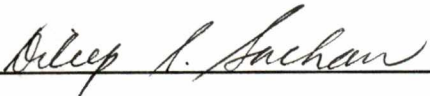


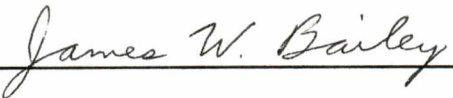
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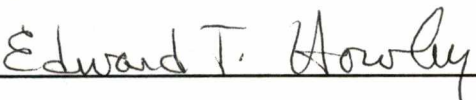
I am submitting herewith a dissertation written by Vivian Haley-Zitlin entitled "Effects of Food Restriction, Exercise, and Aging on the Pituitary-Thyroid Axis in the Rat." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Human Ecology.


Roy E. Beauchene, Major Professor

We have read this dissertation
and recommended its acceptance:







Accepted for the Council:



Associate Vice Chancellor
and Dean of the Graduate School

**EFFECTS OF FOOD RESTRICTION, EXERCISE,
AND AGING ON THE PITUITARY-THYROID AXIS
IN THE RAT**

**A
Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee**

Vivian Haley-Zitlin

May, 1991

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DEDICATION

This dissertation is dedicated, in loving memory, to my grandmother, Frances Camp -- who instilled in me the value of education, a love of learning, and the importance of believing in myself. It is her influence that inspired me to attain this degree.

And to my husband, Mark, and my daughter, Shannon, whose confidence, patience, encouragement, and love have sustained me during this endeavor.

ACKNOWLEDGEMENTS

There are a number of people to whom I would like to express my appreciation and gratitude for their contribution to this project and to my graduate studies. I especially wish to thank:

Foremost, Dr. Roy Beauchene, my committee chairman, who introduced me to the field of nutrition and aging, and was both mentor and friend. His tutelage, patience, encouragement, and expertise served to inspire as well as guide.

Each member of my committee: Dr. Dileep Sachan, Dr. James Bailey, and Dr. Edward Howley, for their guidance and support, and for their willingness to share freely their ideas and knowledge.

The members of our research team: Parvin Darabian, Madeline Dellwo, and David Wright, for their assistance, friendship, support, and humor throughout this study.

Dr. William Sanders for sharing his statistical expertise which was invaluable to this project. His time and attention to detail was greatly appreciated. Also, John Schneider and Alice Beauchene for their assistance with the statistical analyses and graphics generation.

Judith Shaw and Randall Mynatt for their capable technical assistance and friendship.

Dr. Fran Andrews, Dr. Betsy Haughton, Dr. Carol Costello, and Dr. Jean Skinner for their guidance, encouragement, and friendship.

The Agricultural Experiment Station, the College of Human Ecology, and the Department of Nutrition for their financial support of my graduate research and assistantships.

My parents, Mr. and Mrs. G. H. Haley, for their love and encouragement; and my grandfather, Dr. Ephriam Camp, who provided a professional role model for me to emulate.

ABSTRACT

The impact of long term food restriction and exercise on the pituitary-thyroid axis and growth was examined in aging male Wistar rats. Four groups were evaluated: ad libitum-fed, sedentary controls (A); ad libitum-fed, exercised (AE); restricted-fed, sedentary (R); and restricted-fed, exercised (RE). Restriction and exercise regimens were implemented at weaning and continued for the life of the animals. Pituitary-thyroid axis functioning was assessed by serum concentration measurements of thyroid stimulating hormone (TSH), thyroxine (T_4), and triiodothyronine (T_3) at 12, 16, 20, 24, and 28 months of age. Renal cortical NaK ATPase activity was evaluated at concurrent intervals.

Aging resulted in a decline in circulatory levels of T_4 and T_3 , and in the T_4 to T_3 ratio. TSH values increased with age; this increase was delayed by restriction. Restriction also slowed the age-associated decrease in serum T_4 and T_3 levels and lowered absolute T_3 and TSH levels. Exercise resulted in lower absolute serum T_3 concentration, but had little effect on serum T_4 levels. Exercise did not slow the age-associated decline in T_4 or T_3 serum concentration in either ad libitum-fed or restricted-fed groups. Renal cortex NaK ATPase activity decreased with age. This effect seems, in part, attributable to thyroid hormone status.

T_3 serum concentration was positively correlated with body weight; T_4 was not. Food restriction significantly lowered the predicted mature body weight and terminal body weight. Exercise had no effect upon terminal body weight in AE or RE but

increased predicted mature body weight in RE. Food restriction decreased the relative rate of maturation; exercise further decreased the relative rate of maturation in restricted animals.

The results of this study indicate that the pituitary remains capable of mounting an appropriate response with age and suggests that the thyroid gland itself is central to the pituitary-thyroid axis decline with aging. The pituitary-thyroid axis is responsive to alterations in food intake and energy expenditure. Its age-associated decline may be slowed by food restriction.

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CHAPTER I

INTRODUCTION

Interest in the aging process has mushroomed in the last decade as the proportion of older persons in the population has increased. In response to this burgeoning interest, investigators increasingly have begun to study the fundamental causes of aging and to discover if the physiological decrements associated with aging can be circumvented, or at least, delayed. As current researchers build upon the foundation laid in the '30's and '40's, it has become apparent that no definitive answers are in the immediate offing. What has emerged is an appreciation of the complexity of the processes called "aging", as well as a number of proposed theories to account for this phenomenon (1,2,3,4,5,6).

The neuroendocrine theory of aging suggests that the neuroendocrine system may be integral to the aging process (7,8,9). This system is essential not only for homeostasis and maintenance of life (10), but is also responsive to environmental stimuli, such as diet, exercise, or stress (9,11). A recent review by Everitt and Meites (12) suggested that hormones, a product of the neuroendocrine system, may promote aging as well as have anti-aging effects. As evidence that hormones promote aging, they contend that manipulations such as food restriction or hypophysectomy that result in decreased hormone secretion (13) "delay aging of many body tissues... and prolong life" (p. B139) (12). Hormones may, however, delay age-associated

processes; e.g., the age-associated decline in hormone secretion that is thought to contribute to decreased physiological effects with aging may be reversed or inhibited by hormone administration (8,9). Thus, the contrasting actions of hormones have highlighted the importance of a more extensive examination of the effect of the neuroendocrine system on aging.

As a result of the linking of the neuroendocrine system and the aging process, several components of this system have come under scrutiny. One such constituent is the pituitary-thyroid axis. This axis, an important component of the neuroendocrine system, is largely responsible for regulation of tissue metabolism, in general. Like the neuroendocrine system, this axis has been implicated as a factor in the aging process -- acting independently or in tandem with a variety of other suggested causative factors (2,14,15,16).

Age-associated decrements have been noted in the pituitary-thyroid axis. However, there has been disagreement over the pattern of thyroid hormone concentrations, for instance, that have been observed with aging. Also in question is the ability of environmental factors such as food intake or exercise to influence these age-associated changes. The pituitary-thyroid axis regulates energy metabolism, and so is responsive to variations in energy intake and expenditure. It has been shown that severely restrictive energy-intake regimens result in depressed thyroid hormone concentration (17). Food restriction is also the only proven method of extending maximum life span (18,19). Therefore, it has been suggested that the depression of the pituitary-thyroid effects mediated by food restriction decreases "wear and tear"

on the organs and cells of the body (12), thus increasing life span. Also, a central role for the pituitary-thyroid axis as an "aging factor" has also been proposed (20). That is, hormones, through stimulation of their target organs, may accelerate the aging process. The effect of exercise on the pituitary-thyroid axis and longevity is much less conclusive as the research examining these relationships is limited and the results are often equivocal. Thus, it is the purpose of this investigation to examine the effects of age, diet, and exercise on the pituitary-thyroid axis and to determine the effects of dietary restriction and/or exercise on age-associated changes in the pituitary-thyroid axis.

Hypotheses

- 1) Pituitary-thyroid axis function, as indicated by decrements in TSH, T_4 , and T_3 serum concentrations and in NaK ATPase activity, will decrease with age.
- 2) Long-term food restriction will result in decreased absolute levels of serum thyroid hormones: the age-associated decrements in the pituitary-thyroid axis will be retarded with restriction.
- 3) A long-term exercise regimen will result in decreased absolute levels of serum thyroid hormones: the age-associated decrements in the pituitary-thyroid axis will be retarded with exercise.
- 4) Food restriction and exercise will act synergistically to delay the age-associated changes in the pituitary-thyroid axis.

CHAPTER II

REVIEW OF THE LITERATURE

Pituitary-Thyroid Axis: Function and Regulation

Thyroid hormones are widely recognized as regulators of metabolism. As such, they are intimately involved with growth and development, particularly as they mediate energy, carbohydrate, lipid, and protein metabolism. Both energy generating and energy wasting control mechanisms are regulated by thyroid hormones. Decreased thyroid hormone levels can cause the basal metabolic rate (BMR) to fall 40% below normal, while increased levels can increase the BMR by 100% (21).

Virtually every organ system is affected by thyroid hormone actions. Thus, derangement of the pituitary-thyroid axis may result in a wide array of pathophysiological manifestations. This may account for the "tight control" of thyroid hormone concentrations by a negative feedback loop, a prolonged half-life, and thyroid hormone reservoirs that serve to protect serum concentrations.

The feedback mechanism (Figure 1) that maintains the delicate balance between the components of the hypothalamic-pituitary-thyroid axis regulates the blood levels of the thyroid hormones. The hypothalamus produces and secretes thyroid-releasing hormone (TRH) which is transported to the anterior pituitary via the hypothalamic-hypophyseal portal blood. TRH has a direct effect on the cells of

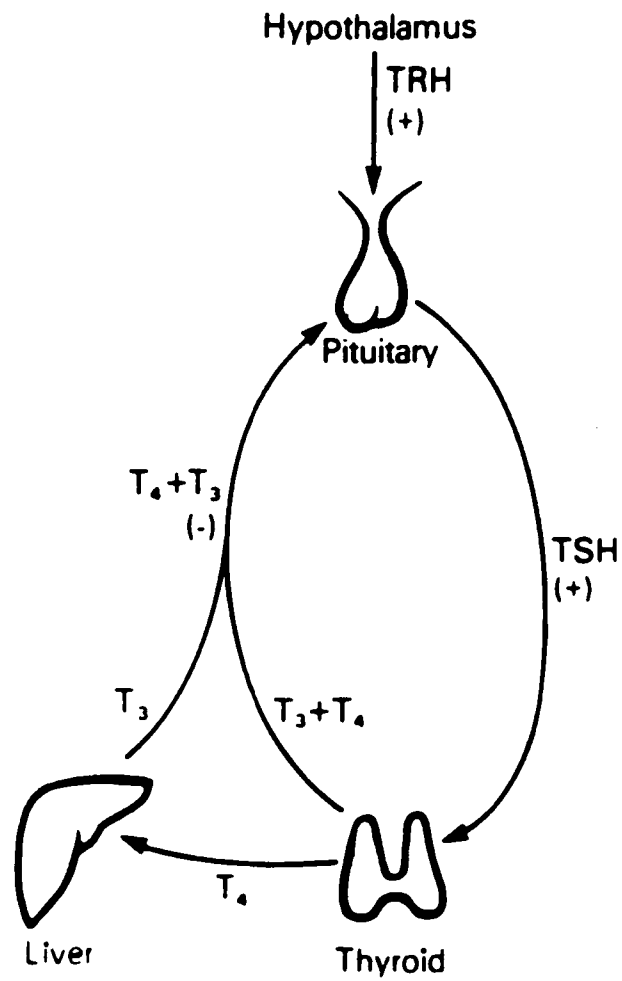


Figure 1. Feedback regulation of thyroid hormone secretion. (+) = stimulation. (-) = inhibition. Adapted from Goodman (22).

the anterior pituitary resulting in secretion of thyroid stimulating hormone (TSH). TRH also increases the synthesis of TSH by a mechanism independent of that mediating TSH secretion. In the event the pituitary is separated from the median eminence of the hypothalamus, the pituitary maintains the ability to secrete TSH at a greatly reduced rate through an as yet unexplained mechanism (23,24).

TSH mediates thyroid hormone synthesis, secretion, and thyroid gland growth. In turn, TSH production and secretion is primarily regulated via feedback inhibition by the thyroid hormones, and by TRH stimulation. TSH secretion may also be a function of the effects of epinephrine, adrenal corticotropin (ACTH), and cortisol, as well as varied physiological states (25,26,27).

The target organ of TSH is the thyroid gland. The thyroid hormones, L-thyroxine (T_4) and L-triiodothyronine (T_3), are produced by the thyroid gland, a bilobular organ located immediately below the larynx and anterior to the trachea, in close proximity to the thyroid cartilage from which the gland derives its name. The thyroid hormones are produced in the follicle of the thyroid gland by the joining of thyroglobulin, tyrosine and iodide. A two to three month supply of thyroid hormones is stored within the colloid of the follicle and is released (upon stimulation by TSH) by a pinocytotic process that involves cleavage of the thyroglobulin with subsequent release of T_3 and T_4 . Thyroid hormones are thought to be released in a T_4 to T_3 ratio of approximately 100:20 (23).

In the blood stream, both T_4 and T_3 are found in bound and free forms, with the large majority of the hormones being bound to albumin, thyroxine binding

globulin (TBG) or thyroxine binding prealbumin (TBPA). The ratio of bound to free hormone has largely been found to be constant, even under a number of physiological conditions (i.e., illness) (28).

The thyroid hormones bound to the plasma proteins serve as a reservoir of extrathyroidal hormone, as three times as much hormone is bound as is secreted and degraded per day. This protects serum hormone concentrations. Additionally, a prolonged half life indicates that thyroid hormone levels are relatively stable, and also indicates that a single blood sample can provide useful clinical information. The above also help insure that serum thyroid hormone levels are maintained within a narrow range (22).

Both T_4 and T_3 are taken up by cells where they modulate a number of biological processes. T_4 to T_3 conversion occurs within a number of extrathyroidal tissues (liver and kidney, especially). This process is accomplished by the deiodination of T_4 by 5'deiodinase. T_4 deiodination may produce either an active (T_3) or an inactive reverse T_3 (rT_3) hormone. Factors responsible for this phenomenon are the subject of several investigations (29,30). Of the T_3 produced per day, approximately 85% is the result of deiodination. This process consumes about 35% of the daily T_4 secretion (22).

T_3 is often labeled the most "potent" of the two hormones as it has been shown to bind with nuclear receptors (28,31). Thyroid hormones are thought to exert their effects primarily at the transcription level (32,33). More than one hundred

enzymes have been shown to be thyroid-hormone dependent, many of which have been shown to be capable of modifying metabolism (34).

Effect of Aging on the Pituitary-Thyroid Axis

There is evidence to support a decline in the pituitary-thyroid axis with aging. However, controversy surrounds the impact of aging on this axis as a variety of patterns of serum thyroid concentrations have been noted with aging (35,36,37,38). Therefore, no consensus has yet been reached on the ability of the body to maintain the axis integrity with aging. To properly address the question, the functioning of vital components of the axis must be studied. These include the thyroid gland itself, thyroid hormone levels, and peripheral effects of thyroid hormone action on target organs.

A number of changes occur in the thyroid gland with time. Andrew and Andrew (39) reported that histological involution of the thyroid gland may occur with aging, as well as fibrosis, atrophy, and degenerative changes. Lansing and Wolfe (40) observed increased thyroidal collagen deposition and size, as well as a generalized decrease in thyroid follicle epithelium height from that of low columnar or cuboidal noted in young rats; an increase in follicle size; and a change in follicular colloid staining reaction indicating decreased activity with age. Frolkis, et al. (16) noted follicular atrophy with a reduction in thyroid weight to body weight ratio, plus connective tissue proliferation and decreased rate of iodine uptake with aging. Data

such as these have led to the suggestion that age-associated decreased thyroidal activity may be related to a reduction in metabolically active thyroid tissue (41,42).

Changes in basal serum TSH, T_4 , and T_3 concentrations with age remain an area of research focus. A number of investigators maintain that TSH levels remain stable with aging (35,36,37). This idea was supported by the work of Huang, et al. (43) who reported no difference in serum TSH levels between young (6 - 8 month) and old (20 - 24 month) Long-Evans rats under basal conditions. In addition, old rats' TSH levels mirrored those of young rats upon exposure to cold stress, following thyroidectomy, and with T_3 or T_4 administration. These data suggested that with aging, the pituitary retains the capacity to respond to feedback inhibition by T_3 or T_4 and to respond to hypothalamic TRH stimulation. No change in the measured basal serum TSH concentrations with time was also reported by Klug and Adelman (35) in 12 or 24 month old male Sprague-Dawley rats; by Donda, et al. (37) in 3 - 5 month (young) and 22 - 24 month (old) male Wistar rats or by Snyder, et al. (44) in male conventional rats. Further, no change in the basal TSH serum concentration has been recorded in dogs (45) or humans (46) with aging.

Agreement that the TSH concentration remains unchanged with aging however, is not universal. Pekary, et al. (47) noted an increase in basal TSH concentration with aging in female Long-Evans rats, while aged male rats of the same strain exhibited a marked decline when compared to controls. Chen, (48), using the same strain, likewise found that the basal TSH concentrations of young male rats were significantly higher than those of old male rats ($p < .05$). However, in contrast

to Pekary (47), no difference in TSH concentrations was noted between young and old female Long-Evans rats (48). It should also be noted that recent research by Pekary (49) recorded no significant difference between basal serum TSH values for young versus old Long-Evans rats. However, a marked difference in serum TSH concentration values were found in the two Pekary (47,49) experiments, even though similar aged strains of rat and the same radioimmunoassay methods were used for both.

With aging, an appropriate TSH secretion response as a result of TRH administration suggests that the aging thyrotroph remains appropriately responsive to TRH. This has been directly tested by measurement of the pituitary's ability to mount an appropriate TSH response to an exogenous TRH dose. Klug and Adelman (35) demonstrated a slightly greater TSH secretion response in 12 month versus 24 month old Sprague-Dawley rats; however, the results were not statistically significant. Pekary, et al. (45) noted no difference in TSH response to TRH-mediated stimulation between young and old rats (47). Chen and Walfish (36) reported no difference in TRH-mediated TSH secretion levels in intact young and old female rats. A later report by Chen (46) noted a significantly higher TSH response to TRH stimulation in young rats when compared to old male or female rats. Chen (48) suggested that the discrepant findings in these two studies were related to age--very old rats (28 - 36 months) were utilized in the later study. Chen proposed that the large incidence of pituitary adenoma found in the older rats was responsible for the differing pituitary responsiveness. In dogs, Gonzales, et al. (45) determined that the TSH response to

TRH stimulation was depressed with age. A similarly dampened pituitary TSH response to an injection of TRH was noted in "old" elderly when compared to "young" elderly men and women (46). Moreover, women had a consistently greater response to TRH stimulation (i.e., higher TSH values) than did men.

It has been suggested that age-associated decrements in the pituitary-thyroid axis begin at an early age (50,51). Azizi (51) proposed that by 5 months of age (in rats) a dual defect exists in the pituitary-thyroid axis: 1) a defect in endogenous thyroid function as indicated by decreased T_3 and T_4 levels following TSH administration, plus 2) a decreased secretory response of the pituitary to stimuli. Azizi found that although basal TSH levels increased significantly during the months 1 through 5, thyroidectomy resulted in a depressed TSH increase in the 5 month old rats.

Thyroid hormone decrements with age have been reported to exhibit varying patterns. A number of investigators have reported a decline in both basal T_3 and T_4 concentration levels with aging (35,37,43). Each of these investigators found this reduction was accompanied by stable TSH values. In contrast, other research efforts have yielded depressed basal T_4 concentrations with normal T_3 values with advancing age (16,36,47,52). Although TSH values again were reported not to change significantly with age in some studies (36,49), that was not always true (47).

Snyder, et al. (44) reported T_4 levels decreased with age in both gnotobiotic (single microbe contaminations) and conventional Lobund-Wistar rats. However, gnotobiotic adult rats (7 - 13 month old) had significantly higher T_4 and T_3 levels than

their conventional counterparts. TSH significantly decreased between middle (16 - 20 months) and old (24 - 28 months) age in gnotobiotic rats.

Gonzales and Quadri (45) observed no significant difference in serum T_4 concentrations between prepubertal and adult dogs, adult and middle-aged dogs, or middle aged and old dogs. However, old dogs' serum T_4 levels were significantly lower than those of adult dogs. This confirmed their earlier finding that T_4 levels gradually decrease with aging. Thyroidal secretion of T_4 in response to TSH administered intravenously was slower in initiation and lower in maximum response in old and middle aged animals when compared to prepubertal and adult dogs. A definitive explanation for the discrepant thyroid hormone patterns with aging and the mechanisms controlling them has not been forthcoming. Huang, et al. (43) asserted that the problem with the pituitary-thyroid axis with aging lies in the thyroid gland itself, as the pituitary in old and young animals responded to thyroidectomy and thyroid hormone replacement in like manner. Magarity, et al. (52) substantiated a suboptimal thyroid state with aging. This state was felt to be a result of an overall alteration in thyroid hormone production and metabolism as indicated by decreased T_4 production with age, accompanied by changes in peripheral tissue binding and processing of thyroid hormone (52). It has also been proposed that depressed serum T_4 levels along with lower free T_4 values in aged rats indicate that a change in T_4 binding protein concentration was not responsible for the decline in total T_4 serum concentration (49). Rather, collective evaluation of the data underscored a marked

decrease in the ratio of T_4 to T_3 secreted from the thyroid with aging, suggesting that the mechanism responsible was increased intrathyroidal conversion of T_4 to T_3 (47).

Sodium potassium adenosine triphosphatase

Thyroid hormone status has been shown to be an important modulator of renal function. Thyroid hormone deficiency results in decreased renal plasma flow (RPF), decreased glomerular filtration rate (GFR), and impaired sodium reabsorption (53,54). Thyroidectomy in rats resulted in decreased renal sodium excretion: this condition was correctable with T_3 administration (55).

A number of renal enzymes are altered with thyroidal changes, also (53). Sodium potassium adenosine triphosphatase (NaK ATPase) -- the enzymatic equivalent of the Na-K pump -- has been shown to be modulated by thyroid hormone status in a number of tissues (56,57). This enzyme system functions to maintain transmembrane chemical and electrical gradients through active transport of Na^+ and K^+ (maintaining Na^+ and K^+ extra- and intra-cellular levels) and net transepithelial salt transport, e.g., in renal tubules (58). Although the topic of numerous investigative efforts since its discovery by Skou in 1957, the mechanism of action, definitive function, and relationship of NaK ATPase to thyroid hormone action remain unknown.

The NaK ATPase enzyme system is present in all cells. Renal NaK ATPase (as well as other tissue NaK ATPases) has repeatedly been shown to be dependent upon thyroid hormone status (59,60). NaK ATPase activity within the kidney is not

uniformly distributed. Renal cortical tissue has proven to be both a sensitive and effective indicator of thyroid hormone action (61,62). Lo, et al. (61) found that a single bolus injection of T_3 in thyroidectomized rats resulted in a 45% increase in NaK ATPase activity in the L-fraction of the renal cortex. Successive T_3 doses elicited further attenuation of the NaK ATPase renal cortical response, but did not significantly affect outer medulla or papilla NaK ATPase response or Mg ATPase activity. Labeled substrate usage and kinetic studies indicated that the thyroid hormone effects observed were the result of a quantitative accumulation of NaK ATPase units (61). Further investigation by Lo and Edelman (63) provided evidence that the increased NaK ATPase activity due to thyroid hormone was the result of increased enzyme (de novo) synthesis, rather than decreased degradation or unmasking of enzyme sites. This finding was supported by the investigations of Oppenheimer, et al. (64) who demonstrated the presence of high affinity, limited capacity nuclear T_3 binding sites in several target organs, as well as the finding that T_3 administration resulted in increased nuclear RNA synthesis followed by increased microsomal and mitochondrial protein (65). Lo and Lo (59) reported that T_3 administration to thyroidectomized rats enhanced de novo synthesis of both subunits of the NaK ATPase molecule as evidenced by methionine incorporation into the subunits.

More recent studies have served to further elucidate the NaK ATPase - thyroid hormone relationship. A dose-dependent increase in NaK ATPase as a result of thyroid hormone administration has been elicited in renal, hepatic, brain, and

skeletal muscle tissue in a number of species (56,66,67,68,69,70).

Katz and Lindheimer (55) observed that thyroidectomy resulted in significantly lowered renal NaK ATPase activity while the renal microsomal enzyme, Mg-ATPase, was not affected. These investigators suggested however that the increased renal NaK ATPase activity with thyroid stimulation was a secondary response to an increased Na^+ load resulting in increased GFR and RPF.

