To the Graduate Council:

I am submitting herewith a dissertation written by Madeline Dellwo entitled "The Effect of Exercise, Diet Restriction and Aging on the Pituitary-Adrenal 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.

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THE EFFECT OF EXERCISE, DIET RESTRICTION AND AGING ON THE PITUITARY - ADRENAL AXIS IN THE RAT

A Dissertation
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Degree
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ABSTRACT

The effects of calorie restriction and exercise on feed consumption, growth, and the pituitary-adrenal axis were studied in aging male Wistar rats. The study consisted of 4 experimental groups: Sedentary, ad libitum fed controls (A); Sedentary, diet restricted by feeding on alternate days (R); Exercised by swimming on alternate days, ad libitum fed (AE); Exercised by swimming on alternate days, diet restricted by feeding on alternate days (RE).

Training effect was verified by heart weight:body weight ratio. Exercise resulted in a significantly lower body weight, in spite of the fact that exercised animals ate more than the sedentary animals. Body weight curves of all the experimental groups were of similar shape, though R and RE were at a lower level than A and AE.

Pituitary-adrenal function was assessed by measuring serum ACTH and corticosterone concentrations, adrenal weight, hepatic glucocorticoid receptor concentration and hepatic tyrosine aminotransferase (TAT) activity. Serum ACTH and corticosterone concentrations increased significantly with age while TAT activity decreased and receptor concentration remained the same. Adrenal weights also increased with age, with the adrenal weights of AE increasing the most dramatically. In general, calorie restriction and exercise didn't have a major influence on the aging axis. An exception was observed in TAT activity, which decreased more gradually with age in calorie restricted rats.

Analyses for relationships between variables revealed a quadratic relationship between serum ACTH and corticosterone

concentrations. Concentrations of both hormones rose as rats aged, though ACTH levels decreased early in life. Additionally, there tended to be an inverse relationship between TAT activity and corticosterone concentration.

In general, aging rats exhibited increased concentrations of ACTH and corticosterone and diminished TAT activity, while receptor concentration remained the same. These observations may be indicative of a loss of feedback loop integrity and possible changes at the cellular level such as in chromatin activity in relation to TAT synthesis. Neither calorie restriction or exercise were able to maintain the integrity of pituitary-adrenal function, though calorie restriction did preserve TAT activity to a small degree.

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CHAPTER I

INTRODUCTION

Traditionally, it has been believed that the function of the pituitary-adrenal axis is to guard the body against stress (1). However, a more plausible theory has been advanced recently by Munck et al. (2), who proposed that stress-induced increases in pituitary-adrenal axis activity protect the body not against stress itself, but rather against the body's reaction to stress. In other words, the axis actually suppresses normal defense mechanisms to prevent them from being the cause of damage to the body. In either case, the ability of an animal to deal with stress depends on the integrity of the pituitary-adrenal axis. The present investigation is concerned with the effect of exercise and/or diet restriction on the capacity of this system to maintain integrity throughout the aging process.

Aging is accompanied by a dramatic decline in the functional capacity of an animal's organs and tissues (3). It is unclear if age-associated changes are strictly programmed, if they occur because of genetic disturbances which increase with age, or if there are some other unrecognized causes (4). The general pattern of age-related changes is a gradual loss of regulation, especially under environmental stress (5). Old animals have particular difficulty in adjusting to stressful stimuli (1). Because of the importance of the pituitary-adrenal axis in adapting to stress, it would be of interest to know whether aging alters the ability of the axis to respond to stressful stimuli. Recent experiments in which alterations in the

pituitary-adrenal axis during aging were examined raise the question whether a decreased response to stress results from decreased pituitary and/or adrenal function, or whether the action of glucocorticoids themselves are modified with age.

Presently, the method most effective for delaying age-associated changes is dietary restriction. It is well documented that feed restriction can increase life expectancy and maximum life span of laboratory animals (6). However, the mechanisms involved in the prolongation of life by feed restriction have not been elucidated clearly. The extension of life span is partially due to a delay in the onset and incidence of age-related diseases. Surprisingly, there has been no research testing the relationship of diet restriction and aging on the onset of decreased pituitary-adrenal function. Likewise, there has been no attempt to evaluate the effects of exercise on age-related changes in this axis. It is, therefore, the purpose of this study to investigate the relationship of diet restriction and/or exercise on age-related changes in the pituitary-adrenal axis.

CHAPTER II

REVIEW OF THE LITERATURE

Pituitary-adrenal axis and assessment of its function

The adrenals (meaning "next to the kidney") lie on the superior pole of the kidneys (7). The outer portion of the adrenal gland, the adrenal cortex, is essential to life. It produces several important steroid hormones which fall into 3 general classes: glucocorticoids, mineralocorticoids, and androgens. The precursor of the adrenal steroids is cholesterol, so it is not surprising that there is a higher concentration of cholesterol in the adrenal cortex than in any other structure of the body, except for the nervous tissue (8).

Adrenal function is regulated by the pituitary hormone corticotropin (ACTH), a straight chain polypeptide. ACTH not only increases the synthesis of corticosteroids by the adrenals but also stimulates their release from the gland. There is a reciprocal relationship between corticosteroid production and ACTH secretion, in that exogenous corticosteroid causes feedback inhibition of ACTH release at the pituitary level (8). Recent research (9) has provided reason to believe the hippocampus acts as an inhibitory influence upon the adrenocortical axis. Hippocampal lesions have been found to result in basal hypersecretion of ACTH and the glucocorticoids. Furthermore, the hippocampus is capable of inhibiting stress-induced activation of the adrenocortical axis, as observed when its destruction produced glucocorticoid hypersecretion after various stressors (9).

Therefore, it is believed that the hippocampus is an important mediating locus of glucocorticoid feedback inhibition.

All of the steroid hormones act primarily at the level of the cell nucleus to bring about the synthesis of RNA and subsequently that of protein (10). Glucocorticoids bind to a receptor protein in the cytosol of the target tissue. Steroid receptors are comparatively specific to a given steroid. The receptors are single polypeptide chains, though in the activated form some may be more complex and dissociate during activation (20). The receptor-hormone complex has increased affinity for DNA and binds to specific binding sites on the DNA upstream from the genes regulated by the hormones. Transcription then increases and mRNA's are translocated to the cytoplasm for translation (10). By these means, cellular protein and enzyme synthesis are modified. It is the changes in the enzyme concentration that actually produce the effects attributable to the hormone.

Target tissue has stereospecific receptors permitting accumulation of the steroid hormone in that tissue against a concentration gradient (20). A key determinant of the extent of binding of a steroid hormone to a target tissue is the hormone's actual blood concentration. The concentration of the steroid in the plasma is dependent on several factors, such as the rate at which the steroid is synthesized and enters the body pool; the rate at which steroid is inactivated by catabolism and removed from the body; and the "tightness" of binding of the steroid to its carrier proteins.

Glucocorticoids, so named because of their influence on the storage of carbohydrate, act on different cells in different ways (7). Often they induce protein synthesis or repress the expression of

certain proteins by transcriptional actions. A general result of raised levels of glucocorticoids is an increase of glycogen storage, especially in the liver. They also increase circulating glucose by direct stimulation of certain rate limiting enzymes in the gluconeogenesis pathway and through negative effects on glucose uptake by peripheral cells. In addition to the shifts in carbohydrate metabolism associated with glucocorticoids, they also increase cardiovascular tone and inhibit growth, the immune and inflammatory responses, and reproduction in order to allow the animal to adapt successfully to acute physical stress (9). Thus, in spite of the fact that these responses are crucial in adapting to stress, excessive exposure to the steroid, as observed during prolonged stress, can potentially be as damaging as the lack of them.

Though glucocorticoids act on many cells, the liver is an important target organ due to the large number of receptors in the hepatocyte (8). Other target tissues include the lymphoid cells, thymus gland, and the kidney. The liver is also the major site of inactivation of the glucocorticoids, with the kidney being a second, somewhat less important site. Liver and kidney enzymes reduce the double bonds of glucocorticoids at carbons 4 and 5, with the resulting metabolites excreted in the urine as glucuronides conjugated to hydroxyl groups.

Measuring the concentration of steroid hormones posed great difficulty until recent years. By means of radioimmunoasasay (RIA), it has been demonstrated that by coupling the steroid chemically to a protein, relatively specific antisera can be obtained (11). However, the assay for steroids, as opposed to protein hormones, was slow in

coming. This was due mainly to the fact that, in spite of the highly successful use of naturally occurring steroid-binding plasma and tissue proteins as assay reagents, this method was not reliable for all steroids, since many steroids did not bind to any easily available protein.

The basis for steroid RIA is the reversible reaction which occurs between the steroid to be measured [S] and the population of antibodies raised against that particular steroid [Pr], for example,

$$[S] + [Pr] \leftrightarrow [S{\cdot}Pr].$$

If [Pr] is fixed, then the [S] present will determine the relative concentrations of all the species present. If these can be determined, then by reference to standard conditions, [S] can be found. The problem of estimating these relative concentrations is solved simply by using a radio-labelled steroid tracer. Thus, by setting up standard amounts of [S] and determining in each case the relative distribution between free steroid [S], and protein-bound steroid [S·Pr], at each point, a standard curve can be constructed from which sample steroid concentrations can be estimated (11).

Pituitary-adrenal function and aging

The effect of age on adrenal function has been examined by several investigators, with inconclusive results. ACTH-mediated adrenocortical secretion *in vivo* (12) and plasma corticosterone levels in rats (13) and humans (14) have been shown to be reduced with age. However, Bolla (15) observed no age-related changes in the concentration of receptor proteins, though there was a decrease in the affinity of the specific receptor proteins for both dexamethasone (a

synthetic glucocorticoid) and corticosterone. Bolla's findings might indicate that the observed age-related decline in receptor protein affinity for steroid hormones is the result of an increased association of the hormones with non-specific receptor proteins and a corresponding decrease in hormones associated with the specific receptor. Bolla concluded that the overall decrease in specific hormone-receptor complex formation may be the major cause of age associated loss or delay of liver response to glucocorticoid hormones.

Roth (16) also examined glucocorticoid receptor concentrations and binding affinity, using 2 rat strains (Sprague-Dawley and Wistar). The epididymal fat pad was used since it is a relatively static postmitotic cell system and allows the same population of cells to be analyzed in both the senescent and young animal. In contrast to the findings of Bolla (15), Roth observed no change in the binding affinity of the receptors. However, there was a gradual reduction with increasing age in the concentration of receptor sites. Roth concluded that the loss of receptors with aging corresponded statistically to the loss in the ability of the hormone to inhibit glucose utilization. Therefore, there appears to be a very close, if not causal, relationship between receptor loss and response loss.

