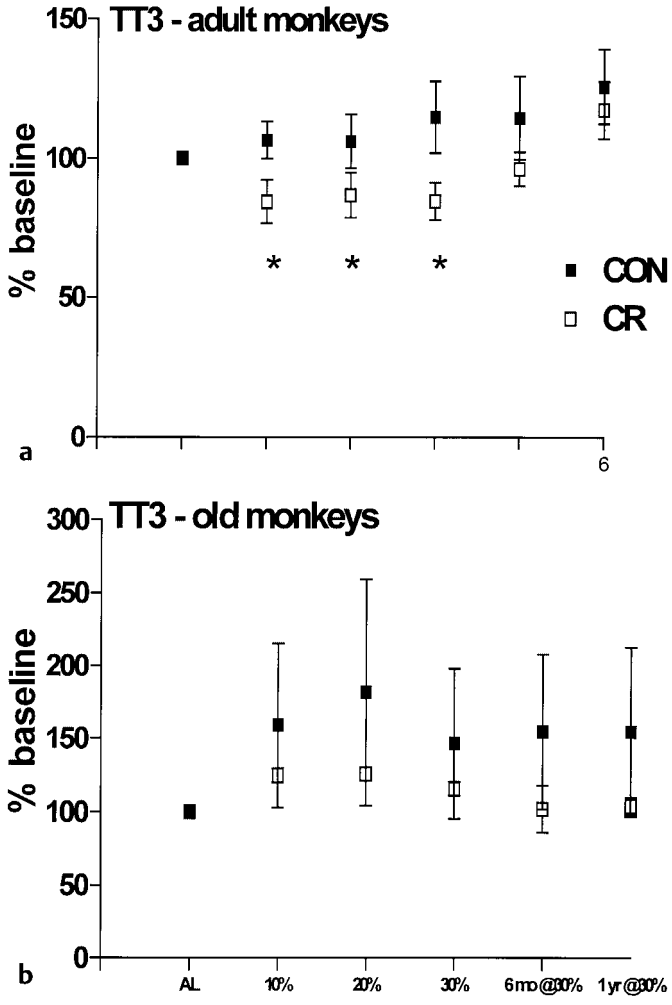

Fig. **4** Effects of shorter term CR on total T_3. (TT_3) in young adult and old monkeys. Total T_3 levels were determined in young adult and old monkeys, subjected to shorter-term CR (and control conditions) as described under **Materials and Methods**. Values were adjusted to 100% at the baseline (*ad libitum*) time point. The percentage change from this level was then calculated and averaged across diet groups at each intake level. Each point represents the mean ± standard error. *Controls (CON) differ from CR at the indicated time points.

Effect of shorter term CR on thyroid hormones

To determine the time-course of CR effects on the thyroid hormone axis, additional cohorts of young and old male monkeys were subjected to gradually phased-in restriction (10% per month for a 3-month period) and sampled up to one year later. Fig. **4** (upper panel) shows that CR lowers plasma T_3 concentrations of young animals within one month and levels remain generally reduced for 6 to 12 months ($p < 0.005$). A similar, but less pronounced effect is apparent in older monkeys (Fig. **4**, lower panel), but this trend was not statistically significant. Part of the problem may be the fact that the old animals selected for CR actually had slightly, but not significantly lower values than age-matched controls at the start of the experiment. Interestingly, significant time effects on T_3 levels were observed for both age groups regardless of diet ($p < 0.03$ and 0.009 for young and old monkeys, respectively), and this phenomenon (increase with time for control, but not CR animals) may contribute to the apparent CR effect for the older monkeys. In agreement with the longer-term CR studies depicted in Fig. **3** (upper panel), no significant short-term effects of CR were observed for total T_4 or TSH in either young adult or old monkeys (data not shown).

Discussion

Results presented here show for the first time that relatively lean rhesus monkeys subjected to defined intake CR exhibit lower plasma levels of T_3. These effects can be observed within one month of initiating CR, even when phased in gradually. There is some evidence (Fig. **4**) that this effect on T_3 may be transient in young males as has been reported for metabolic rate. However, additional studies are needed. In contrast to our T_3 data, no significant effects of CR on T_4 can be detected. However, unlike the case for T_3, T_4 levels appear to decrease progressively over the adult life span. Interestingly, T_4 concentrations were not gender-dependent, while females had higher T_3 levels than males.

Gender differences were not observed for TSH, but plasma concentrations declined with increasing age as observed for T_4. Surprisingly, long-term CR resulted in slightly elevated TSH levels in older monkeys, but had no apparent effect in young adults. This effect might reflect a CR-induced disruption of the thyroid hormone axis and be related to the fact that this intervention elicits a greater reduction in plasma T_3 of younger monkeys than older ones, at least in females. It is well known that the beneficial biological effects of CR are greatest when initiated early in life [1]. If regulation of the thyroid hormone axis plays an early and important role in the mechanism(s) of such CR effects, the present findings offer one possible explanation for the age-dependent response. Unfortunately, it was not possible to detect an effect of short-term CR on TSH in either age group examined. This suggests that CR effects on TSH are probably less important than is the conversion of T_4 to T_3 as has been postulated for rodents on CR [14]. Both the rodent experiments and the present primate results are inconsistent with a previous study in rhesus monkeys, which reported a CR-induced reduction in plasma T_4, but not T_3 [15]. As suggested above, such possible discrepancies may be due to differences in dietary regimens or body composition.