Basal NaK ATPase activity has been shown to decrease with age (62,71). Hypothyroidism induced in 6 week, 6 month, and 20-24 month old rats as evidenced by depressed T_3 and T_4 concentration levels resulted in a significant decrease in renal cortical NaK ATPase activity in all age groups (62). However, the differences in the renal cortical NaK ATPase activities of the hypothyroid animals among the age groups were not significant. T_3 administration resulted in a significant increase in NaK ATPase enzyme activity. A significant decline in NaK ATPase response to the exogenous T_3 was observed with age (62). Other studies suggest that the age-associated decline in NaK ATPase activity may be tissue-specific (72,73). Hennessey (72) determined that NaK ATPase activity of Fisher 344 tail artery and aorta tissue decreased with age, while that of heart did not. In humans, NaK ATPase activity in lymphocytes declined with age (73). Erythrocyte NaK ATPase, however, exhibited only a modest decrease with age in female subjects and no significant differences in males with age (74).

Factors Influencing the Aging Process

A number of parameters have been investigated for their potential to mediate the aging process. These research endeavors have ascertained that food restriction consistently results in extension of the maximum lifespan. To date, this is the only factor that has definitively been established as lifespan extending in vertebrates. However, the mechanism by which food restriction accomplishes this remains an enigma. Exercise -- due to its relationship with energy utilization and intake -- has recently come under scrutiny as another potential anti-aging factor. Although scant, there are data to support a role of exercise in the extension of lifespan. Experimental findings, however, remain inconsistent. In light of the energy--food restriction--exercise relationship, the influence of these experimental variables on the aging process will be evaluated.

Food restriction.

In 1935, McCay, et al. published a landmark work denoting the beneficial effects of food restriction upon lifespan (18). This study served to revolutionize the field of gerontology and has remained the hub to which experimentally induced dietary restriction variables are firmly anchored.

McCay's research (18) examined rat growth and lifespan as a function of the feeding of a nutritionally complete, but calorically restricted diet. His stairstep method of caloric restriction employed 1) at weaning, and 2) two weeks following

weaning, resulted in an increased maximum lifespan for male and female rats on either restriction schedule. Mean age of restricted males far exceeded that of ad libitum-fed (control) males, while the age of females remained constant. Growth, however, was retarded in all restricted animals. The growth-retarded rats did not attain the maximum body size of the rapidly maturing animals; still, growth capacity was retained in restricted animals after 911 days. Male and female rats differed in growth potential (even with restriction), and in caloric intake required for maintenance of growth. McCay (75) verified these results in a later study. From this follow-up study, McCay speculated that a time limit for growth retardation exists beyond which the body loses the capacity to resume growth, as some animals restricted for 1000 days, then fed ad libitum, did not resume growth (75).

Realizing the problems that might accompany experimental use of restricted animals such as McCay's (18,75) in which growth and development were stymied by a staircase method, Nolen (76) fed animals to "produce leaner, longer-lived animals, yet not significantly change most of their biological characteristics" (p. 1478). Nolen, like McCay, et al. initiated food restriction at weaning, and evaluated two levels of food restriction (60 and 80% of ad libitum consumption). His research goal was to evaluate changes in growth and body composition associated with maturation. To this end, he used 12 weeks of age as an end-point for dietary manipulations. Seven groups of Simonsen rats were evaluated: 1) an ad libitum group; 2 and 3) groups restricted to 80 and 60%, respectively, after weaning; 4 and 5) groups fed ad libitum up to 12 weeks, and then restricted to 80 and 60%, respectively; and, 6 and 7) groups

restricted to 80 and 60%, respectively, for 12 weeks, and then fed ad libitum. This selection of dietary regimes was chosen in the hope of attaining a maximum amount of lean body mass (without excessive fat), and to therefore increase lifespan (76).

Unlike others (77), Nolen found that 60% restriction significantly extended lifespan (in male, but not female rats) when compared to 80% restriction. However, both restriction schedules increased maximum lifespan over ad libitum-fed controls. Ad libitum feeding for three months, followed by 60% dietary restriction, resulted in the healthiest and longest lived male rats, while lifetime 60% restriction resulted in subnormal growth, and 80% produced slightly submaximal size. Growth curves of groups 4 and 5 paralleled that of groups 2 and 3. In contrast, rats restricted for three months, then fed ad libitum did not exhibit a growth-stunting response (18,75,78). Rather, final body weights of these restricted-refed animals were not significantly different from ad libitum controls. These results mirror those of other researchers (79).

Barrows and Roeder (80) reported that restriction of adult male Wistar rats to 50% ad libitum intake throughout adulthood resulted in a small, but significant decrease in mean lifespan when compared to ad libitum-fed controls. These findings agree with those of Ross (81) but disagree with those of McCay, et al. (78), who noted that 14 out of 16 groups of male rats restricted in the middle of the life span had a longer life span than ad libitum controls. McCay, et al. (78) however, introduced a number of variables not included in the Barrows study. These included exercise, alternate sources of protein, and varied degrees of restriction. Likewise,

when mice, golden hamsters, and rats were examined, it was reported that animals restricted in adulthood following ad libitum feeding survived longer than ad libitum fed controls (82).

Beauchene, et al. (83) also investigated the differential impact of initiation of food restriction. His groups consisted of ad libitum (A); restricted first year, followed by ad libitum (RA); ad libitum, first year, followed by restriction (AR); and restricted for entire lifespan (R). The percentage of restriction varied by group as a result of the restriction method used; animals were allowed to feed from 5 pm to 8 pm on alternate days. Animals food restricted for life lived the longest, followed by the alternating restricted/ad libitum groups (this was contrary to Nolen's findings (76)); however, the two studies differed on the age of restriction initiation -- 3 mo vs. 12 mo). There was no difference in average lifespan between AR and RA groups. Beauchene, et al. (83) suggested that this supported Goodrick, et al.'s (84) observation that the impact of food restriction on longevity decreases as the age at which restriction is initiated increases. Beauchene found that in the A and RA groups, the later in life the rat attained maximum body weight, the longer they were likely to survive. This was not the case in the R group. These findings seemed to support a relationship between body weight and longevity. The findings of others have reinforced that in rodents the life span of animals can be increased when restriction begins later in life (78,82).

Several innovative means of inducing dietary restriction have been employed. McCay's (18) stairstep method -- a method of restricting growth to approximately 10

grams every 2 to 3 months, with growth allowed only upon any group member's failure as a result of caloric restriction (18); restriction of food intake as a percentage of that consumed by ad libitum fed controls (76,80,85); and every-other-day feeding (83,86) have been employed. All have been shown to be effective.

The effect of restriction methods which involve qualitative rather than quantitative dietary adjustments have also been evaluated. Manipulation of dietary protein and caloric levels, plus forced exercise, resulted in lengthened life for McCay's rats fed a low protein (liver source) restricted diet (78). Summarily, this study like that of Nakagawa, et al. (87) provided little evidence that dietary protein levels have any effect on lifespan, unless the limits are excessive. Nakagawa, et al. (87) and De, et al. (88) found however that a low protein diet results in a lower rate of initial growth in rats and mice respectively, but this effect diminished with time. Moreover, a relationship between increased dietary protein levels and age-associated lesions was in evidence (88).

Davis, Bales, and Beauchene, (89) examined the impact of differential dietary protein and caloric restriction in Wistar rats implemented at 32 days of age. Growth curve shapes were similar for all groups, with ad libitum-fed animals' predicted mature body weights being significantly higher than restricted-fed animals. Kidney weights were lower in restricted rats; no difference was noted as a function of dietary protein level (in contrast to McCay's (78) finding). Also, restricted rats lived longer. It was concluded that restriction of calories in general was more beneficial than restriction of protein in promoting longevity (89).

The mechanism by which food restriction increases lifespan, however, has not been elucidated. Briefly, it has been suggested that food restriction may slow the aging process by retarding maturation (18), decreasing body fat (77), or reducing the metabolic rate per gram of body mass (90). Each of these hypotheses however have been countered with contradictory results (82,91,92). It has also been proposed that the life-prolonging effects of food restriction are the result of a reduction in free radical formation. Free radicals mediate cellular and organ damage that is thought to be instrumental in the aging process (93). Another hypothesis emphasizes the role of protein turnover and DNA synthesis regulation (94). It has been suggested that food restriction, by delaying an age-associated decline in cellular protein turnover, delays aging. Further, food restriction has been associated with significantly decreased tumor and lesion development and a delay in the onset of cardiac, renal, and vascular lesions and tumors (95). Walford, et al. (86) suggested that dietary restriction results in a delay of maturation of the immune system; therefore the immune system remains "young" and capable of mounting an appropriate response to pathological agents, thus ultimately increasing life span. Food restriction has also been shown to decrease hormone levels (96). As previously mentioned, this hormone lowering effect may decrease "wear and tear" on tissues thus delaying age-associated processes (12). This mechanism is an aspect of the proposed neuroendocrine theory of aging.

Exercise

As has been noted, the aging process is characterized by a decline in the functioning of the cellular and structural components of all organ systems (97). Exercise training has been reported to have beneficial effects on the functioning of several organs and organ systems (98,99). This has lead to an examination of the relationship between aging and exercise. As stated by Holloszy (100), the hypothesis to be tested is that "some of the decline in functional capacity with aging results from a decline in the habitual level of exercise in a large segment of our population and can be reversed or prevented by regularly performed exercise" (p. 1823).

Like sedentary aging humans, laboratory rats are essentially bereft of exercise. This has prompted speculation that the establishment of an exercise regimen in rats might readily result in a delaying of the decline in function associated with aging. Several investigators have attempted to systematically evaluate the effects of exercise on physiological functioning and on longevity (99,101).

A number of studies have indicated that exercise results in physiological changes in older animals that more nearly approximate those seen in younger animals. Examples include improved myocardial and cardiovascular function (99,102) and body composition changes (103). Further, exercise training has been shown to decrease fat cell size, and to increase insulin receptors in fat cells, thereby increasing insulin binding (98). Thus, training provides a mechanism for rapid replenishment of energy stores as adipocytes readily take up and oxidize glucose.

VO₂ max is an indicator of cardiovascular function and the functional limits of aerobic metabolism (97,100). Mazzeo, et al. (104) examined the effect of endurance training in 3, 12, and 24 month old rats on maximal oxygen uptake (VO₂ max). Animals were trained and exercised at 75% of their VO₂ max. Following an 8-week training session, all groups exhibited significant improvements in VO₂ max and endurance time. It is noteworthy that the old rats exhibited comparable or greater cardiovascular adaptations with training (as indicated by VO₂ max) than did the younger animals.

A later study by Mazzeo (103) repeated the training level of 75% VO₂ max and the endurance capacity test for rats aged 6, 15, and 27 months. The training response was replicated (104) and the older animals exhibited body composition adaptations similar to those of younger animals. Like the younger animals, the aged animals demonstrated significant decreases in percent body fat with exercise. Total weight loss in older animals was primarily the result of reduced fat body weight, while lean body mass was maintained. Young animals, in contrast, not only lost fat but gained lean body mass. In the older trained animals a significantly higher lean body mass was observed when compared to their older controls. Thus, exercise training resulted in the older trained animals attaining characteristics associated with a younger age.

As mentioned previously, it has been suggested that aging may be delayed by growth retardation. Bloor (105) examined the effect of chronic (daily) and intermittent exercise on young, adult, and old rats. Exercise consisted of one-hour

swims, daily or 2 times per week (for chronic and intermittent groups, respectively) for 10 weeks. His findings indicated that both exercise regimens caused growth retardation in young animals. Further, the delay of growth was proportional to the severity of the exercise. Exercise in the adult animals resulted in smaller weight gain than in the controls. In the older animals, however, it elicited a catabolic response as indicated by a loss of both organ and body weight. Retardation of growth as a result of exercise was also reported by Oscai and Holloszy (106). Paired-weight dietary restriction (food intake restricted to maintain body weights in the same range as the exercised animals) of Wistar rats alone resulted in significant losses of lean body mass as well as fat body mass, while exercised (swimming) animals primarily lost fat body mass. Therefore, the authors suggested that exercise may elicit a lipid mobilizing and protein conserving effect. The ad libitum sedentary animals' weight gain was due almost exclusively to fat weight increases (106).

Goodrick (107), using Wistar rats and a voluntary wheel running design, reported that exercised rats lived significantly longer than sedentary controls. Further, although mean metabolic rate decreased with age in this study, exercised rats did not exhibit this age-related decrease in metabolic rate. This was significant, as increased energy metabolism had been linked with decreased lifespan (108). Cumulatively, these results prompted Goodrick to conclude that "exercise acts to retard the aging process" (p. 29). This study also showed growth duration was positively correlated with longevity, and growth rate was negatively related to longevity. A later study by Goodrick, et al. (109) replicated the growth duration

finding in the ad libitum animals only. When the effects of voluntary wheel running and every-other-day food restriction were evaluated, food restriction was the only variable found to significantly increase longevity. Exercise was allowed throughout the lifespan in this study. No support for the hypothesis that growth rate is inversely related to longevity was found (109). Another study introduced exercise at 10.5 or 18 months (84). No improvement in longevity was observed.

Holloszy and Smith (110) noted that in Goodrick's earlier (107) study, the average survival rate of the sedentary Wistar rats was only 631 days. These investigators state that the normal lifespan for ad libitum-fed Wistar rats is 750 days. Therefore, they questioned Goodrick's conclusion that exercise actually retarded aging in this species. In their own work, Holloszy and Smith (110,111) report that voluntary wheel running increased the survival rate in SPF Long-Evans rats, but showed no significant increase in life span compared to ad libitum-fed and pair-fed (to the runners) animals. Only rats on a paired-weight (to runners) food intake regimen showed a significant increase in lifespan. Holloszy and Smith (101) suggested that the increased survival rate with exercise may be due to counteracting the effects of a sedentary lifestyle (e.g., obesity) rather than slowing primary aging, as growth retardation in exercised and paired-weight (to runners) animals was similar. This suggested that a factor is at work with food restriction that has an effect above and beyond that of growth retardation.

The beneficial effects of food restriction on aging have been previously noted (18,96). Thus, alterations in food intake as a result of exercise should also be

examined. Oscai, et al. (112) noted a sex difference in the feeding response to exercise. Female rats following an exercise regimen increased their food intake to a level commensurate with body weight stability. Male rats, in contrast, lost weight (compared to non-exercised controls) and did not increase their food intake sufficiently to offset their losses (112,113). Higher intensity exercise resulted in a greater depression of food consumption and depressed weight gain than did lower intensity exercise (113). This suggested an appetite suppressive effect of exercise.

While some authors have described the beneficial effects of exercise (101), it should be noted that others have reported shorter life spans with exercise (114,115). These studies, however, had flaws, such as not including SPF animals, and the current general consensus is that the lessened lifespan was due to illness or flawed procedures. Recent research has provided evidence of an avenue by which exercise may cause detrimental effects. For example, Davies, et al. (116) reported that Long-Evans rats, when exercised to exhaustion, had significant increases in free radical concentration in muscle and liver; lipid peroxidation; and damaged mitochondria, sarcoplasmic reticulum, and endoplasmic reticulum. Cellular damage caused by free radical production has been proposed as a mechanism which may induce age-related biological changes (93). Consequently, exercise resulting in increased free radical production may well accelerate the aging process rather than retard it.

Restriction and exercise and body composition.

The impact of food restriction on body composition of experimental animals, and in particular, the percentage of total body fat has been evaluated by numerous researchers (76,83,117). An early study by Berg (118), using dietary restriction levels of 33 and 46% of ad libitum, found 25 and 40% reductions, respectively, in body weight of rats. He observed that these differences were "due largely to absence of excess body fat which developed in unrestricted animals" (p 254). Beauchene, et al. (83) using Wistar rats, found that during the second year of life, ad libitum-fed controls deposited five times more fat than lifetime restricted rats. Tucker (119) suggested that the weight gain of ad libitum-fed animals after one year of age was due to adipose tissue deposition. This was supported by the larger terminal body weight values observed in comparison to the predicted mature body weights and the calculated growth curve.

In contrast, Yu, et al. (120) noted that the percentage of lean body mass increased in both ad libitum and food restricted (60%) sedentary animals, including longer-lived subjects. Reporting on this same study, Bertrand, et al. (92) observed that fat mass also increased in both ad libitum-fed and feed-restricted animals for about 75% of their life span, and then it consistently decreased. It was therefore concluded that although the restricted animals had a lower fat mass than controls, this lower fat mass in and of itself was not related to life prolongation, although food restriction clearly was. This study used barrier maintained SPF Fischer 344 rats, unlike the other laboratories. Furthermore, they used a unique method of

determining fat body mass. Lean body mass was measured by subtracting total fat mass from total body mass. "Fat mass was indirectly determined by measuring the uptake of cyclopropane by the rat" (p 132) (120). The use of this indirect measurement may have played a part in the discrepant findings of these researchers.

The impact of exercise on body composition has also been investigated (121). Crews, et al. (122) noted that forced treadmill exercise resulted in a significant decrease in body weight gain. This was in part due to an appetite suppressing (decreased food intake) effect of the exercise. Exercised rats' body fat was a significantly lower percentage than sedentary controls; in fact, most of the weight difference between the two groups was due to body fat. The percentage of body fat was also lower in sedentary paired-weight (to exercised rats) animals than in the sedentary, free feeding controls. Exercised rats had the least body fat percentage, followed by the sedentary restricted animals, with the sedentary free feeding controls having the greatest percentage of body fat. Lean body mass of exercised animals was greater than that of sedentary animals of comparable weight.

Oscai and Holloszy (106) reported significant decreases in fat, and increased lean body mass with exercise (18 weeks of swimming) while weight loss of sedentary, paired-weight (to exercised animals) Wistar rats was both fat (62%) and LBM (37%). Sedentary, free feeding animals' increased weight during this time was the result of increased body fat. Oscai and Holloszy also report that their adult (10 month old) exercised rats gradually lost weight over the 18 week period as a result of both appetite suppression and increased caloric expenditure. Mazzeo and Horvath (103)

reported that treadmill training significantly reduced the percentage of body fat when compared to controls in 6, 15, and 27 month old animals. Lean body weight was significantly higher for 27 month old trained animals compared to same-aged controls. Six and 15 month old exercised animals were reported to have increased food intake compared to sedentary animals: no increase was noted in the older animals (103).

Exercise has also been found to decrease the percentage of body fat when compared to controls in Sprague Dawley rats (123,124), and in CD1 Swiss mice (125). Ring, et al. (126), however, found that exercise beginning at 50 days of age and continuing for 100 days in Fischer rats resulted in only a slightly lower (nonsignificant) percentage of body fat than that noted in sedentary controls.

Using human subjects, the effects of exercise training on body composition were studied (127). One of each of six pair of male monozygotic twins underwent exercise training for 22 days. A significant decrease in percentage of body fat in trained subjects was noted; however, there was no change in fat-free body mass.

Bouchard, et al. (128) investigated the effects of long-term exercise (cycle ergometer) and caloric restriction in adult males. After a baseline period, subjects were trained for 100 days; during this same period, caloric intake was restricted to result in a 1000 Kcal/day energy deficit. Per cent body fat and fat mass decreased significantly from baseline; no change was observed in the fat-free mass. VO_2 max was not increased by training. Average weight loss was 9%, 82% of which was body fat. The authors suggested that exercise protects the fat-free body mass during energy deficit and weight loss.

Data gathered to date tend to support the observation that both food restriction (76,117,118) and exercise (103,122,123,124,125,127,129) decrease percentage of body fat when compared to ad libitum fed, sedentary controls. Further, exercise protected against lean body mass loss (106,122). However, Yu (120) reported increased lean body mass in both ad libitum (controls) and food restricted groups; and, Ring (126) reported that exercise did not significantly reduce percentage of body fat in their animals. When food restriction and exercise are evaluated within the same study, the finding that food restriction reduces body fat is supported, but the effects of exercise are equivocal. There were other contradictory findings. For example, it was found that exercised rats had lower caloric intake than ad libitum fed sedentary controls (122). However, other researchers found that exercise tended to increase the weekly intake of food, but that cumulative food intake between exercised and sedentary animals was not statistically significant (129). None of the reported studies measured the effects of food restriction and ongoing exercise throughout the lifespan. Further, it has been suggested that body composition changes occur with age (130). This alteration reportedly was the result of decreased LBM and increased body fat (130,131).

Effect of Food Restriction on the Pituitary-Thyroid Axis

It has been suggested that hormone(s) secreted by the pituitary are in effect "aging factors" (132). Therefore, as food restriction has clearly been established as

an effective method to delay age-associated changes, it is of interest to examine pertinent thyroid hormone and food restriction relationships.

Weindruch and Walford (34) have suggested that "the thyroid axis deserves special attention in trying to find the mechanism of dietary restriction's action" (p. 143). This suggestion is based upon the observation that the pituitary-thyroid axis is intimately involved in energy regulation. Energy (kcal) is the essential factor which is limited in lifespan-prolonging dietary restriction (34). Dietary restriction has been shown to depress thyroid axis activity. This has prompted speculation that the pituitary-thyroid axis may be a causative factor in the etiology of age-associated biological changes in the organism and/or, age-associated changes in pituitary-thyroid functioning may be augmented by dietary restriction.

Early studies investigating the relationship between thyroid function and food intake were conducted by Grossie and Turner (133,134,135). They reported that 22% dietary restriction in adult Sprague-Dawley rats resulted in a gradual decrease in T_4 secretion rate (133); thyroidectomy in adult rats significantly reduced mean food intake (134); T_4 replacement therapy (administered 119 days following thyroidectomy) failed to stimulate food consumption equal to that of control animals (134); and T_4 administration to intact female Sprague-Dawley rats resulted in a slight but insignificant increase in food intake (135). These studies clearly support the suggestion that the thyroid - dietary restriction relationship is worthy of investigation.

The classic work of Giles and Everitt (136) examined the causal relationships of thyroid functioning, food intake, and collagen aging. With thyroidectomy, they

found that food intake of the male Wistar rat was decreased significantly (37%) and maturational or aging changes of rat tail collagen fibers were retarded. In fact, thyroidectomy and food restriction yielded comparable maturational changes on collagen fibers in young rats. Thyroxine replacement therapy in thyroidectomized ad libitum-fed rats restored normal food intake and collagen aging in rat tail tendon fibers. When thyroxine-replaced-thyroidectomized rats were food restricted; however, no collagen fiber aging effects were noted. These results led the authors to speculate that the "aging effect of thyroxine is related to the increased food intake in these animals" (p. 69) that accompanies its administration (136). It should be noted, however, that this effect may have been due in part to the overall rate of growth of animals as a result of food intake.

A later study by Everitt, et al. (137) reported a relationship between thyroidectomy and reduced food intake (21%) in older male Wistar rats. As in young rats, thyroxine administration increased mean food intake (39%), and it accelerated collagen aging. However, thyroxine-treated animals showed a mean weight loss 8 times that of control animals. Collagen fiber breaking time was reported to be no different in restricted, thyroidectomized, or control animals. These results led the authors to speculate that the age of intervention may influence the age retarding potential of that intervention.

Total caloric or protein restriction has been shown to result in depressed thyroid hormone concentrations (138). However, the nature of these changes remains controversial. Anterior pituitary function with underfeeding was evaluated

by Campbell et al. (13). Acute (7 day fast) and chronic (7 day fast plus 2 weeks of 1/4 ad libitum intake) starvation of adult male Sprague-Dawley rats resulted in a significant decrease in TSH concentration levels, 69% and 74.4% respectively. Seven days of ad libitum feeding following chronic starvation failed to return TSH values to that of controls -- values approximately 50% of ad libitum were reached by refed rats. The TSH response mounted to LHRH and TRH administration was greater in both acutely and chronically starved groups when compared to "refed" rats fed ad libitum following fasting. However, these refed animals failed to mount a TSH response equivalent to that of control animals. These results indicated that the anterior pituitary is sensitive to food restriction, yet remains capable of responding to hypothalamic stimulation.

The effect of starvation on T_3 and T_4 concentrations has been evaluated by a number of investigators (139,140,141,142). Yeh, et al. (141) found that short term fasting (3 days) resulted in significantly depressed T_3 and T_4 serum concentrations and body weight in adult male Sprague-Dawley rats. Thyroxine administration (40 mg T_4 /day) to fasted rats restored both T_3 and T_4 values to initial values; thyroxine administration to control animals resulted in a significant increase in T_4 levels, with no change noted in T_3 . This level of thyroxine supplementation, however, did not have any effect on body weight losses with fasting, or on weight gain in control rats supplemented with thyroxine.