Roth noted that loss of hormone receptors is progressive over the entire life span. If one compares losses in various species with different mean life spans, binding loss can be expressed as a function of the percentage of life span completed. This may indicate that there is some genetic specificity and the changes have something to do with the life span of the species.

No clear pattern of age-related alterations in either receptor concentration or affinity has been observed. Whereas Roth (18) found a reduction in receptor concentration, though no change in affinity, Kalimi et al. (19) observed no significant age-related change in the concentration of receptor binding sites, affinity or steroid specificities. However, Kalimi and his co-workers did find 50% more receptor-binding sites in adrenalectomized rats than in intact adult animals. By comparison, only a 25% difference was found in old rats.

The findings of Kalimi et al. (19) may reflect a significant increase in unoccupied receptor levels in the cytosol of old intact rats, which could indicate differences in steroid-receptor nuclear binding. Alternatively, it could indicate differences in endogenous glucocorticoid levels in the blood or tissues. According to Kalimi (20), it has yet to be determined whether age-related changes in receptors play an important part in altered sensitivity to glucocorticoids with age. However, Lewis and Wexler (21) found that serum glucocorticoid levels did not decrease in aged Sprague-Dawley rats. This led them to suggest that the change in unoccupied receptor levels in the hepatic cytosol reflects an alteration in hormone tissue distribution or in hormone metabolism in the liver cells of intact rats.

A characteristic of all aging populations is the progressively impaired ability to adapt to an altered or stressful environment (13). To examine the changes in response to stress with age, Sartin et al. (13) subjected male Sprague-Dawley rats of various ages to the stress of starvation for 3-6 days. Young animals adapted to starvation by increasing the circulating levels of corticosterone in the serum. This capability was lost progressively during aging. It was postulated by

Sartin et al. that this observed altered response to an environmental challenge is a very general characteristic of aging and reflects a broad array of endocrine regulatory mechanisms involving many hormones and enzymes.

Intrinsic age-related changes in steroid production were studied by Malamed and Carsia (17) in cells isolated from the adrenal cortex from rats of different ages (3 to 24 months). Corticosterone production in response to stimulation by ACTH and also by cAMP was then measured by radioimmunoassy. It was found that with age, there was a progressive loss in the ability of adrenocortical cells to produce corticosterone. The greatest reduction (approximately 50%) occurred in early life. Because both ACTH- and cAMP-induced steroidogenesis were decreased, it appears that the effect of aging cannot be explained entirely, if at all, on the basis of interference with ACTH-receptor interaction and subsequent adenylate cyclase induction.

In addition to its effect on maximum corticosterone production, age also decreased the adrenocortical cells' sensitivity to ACTH. However, these 2 changes did not occur during the same period, the latter dropping sharply from 12 - 18 months as compared to 6 - 12 months for corticosterone production. Though the mechanisms are not yet clear, a possible explanation for this observation might be that initially there is a loss in the number of receptors without a loss in affinity, followed by a loss in affinity.

There are several possible sites for age-related failures in adrenocortical function. Frolkis (22) demonstrated an age-related reduction in hepatic tyrosine aminotransferase (TAT) induction with

daily administration of hydrocortisone in rats. However, no age-related changes in maximum amounts of induced TAT were found by Adelman and Freeman (23), though they did observe a time-delay in obtaining maximum response. A delay in TAT induction in aged rats has been observed after cold exposure (24), during fasting and after steroid administration (25). This delay is believed to be of endocrine origin. However, a correlation between glucocorticoid binding to specific receptors and enzyme induction has been demonstrated, suggesting that there are some changes in the liver cells themselves. (26).

The expression of genes regulated by the glucocorticoids after physical stress or glucocorticoid induction with aging was examined by Wellinger and Guigoz (24). They observed that aged rats responded more slowly to cold stress, as demonstrated by a delayed increase in TAT activity. A reduction of TAT activity and mRNA concentration also was observed in the aged animals. These results suggest that the increased expression of TAT after stress is preceded by a rise in mRNA coding for TAT. Therefore, the age-related changes are not due to differences in kinetic properties of the enzyme, but rather to regulation of the level of TAT-specific mRNA. Interestingly, with pharmacological doses of hormone, no age-related differences were seen. Thus, aged animals still have the capacity to synthesize maximum amounts of this glucocorticoid-induced enzyme.

Another possible site of age-related failure in adrenocortical response, has been proposed by Sapolsky et al. (9). They observed that the ability of the aged male rat to terminate the secretion of glucocorticoids at the end of stress is impaired. The resulting

increased concentrations of the hormones lead to further pathophysiological consequences. It is their hypothesis that this hormonal excess is due to degenerative changes in the region of the brain which normally inhibits the release of glucocorticoids. Ironically, the degeneration, in turn, is believed to be caused by the cumulative exposure to glucocorticoids.

In a study performed by Sapolsky et al. (27), young and aged male rats were exposed to one hour of immobilization to measure their stress response. While the aged rats compared to the young rats showed no impairments in initiating the adrenocortical stress response, they were dramatically impaired in the ability to terminate it. These investigators reported a loss of approximately 50% of glucocorticoid binding sites in the hippocampal region of the brain in the old rats. They also reported that the receptor loss was partially due to death of the neurons, with surviving neurons having a smaller concentration of receptors. The end result appeared to be a failure in feedback inhibition due to lack of sensitivity to glucocorticoids.

Thus, while there are contradictions in the literature, the consensus among researchers tends to be that the ability of the pituitary-adrenal axis to function at an optimal level decreases with age. However, it is still uncertain if these changes are a result of a loss of receptors or a change in affinity of glucocorticoid receptor binding, a reduction or increase in hormone concentration, decreased responsiveness of the target cell to the hormone, or all of the above. As was emphasized by Gregerman (28), few, if any, conclusions should be drawn from studies which deal only with receptors, or only with the related hormones, since the hormone axis is much more

complex than a single measure of any of these. The number of receptors, for example, is not fixed, but varies with the concentration of the hormone in the cell. This can occur within a matter of hours. Thus, studies that attempt to correlate receptor number with some biological or biochemical event would be more informative than identifying receptor change with age.

Diet Restriction and Aging

It has been said that the only effective experimental method able to extend the life span of an animal is dietary manipulation. The first scientific evidence of this was reported by McCay and co-workers (29) in 1929, when the life span of trout was extended by dietary restriction. Later, McCay et al. (30) demonstrated that restriction of caloric intake by rats consuming diets otherwise nutritionally adequate led to growth retardation and the prolongation of life span. McCay's findings have become a cornerstone of experimental gerontology. They have been confirmed repeatedly in several rodent species and found to be effective whether food restriction is begun at weaning or later in life.

It was once thought that prolongation of life span associated with caloric restriction could be achieved only when animals were restricted early in life and thus growth and maturation were retarded. More recent research has demonstrated that restricting feed intake at a later stage in life, thus allowing normal growth and development, still provides the beneficial effects on life span. In a study of the effects of a 50% dietary restriction during the first and second years of life in hamsters, mice, and rats, Stuchlikova et al. (31)

showed that animals fed ad libitum had the lowest survival rate. By contrast, animals fed ad libitum during the first year of life and restricted thereafter had increased life span. Similar results were obtained in a study performed by Beauchene et al. (32). Thus, it is not necessary to curtail growth and maturation to produce prolongation of life in animals, although the effects of restriction depend upon the severity of the restriction and the age at which it is introduced.

Age changes in physiological processes are primarily considered to be detrimental (33). Feed restriction can delay or prevent a broad spectrum of these changes. Some examples of this are: retardation of the loss of dopamine receptors in the corpus striatum of the rat (34); delay and modulation of the alteration in the immune function in mice (35); and retardation of declining ability of adipocytes of rats to respond to the lipolytic action of hormones (36). The broad range of these effects provides strong evidence that caloric restriction influences primary aging processes which underlie the changes that occur during aging. However, there is still little known regarding how dietary restriction affects the primary aging processes. Of interest is the fact that caloric restriction does not retard all age changes (36). It is possible that age changes not slowed by a restricted diet may also provide insights into the aging processes as well as the mechanism of action of caloric restriction.

In addition to the physiological changes described above, diet restriction has been found to delay the occurrence of most age-associated diseases, such as nephropathy (37), leukemia (38), and autoimmune diseases (39). Again, the breadth of these effects

suggests that this is due to the influence on intrinsic aging processes rather than to the delay in specific diseases. An example in support of this theory was provided by the research of Iwasaki et al. (40). Ad libitum fed male Fischer rats were fed soy protein rather than casein as the source of dietary protein. End stage renal disease was curtailed as effectively with the change in dietary protein as was observed with caloric restriction. However, there was no increase in longevity in the soy protein group as seen with diet restriction. It was therefore concluded that the extension of life span by caloric restriction is due to the retardation of primary aging processes rather than the delay of age-associated diseases.

The question thus arises as to the mechanisms by which food restriction delays the primary aging processes. Much of the current research is focusing on this question in the belief that such knowledge will provide insight into the nature of primary aging processes and, in that way, will provide a base for the development of effective interventions in the aging process.

The three major hypotheses that have been proposed for the mechanisms of action of diet restriction have been ruled out by recent research. First, as mentioned earlier, McCay et al. (30) proposed that life span was increased by retarding growth and development. However, as previously stated, more recent studies (31, 32) demonstrated that feed restriction started in adult life also increased life span. Second, it was believed that the reduction in body fat content was the cause for enhanced longevity (41). Unfortunately, no correlation between body fat content and longevity was observed in a study by Bertrand et al. (42). Finally, it was

proposed by Sacher (43) that a reduced metabolic rate resulting from diet restriction retarded the aging processes. However, Masoro et al. (44) found that there was no decrease in the metabolic rate when measured per unit of lean body mass.

With these three classic hypotheses discarded, alternative hypotheses may be offered. A strong possibility is that caloric restriction is coupled to the aging processes through its actions on the endocrine system. Sapolsky et al. (9) presented evidence that the adrenocortical axis is responsible for many of the degenerative changes observed in aging and that a loss of regulatory control over glucocorticoid secretion results in hyperadrenocorticism, which is often an age-associated process. This was demonstrated when pharmacological concentrations of glucocorticoids produced hippocampal degeneration, and again when cumulative exposure to basal levels of corticosterone over a period of time resulted in hippocampal neuron death in rats (9).