Several additional points should be considered in interpreting the present data. First, in keeping with the current results, it is known that reduced calorie intake results in decreased levels of T_3 and a reciprocal increase in reverse T_3 [18]. Although not measured in the present study, it would be expected that reverse T_3 would increase in CR monkeys. Second, since TSH and T_3 levels exhibit oscillatory patterns, an effect of CR on either secretion or clearance might conceivably shift the phasing of hormonal secretion, resulting in the present findings. A third consideration is that anesthesia may have influenced our findings. However, this latter concern is not likely since all animals received the same weight-adjusted dose of anesthesia, such that any effects would be comparable among the various age and treatment groups.

In any case, several points in addition to the CR effects reported here may be important. First, any attempts to mimic the beneficial effects of CR without reducing food intake, as has been recently proposed as a more practical strategy for humans [4], will require an understanding of the biological mechanism(s) by which restriction works. If thyroid hormones are involved in this process, the present findings could lead to a possible therapeutic

strategy involving their modulation by other means. Indeed, it has already been shown that humans on a reduced caloric intake in the Biosphere II study exhibit similar effects on the thyroid axis as seen here [19]. Second, the age-related reductions observed in plasma T_4 and TSH of rhesus monkeys may provide useful biomarkers of aging. Although these decreases do not appear to be slowed by CR as has been reported for many other age-associated biological changes [1], the possibly more fundamental reduction in T_3 may take precedence. Consequently, it will be valuable to determine whether or not any other candidate 'anti-aging' interventions have any effects on such changes, and whether they can be observed longitudinally in addition to the cross-sectional analyses as performed here. Finally, the possible dissociation of TSH levels from those of T_3 and T_4 by CR in older monkeys may provide some insight into mechanisms by which TSH regulates secretion of T_4 and how the thyroid hormone axis might become disrupted during aging.

Taken together, the present results provide mechanistic information about the aging process, CR, and the thyroid axis, as well as the basis for development of interventions into both aging and endocrine disorders. It is interesting to note that both metabolic rate and body temperature [8] are reported to decrease in rhesus monkeys on CR, possibly as a consequence of an altered thyroid hormone profile. Future studies may now focus on more precise characterization of these CR effects, the possibility of employing thyroid indices as candidate biomarkers of aging, and implementation of the therapeutic strategies suggested above.

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References

[1] Yu BP. Modulation of aging processes by dietary restriction. Boca Raton: CRC Press, 1994
[2] Lane MA, Ingram DK, Roth GS. Beyond the rodent model: Caloric restriction in rhesus monkeys. Age 1997; 20: 45 – 56
[3] Roth GS, Ingram DK, Lane MA. Caloric restriction in primates: Will it work and when will we know? J Am Geriatrics Soc 1999; 46: 896 – 903
[4] Roth GS, Ingram DK, Lane MA. Beneficial effects of caloric restriction and caloric restriction mimetics: Implications for humans. Ann NY Acad Sci 2001; 928: 305 – 315
[5] Yu BP. Free radicals in aging. Boca Raton: CRC Press, 1993
[6] Masoro EJ, Katz MS, McMahan CA. Evidence for the glycation theory of aging from the food-restricted model. J Gerontol Biol Sci 1989; 44: B20 – B29
[7] Sabbatino F, Masoro EJ, McMahan CA, Kuhn RW. Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. J Gerontol Biol Sci 1991; 46: B171 – B179
[8] Lane MA, Baer DJ, Rumpler WV, Weindruch R, Ingram DK, Tilmont EM, Cutler RG, Roth GS. Calorie restriction lowers body temperature in rhesus monkeys consistent with a postulated anti-aging mechanism in rodents. Proc Natl Acad Sci USA 1997; 93: 4159 – 4164
[9] Roth GS, Ingram DK, Lane MA. Slowing aging by caloric restriction. Nature Med 1996; 1: 414 – 415
[10] McCarter RJM, McGee JR. Transient reduction of metabolic rate by food restriction. Am J Physiol 1989; 257: E175 – E179
[11] Norman AW, Litwack G. Hormones. Orlando: Academic Press Inc, 1987: 221 – 262
[12] Sherwin RS. Influence of diet and carbohydrate on thyroid hormone metabolism. In: Blackburn GL, Bray GA (eds). Management of obesity by severe caloric restriction. Littleton: PSG Publishing, 1985: 155 – 163
[13] Blum JW, Schnyder W, Kunz PL, Blom AK, Bickel H, Schurch A. Reduced and compensatory growth: Endocrine and metabolic changes during food restriction and refeeding in steers. J Nutrition 1985; 115: 417 – 421
[14] Merry BJ, Holehan AM. The endocrine response to dietary restriction in the rat. In: Woodhead AD, Blackett AD, Holleander A (eds). Molecular biology of aging. New York: Plenum Press, 1985: 117 – 141
[15] DeLany JP, Hansen BC, Bodkin ML, Hannah J, Bray GA. Long-term calorie restriction reduces energy expenditure in aging monkeys. J Gerontol Biol Sci 1999; 54: B5 – B11
[16] Ingram DK, Cutler RG, Weindruch R, Renquist DM, Knapka JM, April M, Belcher CT, Clark MA, Hatcherson CD, Marriott BM, Roth GS. Dietary restriction and aging: The initiation of a primate study. J Gerontol Biol Sci 1990; 45: B148 – B163
[17] Lane MA, Ingram DK, Cutler RG, Knapka JM, Barnard DE, Roth GS. Dietary restriction in non-human primates: Progress report on the NIA study. Ann NY Acad Sci 1992; 673: 36 – 45
[18] Danforth E, Burger AG. The impact of nutrition on thyroid hormone physiology and action. Annu Rev Nutr 1989; 9: 201 – 227
[19] Walford RL, Mock D, Verdery R, MacCallum T. Calorie-restriction in Biosphere 2: Alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a two-year period. J Gerontol Biol Sci 2002; 57: in press