Goodman, et al. (139) noted a significant decrease in both plasma T_3 and T_4 with fasting in young (8 week old) and older (16 week old) ad libitum fed control

rats. Plasma T_3 levels in 16 week old rats made obese by 8 weeks of high fat dietary intake decreased during the first 5 days of fasting, then increased to values greater than initial basal concentration. Prior to starvation, no significant difference for T_3 or T_4 was noted between groups. Fasting, however, resulted in thyroid hormone differences governed by both age and adiposity. Both T_3 and T_4 decreased more rapidly in the 8 week old group than the 16 week old group, and more rapidly in the control group than the obese group. Further experimentation undertaken by Goodman, et al. (140) suggested that thyroid hormone stability in the obese fed rat with fasting was primarily an effect of obesity and/or fat feeding rather than age. This study suggested that decreased secretion was responsible for the fall in T_3 and T_4 serum concentrations observed (140), indicating that depressed TSH levels such as those seen by Campbell (13) were a causative factor. Additionally, a reduction in extrathyroidal T_4 to T_3 conversion with restriction was indicated in the etiology of dietary influenced thyroid functioning (140). However, no reduction in conversion of T_4 to T_3 by thyroid gland homogenates due to dietary restriction was noted by Ooka (143). However, a sudden increase in T_4 to T_3 conversion in gland homogenates after food restriction started before maturity was replaced by ad libitum feeding was observed (143).

Kinlaw, et al. (142) suggested that the decreased serum T_3 and T_4 seen after 5 days of starvation in young male Sprague-Dawley rats was the result of reduced thyroidal secretion of T_4 . This hypothesis was the outgrowth of greater serum T_4 than T_3 decreases with starvation, an increased T_4 metabolic clearance rate, and a

larger reduction in T_4 than in T_3 turnover rate. Moreover, the findings of this study provide no evidence of a diminished conversion ratio with fasting. This study also led the authors to the conclusion that "essentially all of the extrathyroidal T_3 in the rats is produced by peripheral conversion from thyroxine", a conclusion differing from their own (144), as well as others' previous results.

While noting a marked decrease in serum T_3 concentration in fasting volunteers, Vagenakis, et al. (30) maintained that this effect was the result of altered T_4 peripheral metabolism resulting in depression of T_3 production and increased rT_3 formation. A comparable T_3 pattern was observed by Suda, et al. (145). Moreover, no significant changes were noted by Suda in TSH or T_4 serum concentrations with severe caloric deprivation. Suda, et al.'s results suggested, however, that T_4 and T_3 metabolic clearance rates were lower in the fasting subjects than in control subjects consuming a normal caloric intake (145). Furthermore, fasting was not found to alter T_4 peripheral metabolism as previously suggested (30). Rather, reduced T_3 production and an increased rT_3 concentration was found to be the result of 5'deiodination impairment (145). Tegelman, et al. (146) also noted depressed T_3 values with fasting, while TSH values remained within normal limits in healthy, non-obese male and female subjects.

The effect of fasting has been more extensively investigated (138,141,142,145), while the impact of moderate food restriction on the pituitary-thyroid axis has received less attention (147,148). Moderate food restriction (33% of ad libitum) in young, male athyroidic Wistar rats supplemented with thyroxine resulted in depressed

plasma T_3 concentrations (149). Tissue/plasma ^{131}I T_3 ratios of restricted animals were not significantly different from those of controls. This indicated that, in general, food restriction did not result in altered tissue uptake of T_3 . Kinetic studies suggested that the low serum T_3 values were the result of a decrease in whole body T_4 to T_3 conversion, as well as a slightly increased metabolic clearance rate (MCR) of T_3 . No change in MCR of T_4 was observed with restriction. The work of Merry and Holehan (150) also supported a reduced T_4 to T_3 peripheral conversion with dietary restriction, resulting in reduced (50%) T_3 serum levels and inconsistently depressed T_4 levels. Wrutniak and Cabello (148) have also gathered evidence to support altered peripheral conversion of T_4 into T_3 and rT_3 with food restriction, as well as decreased thyroid hormone secretion (T_3 , T_4 and TSH) in newborn lambs.

Snyder and Wostmann (147) employed mild dietary restriction (12 g/day) from six weeks of age in restricted conventional and gnotobiotic rats. They observed a marked decline in serum T_4 levels in both restricted-fed and ad libitum-fed conventional and gnotobiotic (long-lived, germ-free rats that were monocontaminated) Loribund-Wistar rats with aging, while T_4 was reduced by restriction in conventional rats only. T_3 levels were slightly decreased with restriction in young adult conventional rats; however, T_3 levels in older rats were not affected by restriction in either group. Gnotobiotic, but not conventional rats, exhibited an age-associated decline in T_3 levels. These data do not support a reduced pituitary function as a result of mild dietary restriction.

A recent study by Snyder, et al. (44) indicated that food restriction (30% ad libitum) conferred no effect upon TSH or T_3 levels in adult, or old rats. Likewise, no effect upon T_4 was indicated until old age; then, old restricted Lobund-Wistar rats had less of an age-associated decrease in T_4 than did their ad libitum fed controls. As previously noted, food restriction has been coupled with depressed thyroid function. Thus, Snyder, et al. (44) stressed the importance of the finding of no effect on thyroid function with mild restriction.

Effect of Exercise on the Pituitary-Thyroid Axis

The thyroid hormone system is influenced primarily by long-acting stimuli; therefore, the effects of exercise on this system have often been ignored. Moreover, the results of exercise studies that have been conducted were often contradictory and/or controversial.

Exercise effects on the pituitary-thyroid axis have been evaluated in a number of species (152,153,154,155,156,157) and under a host of conditions (154,158,159,160,161). It is these conditions, e.g., variability in duration and intensity of exercise, trained vs. untrained status of the subjects, time of blood sampling, and age and nutritional status that make interpretation of these studies equivocal.

Exercise effects upon the pituitary-thyroid axis were evaluated by Wirth et al. (162). They suggested that physical training in young male rats produces effects on the pituitary-thyroid axis similar to those produced by moderate energy restriction.

This was based on the finding that both restricted animals, consuming 23% fewer calories than ad libitum-fed controls, and trained animals (treadmill trained for 2 months) had no alterations in basal serum T_4 , T_3 or rT_3 concentrations as a result of their respective treatments; however, both exhibited decreased T_3/rT_3 and TSH levels. Further, both the exercised and the food restricted groups had smaller fat cells and exhibited a norepinephrine stimulated lipolysis that was sensitive to T_3 when compared to ad libitum sedentary controls. Conversely, a single bout of treadmill exercise produced significantly elevated T_4 , T_3 , and T_3/rT_3 concentrations and no change in basal TSH concentration. The results of this study underscore the importance of both duration and intensity of exercise when interpreting its effect on thyroid hormone indices; as, with training, adaptations such as those noted above may occur. Thus, they concluded that peripheral effects are those of importance with chronic exercise.

Winder and Heninger (163) evaluated the effects of chronic exercise training on thyroxine disposal in the rat. Total plasma T_4 concentration was decreased 13% by exercise, while percentage of free T_4 was increased. Half-life studies indicated that the disposal or rate of utilization of thyroxine was increased with exercise, as indicated by the metabolic clearance rate (MCR) and T_4 half-life. These results were similar to those of Irvine (159).

A later study by Winder and Premachandra (151) clarified that the T_4 secretion/degradation rate for the trained and sedentary rats in the 1973 study were essentially identical. Similar results were again verified by Winder, et al. (164) when

no change in either T_4 secretion or degradation occurred. However, in contrast, no change was found in T_4 serum values with exercise. Additionally, thyroid status indicator enzymes, liver mitochondrial glycerophosphate dehydrogenase and citrate synthase, were not increased with exercise. Pair feeding of non-exercised rats to exercised rats' body weight resulted in similar results. These results led the authors to conclude that the exercise training effects evaluated (muscle enzyme increases, etc.) do not require the presence of increased thyroid activity (164).

Tsai, et al., (155) found no effect on serum T_3 and T_4 concentrations as a result of voluntary wheel running for 35 days, or upon discontinuation of exercise in adult female hamsters. Disc running of hamsters did, however, increase food intake 10-20%. Increased food intake was not sufficient to offset the energy expenditure of exercise, however, and weight gain was depressed during the exercise period when compared to the retirement period. Body fat content was also significantly decreased with exercise.

A recent study by Katzeff, et al. (165) investigated the effects of dietary restriction (25% of ad libitum fed animals) and exercise (28 days of voluntary wheel running) in 3 month old male C57/BL6 mice. Caloric intake of exercised, ad libitum-fed mice was 20% more than sedentary ad libitum-fed animals: also a non-significant 5% increase in mean T_3 serum concentration occurred in the exercised group. No significant increase in body weights of the two ad libitum-fed groups was observed. Pair-fed (to ad libitum) controls' intake, exercised mice decreased in body weight -- indicating that energy expenditure was increased with exercise. Sedentary, restricted

fed animals' body weights paralleled those of the pair-fed exercise group, while restricted, exercised animals had a significantly lower body weight when compared to all other groups. Exercise resulted in a significant alteration in T_3 metabolism. T_3 concentration was decreased significantly in the pair-fed exercised and the restricted exercised mice when compared to their diet-matched controls. T_3 production rate, however, was increased significantly in ad libitum exercised mice compared to sedentary controls. This was thought to be the result of increased LBM or increased caloric intake. All other groups had similar significantly depressed T_3 production rates when compared to the ad libitum-fed, sedentary controls. Interestingly, both the pair-fed and restricted, exercised groups had significantly decreased T_3 serum levels when compared to their feed-matched controls. No difference in serum T_4 concentration levels was noted for any group.

Voluntary wheel running was chosen as the exercise method by Katzeff, et al. (165), in this study, in an attempt to reduce in vivo $T_4 \rightarrow T_3$ conversion. In vitro $T_4 \rightarrow T_3$ production, however, was observed in liver homogenates under basal and stimulated conditions. No differences were noted between ad libitum fed groups; however, T_3 production was decreased significantly (50%) under both basal and stimulated conditions in the pair fed (to ad libitum) exercised animals and the restricted, exercised group under stimulated conditions. A 28% decrease in liver 5'deiodinase activity was observed in the restricted only group. The MCR and production rate (PR) of T_3 in vivo were correlated to caloric intake, and an exercise and caloric balance interaction was observed. These correlations led the authors to

suggest that "exercise-induced alterations in T_3 metabolism may be an important factor regulating exercise-induced changes in body composition and energy expenditure" (p. E824) (165).

Exercise and thyroid function have been studied in other domestic animals. In adult domestic ducks a single bout of treadmill exercise for 90 minutes resulted in increased T_3 and decreased T_4 concentrations (152). The author speculated that these results were due to increased T_4 to T_3 conversion. In horses, both serum T_3 and T_4 concentrations were significantly elevated 1 hour after 2 separate swimming bouts. It was posited that an increased circulatory response resulting in movement of these hormones into the intracellular space took place, thus providing the opportunity for increased deiodination to be the mechanism of action (154).

A number of investigators have examined the effects of exercise on thyroid hormone concentrations during or immediately following an exercise bout (166,167,168). A recent study by Sander and Röcker (166) examined the effects of a single bout of strenuous prolonged exercise on thyroid status of well-trained runners. The subjects' TSH, T_3 , and T_4 serum levels were increased immediately after running a marathon, with values falling during the subsequent 22 hours to a value below that of baseline. It was concluded by the authors that prolonged exercise results in an increased TSH secretion and resultant cascade effects. Further, it was suggested that peripheral conversion of T_4 was altered in favor of rT_3 over T_3 , thus producing a "throttle control" which provided protection of the body's energy reserves while allowing synthesis of additional energy resources.

In contrast, Smallridge et al. (167) examined thyroid status in sedentary, jogger, and marathon groups exercised to exhaustion via treadmill. No significant differences were found in serum total T_3 , T_4 , and TSH values pre-exercise, immediately after, or one hour post-exercise in any group. Therefore, these researchers concluded that the pituitary thyroid axis response is not influenced by acute exercise or level of physical fitness. A similar study evaluated nine runners aged 29 - 56 years participating in a non-competitive marathon (160). These subjects did not exhibit any significant change from basal T_3 , T_4 , or TSH values 20 minutes after completion of the run. However, a trend for decreased T_3 values and increased T_4 values was noted (160).

Studies of trained athletes consistently have reported conflicting results regarding thyroid hormone behavior with exercise. Evaluation of thyroid hormone status with maximal physical exercise performed by sport divers and runners determined that TSH levels decreased during exercise, then increased following exercise, while T_3 levels rose, then fell (169). However, submaximal bicycle ergometry resulted in increased TSH values during exercise followed by a decrease, with unchanged T_3 levels during exercise and a fall after exercise. Alterations in pituitary secretion were proposed as underlying TSH changes, while hemoconcentration was suggested for increased T_3 levels. Age or level of fitness may have also played a role in TSH level behavior, as a more pronounced decrease was found in young runners with good endurance training, when compared to the older divers who had moderate endurance training (169).

O'Connel et al. (168) observed that a single bout of moderate prolonged exercise of moderately active non-obese males resulted in a significant decrease in total serum T_3 and an increase in total serum T_4 during exercise. Also reported was an increase in total T_4 concentration during a 30 minute work bout of submaximal exercise utilizing a work-rest protocol in 24-34 year old subjects (170). This change was sustained for 24 hours following exercise. Like Smallridge (167), no change in TSH concentration was noted during or after exercise. The effect of a 9-hour march accomplished at an average speed of 4.6 km/hr on thyroid function in healthy 18 - 22 year old men was examined (171). No significant difference was observed for total T_3 or T_4 serum concentrations in this case; however, average T_3 values tended to decrease with time.

The effects of an endurance training program consisting of training at a 48 km and then an 80 km distance was evaluated in women (172). At 48 km, serum T_3 concentrations had decreased slightly. However, further training (48 km to 80 km) resulted in a return of T_3 to pretraining values, with a significant increase in basal T_4 and TSH values, and a blunting of the TSH response to TRH. It was proposed that the altered thyroid hormone levels were due in part to an increase in lean body mass and a decrease in fat weight, as well as a reaction to the "stress" of repeated and prolonged exercise. Similar results were obtained with an aerobic conditioning program (173). Hohtari, et al. (174) also reported no changes in serum TSH values between female controls, runners or joggers. These results followed 6 months of training and a weekly distance total run of 42 km. Slight decreases in serum T_4 and

T₃ concentrations were seen with exercise, however, prompting the conclusion that thyroid, but not pituitary, secretion may be altered by physical training.

Poehlman, et al. (127) evaluated the effects of a 22-day training (ergocycle) program on thyroid hormone status. One each of male twins trained while the other served as a control. Training reduced plasma levels of T₃, T₄, and free T₄. There was no significant change in rT₃. They suggested that genetic factors take primacy over environmental changes as genotype accounted for about 70% of the response of T₄ and free T₄. A later study conducted by the same laboratory did not duplicate these findings (128). Using adult subjects, diet and cycle ergometric training were regulated for 100 days to obtain a 1000 Kcal/day energy deficit. Measurements were taken at rest, at VO₂ max, and 1 hour past VO₂ max; no significant changes were recorded. No significant change in TSH concentration after TRH stimulation was noted, either. The authors suggested that the lack of change in TSH may be due to the combined effects of training and chronic energy deficit.

These studies suggest that the pituitary-thyroid axis is capable of responding to the effects of exercise -- at least on a short-term basis. As was shown, while some studies have observed no effect of acute exercise on thyroid hormone levels (167), others found significant increases (166), while still others found both increases and decreases -- depending on the hormone measured and the time of sampling (152,165, 172). Chronic exercise exhibited a similar range of effects. As observed, few studies have been undertaken to examine the effects of exercise on the pituitary-thyroid axis in animal models, and the cumulative data from these studies have been inconclusive.

However, as the pituitary-thyroid axis has been implicated as a possible site of regulation of the aging process, it is important that the exercise and pituitary-thyroid relationship be examined.

As is readily apparent from the literature presented, a number of questions remain concerning the pituitary-thyroid axis -- its response to the aging process; its ability to respond to extraneous processes such as food restriction or exercise; and its role (if any) in the extension of lifespan. Previously conducted research has provided the information that this axis is intimately involved in metabolic processes and energy regulation, and that it affects the functioning of every cell in the body. More recent investigations have indicated that the pituitary-thyroid axis is capable of initiating translation and transcription in the cell nucleus. As a result of the diverse range of actions of this system, a more complete understanding of this system and its capacity for regulation is desirable. The following research was undertaken in an attempt to elucidate the age-associated changes that occur in the pituitary-thyroid axis, and to examine the impact of energy restriction (food restriction) and/or energy expenditure (exercise) upon this system.

CHAPTER III

EXPERIMENTAL PROCEDURE

Animals and Housing

Pathogen-free weanling male Wistar rats (Taconic Farms, Germantown, NY) were purchased for this study. All rats were housed in an animal facility accredited by the American Association for the Accreditation of Laboratory Animal Care. The facility was maintained on a 12 hr light/dark cycle (light cycle from 6:00 am to 6:00 pm), and at $72^{\circ} \pm 2^{\circ}\text{F}$ with a relative humidity of 40 - 50%.

The animals were singly housed in suspended stainless steel wire mesh cages (20 cm X 20 cm x by 28 cm) in ventilated cubicles. All animals were fed ground Purina Rodent Chow (#5001, Ralston Purina, St. Louis, MO). Water was available ad libitum.

Protocol

Experimental groups

Rats were divided into treatment groups on the basis of diet and exercise. Subjects were randomly assigned to 1 of 4 experimental groups. The four experimental groups are listed below:

A: sedentary, ad libitum fed animals (n = 35).

AE: exercised, ad libitum fed animals (n = 35).

R: sedentary, dietary restricted animals (n = 35).

RE: exercised, dietary restricted animals (n = 35).

Dietary schedule and monitoring

Dietary restriction consisted of every other day feeding beginning at 6 wks of age. Feed was provided from 9:00 am to 9:00 am. Previous research has shown alternate day feedings to be effective in increasing mean and maximum life span (86). Twenty - four hr food intake was measured monthly from 10 or more representative animals from each group. Food intakes of the food restricted animals were measured on the day the animals were allowed feed; these values were divided by 2 to obtain each animal's average daily intake. Body weight of animals was measured and recorded weekly until 4 mo of age, biweekly to 8 mo, and monthly thereafter.

Exercise apparatus and schedule

Exercised animals swam every other day in 32 gallon plastic barrels (50 cm X 68 cm) fitted with castors for ease of movement. Barrels were filled to a designated fill level with tap water of 33 - 36°C. This temperature range was maintained throughout the exercise period by the addition and removal of water during the swim period. After 1.5 hr of swimming, the rats were transferred to new barrels with fresh water.

Exercised animals began training at 6 wks of age. The initial swim period was 10 min. Thereafter, exercise time was increased by 10 min per day until a maximum swim time of 3 hr at 3 mo of age was reached. This level of exercise was maintained up to 15 mo of age. At that point, duration of the exercise sessions was decreased to 2 hr, then to 1.5 hr at 20 mo and to 1 hr at 26 mo. Group RE subjects swam prior to being fed.

Design

All animals were maintained on their respective feeding and exercise regimens for the duration of the study. This allowed evaluation of the long term effect of food restriction, exercise, and age on the pituitary-thyroid axis. Sacrifice of animals for biochemical analyses occurred at 4 mo intervals beginning at 12 mo and ending at 28 mo. At each interval, 6 randomly chosen rats from each group were weighed, and then sacrificed following a 2 hr fast. Sacrifice was accomplished by delivery of a stunning blow to the head, followed by decapitation. Upon decapitation, blood was collected in conical test tubes and allowed to clot. The clot was loosened from the sides of the tubes, the tubes were centrifuged at 1500 X g at 4°C for 10 min, and the serum was collected. All samples were stored at -70°C for later analyses. The kidneys, hearts, and thyroids were excised, placed on chilled watch glasses on crushed ice, then weighed to the nearest hundredth of a gram.

Thyroid Hormone Assays

Serum Triiodothyronine Assay

The total serum T_3 concentration was determined by the method utilized by Oliver and Held (175). A GammaCoat ^{125}I T_3 radioimmunoassay (RIA) kit, prepared by Clinical Assays (Dade Baxter Travenol Diagnostics, Inc., Cambridge, MA) was utilized. This competitive binding assay uses a classic antigen-antibody reaction whereby unlabeled antigen (standard or subject's serum T_3) competes with ^{125}I labeled T_3 tracer for a limited number of antibody binding sites, allowing quantitation of serum T_3 concentration. These T_3 specific antibody sites are immobilized on the GammaCoat tubes' lower inner wall. ANS (8-anilino-1-naphthalene sulfonic acid) displaces T_3 from its serum binding proteins allowing binding. As the quantity of antibody sites is fixed, the amount of ^{125}I T_3 tracer that binds to antibody is inversely proportional to the concentration of unlabeled T_3 in the subject's serum. Therefore, as the serum T_3 concentration increases, the amount of ^{125}I T_3 binding to the T_3 antiserum decreases.

Reagents.

1. ^{125}I Triiodothyronine Tracer-Buffer: 7 μCi tracer in 6 ml of phosphate buffered saline and ANS. Add the total volume of ^{125}I Triiodothyronine Tracer to the Assay Buffer and mix gently. Rinse the ^{125}I T_3 tracer vial with

the tracer-buffer reagent to maximize ^{125}I T_3 tracer transfer. Store in an amber or foil-wrapped container at 2 - 8°C.

2. Assay Buffer: 120 ml of phosphate buffered saline.
3. T_3 serum blank and standards: 6 concentrations of L-3,3',5-triiodothyronine in processed human serum: 0.0 ng/ml, 0.5 ng/ml, 1.0 ng/ml, 2.5 ng/ml, 5.0 ng/ml, and 8.0 ng/ml.
4. T_3 Control Sera: 2 concentrations of L-3,3',5-triiodothyronine in processed human serum: 1.5 ng/ml and 4.0 ng/ml.
5. T_3 antiserum (rabbit anti-triiodothyronine serum): antibody coated 12 X 75 mm GammaCoat tubes. Store tubes at 2 - 27°C in sealed plastic wrappers to prevent condensation.

All reagents were preserved with 0.02 M sodium azide, and stored at 2 - 8°C.

Procedure. All serum blanks, serum standards, subject serum samples and serum controls were allowed to reach room temperature and were mixed at low speed 20 - 30 seconds (without foaming) on a Genie Vortex Mixer (American Scientific Products, Stone Mountain, GA). The assay reagents were equilibrated to ambient temperature and mixed well prior to analysis. GammaCoat tubes were labeled in duplicate for each serum sample: all samples were run in duplicate. Fifty μl of serum blanks, serum standards, subject serum samples, and serum controls were added to each appropriate duplicate GammaCoat tube. Then, 1.0 ml of ^{125}I triiodothyronine tracer-buffer reagent was pipetted into each tube. Tubes were gently

mixed at low speed on a vortex mixer, then incubated for 1 hr in a $37 \pm 2^\circ\text{C}$ water bath (Precision Scientific Company, Chicago, IL) with the water level above the tube contents, without floating of the tubes.

Following incubation, the liquid was decanted and discarded from all tubes. The decanted tubes were drained in an inverted position for 3 - 5 min. All adhering liquid was removed from the tubes by pressing the inverted tubes against absorbent paper. Tubes were then counted for 1 min in a Beckman Biogamma II Gamma Counter (Beckman Instruments, Inc., Fullerton, CA) with the window adjusted for ^{125}I . A mean of the counts per minute (CPM) was determined from the duplicate tubes for each sample. A standard curve was drawn by graphing the mean CPM of the serum blank and standards (vertical axis) versus T_3 concentration (horizontal axis). The curve was plotted on semi-logarithmic graph paper. Curve accuracy was validated by T_3 control sera.

The concentration of T_3 for each subject serum sample was interpolated from the standard curve defined by the T_3 serum blank and standards. All concentrations were expressed in ng/ml.