This question was further examined by Sapolsky et al. (45) to determine whether prolonged elevation of glucocorticoid concentrations produced a "senescent" hippocampus. Male Sprague-Dawley rats were administered a moderate dose of corticosterone continuously for 3 months. This caused hippocampal receptor number to be down-regulated by 50%. Four months after the end of treatment, there was still no recovery of receptor number. The depletion appeared to be due to the loss of the neurons which bind corticosterone. The changes were nearly identical to those found in the aged hippocampus.

These findings suggest that cumulative exposure to corticosterone leads to the degenerative loss of neurons and glucocorticoid receptors in aging and with chronic stress. It is interesting that the neuron loss was not due to toxins or other insults, but rather to exposure to normal concentrations of an essential hormone. Additionally, it was shown by Landfield et al. (46) that adrenalectomized rats did not exhibit the degenerative changes typical of the senescent hippocampus as they aged. Interestingly, adrenalectomized rats also displayed improved cognition and had decreased tumor growth.

Diet restriction may act by preventing or delaying hyperadrenocorticism and in that way retard aging (47). It is known that undernutrition can depress pituitary function (47). Similarly, many of the effects of a hypophysectomy parallel those of a diet restriction. Both result in an atrophy of the endocrine system, cessation of growth and failure of sexual functions (9). Studies in which hypophysectomies were performed found retarded aging of collagen, kidney, bone and ovary in the rat. This may be the result of reduced secretion of pituitary hormones which, in theory, stimulate these aging processes.

If a prolonged diet restriction alters pituitary-adrenal function, then the changes in the secretion of pituitary hormones may be responsible for the retarded aging and the increased life span of underfed rats. It has been demonstrated that hypophysectomized rats consume approximately 1/3 of the amount of calories of ad libitum fed rats (49), which may explain why these rats show delayed aging processes similar to those observed in diet restricted rats. For example, the aging of collagen and the onset of kidney disease is

retarded in the hypophysectomized rats, as it is in diet restricted rats. Surprisingly, the life span is shortened in hypophysectomized rats, which demonstrates the possibility that the rate of aging of some organs or systems and the life span may vary independently of each other. Clearly, an intact pituitary gland is essential for the life prolonging effect of diet restriction.

Do hormones affect the course of aging? It does appear that the presence of most hormones accelerates the aging process in their target organs as a result of an increase in functional load and greater "wear and tear" of the organs (9,47,48). However, there are no "aging hormones" per se. The delayed aging seen with hypophysectomy and adrenalectomy is most likely due to a reduction in "wear and tear" on the hippocampus as well as other organs. Diet restriction probably increases life span as a result of depression of anterior pituitary function and hypothalamic endocrine function. Support for this argument is provided by the knowledge that continuous exposure to stress increases the levels of ACTH and corticosterone (9). These increases produce lesions similar to old age and result in an enhanced rate of physiological aging and a shortening of the life span (9). The effects of stress are mediated via the hypothalamic-pituitary-adrenal axis. The secretion of many of the hormones of this axis is decreased in diet restricted rats (49) and may lead to an increased life span.

In conclusion, it has been demonstrated quite conclusively that diet restriction prolongs the life span of various species of animals. However, the basic mechanisms associated with this increased life span are not known. It appears that diet restriction delays the onset of many age-related diseases. The question arises as to the

possibility that dietary manipulation may act by reducing hormone secretion and thereby limit "wear and tear" on the body.

Exercise and Aging

It is not known with any certainty if exercise results in a prolongation of life span. Since exercise can cause a retardation in growth and prevent obesity in rats (50) in a manner similar to that observed in calorie restriction, it could be postulated that exercise, too, would increase the life span of experimental animals. Many early studies (50) reported that exercised rats actually had shorter life spans than sedentary rats. In a study by Slonaker (51), rats given access to exercise wheels were shorter lived than those not allowed to exercise. Similarly Benedict and Sherman (52) found that previously untrained middle aged male rats died prematurely when subjected to a training program. The animals could not adjust to the exercise, lost weight and died.

Edington et al. (53) used male rats of various ages who were made to run on a motor driven treadmill. There were no differences observed in the body weights between the exercised animals and their sedentary controls. Nevertheless, exercise was associated with increased survival in the younger animals, but decreased survival in the older ones. It was postulated that there exists a "Threshold Age" above which it is not advantageous for a rat to begin an exercise regime. However, the apparent prolongation of life in the young rats seemed to have been due to a shortened survival of the sedentary controls, while the reduction in survival in the exercised older animals seemed to be attributable to a prolonged survival of their

sedentary controls. Thus, the variability in the control animals' longevity makes it difficult to evaluate the effect that exercise may have had on life span.

Many researchers feel that life span may actually be inversely related to energy expenditure (54). It is postulated that an increased rate of O_2 consumption would give rise to dangerously reactive free radicals, such as the superoxide anion radical and the hydroxyl radical. The resulting oxygen toxicity would, thus, cause injury to the tissues of the body. Such a chain of events is associated with the "free radical" theory of aging (55).

It has, in fact, been reported that exercise increases free radical concentration in the muscle. Higuchi et al., (56) placed male Long Evans rats on a program of running. Increases in mitochondrial superoxide dismutase (SOD) were observed in the leg muscles, but not at a rate great enough to compensate for the rise in superoxide produced by the increased activity of the mitochondrial citrate cycle and respiratory chain enzymes. Thus, there was most probably free radical damage taking place in the exercise-trained muscles. Such damage may not have a significant impact on longevity, since muscle pathology itself is not a significant contributor to mortality and the damage is not observed in other tissues.

Several recent studies (58, 60, 61) have failed to confirm the theory that life span and energy expenditure are inversely related. Instead, there is now evidence indicating that trained rats actually may have a longer average life span than sedentary controls. Goodrick and his co-workers have demonstrated this in several experiments. In one study (57), male Wistar rats given access to

voluntary running wheels survived an average of 4 months longer than controls, while female runners lived 3 months longer than controls. These rats not only increased their average age at death, but also had an increase in their maximum life span, similar to that seen in calorie restriction. This study was later retracted, however, since the control animals had an unusually short life span (60).

In a study designed to provide additional data on the survival effects of exercise, Goodrick et al. (58) housed adult male Wistar rats (10 and 18.5 months of age) in activity-wheel cages. The experimental rats were placed on a regimen of every-other-day feeding, while similarly housed control rats were ad libitum fed. Calorie restriction was observed to increase activity. This was anticipated, since it was hypothesized that dietary restriction would increase survival by inducing spontaneous exercise. Surprisingly, though, when survival rates of the 2 exercised groups were compared to similarly treated nonexercised rats, exercise was found to have no independent effect on survival, while calorie restriction did. These findings seem consistent with the results of Skalicky et al. (59), who reported that an exercise program begun at 6 months of age reduced the beneficial effects of feed restriction on a number of biological parameters in rats.

In another study, Goodrick et al. (60) fed weanling male Wistar rats a calorie restricted diet and allowed voluntary wheel exercise. Calorie restriction was again found to increase substantially survival rate, though their observations did not support the hypothesis that this was due to increased activity. While the calorie restricted group did have higher activity levels later in life, wheel activity was

actually lower in early life compared to the ad libitum fed control group. Thus, they were unable to demonstrate that increased activity mediates the beneficial effects of calorie restriction on life span. Instead, Goodrick et al. suggested that increased longevity was the result of other mechanisms, such as the maintenance of the integrity of the immunological system.

There is other evidence that indicates exercise results in increased survival rates in rats by countering the deleterious effects of a sedentary life. This was demonstrated by Holloszy et al. (61) in a study consisting of 4 groups of male specific-pathogen-free Long Evans rats. The freely eating rats exercised by running had a longer average life span than the sedentary, ad libitum fed controls. There was also a paired-weight group whose food intake was restricted to keep their body weights the same as the runners, and a pair fed group who were fed the same average amount of food as was eaten by the runners. Interestingly, though the runners' average life span was longer than that of the sedentary pair fed group, the average and maximum life span of the runners was significantly shorter than that of the paired weight animals. It thus appeared that wheel running improved survival without slowing the aging process or extending the life span. In other words, exercise diminished the detrimental effects of a sedentary life, such as obesity and associated metabolic abnormalities, and allowed a larger proportion of the animals to attain an older age. However, it did not result in the increased maximum life span seen with calorie restriction.

This observation is very relevant in that it disproves a hypothesis often used to explain the life prolonging effect of diet

restriction, i.e. the persistence of "growth potential" that occurs with underfeeding. In the study performed by Holloszy et al. (61) the growth rates of both the runners and the paired weight sedentary rats were retarded to the same extent. Thus, these two groups retained a similar growth potential, yet the exercised group had a significantly shorter survival. Therefore, calorie restriction apparently provides an as yet unidentified additional life prolonging factor or factors.

Pituitary-Adrenal Function in Exercise and Aging

During exercise, ACTH is secreted in response to the increased stress and to falling blood glucose (10). This, in turn, stimulates the release of glucocorticoids. Proteolysis in the muscle follows, releasing amino acids into the blood to replenish blood glucose subsequently.

It is not surprising that an acute bout of exercise will cause a sharp rise in ACTH, since exercise has long been regarded as stress to an animal (10). In fact, Sapolsky (9) feels that regular exercise training will accelerate the aging process by exacerbating the hyperadrenocorticism caused by stress, as described earlier. The question is, however, whether an animal can adapt to long term exercise. There is, in fact, evidence that with chronic training there appears to be an adaptation of the hypothalamus-hypophyseal region (62). As a result, less ACTH is released in response to exercise. This training effect was observed by Severson et al. (62), who trained male Sprague-Dawley rats of various ages on a motor driven treadmill. Following the training program, the rats were subjected to an acute bout of swimming. The swimming significantly elevated the

adrenal gland response to ACTH in control rats of all ages and in the young swimming rats. However, there was no elevation in the older trained rats.

Similar results were obtained by Tharp and Buuck (63). Serum corticosterone was measured in male rats who had been trained using treadmill running for 2, 4, 6, and 8 weeks. A progressive decline in the release of corticosterone was observed as the length of training time increased.