Serum L-Thyroxine Assay

Total serum T_4 concentration was determined using the method of Oliver and Held (175) and Oliver, et al (176). This method utilized a GammaCoat RIA kit from Clinical Assays (Dade Baxter Travenol Diagnostics, Inc., Cambridge, MA). This assay, like that of T_3 , is a competitive binding radioimmunoassay that utilizes the

principle of antigen-antibody reactivity. T_4 is displaced from its serum binding proteins by ANS and salicylate so that it may bind with the antibody binding sites. ^{125}I labeled T_4 competes with unlabeled subject serum T_4 for a limited number of T_4 specific antibody sites contained on the lower inner wall of the GammaCoat tubes, allowing quantification of serum T_4 concentration levels. Again, as the concentration of the subjects' serum T_4 increases, the amount of ^{125}I T_4 binding to the T_4 antibody sites in the GammaCoat tubes decreases.

Reagents.

1. ^{125}I Thyroxine Tracer-Buffer: 5 μCi tracer in 10 ml of tris buffered saline and 10 ml of T_4 reagent buffer concentrate were added to 90 ml of glass distilled water and mixed. Rinse tracer vial with tracer-buffer reagent to maximize tracer transfer. Store in an amber container or wrap in foil. Store at 2 - 8°C.
2. T_4 reagent buffer concentrate: 10 ml of tris buffered saline, 4 mM ANS, and 6 mM sodium salicylate.
3. T_4 serum blank and standards: L-thyroxine standards in 1 ml of processed human serum with T_4 serum concentrations: 0.0 $\mu\text{g/dl}$, 1 $\mu\text{g/dl}$, 4 $\mu\text{g/dl}$, 8 $\mu\text{g/dl}$, 12 $\mu\text{g/dl}$, and 20 $\mu\text{g/dl}$.
4. T_4 antiserum on coated tubes: 12 X 75 mm GammaCoat tubes coated with rabbit anti- T_4 serum. Store in sealed plastic bags at 2-27°C.

All reagents contained sodium azide as a preservative. Reagents were stored at 2 - 8°C.

Procedure. The assay of all samples and standards was run in duplicate in labeled GammaCoat tubes. Reagents and samples were allowed to reach ambient temperature, then mixed thoroughly without foaming. To 10 μ l of serum samples, standards, and blanks was added 1.0 ml of the ^{125}I tracer-buffer reagent. Tubes were gently mixed on a Genie Vortex Mixer (American Scientific Products, Stone Mountain, GA) then incubated at room temperature (20 - 25°C) for 45 min. After incubation, the tube liquid was quickly decanted and discarded, and the tubes then further drained by inverting for 3 - 5 min. All adhering liquid was removed from the tubes by pressing the inverted tubes against absorbent paper prior to counting the tubes for 1 min in a gamma counter (Beckman Instruments, Inc., Fullerton, CA) with the window adjusted for ^{125}I .

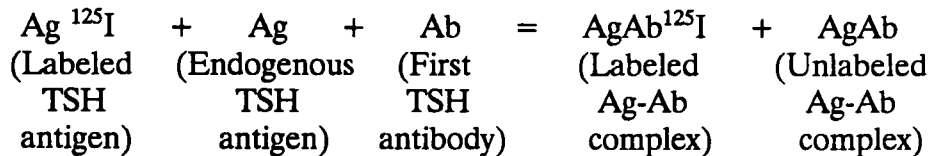
T_4 serum concentrations were expressed in $\mu\text{g}/\text{dl}$. A standard curve was drawn on semi-logarithmic graph paper from the mean CPM of the duplicate blank and standard tubes (vertical axis) versus total T_4 concentration ($\mu\text{g}/\text{dl}$, horizontal axis). Subject serum concentration values were interpolated from the standard curve defined by the serum blank and the 5 serum standards.

Serum Thyroid Stimulating Hormone Assay

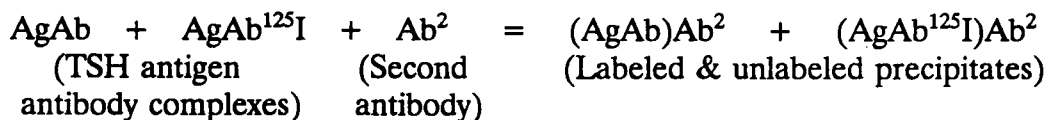
Serum TSH concentration was determined in subject samples using the TSH-Quant RIA kit prepared by Leeco Diagnostics, Inc. (Southfield, MI). This is a classic double antibody radioimmunoassay and is based on the reaction presented below whereby the antibody bound TSH antigen (both labeled and unlabeled) is separated

from unbound TSH by precipitation of the first antibody with a second antibody. The antibody is specific for the beta portion of the TSH molecule which is responsible for the specific biologic activity and the immunoselectivity of the molecule.

Step 1:



Step 2:



The amount of ^{125}I TSH bound to the antibody is inversely proportional to the concentration of TSH present in the subject sample.

Reagents.

1. ^{125}I TSH Tracer Reagent: less than 10 μCi of radiolabeled TSH and 0.05 M Tris-HCl (ph. 7.6); store at 2 - 8°C.
2. TSH Standards: 6 concentrations of thyroid stimulating hormone: 0.0 $\mu\text{IU/ml}$, 1.0 $\mu\text{IU/ml}$, 5.0 $\mu\text{IU/ml}$, 10.0 $\mu\text{IU/ml}$, 25 $\mu\text{IU/ml}$, and 50 $\mu\text{IU/ml}$ in protein stabilized base. Store at -20°C.

3. TSH Control Sera: 2 concentrations of thyroid stimulating hormone: one of normal and one of elevated TSH concentration in protein stabilized base. Store at -20°C.
4. TSH Antiserum (first antibody): Rabbit anti-human TSH serum and 0.05 M Tris-HCl (ph 7.6). Store at 2 - 8°C.
5. Precipitating Antibody Solution (second antibody): Goat anti-rabbit antibody and precipitation accelerator, polyethyleneglycol (PEG); 0.05% BSA. Store at 2 - 8°C.

All reagents were preserved with 0.1% sodium azide.

Procedure. All reagents and samples were brought to room temperature prior to TSH analysis. The serum blanks, serum standards, serum controls and subject serum samples were mixed at low speed, without foaming (to prevent protein denaturation) on a Genie Vortex Mixer (American Scientific Products, Stone Mountain, GA). The samples were run in duplicate in 12 X 75 polypropylene test tubes. Non-specific binding (NSB) tubes were run to evaluate the binding occurring in the absence of TSH antiserum. The counts resulting from NSB were subtracted from all sample values. To 200 μ l of samples, standards, and controls was added 200 μ l of TSH antiserum. Tubes were again mixed on a vortex mixer, then incubated at 37°C for 90 min in a water bath (Precision Scientific Company, Chicago, IL) with the water level above that of the tube contents, without floating of the tubes.

Following incubation, 200 μ l of ^{125} I TSH tracer was added to all tubes. The tube's contents were mixed thoroughly using a vortex mixer, then incubated at 37°C for 60 min under the previously described conditions. After this second incubation, 500 μ l of precipitating antibody solution was added to all tubes and the samples were mixed well by vortexing. The tubes were then centrifuged (Model TJ-6, Beckman Instruments, Palo Alto, CA) at 1500 g for 25 min at 4°C until a firmly packed pellet was obtained.

The supernatant was carefully decanted to avoid any loss of protein precipitate, then discarded. The tubes were then inverted on absorbent paper to assure removal of all liquid present. The antibody-bound tracer in the precipitate was counted in a gamma counter (Beckman Instruments, Inc., Fullerton, CA) with the window adjusted for ^{125} I for 1 min.

The average CPM was determined for each pair of duplicate tubes. The standard curve was prepared by graphing the percentage of bound/unbound TSH (% B/Bo) of each TSH standard against the TSH concentration in μ IU/ml plotted on semi-logarithmic graph paper. The %B/Bo was determined for each pair of duplicate tubes. The TSH concentration of subject samples was interpolated from the standard curve and expressed as μ IU/ml. The % B/Bo was derived as follows:

$$\% \text{ B/Bo} = \frac{\text{Mean Sample CPM} - \text{Mean Non-Specific Binding CPM}}{\text{Mean CPM of zero standard tube} - \text{Mean NSB CPM}} \times 100$$

NaK ATPase Analyses

Renal cortex L-fraction isolation procedure

NaK stimulated ATPase activity was determined in the "plasma membrane-rich" L-fraction (61) of the rat renal cortex. This renal cortex L-fraction has been shown by electron microscopy to contain mostly microsomal membrane vesicles (61). The NaK ATPase containing L-fraction was obtained by a differential centrifugation method of Lo, et al. (61) as outlined in Figure 2. This method of isolation is a modification of the methods of Jorgensen and Skou (177) and Kyte (178). Differential centrifugation and sucrose gradient centrifugation have been shown to increase measurable NaK ATPase activity as a result of better separation of the microsomal fraction from contaminant proteins and cellular particles (177,178).

Homogenizing Solution Reagents.

1. DL-Histidine, 0.09 M: 3.492 g of DL-Histidine was dissolved in glass distilled water and brought to a final volume of 250 ml.
2. Ethylenediaminetetraacetic Acid (EDTA, free acid), 0.015 M: 1.095 g of EDTA was brought to a final volume of 250 ml. Some EDTA may not dissolve, but upon mixing with companion solutions dissolution will result.
3. Sucrose, 0.75 M: 64.181 g of sucrose was dissolved in glass distilled water and brought to a final volume of 250 ml.

The homogenizing solution was prepared by mixing the DL-Histidine, EDTA, and

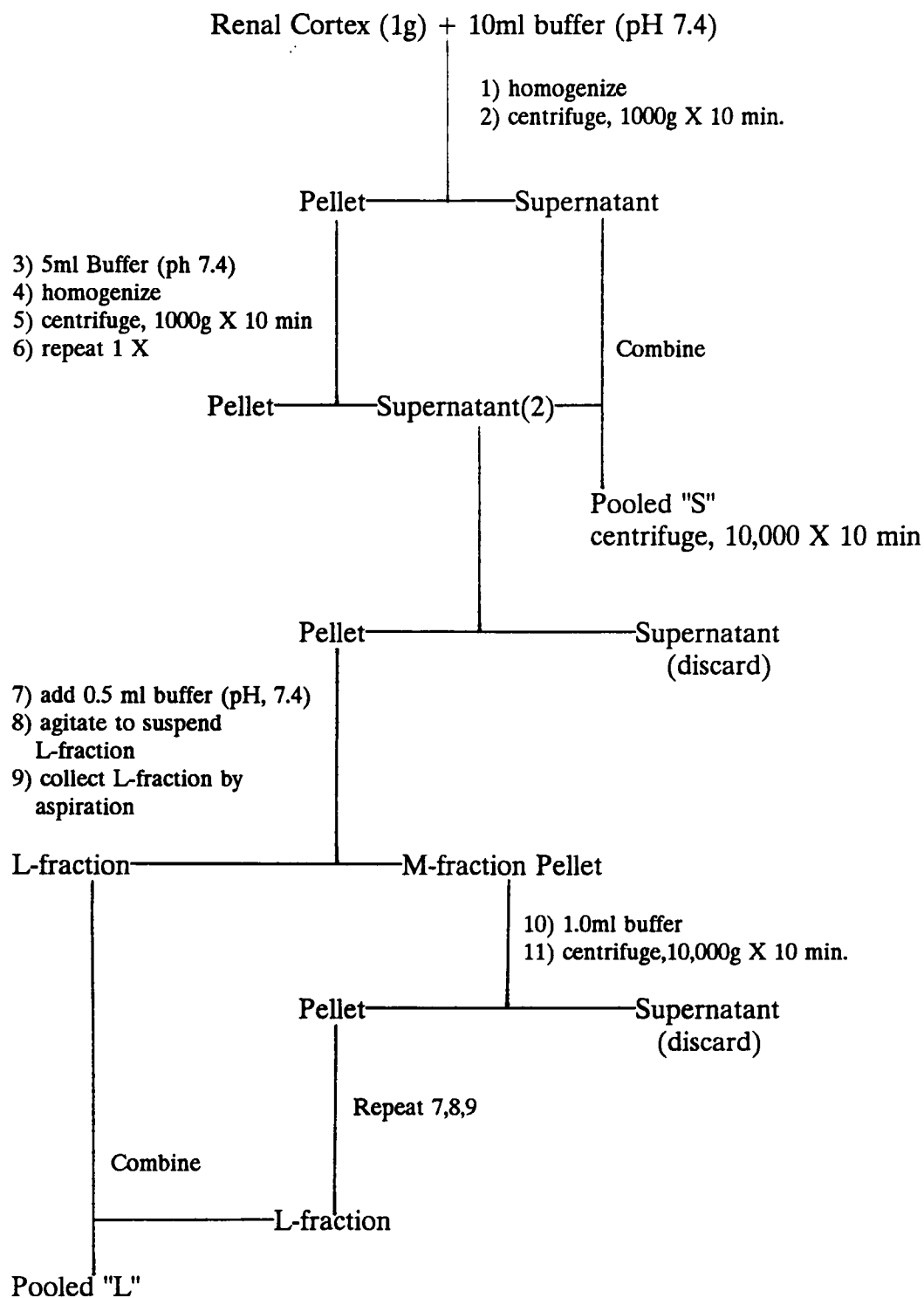


Figure 2. Schematic of Renal Cortex Membrane-Rich Fraction Isolation [adapted from Lo, et al. (61)].

sucrose in equal volumes and adjusting the pH to 7.4. The complete solution of final volume 750 ml was stored frozen in polyethylene bottles until needed. The solution was brought to room temperature prior to analysis.

Procedure. (All steps of the following procedure were carried out at 0 - 4°C) and are detailed in Figure 2. Following decapsulation of the kidneys, the renal cortex was collected by sharp dissection and a 1 g sample homogenized (10 strokes) in 10 ml of homogenizing solution (1:10 w/v) with a Teflon pestle and a glass homogenizing vessel. The renal cortex homogenization was carried out on ice using a Brinkman polytron homogenizer (Brinkman Instruments, Inc., Palo Alto, CA). The tissue homogenate was placed in polycarbonate bottles (Beckman Instruments, Inc., Palo Alto, CA), capped, and centrifuged at 1000 X g for 10 min. at 2°C (Beckman Model J2 - 21M, Beckman Instruments, Palo Alto, CA). Upon completion of the centrifugation cycle, the supernatant was carefully aspirated using a Pipetman automatic pipet, and saved. Resuspension of the remaining pellet in 5 ml of homogenizing solution followed by a further cycle of homogenization (5 strokes), centrifugation, and supernatant collection yielded a pellet to which this procedure was repeated.

The pooled supernatant fractions in capped polycarbonate bottles were centrifuged at 10,000 X g for 10 min at 2°C resulting in formation of a bilayered pellet. The supernatant was carefully removed and discarded leaving a bilayered pellet. The L-fraction (the upper white loose layer of the pellet) was resuspended

in 0.5 ml of homogenizing solution and collected with a Pipetman pipet then stored in 12 X 75 mm polypropylene tubes. The remaining pellet was resuspended in 1.0 ml of homogenizing solution, respun at 10,000 X g at 2°C for 10 min, and the L-fraction collection step repeated, again leaving behind the lower, brown mitochondrial layer (61). Pooled L-fraction samples were stored in capped 12 X 17 mm polypropylene tubes and further sealed with parafilm in an ultra-cold freezer at -90°C for later analysis of protein and enzymatic activity.

Tissue Protein Assay

L-fraction protein was determined after its precipitation with PCA. The L-fraction protein was precipitated with acid to ensure no interference by the DL-Histidine in the L-fraction protein level quantitation. Protein content was then determined by a spectrophotometric assay developed by Ohnishi and Barr (179). This dilution method is a modification of the Lowry (180) method. It is based upon the formation of complex ions between protein and copper in an alkaline solution followed by the reduction of the phenol reagent by the copper-treated protein to yield a molybdenum blue color which can be measured spectrophotometrically at 550 nm. The Ohnishi and Barr modifications yield stable color development and good reproducibility (179). Bovine serum albumin was used as the protein standard.

Reagents.

1. Sodium Chloride, 5% (w/v): 12.5 g of NaCl was dissolved in glass distilled water and diluted to 250 ml total volume.
2. Perchloric Acid (PCA), 0.14% (w/v): 0.4 ml of 70% HClO₄ was diluted to 200 ml with acetone. Prepared immediately before use.
3. Trichloroacetic Acid (TCA), 5% (w/v): 25 g of Cl₃CCO₂H was dissolved in glass distilled water and diluted to 500 ml.
4. Sodium Hydroxide, 0.4 N: 16 g of NaOH was dissolved in glass distilled water and diluted to a total volume of 1 liter.
5. Sodium Carbonate, 2.3% (w/v): 2.3 g of Na₂CO₃ was dissolved in glass distilled water and diluted to 100 ml.
6. Phenol Reagent, 2N: purchased from Fisher Scientific Company (Fair Lawn, NJ) ready to use.
7. Biuret Reagent: 1.5 g of CuSO₄ · 5H₂O, 6.0 g of NaKC₄H₄O₆ · 4H₂O, 30 g of NaOH, and 1.0 g of KI were dissolved in sequence in glass distilled water and diluted to a final volume of 1 liter. Working biuret: biuret reagent diluted 8 times with 2.3% Na₂CO₃ (i.e., 1 volume of biuret reagent plus 7 volumes of Na₂CO₃). Prepare at the time of analysis.
8. Bovine Serum Albumin Stock Standard: 1 g of bovine serum albumin (BSA) was dissolved in 5% NaCl and diluted to 100 ml. From this solution a working standard was prepared by diluting 1 ml stock BSA with 5% NaCl to a final

volume of 50 ml. Standard curves were prepared for each run by bringing 0.0 - 1.0 ml of the working standard up to 1 ml with 5% NaCl.

Procedure. All samples were analyzed in duplicate and a standard curve was prepared for each run. Twenty μ l of L-fraction was brought to a final volume of 1 ml with 5% NaCl. The sample protein was precipitated by the addition of 4 ml of 0.14% PCA to each sample or standard in conical test tubes. The tubes were mixed on a Genie Vortex Mixer (American Scientific Products, Stone Mountain, GA) and incubated for 20 min in a 50°C water bath (Precision Scientific Company, Chicago, IL) with the water level above that of the tube contents, without floating the tubes.

Following incubation, all tubes were centrifuged at 1000 X g for 10 min at 2°C in a Beckman centrifuge until a firm precipitate pellet was formed. The supernatant was decanted and discarded. The remaining protein pellet was resuspended in 2 ml of 5% TCA to wash the pellet. A second centrifugation cycle of 1000 X g for 10 min at 2°C was conducted and a second firm protein pellet formed. The supernatant was decanted and the precipitate saved for protein analysis by the method of Ohnishi and Barr (179).

Per the Ohnishi and Barr method, the protein pellets for samples and standards were resuspended in 1 ml of 0.4 N NaOH, and vortexed. Four ml of working biuret reagent was added to each tube and mixed. All tubes were then allowed to stand at room temperature for 10 min at which time 0.125 μ l of 2N phenol reagent (undiluted) was added to each tube while mixing on a vortex mixer.

Color development was allowed for 30 min. The absorbances of samples and standards were read at 550 nm with a Beckman spectrophotometer (Model 34, Beckman Instruments, Fullerton, CA). The standard curve of protein content was linear to 200 $\mu\text{g/ml}$. The protein concentration of samples were calculated by the equation:

$$\frac{\mu\text{g Protein}}{\mu\text{l L-fraction}} = \frac{\text{Concentration of Standard}}{\text{Absorbance of Standard}} \times \text{Absorbance of Sample} \times 50$$

L-fraction protein on a wet weight basis of renal cortex was calculated by the equation:

$$\frac{\mu\text{g L-fraction Protein}}{\text{g renal cortex (wet weight)}} = \frac{\mu\text{g L-fraction protein}}{\mu\text{l L-fraction}} \times 1000$$

Enzyme Assays

The method used to determine NaK ATPase activity was based on that of Jorgenson (177) and Lo, et al. (61). NaK ATPase activity was derived from the activity generated in a complete system containing Na^+ , K^+ , and Mg^{++} minus the activity from a system omitting Na^+ and K^+ . The system devoid of Na^+ or K^+ represented Mg ATPase activity. NaK ATPase activity is defined by the hydrolysis of ATP in the presence of Na^+ , K^+ , and Mg^{++} minus the activity of Mg ATPase.

NaK ATPase activity was expressed as μ moles Pi/mg L-fraction protein/hr.

Reagents.

1. Sodium Chloride, 0.5 M: 7.305 g of NaCl was dissolved in glass distilled water and brought to a final volume of 250 ml.
2. Potassium Chloride, 0.010 M: 1.864 g of KCl was dissolved in glass distilled water and brought to a final volume of 250 ml.
3. Magnesium Chloride, 0.015 M: 1.167 g of $MgCl_2$ was dissolved and brought to a final volume of 250 ml with glass distilled water.
4. Tris, 0.5 M: 15.14 g of Tris was dissolved and brought to a final volume of 250 ml with glass distilled water.
5. Ethylenediamine tetraacetic acid (EDTA), 0.005 M: 0.365 g of EDTA was brought to a final volume of 250 ml with glass distilled water. Complete dissolution will result upon mixing with companion solutions and pH adjustment.
6. ATP (Tris salt), 0.03 M: 250 mg of Adenosine 5'-Triphosphate (Tris Salt) (Sigma Chemical Company, St. Louis, MO) was dissolved in 9.74 ml of glass distilled water. One ml aliquots were frozen for storage at $-90^{\circ}C$.
7. 30% Trichloroacetic Acid (TCA): 3 ml of 100% TCA was brought up to a final volume of 10 ml with glass distilled water.

Procedure. A complete system solution (pH 7.4) was prepared by mixing equal parts of the NaCl, KCl, MgCl, Tris, and EDTA aqueous solutions. A solution devoid of Na and K (pH 7.4) was prepared replacing the solutions of NaCl and KCl with equal volumes of water. The solutions were frozen in polyethylene bottles until needed for analysis.

For each sample, aliquots of the L-fraction were diluted with the homogenizing media to a final concentration of 250 μg protein/ml. From this diluted solution, 0.1 ml duplicate samples were collected and added to 0.9 ml of incubation medium containing a complete system (+ Na^+ , K^+ , and Mg^{++}). Additional 0.1 ml duplicate samples were added to 0.9 ml of the same medium devoid of NaCl and KCl (+ Mg^{++}). The tubes were vortexed gently on a Genie Vortex Mixer (American Scientific Products, Stone Mountain, GA) and 0.1 ml of 0.03 M ATP (Tris salt) was added to each tube to start the reaction. The contents of the reaction mixture tubes were again vortexed on a vortex mixer, then incubated in a water bath (Precision Scientific Co., Chicago, IL) at 37° for 15 min.

At the end of the incubation period, the reaction was terminated by immediately adding 0.2 ml of ice cold 30% TCA to each sample, then vortexing. Inorganic phosphate (Pi) was then determined by the method of Fiske and Subbarow (181). This NaK ATPase assay methodology provided a linear increase in Pi generation with membrane concentrations up to 75 μg protein/ml and a linear rate of production of Pi for 15 min.

Inorganic phosphate assay

The procedure of Fiske and Subbarow (181) was utilized to determine the inorganic phosphate content of the L-fraction. This method consists of the reaction of the L-fraction supernatant with ammonium molybdate in an acid solution to form phosphomolybdate. The phosphomolybdate is then reduced with a mixture of 1-amino-2-naphthol-4-sulfonic acid, sodium sulfate, and sodium bisulfite to form a blue phosphomolybdenum complex which is measured spectrophotometrically at 660 nm. Pi was determined following a timed incubation of both the complete (+ Na⁺, K⁺, and Mg⁺⁺) and incomplete systems (+ Mg⁺⁺) with ATP as described above.

Reagents.

1. Phosphate Standard: 1.098 g of potassium phosphate monobasic (Sigma Chemical Company, St. Louis, MO) was dissolved in 2.5 ml of 10 N sulfuric acid, then diluted to 250 ml with glass distilled water. Three ml of this stock standard was diluted to a final volume of 100 ml to give a working standard. A standard curve was prepared for each run by diluting 0 - 0.8 ml of working standard to a total volume of 2.5 ml with glass distilled water.
2. Acid Molybdate Reagent: 12.5 g of ammonium molybdate was dissolved in glass distilled water and brought to a volume of 100 ml. To this solution, 150 ml of 10 N sulfuric acid was slowly added. This mixture was then diluted to a final volume of 500 ml with glass distilled water.

3. 10 N of sulfuric acid:
4. Fiske and Subbarow Reducer purchased from Sigma Diagnostics (St. Louis, MO): 1-amino-2-naphthol-4 sulfonic acid, 0.8%, sodium sulfite and sodium bisulfite. Reconstitute 5 g in 31.5 ml of glass distilled water. Filter and store in an amber bottle at temperature 18 - 26°C.

Procedure. All glassware underwent a repetitious wash and rinse regimen consisting of 2 separate washes followed by 7 rinse cycles to ensure elimination of error introduced by phosphate residues. A 1.0 ml aliquot of the contents of the reaction mixture was pipetted from each tube and diluted to 5 ml with glass distilled water. From this diluted sample, a duplicate 2.5 ml sample was pipetted. To each tube, 0.5 ml of molybdate reagent was added. The contents were mixed by gentle vortexing on a Genie Vortex Mixer (American Scientific Products, Stone Mountain, GA). Two ml of Fiske and Subbarow solution was then pipetted into sample and standard tubes, and the tubes again vortexed. Tube contents were diluted with 3 ml of glass distilled water and allowed to stand for 5 min for color development. The absorbances were read at 660 nm with a Beckman Spectrophotometer (Model 34, Beckman Instruments, Fullerton, CA). Inorganic phosphate was calculated by the equation:

$$\mu \text{ moles Pi/mg L-fraction protein/hr} = \frac{\text{Concentration of Standard}}{\text{Absorbance of Standard}} \times$$

$$[\text{Ab. of complete System (Na}^+, \text{K}^+, \text{Mg}^+) - \text{Ab. of Mg ATPase System (Mg}^+)] \times 208$$

Growth Curves

Exponential growth curves were generated by computer for each animal using the Brody Equation (182). Values used to define this equation were 14 body weight points taken at regularly spaced intervals during the first year of life. Mathematically, the equation is expressed as:

$$W = A - Be^{-kt}$$

Where W = body weight in grams at any given age (t)

A = predicted mature body weight in grams

B = an integration constant

e = base of natural logarithms

k = relative growth rate with respect to growth yet to be made
(relative rate of maturation, proportionality constant)

t = age measured in weeks

A, B, and K values were calculated for each animal. Experimental group mean values for A, B and K were used to draw the mean growth curve for each group.

Statistical Methods

Data were analyzed by the General Linear Model (GLM) procedure of SAS (SAS Program, University of Tennessee Computing Center, 1989) (183). The class

variables of age, diet, and exercise were examined for main effects and interactions on each parameter evaluated. The regression equation was modeled to examine the continuous variable effects that were not accounted for by the class variables. Relationships between the class variables and the continuous variables were tested using Type I (sequential) and Type III (partial) sum of squares (SS) in multiple regression analysis. Non-linear relationships were examined by modeling for quadratic, or cubic effects. Path analysis was employed to answer the following questions, where Y equals the dependent variable and X equals the independent variables: 1) Was there an effect of X on Y (Type I); 2) Was there an effect of age, diet, or exercise on Y (Type I); 3) Was there a unique contribution of X on Y above and beyond that of age, diet, or exercise (Type III); and, 4) Was there a unique contribution of age, diet, or exercise on Y above and beyond that of X (Type III). Statistical "Model A" will model the equation: $Y = \text{age diet exercise X}$. "Model B" will model the equation: $Y = X \text{ age diet exercise}$. The significance of differences between groups for predicted relative growth rate and relative rate of maturation were evaluated using Duncan's multiple range test (184). Statistical significance was set at a probability level of 0.05 or less. Trends were evaluated using a probability level of less than 0.1.

CHAPTER IV

RESULTS

Body Weight

Calculated mean growth curves for all groups (A, AE, R, and RE) are shown in Figure 3. These curves were drawn using Brody's equation (182) -- substituting mean values of predicted mature body weight (A), an integration constant (B), and relative rate of maturation (k) calculated for each group into the equation. Table 1 presents mean mature body weight (A) and rate of maturation (k) for each group. Mean terminal body weights for each group are plotted at 12, 16, 20, 24, and 28 months of age in Figure 3 and are presented in Table 1. Statistical analyses of terminal body weights are shown in Table 2.

The general shapes of the calculated growth curves are very similar for A, AE, and R groups. The curve of group A is slightly higher than that of AE, and that of AE is higher than R or RE. The shapes of the growth curves of R and RE are different. The lower k value (.034) in combination with a higher predicted mature body weight (491.1 g) for RE rats compared to these values for R rats ($k = .052$; $A = 441.2$ g) is responsible for the difference in these growth curves ($p < .05$). The rate of approach to maturation in R rats was 1.5 times ($.052/.034$) that of RE. The mean predicted mature body weight (A) and maturational rates (k) for A and AE

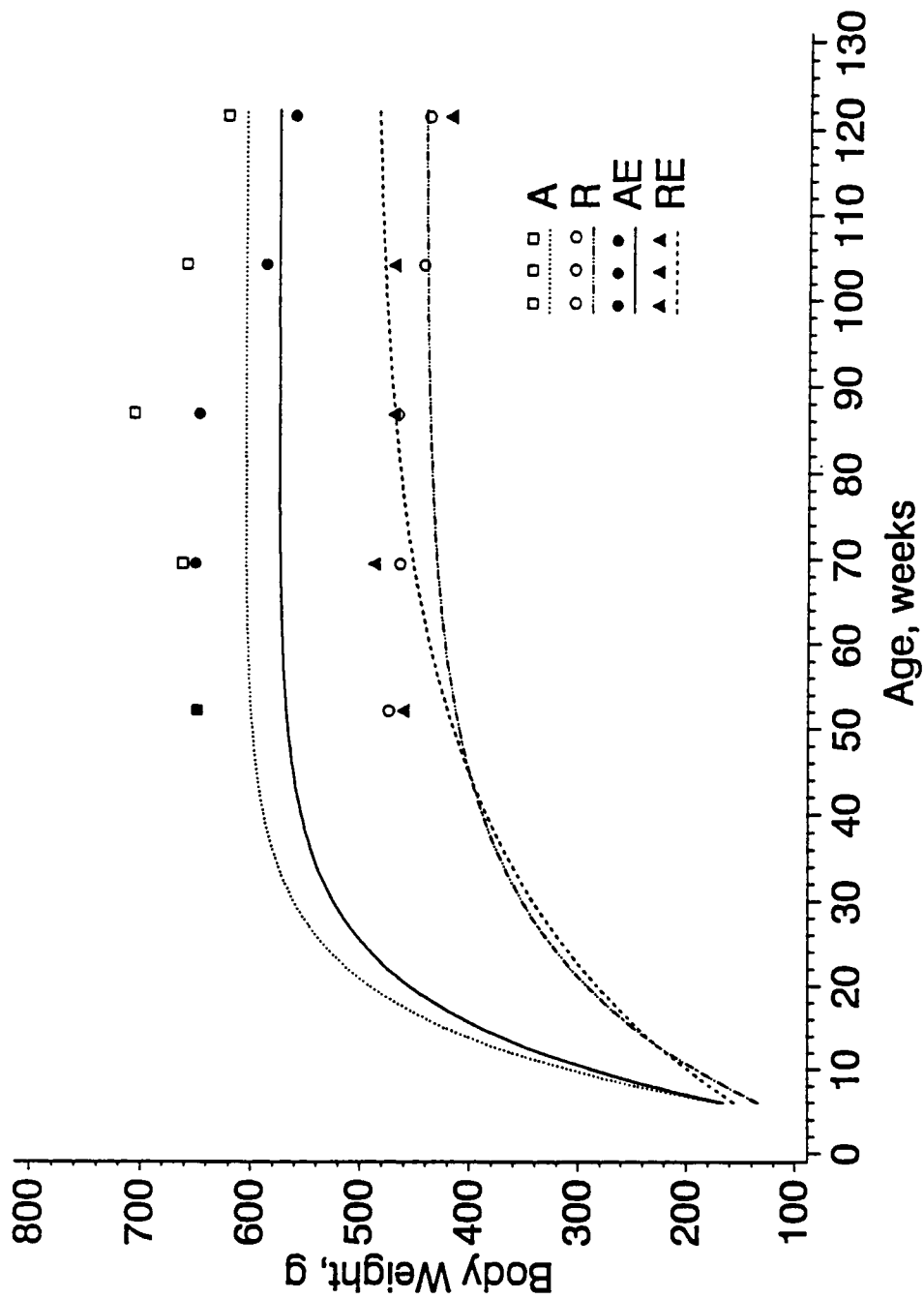


Figure 3. Effect of age, diet, and exercise on body weight. Curves shown represent calculated growth curves for each group. A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Symbols represent average group terminal body weight at 12, 16, 20, 24, and 28 months; in general, $n = 6$.

Table 1. Influence of Diet and Restriction on Growth Curve Parameters and Terminal Body Weight¹

Group	Growth Curve Parameters ²		Terminal Body Weight ³				
	Predicted Mature Body Wt. (g)	Relative Rate of Maturation	Age in Months				
			12	16	20	24	28
A ⁴	606.3 ± 16.8 ^a	.095 ± .005 ^a	649.2 ± 42.3	662.8 ± 35.3	707.7 ± 31.4	659.7 ± 61.9	622.7 ± 39.2
R ⁵	441.2 ± 5.6 ^b	.052 ± .002 ^b	474.0 ± 11.6	463.8 ± 11.8	466.0 ± 16.9	442.2 ± 8.4	437.7 ± 17.4
AE ⁶	575.9 ± 10.2 ^a	.087 ± .004 ^a	649.2 ± 10.8	651.2 ± 24.6	648.0 ± 30.5	587.2 ± 22.8	561.3 ± 31.5
RE ⁷	491.1 ± 22.5 ^c	.034 ± .002 ^c	459.0 ± 20.8	485.5 ± 19.0	467.8 ± 24.0	467.8 ± 37.4	416.5 ± 12.1

¹ Values are means ± SEM.

² Predicted mature body weight (A) and relative rate of maturation (k) in Brody's (182) equation. Means in a column with a common superscript are not significantly different from one another using Duncan's Multiple Range Test (p > .05).

³ In general, n = 6.

⁴ A = Ad libitum-fed, non-exercised.

⁵ R = Restricted-fed, non-exercised.

⁶ AE = Ad libitum-fed, exercised.

⁷ RE = Restricted fed, exercised.

Table 2. ANOVA Table for Terminal Body Weight

Variable					
Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age		4	53138.93	2.78	0.0310
	Linear	1	40543.01	8.76	0.0038
	Quadratic	1	24644.24	5.32	0.0230
	Cubic	1	15.37	0.00	0.9542
Diet		1	935927.05	195.95	0.0001
Exercise		1	10451.33	2.19	0.1423
Age*Diet		4	7287.24	0.38	0.8214
Age*Exercise		4	7286.18	0.38	0.8215
Diet*Exercise		1	13470.76	2.82	0.0963
Age*Diet*Exercise		4	10247.20	0.54	0.7093
Residuals		96	458522.50		

were significantly greater than those of R and RE ($p < 0.05$). The difference in predicted mature body weight or in relative rate of maturation between A and AE was not significant ($p > 0.05$). Food restriction of animals decreased the predicted mature body weight and rate of attaining mature body weight when compared to ad libitum feeding. Exercise resulted in a 35% decrease ($p < 0.05$) in the relative rate of maturation of the restricted-fed animals, but only an insignificant 8% decrease in ad libitum-fed animals.

Mean maximum terminal body weights of the ad libitum-fed animals, A and AE, 707.6 g and 651.2 g, respectively, were on average 15% higher than their predicted values. In contrast, the average R and RE mean maximum terminal body weights, 474.2 g and 485.5 g, respectively, more closely agreed with calculated values. Interestingly, the 12 to 28 month mean terminal body weight of RE animals were consistently lower than the predicted mature body weight (see Table 1). As indicated in the ANOVA table (Table 2), the terminal body weights of all groups decreased with age ($p < 0.05$). The age effect on body weight was primarily linear ($p < 0.005$), with a small quadratic component ($p < 0.05$). The loss in body weight began between 20 and 24 mo of age in non-exercised groups (A and R, respectively) and between 16 and 20 months in exercised groups (AE and RE, respectively). The terminal body weights of R and RE groups were significantly less than those of the A and AE groups ($p < 0.0001$). Exercise had no effect on terminal body weight.

Serum Thyroid Stimulating Hormone (TSH)

Serum TSH concentrations are graphed in Figure 4: Statistical analyses of these values are shown in Table 3. TSH concentration changed significantly with age ($p < 0.0001$). This change was expressed by a quadratic function ($p < 0.0001$). That is, TSH levels were higher in the 12- and 28-month groups than at the intermediate ages. Food restriction, in general, resulted in lower TSH values ($p < 0.005$). Mean TSH values for A and AE at 12 months were $2.13 \mu\text{IU/ml}$ while those of R and RE were 1.38 . At 24 months, mean values for ad libitum-fed (A and AE) animals and restricted-fed (R and RE) animals were $1.34 \mu\text{IU/ml}$ and $0.50 \mu\text{IU/ml}$, respectively. TSH values were higher at 28 than at 24 months. This increase was delayed by restriction. No effect of exercise on TSH serum levels was noted.

Thyroid Weight

Absolute values of mean thyroid weights (both glands) for all groups are shown in Table 4. Statistical analyses of these data are in Table 5. The most significant factor influencing thyroid weight was body weight ($p < 0.05$). There was a trend for mean thyroid mass to increase in linear fashion with age ($p < 0.1$). The lowest mean thyroid weight noted was 0.74 g (for R, at age 12 mo); the highest was 0.90 g (for AE, at age 20 mo).

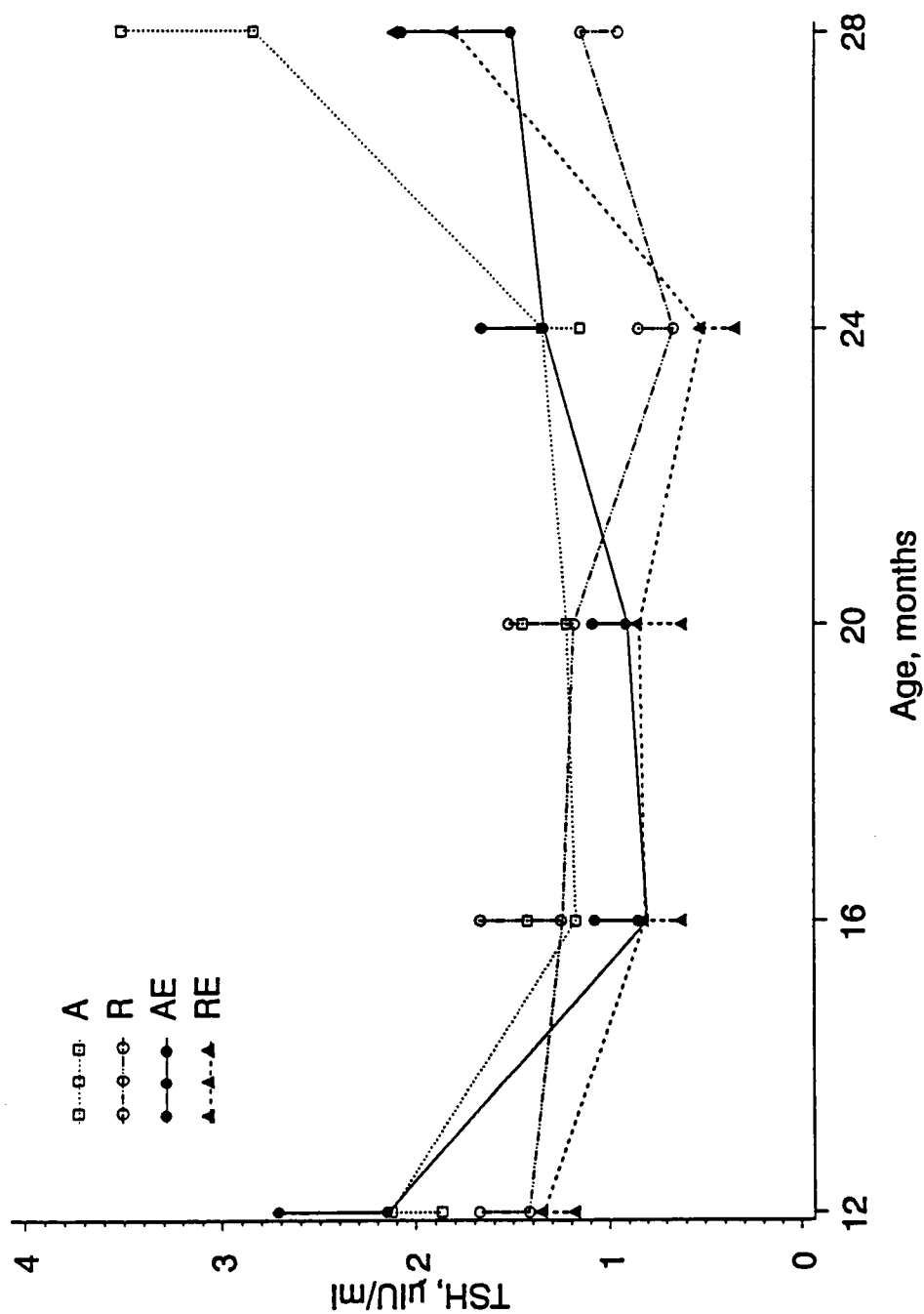


Figure 4. Effect of age, diet, and exercise on serum thyroid stimulating hormone concentration: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effects: age, $p < 0.0001$; diet, $p < 0.005$ in multiple regression analysis.

Table 3. ANOVA Table for Thyroid Stimulating Hormone

Variable					
Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age		4	16.058234	6.78	0.0001
	Linear	1	0.007520	0.01	0.9115
	Quadratic	1	16.041403	26.50	0.0001
	Cubic	1	0.129368	0.21	0.6449
Diet		1	5.533009	9.35	0.0029
Exercise		1	1.466533	2.48	0.1189
Age*Diet		4	3.536798	1.49	0.2105
Age*Exercise		4	0.635238	0.27	0.8977
Diet*Exercise		1	0.720826	1.22	0.2727
Age*Diet*Exercise		4	4.144884	1.75	0.1456
Residuals		93	55.054263		

Table 4. The Effect of Dietary Restriction, Exercise and Age on Thyroid Weight¹

Group	Age in Months			
	12	16	20	24
	g	g	g	g
A ²	0.77 ±0.06 ⁶	0.83 ±0.02	0.89 ±0.04	0.80 ±0.06
R ³	0.74 ±0.06	0.76 ±0.05	0.82 ±0.06	0.74 ±0.04
AE ⁴	0.84 ±0.06	0.88 ±0.06	0.90 ±0.03	0.82 ±0.04
RE ⁵	0.76 ±0.04	0.78 ±0.04	0.88 ±0.06	0.88 ±0.04
				0.84 ±0.06

¹ Weight represents both glands; $p < 0.05$ for body weight.

² A = Ad libitum-fed, non-exercised.

³ R = Restricted-fed, non-exercised.

⁴ AE = Ad libitum-fed, exercised.

⁵ RE = Restricted-fed, exercised.

⁶ Values are means ± SEM; in general, $n = 6$.

Table 5. ANOVA Table for Thyroid Weight

Variable					
Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age		4	0.1217675	2.12	0.0846
	Linear	1	0.0444063	3.16	0.0786
	Quadratic	1	0.0228320	1.62	0.2056
	Cubic	1	0.0195196	1.39	0.2416
Diet		1	0.0040241	0.28	0.5978
Exercise		1	0.0303202	2.11	0.1496
Body Weight		1	0.0721037	5.02	0.0274
TSH		1	0.0339792	2.37	0.1274
	Linear	1	0.0472389	3.30	0.0725
	Quadratic	1	0.0000132	0.00	0.9758
	Cubic	1	0.0207887	1.45	0.2311
Age*Diet		4	0.0268782	0.47	0.7590
Age*Exercise		4	0.0416075	0.72	0.5773
Diet*Exercise		1	0.0006638	0.05	0.8302
Age*Diet*Exercise		4	0.0248461	0.43	0.7846
Residuals		91	1.3062147		

Thyroid weight expressed as a percentage of terminal body weight is shown in Figure 5; statistical analyses follow in Tables 6 and 7. A significant effect of age was seen on the thyroid to body weight ratio ($p < 0.005$). The relationship was linear ($p < 0.0001$) and resulted in a mean increase of 24% in the R and RE animals' percentage, while that of A and AE increased 16% between the ages of 12 and 28 months. A significant diet effect was noted as well ($p < 0.0001$), with the percentage clearly larger for R and RE groups, than for A and AE. As observed in the sequential analysis of the parameters influencing the thyroid weight to body weight ratio (Table 7 - Type I SS), there is a significant effect of TSH on the ratio of thyroid weight to body weight that is linear in nature ($p < 0.0005$). However, there was no statistically significant effect of TSH on the thyroid to body weight ratio beyond the main effects of age, diet, and exercise (Table 6 - Type III SS). Thus, the effect of TSH on the ratio of thyroid weight to body weight is a function of the significant effects of age and diet. Also noteworthy is a slight influence of exercise ($p < 0.1$) upon the thyroid-body weight ratio. Animals that participated in the exercised regimen had, in general, a higher thyroid/body weight ratio than did sedentary animals.

Serum Thyroxine (T_4)

Figure 6 depicts the effects of age, diet, and exercise on basal serum thyroxine concentration: Tables 8 and 9 show the statistical analyses (ANOVA) of the data.

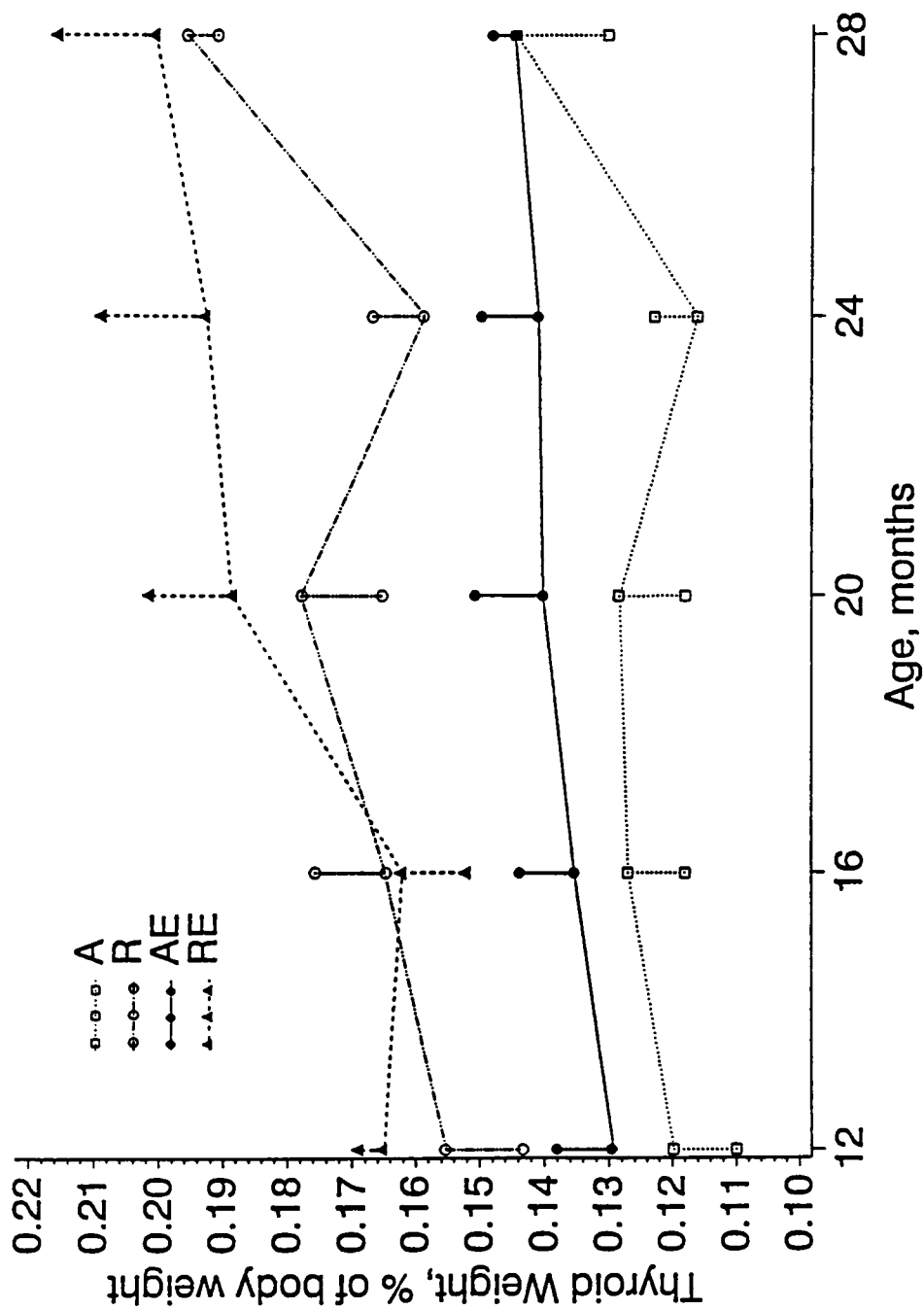


Figure 5. Effect of age, diet, and exercise on thyroid weight expressed as a percentage of body weight: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effects: age, $p < 0.005$; diet, $p < 0.0001$ in multiple regression analysis.