Many interpretations of these results have been suggested. Frenkle and Csalay (64) believed this to mean that the adrenals are approaching exhaustion, i.e., prolonged exercise may be causing deterioration of the adrenals. Another possibility is that the pituitary-adrenal axis is undergoing adaptation to the stress of exercise. Such an adaptation has been described by Selye (1) as the General Adaptation Syndrome.

Severson et al. (62) postulated that the change in response to acute exercise is the result of improved efficiency in energy metabolism, leading to a decreased demand for energy compounds, such as glucose and fatty acids. This decrement may be due to the enhanced capacity of skeletal muscle to generate ATP aerobically. These alterations probably do not occur until after maximal growth, as observed by Severson et al. (62)

Epinephrine has also been suggested to play a role in the activation of the pituitary-adrenal system (65). Activity of the central nervous system appears to be lower in trained than in nontrained rats. Chin et al. (65) postulated that exercise trained rats become accustomed to regular physical stress. When subjected to

another physical stress, even though it may be a novel experience, trained rats respond with a smaller release of epinephrine than did controls.

Thus, it has been shown that animals are able to adapt to the stress of exercise as demonstrated by decreases in the amounts of corticosterone, ACTH and epinephrine released in response to training. Therefore, exercise would not exacerbate aging by hyperadrenocorticism, as once believed. Nevertheless, it has yet to be shown that exercise can enhance longevity by alterations in the adrenocortical response.

In conclusion, the consensus among researchers is that caloric restriction increases life span, though the mechanism remains to be elucidated. It has been hypothesized that the enhanced longevity associated with caloric restriction is mediated via the pituitary-adrenal axis. This has been difficult to demonstrate since age-related changes in this axis are unclear and appear to be dependent on the animal model used, tissues analyzed, and/or the conditions under which the tissues were analyzed. Nevertheless, it is apparent from the literature that caloric restriction depresses pituitary function, which results in a diminished "wear and tear" on the organs of the body and possibly leads to an increased life span. The evidence regarding the role of exercise in longevity is not conclusive. It appears that exercise will increase median life span by allowing more animals to reach old age. However, the ability of exercise to extend the life span remains controversial.

None of these studies provided specific information about the effect of diet restriction and aging on the onset of age-related changes in the pituitary-adrenal axis. They also did not examine the effects of exercise and age on the axis. Therefore, it is the purpose of the present study to explore the impact of both diet restriction and/or exercise on the aging pituitary-adrenal axis.

CHAPTER III

EXPERIMENTAL PROCEDURE

Basic Design

In this study, serum levels of corticosterone and ACTH, as well as the concentration of hepatic glucocorticoid receptors and activity of tyrosine aminotransferase were measured in animals which were calorie restricted or fed ad libitum and which were exercised by swimming or were sedentary. Pathogen-free weanling male rats of Wistar origin were obtained from Taconic Laboratories, Germantown, New York. Animals were housed individually in wire mesh stainless steel cages (20 cm x 20 cm x 26 cm). The animal facility was maintained at 22°±2° C, with a 12 hour light - 12 hour dark cycle. Water was provided ad libitum.

There were four experimental groups:

A: Non-exercised; ad libitum fed controls (n=35)
R: Non-exercised; diet restricted by intermittent feeding (n=35)
AE: Exercised by swimming for 3 hours on alternate days; ad libitum fed (n=35)
RE: Exercised by swimming for 3 hours on alternate days; diet restricted by intermittent feeding (n=35)

The animals were weighed weekly until 6 months of age, bi-weekly until 12 months, and monthly thereafter. Ground Purina Rodent Chow was provided as feed. Diet restriction was achieved by feeding the animals on alternate days. Feed cups were placed in the cages at 9:00 a.m. and removed at 9:00 a.m. the following day. Ten or more animals from each experimental group were monitored for feed

intake by measuring feed consumption for 24 hours at monthly intervals. The intake of the diet restricted animals was divided by 2 to determine the average 24 hour intake in a 48 hour period.

The exercised animals were swum in groups of 5 in 32 gallon plastic barrels (50 cm x 68 cm) for 3.0 hours in the morning on alternate days. Water temperature was maintained between 33°C - 36°C. The exercise regimen was begun at 6 weeks of age, initially for a 10-minute period and increased 10 minutes daily until 3.0 hours was reached at 3 mo of age. The RE rats were swum prior to being fed. Swimming was continued throughout the experimental period, i.e., until the the animals were killed for biochemical analyses. As the animals aged, swimming time was decreased to 2.0 hours at 15 months of age, 1.5 hours at 20 months and 1.0 hour at 26 months. At pre-planned points in the experiment, i.e. at 12-, 16-, 20-, 24- and 28-months of age, six rats, randomly selected from each of the experimental groups, were killed for biochemical determinations, though, to allow for natural or accidental deaths, the groups had 35 animals initially.

The R and RE rats were allowed feed the day and night before they were killed, though no rats were swum on that day. Feed cups were removed from all cages two hours before sacrifice. Rats were stunned by a blow to the head, followed by decapitation. Serum was collected and frozen for later analysis of corticosterone and ACTH. The livers, hearts and adrenals were removed and weighed and the liver was analyzed for hormone receptors and enzyme activity.

Measurement of Glucocorticoid Receptor Concentration

The method used to measure the concentration of hepatic glucocorticoid receptors was that described by Kalimi and Hubbard (66) whereby the cytosol is treated with [^{3}H] dexamethasone, with and without cold dexamethasone. The complete exchange of free and steroid-bound receptors occurs by means of the synergistic effects of molybdate and dithiothreitol (DTT). This assay allows the accurate quantification of both free and steroid-bound receptors in the tissues of animals, and alleviates the need to perform adrenalectomies before receptors can be measured.

Reagents.

1. Tris-HCl Buffer (10 mM): 1.21 g Tris was mixed with 500 ml water. Approximately 76 ml 0.1 N HCl was added to obtain a pH of 7.5. Finally, 85.5 g sucrose (0.25 M) was dissolved into the solution. (A separate Tris-HCl buffer was prepared without sucrose and used for preparation of the dexamethasone reagent.)

2. Dithiothreitol (5 mM): 462.72 mg DTT was dissolved in 10 ml Tris-HCl buffer.

3. Molybdate (Mo, 10 mM): 1.4518 g Na_2MoO_4 was dissolved in enough Tris-HCl buffer to make 10 ml.

4. [^{3}H]-Dexamethasone (5 x 10^{-8}M, 49.9 Ci/mmol): 25 ul of ^{3}H-sigma diluted to 1.84 ml with Tris-HCl buffer without sucrose. This was added to a mixture in which 0.366 ml DTT and 0.366 ml Mo had been mixed with 1.08 ml buffer without sucrose.

5. Cold dexamethasone (10^{-5} M): 11.775 mg cold dexamethasone was brought up to 250 ml using the Tris-HCl buffer without sucrose.

6. Dextran-coated charcoal (DCC): 3.75 g activated charcoal and 0.375 g dextran-500 were dissolved in Tris-HCl buffer to a final volume of 100 ml.

Procedure. Immediately following excisions of livers, they were blotted to remove excess blood, and weighed. Liver tissue (3 g) was homogenized with 12 ml Tris-HCl buffer (1:4 w/v), using a Polytron (Brinkman Instruments, Westberg, New York). Liver samples were kept in an ice bath at all times during the procedure. The homogenates were then centrifuged at 100,000 x g for 60 minutes at 4°C. The upper fatty layer was aspirated and discarded.

A 0.2 ml aliquot of the cytosol was removed. The remainder of the cytosol was saved for later measurements of tyrosine aminotransferase (TAT) activity and protein concentration. ^{3}H-dexamethasone (0.02 ml) was added to all samples, while 0.02 ml of cold dexamethasone was only added to half of the samples. The same amount of buffer was added to those without cold dexamethasone to maintain volume parity. All samples were mixed using a vortex mixer and then incubated for 48 hours at 4° C. After incubation, the samples were treated with 100 ul DCC, mixed for 10 seconds and allowed to stand in an ice bath for 10 minutes. The samples were centrifuged at 3000 x g for 10 minutes at 2°C. A 200 ul aliquot of the supernatant was transferred to a minivial containing 10 ml Aquasol

scintillation fluid. Radioactivity was determined with a Beckman liquid scintillation counter and recorded as counts per minute (cpm). All assays were run in duplicate.

Determination of Tissue Protein Levels

The protein content of the liver homogenate supernatant was determined by the method of Ohnishi and Barr (67). This method is a modification of the Lowry method (68) whereby the protein sample is mixed first with a diluted biuret reagent and then with 2 N phenol reagent for color development.

Reagents.

1. Biuret reagent: 1.5 g $CuSO_4 \cdot 5H_20$, 6 g $NaKC_4H_4O_6 \cdot 4H_2O$, and 30 g NaOH and 1 g KI were dissolved, in order, and diluted to 1 liter with distilled water and stored in a polyethylene bottle. This was diluted eight times with 2.3% w/v Na_2CO_3 (i.e., 1 volume of biuret reagent plus 7 volumes of Na_2CO_3) at the time of analysis.

2. 2 N phenol reagent.

3. Bovine serum albumin stock standard: 1 g bovine serum albumin was dissolved in and diluted to 100 ml with 0.4 N NaOH. A working standard was prepared daily by diluting this to a concentration of 0.02 g bovine serum albumin per 100 ml of 0.4 N NaOH.

Procedure. To 0.8 ml of supernatant, 3.2 ml of the diluted biuret solution was added. After mixing, the solution was allowed to stand for 10 minutes at room temperature. One-tenth ml of 2N

phenol reagent (undiluted) was added while the solution was mixed using a vortex mixer. The protein standard containing bovine serum albumin received the same treatment as the supernatant from the samples.

After 30 minutes, the absorbance (A) of the samples and standards were read in a Beckman spectrophotometer (Model 34, Palo Alto, California) at 550 nm. The protein concentrations of the samples were calculated by the following formula:

mg protein/ml supernatant fluid=

$$\frac{\text{A sample}}{\text{A standard}} \times 0.2 \text{ mg protein/ml} \times 10 \text{ (dilution factor)}$$

Methods for Enzyme Assay

Tyrosine aminotransferase (TAT) activity of rat liver supernatant fraction was determined by the method of Diamondstone (69). The basis of this method is the spectrophotometric measurement of the rate of 4-hydroxybenzaldehyde formation from the increase in absorbance at 331 nm.

Reagents.