Table 6. ANOVA Table for Thyroid Weight to Terminal Body Weight Ratio -- Model A

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
Age	4				0.0107901	4.06	0.0044
Linear	1	0.0103282	16.16	0.0001			
Quadratic	1	0.0001018	0.16	0.6907			
Cubic	1	0.0010627	1.66	0.2002			
Diet	1	0.0523453	78.88	0.0001	0.0416848	62.81	0.0001
Exercise	1	0.0031954	4.82	0.0307	0.0020556	3.10	0.0817
TSH	1				0.0016472	2.48	0.1186
Linear	1	0.0015383	2.34	0.1294			
Quadratic	1	0.0000762	0.12	0.7341			
Cubic	1	0.0023712	3.61	0.0606			
Age*Diet	4	0.0020829	0.78	0.5380	0.0022660	0.85	0.4950
Age*Exercise	4	0.0014208	0.54	0.7102	0.0014677	0.55	0.6974
Diet*Exercise	1	0.0000206	0.03	0.8607	0.0000375	0.06	0.8125
Age*Diet*Exercise	4	0.0006264	0.24	0.9174	0.0006264	0.24	0.9174
Residuals	92	0.0610528					

Table 7. ANOVA Table for Thyroid Weight to Terminal Body Weight Ratio -- Model B

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
TSH	1				0.0016472	2.48	0.1186
Linear	1	0.0091340	13.91	0.0003			
Quadratic	1	0.0001283	0.20	0.6595			
Cubic	1	0.0022392	3.41	0.0681			
Age	4				0.0107901	4.06	0.0044
Linear	1	0.0101523	15.88	0.0001			
Quadratic	1	0.0030446	4.76	0.0314			
Cubic	1	0.0013832	2.16	0.1444			
Diet	1	0.0412473	62.16	0.0001	0.0416848	62.81	0.0001
Exercise	1	0.0025848	3.90	0.0514	0.0020556	3.10	0.0817
Age*Diet	4	0.0020829	0.78	0.5380	0.0022660	0.85	0.4950
Age*Exercise	4	0.0014208	0.54	0.7102	0.0014677	0.55	0.6974
Diet*Exercise	1	0.0000206	0.03	0.8607	0.0000375	0.06	0.8125
Age*Diet*Exercise	4	0.0006264	0.24	0.9174	0.0006264	0.24	0.9174
Residuals	92	0.0610528					

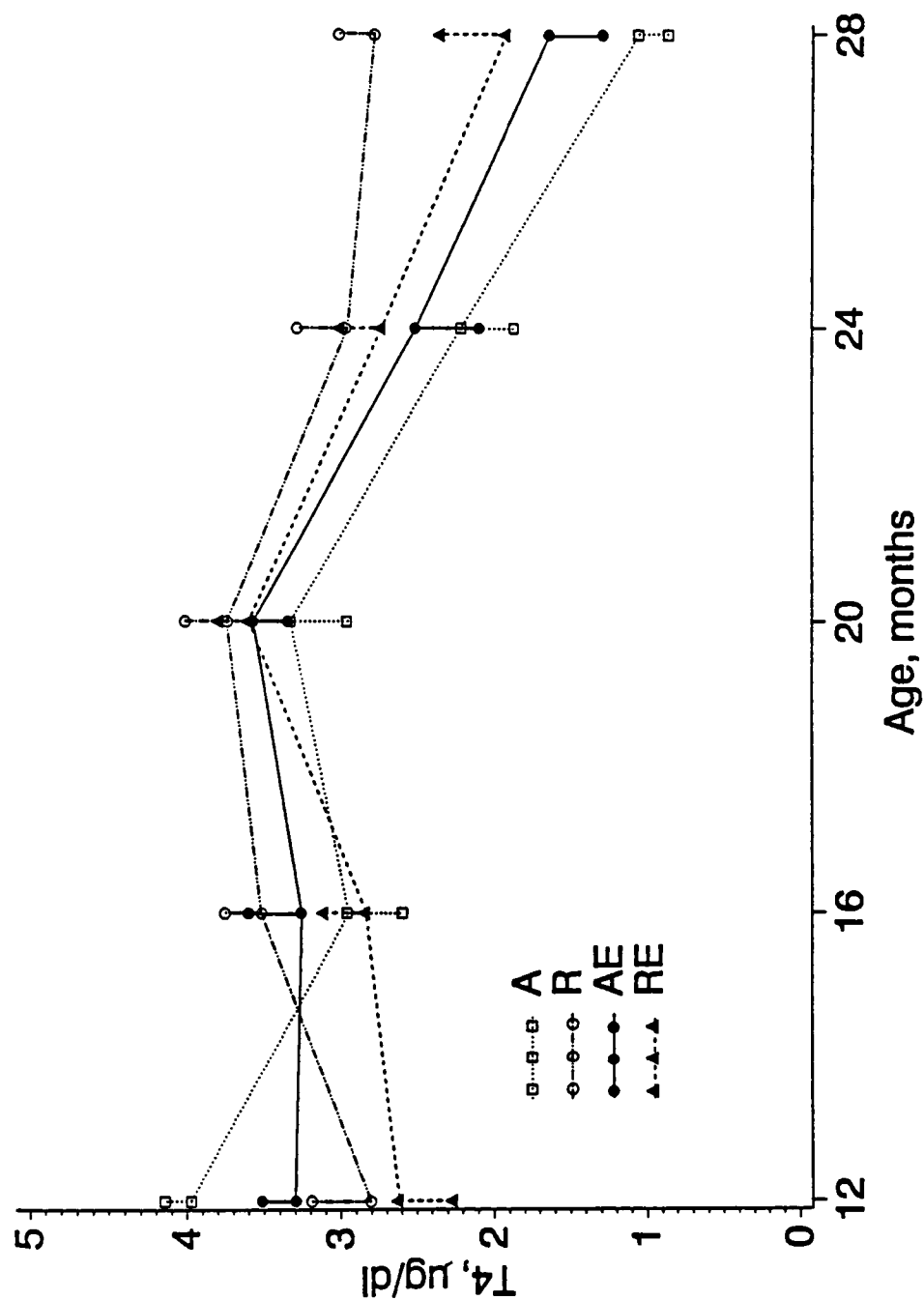


Figure 6. Effect of age, diet, and exercise on serum thyroxine concentration: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effects: age, $p < 0.0001$; age*diet, $p < 0.001$, in multiple regression analysis.

Table 8. ANOVA Table for Thyroxine (T_4) -- Model A

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
Age	4				29.493087	13.48	0.0001
Linear	1	18.261308	31.08	0.0001			
Quadratic	1	11.638027	19.81	0.0001			
Cubic	1	0.073813	0.13	0.7226			
Diet	1	0.658225	1.20	0.2756	0.003599	0.01	0.9355
Exercise	1	0.699180	1.28	0.2613	0.496013	0.90	0.3450
TSH	1				0.959914	1.75	0.1887
Linear	1	1.674136	3.02	0.0857			
Quadratic	1	0.024235	0.04	0.8348			
Cubic	1	1.621486	2.93	0.0907			
Thyroid Weight	1	1.975972	3.61	0.0606	2.221218	4.06	0.0469
Body Weight	1	0.335032	0.61	0.4359	0.067266	0.12	0.7267
Age*Diet	4	12.308351	5.63	0.0004	11.552196	5.28	0.0007
Age*Exercise	4	1.237976	0.57	0.6881	1.016894	0.46	0.7614
Diet*Exercise	1	1.286805	2.35	0.1286	1.603761	2.93	0.0903
Age*Diet*Exercise	4	2.189605	1.00	0.4116	2.189605	1.00	0.4116
Residuals	89	48.682908					

Table 9. ANOVA Table for Thyroxine (T_4) -- Model B

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
TSH	1				0.959914	1.75	0.1887
Linear	1	5.514027	9.95	0.0022			
Quadratic	1	0.073561	0.13	0.7164			
Cubic	1	2.009609	3.63	0.0601			
Thyroid Weight	1	2.861452	5.23	0.0246	2.221218	4.06	0.0469
Body Weight	1	0.995862	1.82	0.1807	0.067266	0.12	0.7267
Age	4				29.493087	13.48	0.0001
Linear	1	15.834132	26.95	0.0001			
Quadratic	1	7.558518	12.86	0.0005			
Cubic	1	0.015715	0.03	0.8704			
Diet	1	0.094936	0.17	0.6780	0.003599	0.01	0.9355
Exercise	1	0.794748	1.45	0.2313	0.493013	0.90	0.3450
Age*Diet	4	12.308351	5.63	0.0004	11.552196	5.28	0.0007
Age*Exercise	4	1.237976	0.57	0.6881	1.016894	0.46	0.7614
Diet*Exercise	1	1.286805	2.35	0.1286	1.603761	2.93	0.0903
Age*Diet*Exercise	4	2.189605	1.00	0.4116	2.189605	1.00	0.4116
Residuals	89	48.682908					

T_4 values decreased significantly as a result of age ($p < 0.0001$) in all groups. Noted was a linear ($p < 0.0001$) as well as quadratic ($p < 0.0001$) component of this relationship. Group A's T_4 values decreased from 4.0 $\mu\text{g/dl}$ at 12 months to 1.0 $\mu\text{g/dl}$ at 28 months, while AE decreased from 3.3 $\mu\text{g/dl}$ to 1.6 $\mu\text{g/dl}$ with the same age increment. AE values remained constant until 20 months of age, then decreased. Restricted-fed (R and RE) animals' T_4 values increased slightly from a mean of 2.7 $\mu\text{g/dl}$ at 12 months to 3.7 $\mu\text{g/dl}$ at 20 months. This was followed by a slight decrease to 2.8 $\mu\text{g/dl}$ at 28 months in R animals, and 1.9 $\mu\text{g/dl}$ in RE's. Therefore, T_4 values were essentially the same at 12 and 28 months for R, but decreased 27% in RE. There were no main effects of diet or exercise on T_4 concentration levels; however, a significant age by diet interaction was observed ($p < 0.001$). That is, as age increased, T_4 decreased faster in ad libitum-fed animals than in restricted animals. A trend ($p < 0.1$) for a diet by exercise influence on T_4 was also observed, i.e., the magnitude of the decrease in T_4 with age related to diet was dependent on the exercise levels. Also of note is a significant effect of thyroid weight on T_4 concentration ($p < 0.05$).

Examination of the Type I sum of squares (Table 9) reveals a significant linear effect of TSH on serum T_4 levels ($p < 0.005$). This analysis examined the relationship of TSH on T_4 independent of the other main factors examined in this study -- age, diet, and exercise. When the three main factors, however, are considered (Table 8 - Type III SS) it is shown that the influence of TSH on T_4 is mediated by the influence of age, diet, and exercise as the level set for significant

effects is no longer met by TSH. That is, there is no significant independent component of the relationship between TSH and T_4 that is not a direct result of age, diet, or exercise. Like TSH, a significant effect ($p < 0.05$) of thyroid weight upon T_4 concentration is observed in Table 9 (Type III SS). No influence of body weight on T_4 concentration level was observed.

Serum Triiodothyronine (T_3)

The relationship between age, diet, exercise and basal triiodothyronine (T_3) serum concentration is shown graphically in Figure 7, with the statistical analyses of the data shown in Tables 10 and 11. In general, aging (12 mo to 28 mo) was associated with decreased T_3 levels for all groups ($p < 0.0005$). T_3 concentrations of ad libitum-fed (A and AE) animals remained significantly higher than those of restricted-fed (R and RE) throughout the study ($p < 0.0001$). Overall, ad libitum T_3 values were 42% higher than those of the restricted group. The only age at which ad libitum-fed values were not greater than those of restricted-fed animals was at 28 months, where overlap of R and AE T_3 levels occurred. Age effects upon T_3 had significant ($p < 0.05$) quadratic and cubic components. Exercise resulted in significantly ($p < 0.0001$) lower serum T_3 concentration levels for both AE and RE when compared to their non-exercised controls. T_3 levels of AE and RE groups were 22 and 25% lower than those of A and R groups, respectively. Terminal body weight was shown to have a significant relationship with T_3 concentration ($p < 0.05$); that

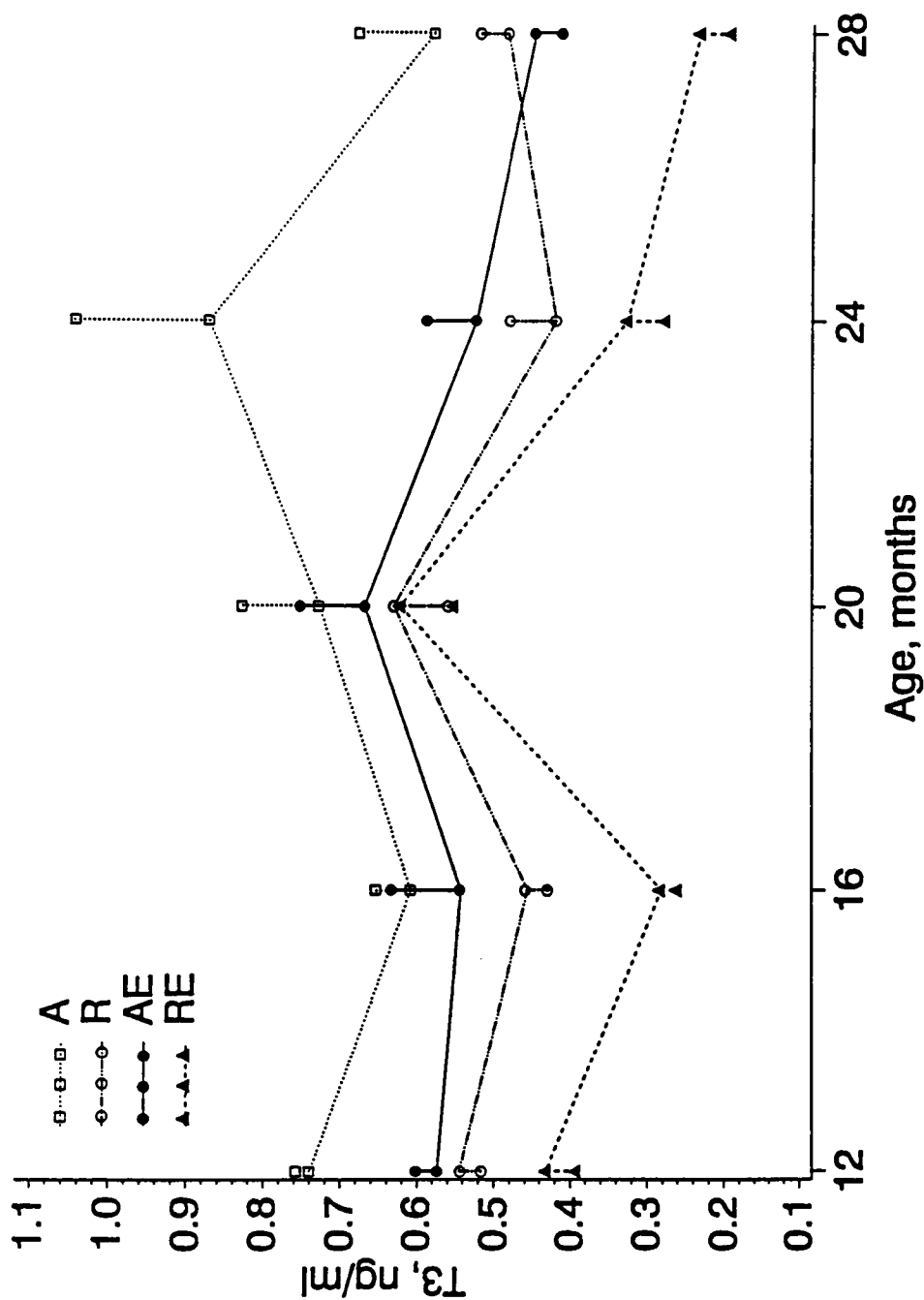


Figure 7. Effect of age, diet, and exercise on serum triiodothyronine concentration: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effects: age, $p < 0.0005$; diet, $p < 0.0001$; exercise, $p < 0.0001$ in multiple regression analysis.

Table 10. ANOVA Table for Triiodothyronine (T₃) -- Model A

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
Age	4				0.4982583	5.57	0.0005
Linear	1	0.0474857	1.74	0.1902			
Quadratic	1	0.1109656	4.07	0.0465			
Cubic	1	0.1285948	4.71	0.0324			
Diet	1	1.1325751	50.61	0.0001	0.8224045	36.75	0.0001
Exercise	1	0.6328949	28.28	0.0001	0.5414551	24.20	0.0001
T ₄	1				0.0114612	0.51	0.4761
Linear	1	0.0199963	0.89	0.3489			
Quadratic	1	0.0021144	0.09	0.7601			
Cubic	1	0.0082223	0.36	0.5474			
TSH	1				0.0226480	1.01	0.3172
Linear	1	0.0434805	1.91	0.1704			
Quadratic	1	0.0040355	0.18	0.6746			
Cubic	1	0.0257225	1.13	0.2906			
Thyroid Weight	1	0.1901593	8.50	0.0045	0.1470885	6.57	0.0120
Body Weight	1	0.0991914	4.43	0.0381	0.1306743	5.84	0.0177
Age*Diet	4	0.1554129	1.74	0.1491	0.1393032	1.56	0.1931
Age*Exercise	4	0.1774031	1.98	0.1042	0.1793429	2.00	0.1009
Diet*Exercise	1	0.0485155	2.17	0.1445	0.0393850	1.76	0.1880
Age*Diet*Exercise	4	0.1642451	1.83	0.1292	0.1642451	1.83	0.1292
Residuals	88	1.9691580					

Table 11. ANOVA Table for Triiodothyronine (T₃) -- Model B

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
T ₄	1				0.0114612	0.51	0.4761
Linear	1	0.1730412	7.68	0.0068			
Quadratic	1	0.0740525	3.29	0.0734			
Cubic	1	0.0070954	0.31	0.5762			
TSH	1				0.0226480	1.01	0.3172
Linear	1	0.3294955	14.49	0.0003			
Quadratic	1	0.0225796	0.99	0.3219			
Cubic	1	0.0809248	3.56	0.0626			
Thyroid Weight	1	0.0364283	1.63	0.2053	0.1470885	6.57	0.0120
Body Weight	1	0.5798697	25.91	0.0001	0.1306743	5.84	0.0177
Age	4				0.4982583	5.57	0.0005
Linear	1	0.0316699	1.16	0.2840			
Quadratic	1	0.0963097	3.53	0.0632			
Cubic	1	0.0875124	3.21	0.0765			
Diet	1	0.6220324	27.80	0.0001	0.8224045	36.75	0.0001
Exercise	1	0.5282455	23.61	0.0001	0.5414551	24.20	0.0001
Age*Diet	4	0.1554129	1.74	0.1491	0.1393032	1.56	0.1931
Age*Exercise	4	0.1774031	1.98	0.1042	0.1793429	2.00	0.1009
Diet*Exercise	1	0.0485155	2.17	0.1445	0.0393850	1.76	0.1880
Age*Diet*Exercise	4	0.1642451	1.83	0.1292	0.1642451	1.83	0.1292
Residuals	88	1.9691580					

is, as body weight decreased so did T_3 . This effect was independent of any other factor acting upon T_3 (Table 10 - Type III SS). A significant effect of thyroid weight on T_3 serum levels was also noted ($p < 0.05$).

As shown by the Type I and Type III sum of squares (Tables 10 and 11), age, diet, and exercise had significant independent effects on T_3 serum levels. In contrast, T_4 (Table 11 - Type I SS) had a significant linear effect on T_3 when it was assessed independently of the effects of age, diet, and exercise ($p < 0.01$). A trend for a quadratic ($p < 0.1$) component was noted as well. In general, as T_4 decreased, T_3 decreased as well. This effect, however, was negated when age, diet, and exercise effects were included in the analyses (Table 11 - Type III SS). The TSH relationship with T_3 was similar to that of T_4 . A significant linear effect ($p < 0.0005$) on T_3 levels by TSH was observed (Table 11 - Type I SS) even after the effect of T_4 on T_3 was considered, and when age, diet, and exercise effects were not yet considered. When the effect of the main experimental variables of age, diet, and exercise were considered, however, TSH and T_4 effects were not significant (Table 11 - Type III SS).

Serum T_3/T_4 Ratio

The T_3/T_4 concentration for each animal was calculated and a mean value for each group was derived. These group means are shown in Table 12. Little change in the ratio of T_3 to T_4 was noted for R and RE with advancing age. Ad libitum-fed animals' (A and AE) ratios, however, increased significantly with age ($p < 0.05$).

Table 12. The Effect of Dietary Restriction, Exercise and Age¹ on the Ratio of Triiodothyronine (T₃) to Thyroxine (T₄) Concentrations

Group	Age in Months					
	12	16	20	24	28	
A ²	.19 ±.01 ⁶	.22 ±.03	.22 ±.03	.43 ±.10	.75 ±.16	
R ³	.23 ±.05	.14 ±.02	.18 ±.03	.15 ±.03	.18 ±.02	
AE ⁴	.18 ±.01	.18 ±.04	.19 ±.02	.24 ±.05	.31 ±.09	
RE ⁵	.17 ±.02	.10 ±.01	.17 ±.02	.12 ±.02	.15 ±.03	

¹ p < 0.05 for diet, exercise, and age.

² A = Ad libitum-fed, non-exercised.

³ R = Restricted-fed, non-exercised.

⁴ AE = Ad libitum-fed, exercised.

⁵ RE = Restricted-fed, exercised.

⁶ Values are means ± SEM.

Sedentary, ad libitum-fed animals' T_3/T_4 ratio was 0.19 at 12 months and increased to 0.75 at 28 months. Ad libitum-fed, exercised animals' values were 0.18 and 0.31 at 12 and 28 months, respectively. An exercise effect was also observed -- exercised animals' values were lower than those of non-exercised animals.

Kidney Weight

Table 13 shows the mean renal mass of both kidneys in the experimental groups; results of statistical analyses of the data are shown in Table 14. Dietary restriction resulted in a reduction in renal mass in the R and RE groups when compared to A and AE ($p < 0.01$). Group A's mean renal mass was 20% greater than R at 12 months and was 47% greater at 28 months of age. Renal mass of AE was 32% greater than that of RE at 12 months and 44% greater than RE at 28 months. Kidney weights increased linearly with age in all groups ($p < 0.0001$). Age was associated with a 14% increase in restricted animals' (R and RE) renal weight from 12 to 28 months, a 24% increase in AE's, and a 41% increase in A's during the same time period. Exercised animals, AE and RE, had an overall increase in renal mass of 7% ($p < 0.01$) when compared to non-exercised animals, A and R.

A significant relationship existed between renal mass and body weight ($p < 0.005$, Table 14). In Figure 8, renal weight as a percentage of body weight is plotted versus age. Statistical analyses of these data are shown in Table 15. The renal weight to body weight percentages were significantly greater ($p < 0.005$) in the

Table 13. The Effect of Dietary Restriction, Exercise and Age on Kidney Weight¹

Group	Age in Months			
	12	16	20	24
	g	g	g	g
A ²	4.04 ±0.11 ⁶	4.40 ±0.24	4.82 ±0.20	4.75 ±0.29
R ³	3.38 ±0.09	3.35 ±0.20	3.60 ±0.19	3.50 ±0.17
AE ⁴	4.56 ±0.06	4.62 ±0.18	4.67 ±0.28	5.21 ±0.36
RE ⁵	3.46 ±0.23	3.68 ±0.13	4.03 ±0.25	4.37 ±0.41
				3.94 ±0.33

¹ Weight of both kidneys; $p < 0.05$ for diet, exercise, and age.

² A = Ad libitum-fed, non-exercised.

³ R = Restricted-fed, non-exercised.

⁴ AE = Ad libitum-fed, exercised.

⁵ RE = Restricted-fed, exercised.

⁶ Values are means ± SEM; in general, $n = 6$.

Table 14. ANOVA Table for Kidney Weight

Variable					
Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age		4	14.688732	9.15	0.0001
	Linear	1	9.497747	24.87	0.0001
	Quadratic	1	0.003488	0.01	0.9241
	Cubic	1	0.015580	0.04	0.8403
Diet		1	2.967745	7.40	0.0078
Exercise		1	3.251901	8.11	0.0054
Body Weight		1	4.427063	11.04	0.0013
Age*Diet		4	3.417152	2.13	0.0831
Age*Exercise		4	1.230576	0.77	0.5494
Diet*Exercise		1	0.005474	0.01	0.9073
Age*Diet*Exercise		4	0.454662	0.28	0.8881
Residuals		95	38.106407		

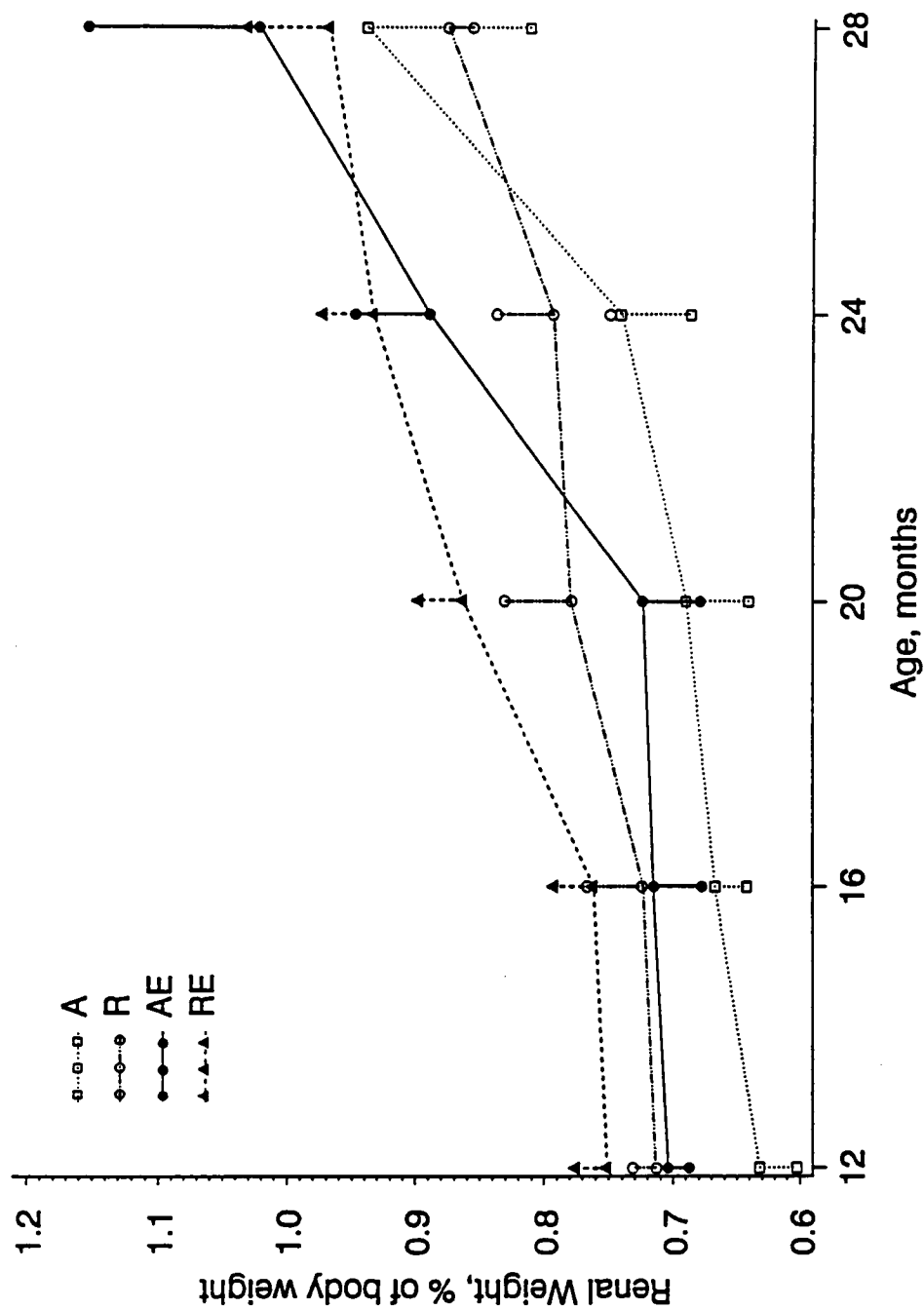


Figure 8. Effect of age, diet, and exercise on renal weight expressed as a percentage of body weight: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effects: age, $p < 0.0001$; exercise, $p < 0.005$ in multiple regression analysis.

Table 15. ANOVA Table for Kidney Weight to Terminal Body Weight Ratio¹

Variable					
Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age		4	0.8530927	14.28	0.0001
	Linear	1	0.7717272	54.78	0.0001
	Quadratic	1	0.0526035	3.73	0.0561
	Cubic	1	0.0002851	0.02	0.8872
Diet		1	0.0478539	3.20	0.0766
Exercise		1	0.1603594	10.74	0.0015
Age*Diet		4	0.0961699	1.61	0.1781
Age*Exercise		4	0.0390207	0.65	0.6260
Diet*Exercise		1	0.0001003	0.01	0.9348
Age*Diet*Exercise		4	0.0059904	0.10	0.9821
Residuals		96	1.4337471		

¹ ratio expressed as a percentage.

exercised groups (AE and RE) than in the non-exercised groups (A and R). In general, the renal weight to body weight ratio increased linearly with age ($p < 0.0001$). The effects of diet were marginally significant ($p < 0.1$) with ad libitum-fed groups' kidney to body weight ratios in general being less than those of restricted-fed groups.

Sodium Potassium Adenosine Triphosphatase (NaK ATPase)

Renal cortex L-fraction protein

The membrane-rich renal cortex L-fraction protein (mg/g wet weight renal cortex) content is shown in Figure 9: statistical analyses follows in Tables 16 and 17. L-fraction protein decreased linearly with age ($p < 0.0001$) as shown in Table 16. The mean L-fraction protein content for all groups was 10.35 mg/g wet weight renal tissue at 12 months of age. This value decreased to mean values of 8.55 and 6.76 mg/g wet weight renal tissue at 28 months for restricted and ad libitum-fed animals, respectively. There were no significant effects of diet or exercise on L-fraction protein.

Evaluation of the Type I sum of squares revealed a significant effect of T_3 and T_4 on L-fraction protein content (Table 17). The significant effect of T_3 on protein content was expressed by a quadratic ($p < 0.05$) and cubic ($p < 0.05$) relationship; while that of T_4 was linear in nature ($p < 0.05$). As shown in Table 16 there was no significant effect of T_4 on protein content over and above that of the main effects of

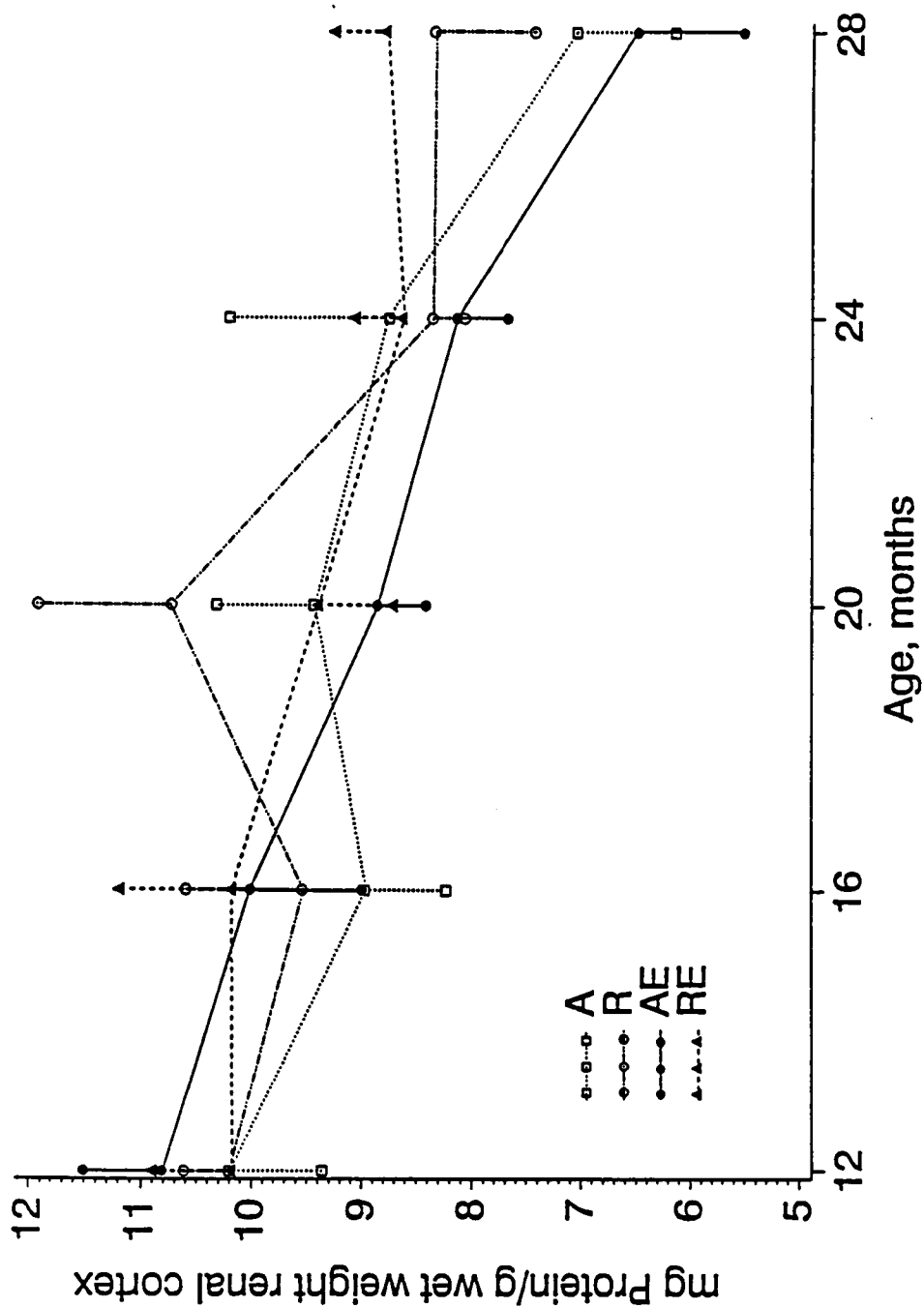


Figure 9. Effect of age, diet, and exercise on renal cortex L-fraction protein content: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effect: age, $p < 0.01$ in multiple regression analysis.

Table 16. ANOVA Table for Renal Cortex L-Fraction Protein -- Model A

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
Age	4				64.429907	3.95	0.0053
Linear	1	77.098605	19.41	0.0001			
Quadratic	1	0.764158	0.19	0.6619			
Cubic	1	0.006150	0.00	0.9687			
Diet	1	5.211879	1.28	0.2610	2.338419	0.57	0.4506
Exercise	1	0.002572	0.00	0.9800	0.634880	0.16	0.6939
T ₃	1				1.276259	0.31	0.5770
Linear	1	0.678086	0.18	0.6740			
Quadratic	1	4.515080	1.19	0.2790			
Cubic	1	23.999186	6.30	0.0138			
T ₄	1				0.016539	0.00	0.9493
Linear	1	0.282325	0.07	0.7942			
Quadratic	1	2.595170	0.63	0.4298			
Cubic	1	0.004412	0.00	0.9740			
Age*Diet	4	10.378722	0.64	0.6374	10.478739	0.64	0.6331
Age*Exercise	4	9.799753	0.60	0.6625	10.134786	0.62	0.6480
Diet*Exercise	1	0.036164	0.01	0.9251	0.153620	0.04	0.8465
Age*Diet*Exercise	4	5.929769	0.36	0.8337	5.929769	0.36	0.8337
Residuals	91	370.66695					

Table 17. ANOVA Table for Renal Cortex L-Fraction Protein -- Model B

Variable	df	Type I			Type III		
		Sum of Squares	F value	p-value	Sum of Squares	F value	p-value
T ₃	1				1.276259	0.31	0.5770
Linear	1	0.000218	0.00	0.9978			
Quadratic	1	19.371377	5.09	0.0265			
Cubic	1	17.904923	4.70	0.0328			
T ₄	1				0.016539	0.00	0.9493
Linear	1	25.773793	6.25	0.0143			
Quadratic	1	5.207645	1.26	0.2642			
Cubic	1	0.001905	0.00	0.9829			
Age	4				64.429907	3.95	0.0053
Linear	1	54.863529	13.82	0.0003			
Quadratic	1	0.288818	0.07	0.7880			
Cubic	1	0.009129	0.00	0.9619			
Diet	1	2.353433	0.58	0.4492	2.338419	0.57	0.4506
Exercise	1	0.132357	0.03	0.8573	0.634880	0.16	0.6939
Age*Diet	4	10.378722	0.64	0.6374	10.478739	0.64	0.6331
Age*Exercise	4	9.799753	0.60	0.6625	10.134786	0.62	0.6480
Diet*Exercise	1	0.036164	0.01	0.9251	0.153620	0.04	0.8465
Age*Diet*Exercise	4	5.929769	0.36	0.8337	5.929769	0.36	0.8337
Residuals	91	370.66695					

age, diet, and exercise. However, even after the sequential effects of age, diet, and exercise were taken into consideration, there was still a significant effect of T_3 on protein content that is expressed by a cubic relationship ($p < 0.05$).

NaK ATPase activity

Mean NaK ATPase activity values expressed as $\mu\text{M Pi/mg L-fraction protein/hour}$ are recorded in Table 18. The statistical analyses are shown in Table 19. No main effects of age, diet, or exercise upon NaK ATPase were observed; however, a significant age by diet interaction was observed ($p < 0.05$). The trend ($p < 0.1$) for a main age effect upon enzyme activity (Type III SS) was shown to be an expression of a significant quadratic ($p < 0.05$) age effect upon NaK ATPase. A significant quadratic T_4 effect ($p < 0.005$) upon NaK ATPase was observed.

NaK ATPase activity expressed as $\mu\text{M Pi/g wet weight renal cortex/hour}$ is shown in Figure 10. Statistical analyses are shown in Table 20. NaK ATPase activity, expressed per wet weight of cortex tissue, decreased linearly with age ($p < 0.0005$). Neither diet nor exercise had a significant effect on enzyme activity. By 20 months of age, the renal enzyme activity of ad libitum-fed animals (A and AE) decreased 60% of its 12 month value. During the same time period, the restricted animals' NaK ATPase activity decreased only 8%. NaK ATPase activity remained low in ad libitum-fed animals for the remainder of the study, whereas, the enzyme activity of restricted (R and RE) animals did not exhibit further decline until 28 months. At 28 months, both restricted and ad libitum-fed animals had similar low values of 114.5

Table 18. The Effect of Dietary Restriction, Exercise and Age on NaK ATPase Activity¹

Group	Age in Months				
	12	16	20	24	28
	$\mu\text{M Pi/mg protein/hr}$				
A ²	17.70 $\pm 2.86^6$	14.47 ± 1.24	11.43 ± 1.18	12.92 ± 1.74	17.15 ± 2.03
R ³	15.61 ± 2.43	14.02 ± 1.28	12.42 ± 0.96	16.40 ± 1.08	12.94 ± 1.94
AE ⁴	16.33 ± 1.70	15.43 ± 1.32	12.30 ± 1.57	13.94 ± 1.12	17.80 ± 2.20
RE ⁵	14.88 ± 1.88	13.56 ± 0.46	15.26 ± 0.83	16.99 ± 0.92	13.52 ± 1.21

¹ expressed as $\mu\text{M Pi/mg L-fraction protein/hr}$.

² A = Ad libitum-fed, non-exercised.

³ R = Restricted-fed, non-exercised.

⁴ AE = Ad libitum-fed, exercised.

⁵ RE = Restricted-fed, exercised.

⁶ Values are means $\pm$ SEM; in general, n = 6.

Table 19. ANOVA Table for NaK ATPase Activity -- Model I¹

Variable		Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age				4	123.44451	2.05	0.0946
	Linear			1	11.00088	0.77	0.3837
	Quadratic			1	72.79587	5.07	0.0266
Diet				1	24.80972	1.73	0.1919
	Cubic			1	0.94713	0.06	0.8000
Exercise				1	4.33798	0.29	0.5931
	Linear			1	0.39101	0.03	0.8730
	Quadratic			1	0.13648	0.01	0.9248
T ₃				1	1.83262	0.12	0.7295
	Cubic			1			
T ₄				1	0.30004	0.02	0.8847
	Linear			1	130.54756	9.21	0.0032
	Quadratic			1	4.71801	0.33	0.5655
Age*Diet				4	175.00817	2.90	0.0262
	Cubic			4	29.73812	0.49	0.7410
Age*Exercise				1	0.13049	0.01	0.9261
	Quadratic			1	7.49069	0.12	0.9734
	Cubic			1			
Diet*Exercise				4			
	Cubic			4			
	Quadratic			4			
Age*Diet*Exercise				4			
	Cubic			4			
	Quadratic			4			
Residuals				90	1357.73342		

¹ NaK ATPase activity expressed as $\mu\text{M Pi/mg L-fraction protein/hr.}$

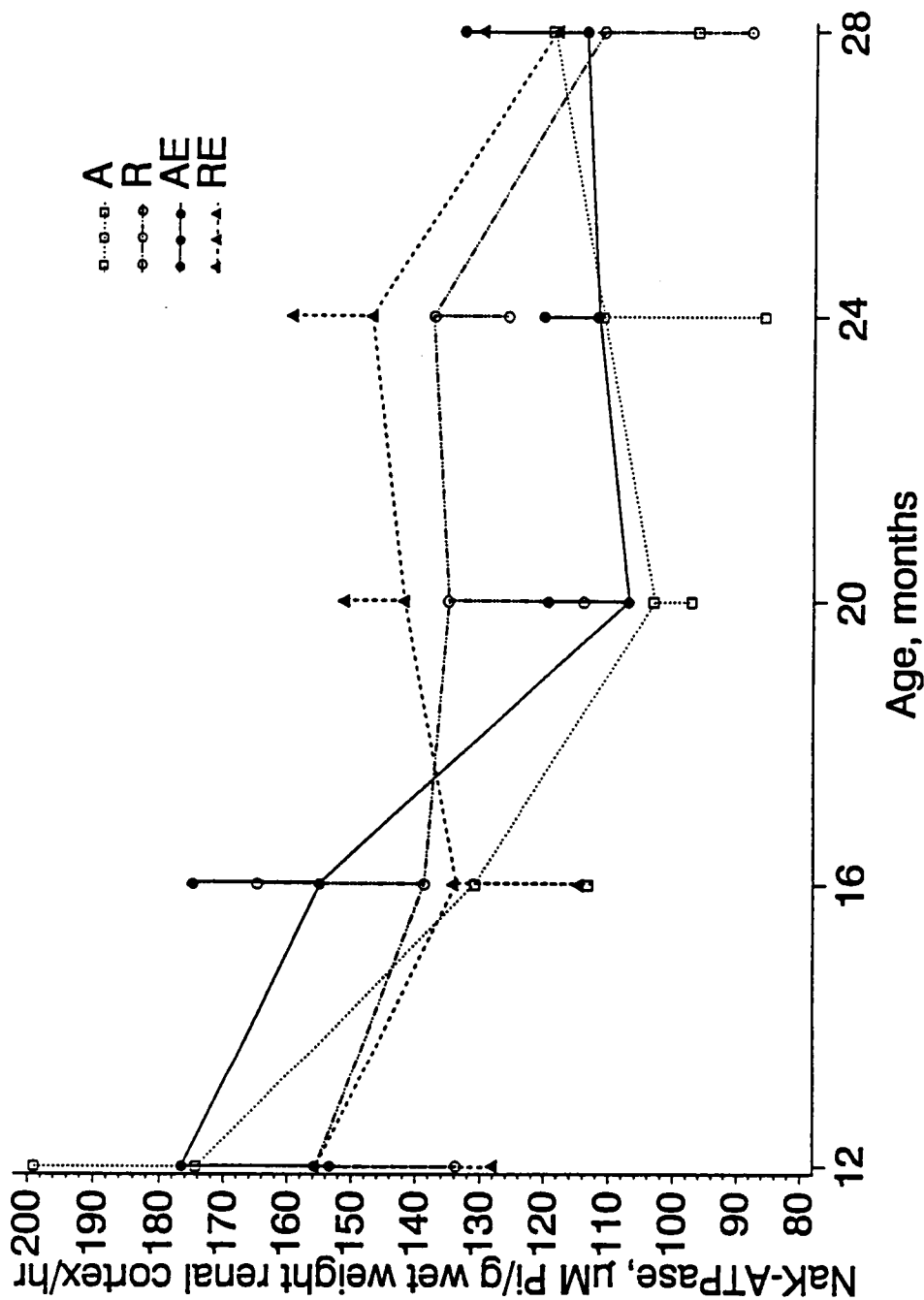


Figure 10. Effect of age, diet, and exercise on sodium potassium ATPase activity: A = ad libitum-fed controls; R = restricted-fed, non-exercised; AE = ad libitum-fed, exercised; RE = restricted-fed, exercised. Values are means $\pm$ SEM, in general $n = 6$. Significant effect: age, $p < 0.005$ in multiple regression analysis.

Table 20. ANOVA Table for NaK ATPase Activity -- Model II¹

Variable					
Type III SS	Type I SS	df	Sum of Squares	F Value	p Value
Age		4	32032.117	3.57	0.0094
	Linear	1	27054.580	12.89	0.0005
	Quadratic	1	5047.763	2.40	0.1243
	Cubic	1	1722.752	0.82	0.3673
Diet		1	350.363	0.16	0.6935
Exercise		1	12.139	0.01	0.9415
T ₃		1	351.237	0.16	0.6923
	Linear	1	1167.895	0.52	0.4710
	Quadratic	1	5642.838	2.53	0.1151
T ₄		1	423.010	0.19	0.6634
	Linear	1	6400.125	2.88	0.0929
	Quadratic	1	1100.909	0.50	0.4830
	Cubic	4	11950.541	1.33	0.2639
Age*Diet		4	326.345	0.04	0.9974
Age*Exercise		4	29.305	0.01	0.9092
Diet*Exercise		1	1192.800	0.13	0.9699
Age*Diet*Exercise		4			
Residuals		90	201754.347		

¹ NaK ATPase activity expressed as $\mu\text{M Pi/g}$ wet weight renal cortex/hr.

and 116.5 μ M Pi/g wet weight renal cortex/hour, respectively. A trend ($p < 0.1$) for a quadratic relationship between T_4 and Nak ATPase activity was noted (see Table 20).

CHAPTER V

DISCUSSION

The results of the present study support the hypotheses that the components of the pituitary-thyroid axis undergo a decrement in function with age. Support for this finding is provided by the decreased T_4 and decreased T_3 serum concentrations noted with aging in both ad libitum-fed and restricted-fed groups. A decline in Nak ATPase activity also supports the hypothesis. The finding of decreased T_3 and T_4 serum concentrations agrees with the results of several investigators (35,37, 43), while disagreeing with those of others (49,52). Reasons for these discrepant findings may be the result of differing experimental models, protocol implementation variability, or varied measurement methodologies.

Ad libitum-fed sedentary control animals had the highest serum T_4 and T_3 levels of the groups studied at 12 months of age. A and AE groups had the highest TSH serum levels at 12 months, as well as the largest thyroid gland weights. By 28 months of age, the ad libitum-fed, sedentary animals showed a 70% decrease in serum T_4 levels -- the largest decrement of any group evaluated. T_3 values of A and AE declined 22% during the same time period. In contrast, T_4 serum concentration levels decreased, on average, 13% between 12 and 28 months in the restricted groups. During this same time span, R serum T_3 levels declined only 11%. Thus, in

general, peripheral thyroid hormone concentration of ad libitum-fed animals exhibited a significantly greater decrement with advancing age than restricted-fed animals.