1. Phosphate buffer (0.2 M): 6.96 g K_2HPO_4 was dissolved in 200 ml water. KH_2PO_4 (2.18 g) was dissolved in 80 ml water. The K_2HPO_4 solution was stirred constantly while adding enough KH_2PO_4 to bring the pH to 7.3.

2. 2-oxoglutarate (0.3 M): 554 mg monopotassium salt of 2-oxoglutarate was dissolved in 20 ml phosphate buffer.

3. Pyridoxal phosphate (1.33 M): 3 mg pyridoxal phosphate was dissolved in 10 ml of phosphate buffer.

4. Tyrosine (6.85 mM): 62 mg tyrosine was slowly stirred into 50 ml phosphate buffer. The mixture was heated below the boiling point until all tyrosine was dissolved and held in a 37°C water-bath until used. The mixture was prepared fresh daily.

5. Diethyldithiocarbamate (0.06 M): 200 mg was dissolved in 200 ml water.

6. Sodium hydroxide (10 M): 4 g NaOH was slowly added to 6 ml water until a 10 ml final solution was achieved.

Procedure. The cytosolic fraction of the liver tissue (purified for the receptor assay) was utilized for assay of TAT activity. The reaction mixture was prepared to contain 2.8 ml tyrosine solution, 0.1 ml pyridoxal phosphate solution, and 0.2 ml of the sample. This solution was mixed and allowed to stand in a water-bath (37°C) for 1 hour. After 1 hour, 0.1 ml 2-oxoglutarate solution was added and mixed. A reaction time of 10 minutes was allowed. One-tenth ml NaOH was added at the end of the reaction time and the solution mixed immediately. The samples were then allowed to stand at room temperature for 30 minutes. The absorbance of the sample was measured at 331 nm against a blank containing all the reagents, but no cytosol.

The enzyme activity of TAT was expressed as U/g wet weight was calculated using an absorption coefficient for 4-hydroxybenzaldehyde under the conditions of the assay as 1.991 x $mmol^{-1}$ x mm^{-1}.

The catalytic activity of the sample was calculated by the following equation:

$$\text{TAT activity} = \frac{A_{331}}{t} \times \frac{3.3 \times 1.03 \times 1000}{1.99 \times 10 \times 0.2}$$

$$= 854 \times \frac{A_{331}}{t} \text{ U/ml}$$

Method for Serum ACTH Assay

The concentration of serum ACTH was determined by the method of Orth (70). A RIA kit prepared by Diagnostic Systems Laboratories, Inc., Webster, Texas, was used for this assay. The basis of this method is radioimmunosassy (RIA) whereby there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of [^{125}I] ACTH bound to the antibody is inversely proportional to the concentration of unlabeled ACTH in the plasma.

Reagents.

1. ACTH Standards (0 - 1000 pg/ml of ACTH in buffered ACTH free human plasma): Reconstituted with 1.0 ml ACTH buffer.

2. ACTH [I^{125}] Reagent (1 uCi): Reconstituted with 10 ml ACTH buffer.

3. ACTH-Antiserum (rabbit antihuman ACTH serum): Reconstituted with 10 ml deionized water.

4. Precipitating Reagent (goat anti-rabbit gamma globulin serum and polyethylene glycol): Provided ready to use in kit.

5. ACTH Controls (2 vials containing a low and a high ACTH concentration in buffered ACTH free human plasma): Reconstituted with 1.0 ml ACTH buffer.

Procedure. The assay was done in duplicate and all reagents and samples were kept in an ice bath at all times during the analysis. To 200 ul of standard or sample, 100 ul ACTH antiserum was added. Non-specific binding (NSB), or counts obtained bound in the absence of antiserum, was controlled for by omitting the ACTH antiserum from the NSB tubes and then subtracting these counts from the samples. ACTH [^{125}I] reagent (100 ul) was then added to all tubes. Tubes were mixed and then incubated at 4°C for 24 hours.

After incubation, 1.0 ml of precipitant reagent was added to all tubes. The contents of the tubes was mixed thoroughly with a mechanical mixer until a homogeneous color was observed and then allowed to stand at room temperature 15 minutes. The samples were centrifuged at 1500 x g 10 minutes. The supernatant was decanted and the remaining precipitate counted in a gamma counter for 1 minute.

The amount of serum ACTH was reported as pg/ml. To determine this, a curve of % bound/unbound hormone (%B/Bo) for the ACTH standards against the ACTH concentration was plotted on linear-log graph paper. The ACTH concentration of the samples was determined from the standard curve. The %B/Bo was calculated by the following equation:

$$\%B/Bo = \frac{\text{CPM (sample) - CPM Non-specific Binding (NSB)}}{\text{Average counts of zero standard - NSB}}$$

Measurement of Serum Corticosterone

An RIA kit from Radioassay Systems Laboratories, Inc., Carson, CA, was used to determine the concentration of serum corticosterone. The method is based on that of Shimuza et al. (71), which used a labelled antigen (^{125}I) added to the serum. As with serum ACTH, RIA was applied to quantitate the amount of antigen (i.e. hormone) by determining the extent to which it combines with its antibody.

Reagents. (All were provided by the manufacturer as indicated below):

1. Steroid diluent: Phosphosaline gelatin buffer (pH 7.0) containing rabbit gamma globulins.

2. Anti-Corticosterone: Corticosterone-3-carboxymethyloxime: Bovine serum albumin.

3. Corticosterone Calibrators: 6 calibrators at the following concentrations: 25 ng/ml, 50 ng/ml, 100 ng/ml, 250 ng/ml, 500 ng/ml, and 1000 ng/ml.

4. Precipitant Solution: Contained a mixture of PEG and goat anti-rabbit gamma globulins.

5. Corticosterone-^{125}I derivative: Contained less than 7 uCi per kit.

Procedure. Rat serum was diluted 1:200 with the steroid diluent. One-tenth ml of the diluted serum was added to 10 x 75 cm glass test tubes to which 0.2 ml of corticosterone -^{125}I and then 0.2 ml of anti-corticosterone were added. Samples were then mixed mechanically and allowed to incubate for 2 hours at room

temperature. After incubation, 0.5 ml of the precipitant solution was added to all tubes, after which all tubes were mixed. The assay tubes were centrifuged at 1000 x g 15 for minutes. The supernatant was decanted, and the remaining precipitate was counted in a gamma counter for 2 minutes.

The serum corticosterone was reported as ng/ml. To determine the concentration, a curve of % bound/unbound hormone (%B/Bo) was plotted on linear-log graph paper, as was described for the ACTH assay. The corticosterone concentration of each sample was determined from the standard curve. %B/Bo was calculated by the following equation:

$$\%B/Bo = \frac{CPM\ (Sample) - CPM\ (NSB)}{CPM\ (0\ calibrator) - CPM\ (NSB)} \times 100$$

Statistical Methods

The general linear model (GLM) procedures of SAS 1987 (72) were utilized to evaluate the effect of diet restriction, exercise and aging on the pituitary-adrenal function. Type I and Type III sum of squares in multiple regression analysis were calculated to test these relationships. In each model statement the class variables age, diet and exercise were entered first, followed by the continuous variables. This was done to assess whether there was a relationship between the continuous variables that could not be accounted for by the class variables.

Type III sum of squares was used to analyze the effects of the class variables (diet, age, exercise) and their interactions on the test

responses indicative of pituitary-adrenal function. Relationships between the continuous variables were assessed by calculating the Type I sum of squares. To measure nonlinear relationships, the quadratic and cubic equations of multiple regression were employed. A probability level of less than 0.05 was considered statistically significant.

CHAPTER IV

RESULTS

Body weights of each experimental group at the time of sacrifice are presented in Table 1, with the results of the statistical analyses shown in Table 2. The mean maximum body weights of the two calorie restricted groups were significantly less than those of the 2 ad libitum fed groups (R vs A, 33%; RE vs AE, 26%; $p<0.0001$). Body weights decreased as the rats aged ($p<0.03$). The ad libitum fed control group (A) reached 707 g at 20 mo and decreased 12% to 622 g at 28 mo. AE's maximum weight (651 g) was approximately 8% less than that of A (NS), and decreased 14% to 561 g at 28 mo. The body weights of R and RE decreased more gradually with age, and their weights varied around each other so that there were no significant differences between R and RE. There was, however, a trend for an interaction between diet and exercise ($p<0.1$) whereby AE body weights were less than A while there were no differences between R and RE.

Mean feed consumption was greater in the exercised than in the non-exercised animals. At 6 mo of age, A and AE mean feed intakes were 24 and 27 g, respectively. Intake decreased to an average of 20.5 (A) and 25 g (AE) at 28 mo of age. In contrast, R and RE animals increased their feed consumption from an average of 16 and 19 g respectively at 6 mo of age to an average of 18.5 and 20.5, respectively at 28 mo. At midpoint in the experiment (16 mo of age),

Table 1. Effect of exercise, diet restriction and age on body weight[1]

GROUP	AGE IN MONTHS 12	16	20	24	28
	g	g	g	g	g
A[2]	649.2 ±42.3(6)[6]	662.8 ±35.3(6)	707.6 ±31.4(6)	659.7 ±61.9(6)	622.0 ±39.2(6)
R[3]	474.0 ±11.6(6)	463.8 ±11.8(6)	466.0 ±16.9(6)	442.2 ±8.4(6)	437.6 ±17.4(6)
AE[4]	649.0 ±10.8(6)	651.2 ±24.6(6)	648.0 ±27.8(5)	587.2 ±22.8(6)	561.3 ±22.3(3)
RE[5]	459.0 ±20.8(6)	485.5 ±18.9(6)	467.8 ±24.0(6)	467.8 ±37.4(6)	416.5 ±12.1(6)

[1]$p<0.05$ for age and diet in multiple regression analysis for all groups.

[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 for the number of animals shown in parentheses.

Table 2. ANOVA table for body weights

Body Weight	df	SS	F Value	P Value
TYPE III SS				
Age	4	53138.9	2.78	0.0310
Diet	1	935927.0	195.95	0.0001
Exercise	1	10451.3	2.19	0.1423
Age*Diet	4	7287.2	0.38	0.8214
Age*Exercise	4	7286.2	0.38	0.8215
Diet*Exercise	1	13470.8	2.82	0.0963
Age*Diet*Exercise	4	10247.2	0.54	0.7093
RESIDUAL	96	458522.5		

AE ate approximately 12% more feed than A, and RE ate approximately 11.5% more than RE. R and RE ate about 26% less than A and AE respectively at 20 mo of age.

Heart and Adrenal Weights

The mean heart weight to body weight ratios of all groups are plotted versus age in Figure 1 and the ANOVA Table is presented in Table 3. The ratios of the exercised groups (AE and RE) were, in general, significantly larger ($p<0.0001$) than those of sedentary animals (A and R). All ratios increased significantly with age ($p<0.0001$). Absolute heart weights for A and AE (not presented) tended to increase with age ($p=0.1$), with means ranging from approximately 1.8 g at 12 mo to 2.1 g at 28 mo. By contrast, R and RE heart weights stabilized at about 20 mo. Heart weights of R and RE varied from approximately 1.4 g at 12 mo to 1.6 g at 28 mo. Exercise resulted in significantly larger hearts in both AE and RE when compared to the sedentary groups, A and R ($p=0.01$), and calorie restriction resulted in smaller hearts, i.e., A>R and AE>RE ($p<0.0001$).

Adrenal weight also increased ($p<0.0001$) with age (Figure 2 and Table 4) with AE having the sharpest rise from 20 mo to 28 mo. In addition, exercise resulted in enlarged adrenals ($p=0.0009$) while caloric restriction significantly reduced adrenal weights ($p<0.05$). An age*diet interaction was observed ($p=0.01$) whereby the adrenal mass of the ad libitum fed animals increased more with age than those of the feed restricted animals. At 28 mo of age, the adrenal mass of AE was 43% larger than that of A, while the adrenal mass of RE was 23%

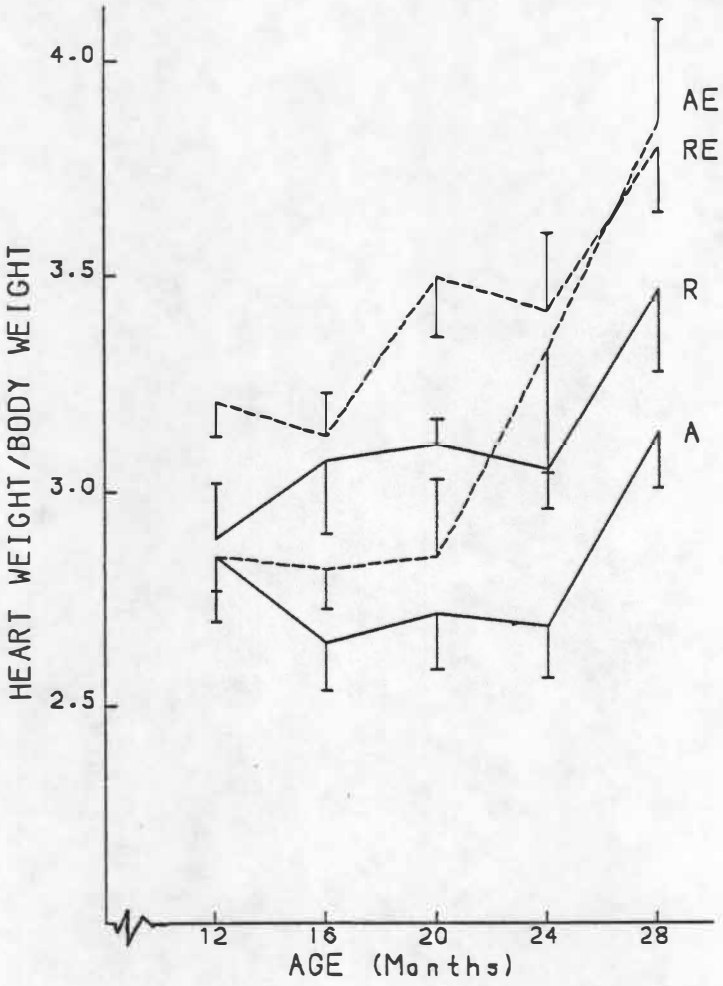

Figure 1. Ratio of heart weight and body weight, plotted versus age. (A=Ad libitum-fed controls; R=Restricted-fed, non-exercised; AE=Ad libitum-fed, exercised; RE=Restricted-fed, exercised. Values are means ± SEM; in general n=6. Heart weight/body weight ratio is related to age, diet and exercise in multiple regression analyses, $p<0.0001$.)

Table 3. ANOVA table for the ratio of heart weight to body weight

HW:BW	df	SS	F Value	P Value
TYPE III SS				
Age	4	5.6028	11.02	0.0001
Diet	1	2.3451	18.45	0.0001
Exercise	1	2.7534	21.66	0.0001
Age*Diet	4	0.5174	1.02	0.4021
Age*Exercise	4	0.8241	1.62	0.1752
Diet*Exercise	1	0.0082	0.06	0.7998
Age*Diet*Exercise	4	0.5828	1.15	0.3395
RESIDUAL	96	12.2009		

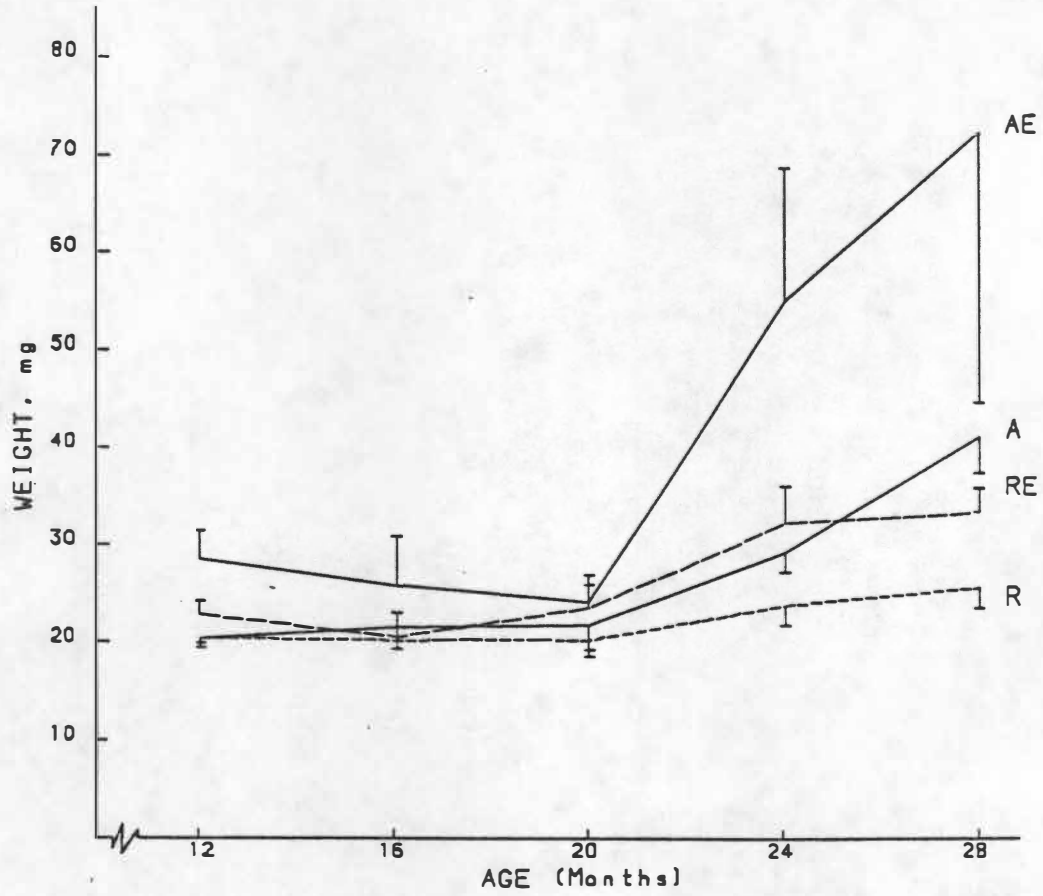

Figure 2. Adrenal weight (both glands) in g, plotted versus age. (A=Ad libitum-fed controls; R=Restricted-fed, non-exercised; AE=Ad libitum-fed, exercised; RE=Restricted-fed, exercised. Values are means ± SEM; in general n=6. Adrenal weight is related to age ($p<0.0001$), diet ($p<0.0001$), exercise ($p=0.0009$), age*diet ($p=0.01$), in multiple regression analyses.)

Table 4. ANOVA table for adrenal weights

Adrenals	df	SS	F Value	P Value
TYPE III SS				
Age	4	1689.6	8.80	0.0001
Diet	1	789.5	16.45	0.0001
Exercise	1	564.5	11.76	0.0009
Body Weight	1	72.9	1.48	0.2275
Age*Diet	4	671.5	3.50	0.0104
Age*Exercise	4	431.4	2.25	0.0698
Diet*Exercise	1	176.6	3.68	0.0581
Age*Diet*Exercise	5	305.8	1.27	0.2815
TYPE I SS				
ACTH				
Linear	1	9.3	0.19	0.6654
Quadratic	1	2.4	0.05	0.8211
Cubic	1	179.4	3.74	0.0562
RESIDUAL	93	4462.2		

greater than R. Mean adrenal weights (both glands) ranged from 20.1 mg in the 12 mo R animals to 72.5 mg in the 28 old AE animals.

Serum ACTH

There was a significant nonlinear increase in serum ACTH concentrations with age ($p=0.01$, Tables 5 and 6). With the exception of RE, ACTH concentrations decreased between 12 and 20 mo. However, the concentrations increased sharply thereafter. There were no significant differences in the concentration of serum ACTH resulting from exercise or dietary treatments. ACTH concentrations of A ranged from 247 pg/ml at 12 mo to 608 pg/ml at 28 mo, while R ranged from 463 pg/ml at 12 mo, reached a low of 128 pg/ml at 20 mo, and then increased to 335 pg/ml at 28 mo.

Serum Corticosterone

Figure 3 and Table 7 present the effects of diet, exercise and age on serum corticosterone concentration. Table 5 also presents the relationship between ACTH and corticosterone. All groups had significant linear increases in serum concentrations with age ($p<0.05$). Serum levels were fairly constant up to 20 mo of age with a sharp rise appearing thereafter. Corticosterone concentration was not significantly affected by diet or exercise, though there was a relationship with body weight ($p=0.0029$) whereby both body weight and serum corticosterone concentration increased with age. A similar relationship was observed for adrenal weight and corticosterone concentration ($p<0.05$). There was also a quadratic relationship between ACTH and corticosterone ($p<0.05$), i.e., before 20 mo of

Table 5. Effect of exercise, diet restriction and age[1] on serum ACTH concentration

GROUP	AGE IN MONTHS 12	16	20	24	28
	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml
A[2]	247 ±151(6)[6]	69 ±19(6)	182 ±97(6)	203 ±65(6)	608 ±119(6)
R[3]	463 ±131(6)	398 ±109(6)	128 ±25(6)	255 ±71(6)	335 ±44(6)
AE[4]	468 ±149(6)	272 ±72(6)	268 ±122(5)	345 ±141(6)	450 ±89(3)
RE[5]	362 ±139(6)	392 ±129(6)	608 ±234(6)	164 ±53(6)	474 ±105(6)

[1]p=0.05 for age in multiple regression analysis for all groups.