Interestingly, TSH concentration levels in ad libitum-fed sedentary controls remained at a consistently higher absolute level than those of the restricted-fed groups throughout the duration of the study. While there was little variability in TSH values for all groups at 16 and 20 months of age, TSH values of ad libitum-fed groups (A and AE) began to increase at 20 months of age. This increase in values was delayed by restriction in R and RE groups. To the author's knowledge, no other studies have examined, at regularly scheduled intervals, TSH serum values with aging over time. However, a number of investigators have observed no change in TSH serum levels with age in cross sectional studies (35,37,43,45,46). Others have found either increased (47) or decreased (44,48) values. The present study suggests that the pituitary-thyroid axis feedback system remains intact with aging. This is substantiated by the pituitary's ability to respond to stimuli throughout the aging process, as indicated by the increase in TSH values at 24 or 28 months. This finding has been substantiated by others (36). The nature of the stimuli upon the pituitary that is evoking this response may be centrally (TRH) or peripherally (T_4 , T_3) mediated. Possibilities include a feed-forward reaction to the depressed feedback inhibition by serum T_4 or T_3 . This appears to be the mechanism at work in this instance -- as the decreased serum T_4 and T_3 levels appear to be accompanied by an increase in TSH values.

It must be acknowledged that a concomitant increase in T_4 and T_3 serum levels due to the increased TSH levels did not occur. A possible explanation may be that the TSH that is produced with advancing age consists largely of biologically inactive "big TSH" as suggested by Klug and Adelman (185). If indeed this is the case, the immunoassayable TSH concentration may well be increased, but will not be representative of biologically active TSH that may decrease with age. Another possibility is that the functionality of the thyroid gland itself may decrease with age, resulting in decreased thyroïdal activity (41). There is evidence to support this finding, as the thyroid gland does not appear to be making an appropriate response to the increased TSH serum concentration levels observed. There was no difference in thyroid weight of the different groups due to age, food restriction, or exercise. Thyroid weight changes during the course of this study varied unevenly. Body weight was the only parameter shown to have a significant effect upon absolute thyroid weights. When thyroid weights were expressed as a percentage of body weight, restricted-fed animals clearly had more thyroid mass per unit body weight than did ad libitum-fed animals. This finding was unexpected, but is very interesting. Ad libitum-fed, sedentary (A) rats had the smallest thyroid mass per gram of body weight. Both increased (40) and decreased (16) thyroid mass with aging have been reported. Collagen deposition was cited as a primary causative agent in age-associated thyroid hypertrophy: a causal relationship between decreased thyroid function and increased collagen deposition has been suggested (16,40). Histological evaluation of the thyroids was not performed as an aspect of this study. This is

unfortunate, as the thyroid weight data, when considered in conjunction with the serum thyroid hormone levels suggests that the information that may have been attained would help to further explain the control mechanism at work.

Path analysis was employed to evaluate the effect of all parameters examined on the pituitary-thyroid axis. Therefore, the experimental variables of age, diet, and exercise as well as the components of the pituitary-thyroid axis could be analyzed simultaneously. As the literature has often reported, there was a causal relationship between the components (TSH, T_4 , and T_3) of the pituitary-thyroid axis. The relationship was confirmed in the present study. That is, TSH had a significant effect on T_4 and T_3 values. This relationship was expected as TSH is directly responsible for T_4 and T_3 synthesis and secretion: T_4 had a significant effect on T_3 values as well, as was expected by T_4 to T_3 conversion (31). Although there was no significant effect of TSH on thyroid weight, there was a significant effect of TSH on the thyroid weight/body weight ratio.

There was, as well, a significant relationship of thyroid weight on T_3 and T_4 values above and beyond that of the main effects of age, diet, and exercise. It is known that the thyroid gland is the site of synthesis of T_4 (in totality) and T_3 (in part) through thyroglobulin, iodide, and tyrosine binding, as discussed in the review of the literature. Further, it has been noted that a supply of these hormones are stored in the thyroidal colloid following production. Therefore, the actual amount of stored hormones may be responsible for the relationship noted and/or the relationship noted may be an expression of an absolute amount of thyroid gland necessary in order to

produce the T_3 and T_4 serum levels present. Evaluation of the Type I SS (for T_3) suggests that the relationship of thyroid weight on T_3 serum levels is largely the result of T_4 and TSH serum concentration, as after T_4 and TSH input is considered, the effect of thyroid weight on T_3 levels is negated. The influence of TSH alone, however, was not sufficient to negate the effect of thyroid weight upon T_4 serum levels. These data appear to agree with Degroot's (186) observation that the size of the thyroid gland is controlled primarily by the gland's capacity to produce hormone.

Although significant causal relationships were noted between TSH, T_4 , and T_3 , the influence of these pituitary-thyroid axis parameters in the present study were a direct result of the main effectors age, diet, or exercise, as will be explained below. The parameters of the pituitary-thyroid axis -- TSH, T_4 and T_3 -- changed significantly with age. There was an age-associated decrease in T_4 and T_3 concentration levels in all groups. This pattern has been shown previously by a number of researchers (35,37,43). Others, however, have noted depressed basal T_4 concentrations with normal T_3 values with advancing age (49, 52). TSH and T_3 values were shown to be influenced significantly by the independent effects of food restriction; while T_4 was significantly influenced by an age by diet interaction. There was also a trend for a diet by exercise effect on T_4 serum levels. Serum T_3 levels were influenced significantly by all three main effects -- age, diet, and exercise.

The ratio of T_3 to T_4 increased significantly during the course of the study in both A and AE groups. No concurrent increase was noted for R and RE animals. Interestingly, the increase in the A ratio was greater than twice that of AE. The

literature has recounted several instances of an increased T_3/T_4 ratio with age (42,47). Explanations of this phenomenon include increased intrathyroid conversion of T_4 to T_3 , resulting in a decrease in the ratio of T_4 to T_3 secreted from the thyroid with aging (47), or changes in the peripheral metabolism of T_4 (42). Moreover, the increased T_3 to T_4 ratio in A when compared to AE and the restricted-fed groups is consistent with the finding of decreased 5'-deiodinase activity with exercise and/or underfeeding (165).

TSH is produced in the pituitary as a result of stimulation by hypothalamic produced TRH and controlled by negative feedback of T_4 and T_3 . Once produced, TSH acts directly upon the thyroid gland to produce all of the T_4 present in the serum and a portion of the T_3 as well. Just as age and diet were also shown to have a significant influence on TSH serum concentration levels, age and diet were shown to have a significant effect on the amount of T_4 and T_3 present, as was expected. However, in the periphery, another factor -- exercise -- was shown to exert an influence on thyroid hormone levels (T_4 and T_3). This was much more pronounced for T_3 which exhibited a large main effect due to exercise ($p < 0.0001$) than it was for T_4 which exhibited a trend for a diet by exercise interaction effect. These data support a causal role for exercise upon peripheral thyroid hormone metabolism. Exercise may influence the conversion of T_4 to T_3 (166) or possibly speed up the degradation of T_3 (165) resulting in a decreased T_3 serum concentration. These effects could be mediated by 5'-deiodinase activity, or through T_3 catabolic pathways, respectively. As there was a diet by exercise interaction effect noted for T_4 , it would

appear that exercise is having an effect on the thyroid gland itself. Evaluation of possible changes at the gland site is beyond the scope of this investigation; however, as was previously mentioned, it is an area worthy of further investigation.

Thyroid hormones are capable of stimulating NaK ATPase activity (63,66). The kidney has been shown to be an appropriate organ in which to examine thyroid induced NaK ATPase enzymatic alterations. Expected increases in renal weight with age were noted along with decreased kidney weight in restricted-fed animals when compared to controls (89), and an increased renal weight/body weight ratio with age.

Renal NaK ATPase activity was assessed to evaluate the effect of the thyroid hormones on their target organ. When NaK ATPase activity was expressed as $\mu\text{M Pi/mg L-fraction protein/hr}$, an age by diet interaction (Type III SS) was found to be related significantly to its activity level. A significant (quadratic) effect of T_4 upon NaK ATPase was also noted. There was little change in the mean values observed with age. L-fraction renal protein per gram wet weight renal cortex was thus examined to determine if NaK ATPase enzymatic activity was being masked by its expression per mg L-fraction protein/hr. Renal cortex L-fraction protein was observed to decrease linearly with age. It was not affected by diet or exercise. L-fraction renal protein did, however, vary with T_3 concentration level as well as that of T_4 . As thyroid hormone decreased, L-fraction renal protein decreased. Extrathyroidal T_4 to T_3 conversion occurs in the kidney. High affinity, limited capacity nuclear T_3 receptors regulate protein synthesis by receptor binding, thus initiating events that result in transcription and translation and culminating in protein

production (28,32). This might be the process which resulted in decreased L-fraction protein concentration in the renal cortex with age.

When NaK ATPase activity was expressed on the basis of μM Pi released per unit of renal mass, the enzyme activity was significantly decreased with age. Further analysis revealed that the enzymes's decrement was related to a significant decline in L-fraction protein per unit of renal cortex. Interestingly, and unexpectedly, there was no effect of T_3 upon NaK ATPase activity. Nor was there a relationship of NaK ATPase activity with dietary restriction or exercise. There was, however, a trend ($p < 0.1$) for an effect of T_4 upon NaK ATPase activity. Again, as T_4 decreased, so did NaK ATPase activity. The decrease in enzyme activity with age agrees with the results of others (71,72,73).

As was mentioned above, an effect of T_3 upon NaK ATPase was expected as thyroid hormones have been suggested to be modulators of NaK ATPase activity (59,60). Several investigators have noted that injection of exogenous T_3 into thyroidectomized rats elicited a response of increased NaK ATPase activity (55,61). Gambert (62), however, observed a significant decline in NaK ATPase response to exogenous T_3 administration to hypothyroid animals with age. Thus, the phenomenon occurring in this study may characterize T_3 modulation of a sequence of events whereby protein production ultimately contributes to NaK ATPase activity. Further, this study examined basal thyroid hormone levels, whereas the studies examined utilized exogenous T_3 administration (56,59, 61,63). Therefore, the failure to demonstrate a significant T_3 and NaK ATPase relationship in this experiment may

be related to the sensitivity of the in vitro assay systems and their ability to detect the relationship which may exist in vivo.

The hypothesis that long-term food restriction will result in decreased absolute levels of thyroid hormone serum concentrations and will slow the age-associated changes in the pituitary-thyroid axis was, in general, substantiated by the results of this study. Restricted-fed (R and RE) animals had lower basal TSH, T_4 and T_3 values at 12 months of age than did ad libitum-fed animals. R and RE groups' T_3 serum levels remained consistently lower than those of the A and AE groups for the duration of the study. The average serum TSH values for R and RE rats were lower than those of A and AE. Serum T_4 values, although initially lower in the R and RE groups, were by 20 months of age, greater than those of A and AE groups. This effect was the result of decreasing T_4 serum levels in A and AE as well as slightly increased levels in R and RE. Twenty-four and 28 month T_4 levels for all groups decreased with age, with A and AE levels remaining consistently lower than those of R and RE. Depressed T_3 and T_4 serum concentrations agree with the findings of several investigators (35,37). Some investigators, in contrast, have observed differing patterns of decrease.

It is also significant that food restriction served to slow the age-associated decrease in the peripheral thyroid hormone levels. While the R group exhibited a slight (11%) T_3 decline with aging, the A and AE groups' decline was twice as much. For completeness, it should be noted that RE T_3 serum levels decreased 46% with advancing age. Clearly, exercise contributed to this significant decline. As stated

above, a similar slowing of the age-associated decrement in T_4 values was noted in restricted-fed (R and RE) animals.

A number of explanations have been offered for these effects. It has been suggested that T_4 secretion may decrease with food restriction (142) or that peripheral $T_4 \rightarrow T_3$ conversion is decreased as a result of 5'deiodinase (148,149). It must be remembered that in this study food restriction was imposed essentially for a lifetime. The consistently lower T_3 values in the face of relatively stable then decreasing T_4 values, as well as considerably lower TSH values and a responsive pituitary, suggests that thyroid hormone levels are being mediated both centrally (at the pituitary) as well as peripherally by food restriction.

Research has suggested that the pituitary-thyroid axis may be instrumental in the aging process (34). Food restriction is the only method that has been shown to extend maximum lifespan (18). The lifespan-prolonging effect of food restriction was again confirmed in the present study -- R and RE rats had a longer lifespan than A and AE animals (187). No effect of exercise on longevity was observed -- lifespans of A and AE were comparable, as were those of R and RE.

As was previously noted, food restriction resulted in significantly decreased absolute TSH and T_3 serum concentration levels. T_4 serum levels were significantly influenced by an age by diet interaction and a trend ($p < 0.1$) for a diet by exercise interaction. These effects of diet were independent (above and beyond) the effect of TSH on T_4 and T_3 , or T_4 on T_3 . This means, in essence, that there was no significant independent component of the relationship between TSH and T_4 or TSH

and T_3 that was not a direct result of diet (and age). A similar relationship exists for T_4 and T_3 . Recognition and understanding of this hierarchy is essential for an accurate portrayal of the physiological processes being investigated.

Food restriction (severe) has been shown to decrease thyroid hormone levels (13,138,140,141). This study found that long-term, moderate food restriction resulted in decreased basal serum thyroid levels. As was addressed earlier, TSH, T_4 , and T_3 serum levels of R and RE rats were lower than those of ad libitum-fed animals at 12 months of age. During the first year of life, T_3 levels of restricted-fed rats have been observed to be 50% lower than those of ad libitum-fed controls (188). It may therefore be assumed that the concentration patterns noted were in effect for the first year of life. The lower T_3 levels were maintained throughout the duration of the study: lower average TSH values for R and RE groups were also maintained. Significantly lower T_4 serum values were not maintained with advancing age; however, this may not be of great importance as T_4 is often considered to be a "prohormone" (31) that is primarily responsible for T_3 production and it is T_3 that has pronounced activity at the cellular level (28). These lowered serum hormone concentrations, the author suggests, were possibly instrumental in the life-prolonging conditions that resulted in a significantly longer lifespan for R and RE animals. It has been suggested that lower serum hormone concentrations decrease "wear and tear" on cells and tissues which is consistent with a physiologically younger animal (12). It is therefore reasonable to deduce that this process was in effect in the present study.

Taken together -- the overriding influence of diet upon serum thyroid hormone concentrations and the "anti-aging" effect of lower hormone levels throughout the duration of the lifespan -- it appears that thyroid hormones may indeed serve as agents through which food restriction exerts a life-prolonging effect. These findings are consistent with recent observations (189) as well as with the neuroendocrine theory of aging.

It has been suggested that because of the energy expenditure during exercise, exercise should have physiological effects similar to those accompanying restriction of energy intake. This was not found in the present study. Adherence to the long-term exercise regimen did not result in an overall decrease in the thyroid hormones as was hypothesized. T_3 alone exhibited a significant independent exercise effect. Exercised animals did have significantly lower (22% to 25%) absolute T_3 serum concentration levels than non-exercised animals. Exercise did not have a significant effect on serum T_4 values. This agrees with the findings of others (155,164). A trend for a diet by exercise effect was noted for T_4 . Interestingly, the T_4 values for RE were consistently less than those of R: this difference was not observed between AE and A animals. The reason for the differences in these results is unclear. Further, exercise did not retard the age-associated decline in serum T_3 concentration for either ad libitum-fed or restricted-fed animals. In fact, exercise exacerbated the age-associated decrease in RE animals. Nor did exercise retard the age-associated decline in T_4 serum values for RE. The decline, however, for AE was less than that of A with aging. Few studies have examined the effect of exercise upon thyroid

hormone in rats (156,157). No studies of which the author is aware have evaluated the effect of long-term (lifetime) exercise upon the pituitary-thyroid axis. As previously noted, results of exercise studies reported in the literature have been conflicting and contradictory. The results of the present study agree in part with the findings of Katzeff, et al. (165), who noted no change in serum T_4 with exercise, but noted significantly decreased serum T_3 levels in the restricted, exercised group.

Hohtari, et al. (174) has reported that thyroid secretion may be altered by physical training. Sander and Röcker (166) suggested that prolonged exercise results in an altered peripheral conversion of T_4 in favor of rT_3 over T_3 , thus protecting the body's energy reserves (allowing the body to synthesize additional energy resources). The hypothesis of Sander and Röcker is much more in keeping with the findings of the present study. Altered thyroid hormone secretion would decrease both T_4 and T_3 levels, while altered peripheral conversion would only decrease serum T_3 concentrations. Such decreases were observed in the present study. This decrease in AE and RE T_3 absolute concentration levels is the only data in support of a possible age-retarding component of exercise. Exercise was found to provide no protection against the age-associated decrements in the pituitary-thyroid axis, thus it is concluded that food restriction and exercise do not act synergistically to delay the age-associated changes in the pituitary-thyroid axis.

The mean terminal body weight of all animal groups decreased with age. These results are similar to the weight loss frequently associated with senescence as noted by Yu, et al. (120) and those seen in other aging mammals (85,131). No

known mechanism for this age-associated decrement in body weight has been elucidated. It has been suggested that this phenomenon may occur as a result of decreased appetite, or LBM (131).

Food restriction resulted in a significant lowering of predicted mature body weight as well as terminal body weight. Food consumption of R was 25% that of A. The body weight difference of restricted-fed and ad libitum-fed rats is reportedly due to adipose tissue deposition (77, 119). Food restriction also decreased the relative rate of maturation of R and RE groups.

Exercise has been shown to increase or maintain LBM and decrease body fat (103); however, in this study exercise had no significant effect on terminal body weight. Exercise did result in slightly increased food intake (15% and 9.5%, respectively) in exercised animals (AE and RE) when compared to sedentary animals (A and R); however, this increased consumption apparently failed to offset the increased energy expenditure of exercise after 16 months of age. The inability of male rats to increase their food consumption commensurate with the energy expenditure of exercise has been previously noted (112,113). The trend for a diet by exercise effect upon body weight was therefore not surprising. Interestingly, although RE animals' food intake was slightly greater than that of R's, the two groups' terminal body weights varied little throughout the study.

The lack of exercise effect upon terminal and predicted mature body weight may call into question whether the animals in this study were in fact, trained. The timed exercise sessions speak to the regularly scheduled, endurance nature of the

exercise protocol; however, the intensity of the exercise was not measured directly. Swimming regimens have however been shown in previous studies to be adequate at eliciting a training response (106,154). One parameter indicative of a training response is cardiac hypertrophy -- resulting in increased heart weight to body weight ratios (112). Exercised animals in this study exhibited cardiac hypertrophy indices characteristic of those found in trained animals that also exhibited a training effect (112,190). It may thus be conjectured that exercised groups were indeed trained.

Comparison of the relative rate of maturation of ad libitum-fed animals suggests that exercise may slow maturation -- although to an insignificant degree in ad libitum-fed animals ($A = .095$; $AE = .087$). In contrast, the rate of maturation of RE animals was significantly lower than the sedentary, restricted-fed animals. Bloor (105) noted that retardation of growth was proportional to exercise severity. This was not substantiated by the present study as both RE and AE followed the same exercise regimen. A possible explanation for the discrepancy may be that food restriction and exercise, in combination, are synergistic in their effects upon relative rate of growth. Slowing of the rate of maturation (or maintaining growth potential longer) has been suggested as a method by which lifespan may be extended (18). This hypothesis however, was not supported by the present study as neither AE nor RE had a maximum lifespan longer than A or R groups, respectively (187).

Calculated growth curves from Brody's equation have been utilized to predict degree of adiposity (119). The calculated growth curves in this study were based on body weights during the first year of life; i.e., during the growth phase and subsequent

adult stabilization of body weight. Terminal body weights of R and RE animals fell close to their calculated growth curves; those of A and AE, in general, fell above their growth curves. It has been suggested that ad libitum-fed weight gains above that of their calculated growth curves are due to adipose tissue deposition (119). This increased adiposity during the second year of life was reportedly the reason the Brody group equation failed to predict the ad libitum-fed animals' maximum body weights after one year of life (83).

Both food restriction (83) and exercise (103,106,122) decrease the percentage of body fat when compared to ad libitum-fed sedentary controls. Evidence from the present study supports this. Further, exercise has been shown to protect against LBM loss (106,122). Therefore, it may be hypothesized that the difference between the A and AE calculated growth curves is due to adipose tissue mass. It may also be speculated that the R and RE difference is the result of increased LBM in exercised animals, while the difference between restricted (R and RE) and ad libitum (A and AE) growth curves, respectively, is due primarily to increased fat mass in the ad libitum-fed animals.

CHAPTER VI

SUMMARY

This study was undertaken to examine the effects of age, diet, and exercise on the pituitary-thyroid axis in the male Wistar rat and to ascertain the age-inhibiting potential of food restriction and/or exercise on the aging pituitary-thyroid axis. The cumulative effect of aging on the pituitary-thyroid axis was a resultant decline in activity as expressed by a significant decrement in T_4 and T_3 concentration levels in both ad libitum-fed and restricted-fed groups. Decreased NaK ATPase activity in the renal cortex with age was also indicative of the axis decline.

Path analysis was utilized to simultaneously analyze the effects of age, diet, exercise, and the components of the pituitary-thyroid axis on the parameters examined. A causal relationship between the components of the pituitary-thyroid axis was confirmed. That is, a significant relationship of TSH with T_4 and T_3 serum levels, and of T_4 with T_3 was observed. Thyroid weight changes were minimal through the course of the study. The thyroid weight to body weight ratio was larger in restricted-fed animals than in ad libitum-fed animals.

The influence of the pituitary-thyroid axis parameters noted above were a direct result of the main effectors age, diet and exercise. Long-term food restriction resulted in decreased absolute levels of TSH, T_4 and T_3 at 12 months of age. This effect was sustained for T_3 serum levels and, on average, for serum TSH levels.

Although T_4 absolute serum levels were not consistently lower as a result of food restriction, food restriction did slow the age-associated decline in T_4 serum levels when compared to ad libitum-fed controls. A slowing of the age-associated T_3 decline was present in the R group as well. TSH values of ad libitum-fed animals began to increase at 20 months of age. This increase was delayed by food restriction in both R and RE groups. The increase signified the ability of the pituitary to respond to stimuli throughout the aging process.

Exercise did not contribute significantly to a slowing of T_3 decrements with age in either RE or AE; however, exercised animals' absolute T_3 serum concentration levels were significantly lower than those of non-exercised animals. There was no effect of exercise on T_4 or TSH serum concentration levels. As exercise provided no protection against the age-associated decrements in the pituitary-thyroid axis, it was concluded that food restriction and exercise do not act synergistically to delay the age-associated changes in the pituitary-thyroid axis.

Additional findings include a loss in terminal body weight with age. Food restriction also decreased the predicted mature body weight and the relative rate of maturation. There was no significant effect of exercise on terminal body weight; however, exercise appeared to retard growth in both AE and RE groups. Terminal body weights of restricted-fed animals (R and RE) fell close to their predicted body weights as indicated by their respective growth curves. A and AE terminal body weights fell above their predicted values. Distance above the curve is associated with degree of adiposity. As exercise has been shown to protect against loss of LBM, the

body composition changes with food restriction and exercise may be projected using the calculated growth curves. Exercise increased food consumption in both AE and RE, when compared to A and R, respectively. This increased energy intake was not sufficient to offset the energy expenditure of exercise after 16 months of age as indicated by decrements in body weight.

In summary, pituitary-thyroid axis function declined with age. However, the pituitary-thyroid feedback system remained intact, as indicated by the ability of the pituitary to mount an appropriate response to depressed T_3 and T_4 serum concentrations. Long-term food restriction retarded the age-associated decrements in the pituitary-thyroid axis, and, in general, resulted in lower absolute levels of serum thyroid hormones. Exercise, in contrast, did not retard the age-associated pituitary-thyroid axis decrements, nor did it result in decreased absolute thyroid hormone concentrations of TSH or T_4 . Long-term exercise did, however, result in lower serum T_3 concentration levels.

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VITA

Vivian Haley-Zitlin was born in Clarkesville, Tennessee. She received her elementary education in Trenton, Kentucky, and her secondary education at Todd County Central High School where she graduated Valedictorian of her class. She graduated from the University of Kentucky in 1977 in the Honors Program with a Chemistry, Biology, and Animal Science triad. She later worked as a senior research assistant in the Cardiovascular Psychophysiology Laboratory at the University of Tennessee, Knoxville.

In 1986, she entered the doctoral program in Nutrition and Food Science at the University of Tennessee, Knoxville. While a doctoral student, she served as a graduate teaching assistant, and as an undergraduate advisor. She is a member of Sigma Xi, the Gerontological Society of America, Kappa Omicron Nu Honor Society, and the American Dietetic Association. She has been recognized by the Department of Nutrition and Food Science for outstanding graduate research; and she has received the Chancellor's Citation for Extraordinary Professional Promise. The Ph.D. was subsequently awarded in May, 1991, at which time she began a postdoctoral fellowship in Gerontology at the University of Texas Health Sciences Center in San Antonio.