[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 for the number of animals shown in parentheses.

Table 6. ANOVA table of serum ACTH concentration

ACTH	df	SS	F Value	P Value
TYPE III SS				
Age	4	787694.1	2.44	0.0526
Diet	1	88092.3	1.09	0.2993
Exercise	1	292519.9	3.62	0.0603
Body Weight	1	9147.8	0.11	0.7437
Age*Diet	4	537961.8	1.66	0.1651
Age*Exercise	4	283926.2	0.88	0.4804
Diet*Exercise	1	1424.7	0.02	0.8943
Age*Diet*Exercise	4	646720.8	1.60	0.1679
TYPE I SS				
Age^2	1	557187.4	6.89	0.0101
RESIDUAL	93	7519689.7		

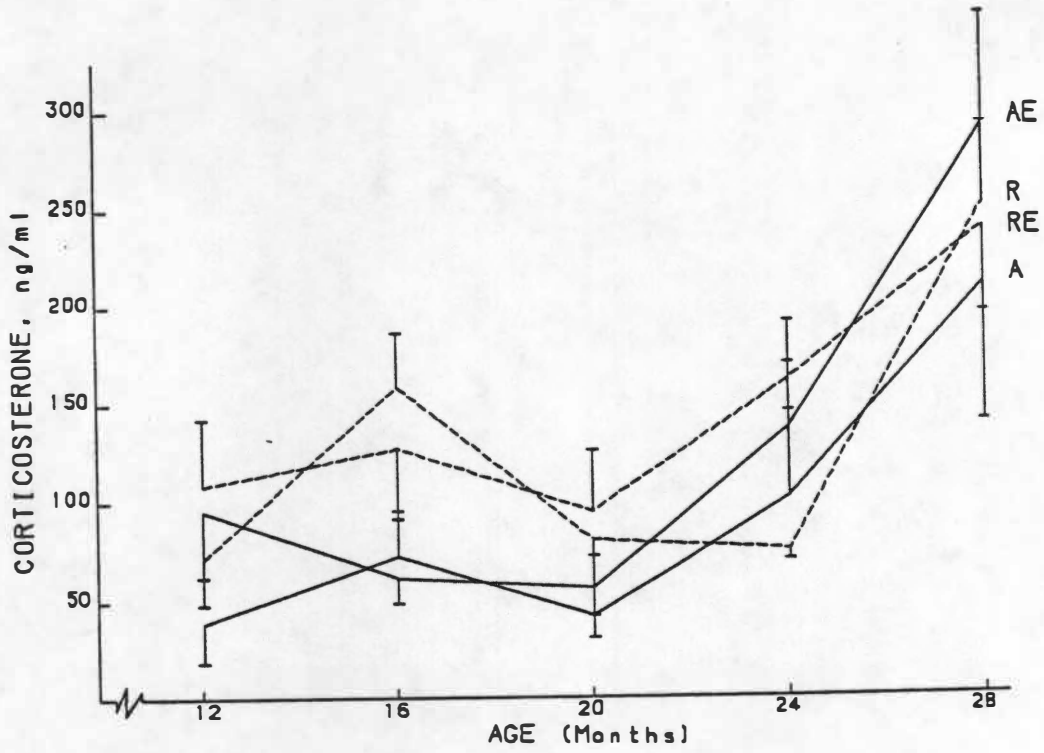

Figure 3. Serum corticosterone concentrations plotted versus age. (A=Ad libitum-fed controls; R=Restricted-fed, non-exercised; AE=Ad libitum-fed, exercised; RE=Restricted-fed, exercised. Values are means ± SEM; in general n=6. Serum corticosterone is related to age ($p<0.0001$), body weight ($p=0.0029$), and adrenal weight ($p=0.0444$) in multiple regression analyses.)

Table 7. ANOVA table for serum corticosterone concentration

Corticosterone	df	SS	F Value	P Value
TYPE III SS				
Age	4	270785.6	9.93	0.0001
Diet	1	13817.8	2.03	0.1578
Exercise	1	24153.5	3.54	0.0629
Body Weight	1	58633.8	9.26	0.0029
Adrenal Weight	1	26198.2	4.14	0.0444
Age*Diet	4	32196.2	1.18	0.3242
Age*Exercise	4	31819.9	1.17	0.3303
Diet*Exercise	1	289.1	0.04	0.8372
Age*Diet*Exercise	4	11291.9	0.41	0.7980
TYPE I SS				
ACTH				
Linear	1	12284.2	1.80	0.1826
Quadratic	1	37531.8	5.51	0.0211
Cubic	1	2281.5	0.33	0.5642
RESIDUAL	93	633701.2		

age, ACTH concentration decreased while corticosterone concentration remained fairly stable, whereas after 20 mo, both hormone concentrations increased.

Glucocorticoid Receptor Concentration

The mean concentrations of hepatic glucocorticoid receptors are shown in Table 8. The concentrations varied greatly and there were no significant differences resulting from diet, exercise, or age (Table 9). Additionally, the relationship between serum corticosterone and liver receptors was non-significant.

Tyrosine Aminotransferase Activity (TAT)

There was a significant linear decrease in liver TAT activity with age for all groups ($p<0.0001$, Figure 4, Table 10). Ad libitum feeding resulted in significantly less TAT activity than did restricted feeding ($p=0.0004$). However, there were no differences in TAT activity resulting from exercise. Also, no significant relationships were observed between serum corticosterone and TAT activity or between hepatic receptors and TAT activity.

Table 8. Effect of exercise, diet restriction and age on liver glucocorticoid receptor concentration

GROUP	AGE IN MONTHS 12	16	20	24	28
	cpm x 10^{-3}/g				
A[1]	11.4 ±3.1(6)[5]	11.6 ±1.4(6)	11.5 ±1.6(6)	14.2 ±4.1(6)	14.4 ±1.3(6)
R[2]	14.4 ±1.8(6)	8.7 ±1.5(6)	14.4 ±1.1(6)	11.6 ±1.5(6)	12.6 ±1.5(6)
AE[3]	12.9 ±3.3(6)	12.6 ±1.3(6)	11.9 ±1.4(5)	11.6 ±1.9(6)	10.2 ±0.5(3)
RE[4]	12.1 ±14.6(6)	11.8 ±1.1(6)	11.5 ±0.9(6)	13.1 ±1.3(6)	12.4 ±1.1(6)

[1]A = Ad libitum-fed, non-exercised.

[2]R = Restricted-fed, non-exercised.

[3]AE = Ad libitum-fed, exercised.

[4]RE = Restricted-fed, exercised.

[5]Values are means ± SEM for the number of animals shown in parentheses.

Table 9. ANOVA table for hepatic glucocorticoid receptor concentrations

Receptor	df	SS	F Value	P Value
TYPE III SS				
Age	4	53094163.7	0.59	0.6713
Diet	1	1008558.6	0.04	0.8329
Exercise	1	11597572.9	0.51	0.4749
Age*Diet	4	31141247.2	0.35	0.8465
Age*Exercise	4	57004052.1	0.63	0.6405
Diet*Exercise	1	5778888.1	0.26	0.6137
Age*Diet*Exercise	4	65081529.3	0.72	0.5789
TYPE I SS				
Corticosterone				
Linear	1	1579941.7	0.07	0.7917
Quadratic	1	1421454.9	0.06	0.8022
Cubic	1	863468.6	0.04	0.8452
RESIDUAL	93	2095249674.6		

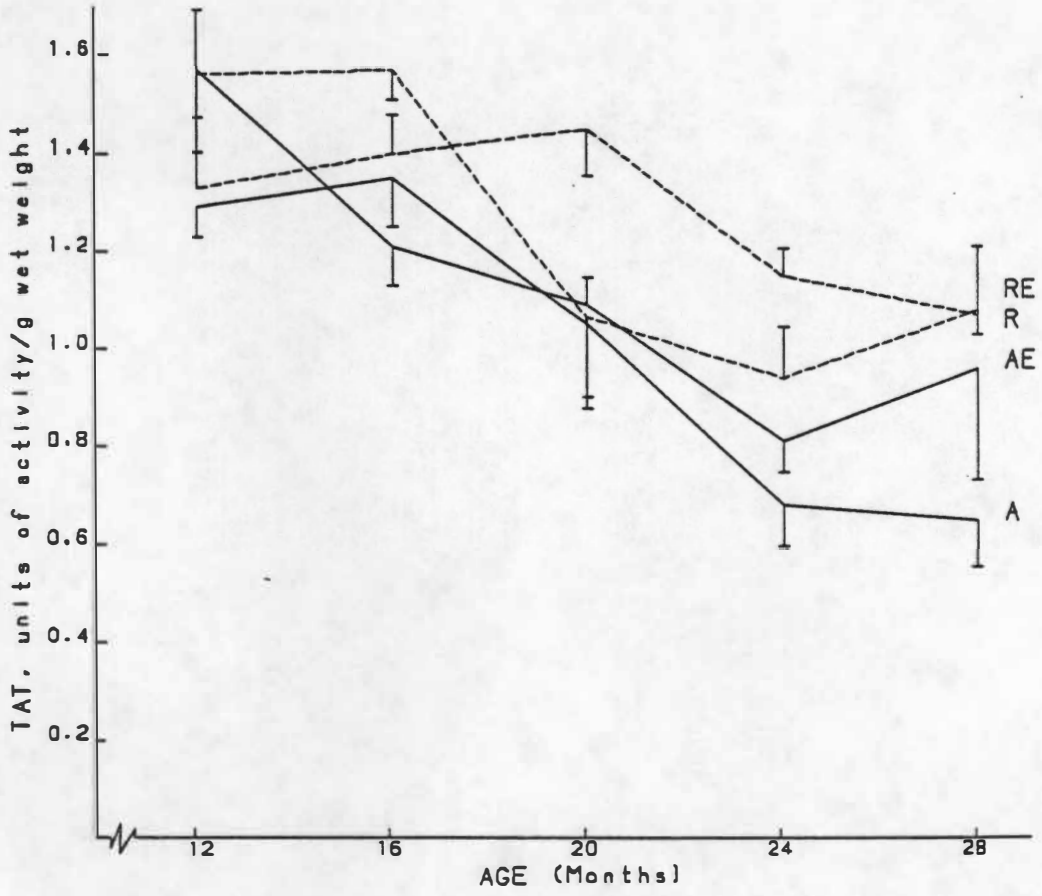

Figure 4. Hepatic tyrosine aminotransferase activity plotted versus age. (A=Ad libitum-fed controls; R=Restricted-fed, non-exercised; AE=Ad libitum-fed, exercised; RE=Restricted-fed, exercised. Values are means ± SEM; in general n=6. TAT activity is related to age ($p<0.0001$) and diet ($p=0.0004$) in multiple regression analyses.)

Table 10. ANOVA table for hepatic tyrosine aminotransferase activity

TAT Activity	df	SS	F Value	P Value
TYPE III SS				
Age	4	5.0395	17.77	0.0001
Diet	1	0.9666	13.63	0.0004
Exercise	1	0.1699	2.40	0.1250
Body Weight	1	0.0462	0.60	0.4399
Age*Diet	4	0.2675	0.94	0.4427
Age*Exercise	4	0.4876	1.85	0.1098
Diet*Exercise	1	0.0502	0.71	0.4002
Age*Diet*Exercise	4	0.6577	1.85	0.1098
TYPE I SS				
Corticosterone				
Linear	1	0.0291	0.41	0.5229
Quadratic	1	0.0031	0.04	0.8343
Cubic	1	0.0974	1.37	0.2443
Receptor				
Linear	1	0.0209	0.27	0.6041
Quadratic	1	0.0476	0.62	0.4347
Cubic	1	0.0713	0.92	0.3395
RESIDUAL	93	6.5954		

CHAPTER V

DISCUSSION

The hypothesis that the enhanced longevity observed with caloric restriction may be mediated via the pituitary-adrenal axis (9,48) was supported only partially in the present investigation. The results did indicate, however, that aging alone produced many changes in the axis. While serum corticosterone concentration and adrenal weight increased significantly with age, hepatic TAT activity decreased steadily. There was a nonlinear change in serum ACTH concentration with age whereby the concentration decreased up to 20 mo of age and increased thereafter. Liver receptor concentration remained unchanged. These findings are similar to those of some researchers (9,15,19,24,25,27), though they are inconsistent with the findings of others (12,13,16,17,18). The many discrepancies found between these studies may be due to the use of different tissues or cells, examining tissues under different conditions, or the possibility that many results may be species-specific.

Exercised animals exhibited a slightly diminished weight gain when compared to the non-exercised animals. Even though the exercised animals ate approximately 12% calories more, the maximum body weights of AE at sacrifice were approximately 8% less than A and there were no real differences between R and RE. This contradiction between feed consumption and body weight is consistent with most findings (73,74), though similar findings have not been observed in studies using female animals (75,76). Male rats

tend to lose weight with exercise even when feed intake is increased, whereas females rats tend to boost their feed intake enough to maintain body weight to the level of sedentary animals (75,76). It is interesting to note that, while the exercised animals demonstrated a small decrease in the rate of growth, there was no concomitant increase in longevity in those animals allowed to live out their life span (85). This finding is inconsistent with the theory that increased growth potential is the cause of increased longevity in diet restricted rats (30).

Neither feed restriction nor exercise, alone or in combination, resulted in significant differences when compared to control values of most of the parameters measured. The feed restricted animals and ad libitum-fed exercised animals all had increases similar to A in serum corticosterone concentration, a similar nonlinear change in serum ACTH concentration, and no change in receptor concentration. However, the one exception was TAT activity. Feed restriction prevented TAT activity from decreasing as rapidly with age.

To interpret the latter observation, it is necessary to understand the mechanism of diminished TAT activity with age. There are many possible explanations. It has been postulated that the age-related decrease in TAT activity may by the result of an impairment in the regulation of TAT synthesis (77). Adelman (77) reported that it is most likely due to a delay in the incorporation of amino acids into the enzyme. It has also been suggested that there is a decrease in hepatic blood flow with age, resulting in a diminished TAT response (25). Disturbances in extrahepatic endocrinological regulatory mechanisms may also exist (77). Jacobus and Gershon (25) advanced the

possibility that alterations in the enzyme molecules accumulate with age. These modifications would most likely be minor conformational changes which result in inactive TAT molecules that are catalytically impaired but antigenically intact, abbreviated as CRM (cross-reacting material). Considerable amounts of CRM have been observed in old mice for both basal and induced phases of TAT. This suggests an impairment in the processing of precursor molecules in old animals which makes it necessary for the cells of old animals to have a larger number of enzyme molecules in an effort to maintain TAT activity. Though the mechanism is not known, diet restriction, as observed in the present study, may help preserve TAT activity by intervening in any of the above possibilities.

In discussing the results of the present study, it must be emphasized that all the analyses were performed on animals in the basal state. While this was a necessity due to the limitations of the investigation (i.e., to prevent the introduction of other variables into the study), measuring induced levels of TAT might have revealed something quite different. In this study, basal TAT activity decreased with age. This finding is in agreement with that of Jacobus and Gershon (25) who observed considerable age-related decreases in basal TAT activity. However, it has been reported by Adelman (77) that, though the period of time required to induce maximal levels of TAT was significantly increased in aged animals, its peak activity was eventually the same for young and old animals.

As mentioned above, exercise resulted in no differences in the pituitary-adrenal axis responses. The question, thus, arises as to whether the animals were effectively trained by the exercise

regimen. While there are several methods used to verify a training effect (measuring mitochondrial enzymes, for example), the ratio of heart weight to body weight (HW:BW) is recognized as an appropriate test (78). There may be some inaccuracies inherent in this method, such as varying amounts of blood remaining in the heart valves. However, these are considered to be minor. In the current study, it was verified that the swimming did, in fact, result in a training effect as indicated by HW:BW. The exercised animals had significantly greater ratios which strongly suggests that training did occur in these rats (78). The mechanisms for the cardiac hypertrophy observed with training are not clear. Kurowski et al. (79) postulated that, though the glucocorticoids are usually catabolic on skeletal muscle, they may be anabolic in the cardiac muscle. In fact, they observed an additive effect of exercise and glucocorticoids on cardiac mass. Hickson et al. (78) demonstrated that it is the glucocorticoids and not the androgens which undergo adaptive changes leading to exercise-induced cardiac hypertrophy.

In spite of the fact that a training effect was observed in the present study, it appears that exercise did not provide any benefit to the aging pituitary-adrenal axis. It is possible that this was due to the stress caused by the exercise regimen. The chosen form of exercise, i.e. swimming, was very likely more stressful than voluntary wheel running. This was evidenced by the fact that the animals struggled with each other while swimming, resulting in many cuts and gashes and near drownings. The swimmers also had significantly larger adrenal glands, indicating a stress to the adrenocortical system (48). Though AE had markedly enlarged adrenals during the latter

part of the life span, RE did not have such major changes. This finding suggests that feed restriction may have protected the RE rats somewhat from the stresses of swimming.

Some adaptation to exercise stress may have occurred. With time, the exercised rats appeared to relax more while swimming and to struggle less with the other rats. Though the present study did not measure serum hormones at the time of exercise to assess the amount of stress that exercise was causing, the present observations are consistent with many studies which were able to make such measures. Rees et al. (80) exercised ducks on a treadmill for 28 days and reported decreases in ACTH and corticosterone secretion during training. Tharp and Buuck (63), Kurowski et al. (79) and Severson et al. (62) observed similar decreases in exercised rats. Various explanations for these observations were given. Rees et al. (80) postulated that an habituation to the exercise occurred, thereby causing less stress than when the exercise was first initiated. Tharp and Buuck (63) suggested that there is an adaptation of both the adrenal gland and the hypothalamus-hypophyseal axis that occurs with exercise. Finally, Severson et al (62) postulated that the reason exercise-training resulted in an adaptation of the axis was because of an improved efficiency in aerobic energy metabolism, creating a diminished demand for compounds such as glucose. The above findings may demonstrate the General Adaptation Syndrome (G.A.S.) described by Selye and Tuckweker (48) whereby after an initial alarm reaction to stress, characterized by weight loss, adrenal enlargement, decreased lipid storage, and so forth, the animals seem to adjust to the stressor and physiological functions return to normal.

However, after prolonged exposure, or as the animal ages, there is a loss of this adaptation (48).

While the researcher knows of no studies which have examined the entire pituitary-adrenal pathway, Gregerman (28) has argued that it is important to examine more than just one aspect of the axis since it is very complex and all the components act together. In the present study, an attempt was made to do this by examining several steps of the adrenocortical axis, from serum ACTH and serum corticosterone to the glucocorticoid receptors and finally to hepatic tyrosine aminotransferase. The relationships between variables were examined to determine whether a change in one was related to a change in another. Though not many significant relationships were observed, there were some interesting findings. The strongest relationship was observed between ACTH and corticosterone. Though ACTH concentrations decreased early in life, concentrations of both ACTH and corticosterone increased dramatically after 20 mo of age. These changes were very likely to be due to a decrease in feedback inhibition, as reported by Sapolsky et al. (9) and Adelman (81). Sapolsky et al. observed that aging rats displayed elevated basal corticosterone and ACTH levels that were resistant to the actions of dexamethasone. Accompanying this was a diminished adrenal sensitivity to ACTH. These alterations have been proposed by Sapolsky (9) to be due to degenerative changes in the hippocampal region of the brain. The closed-loop feedback system which normally would act by inhibiting subsequent release of ACTH once corticosterone levels rise experiences a malfunction and a "feed-forward cascade" results. In this manner, with the cumulative stress

of aging, the number of corticosterone receptors in the hippocampus is down regulated to the point that hippocampal feedback inhibition of the adrenocortical axis is dampened, resulting in further corticosterone hypersecretion. Damage to the hippocampal neurons becomes permanent and an irreversible cascade begins. This phenomenon was indirectly observed in the present study which showed increases in both ACTH and corticosterone with age. Neither exercise nor calorie restriction appeared to alter this situation in the current investigation. It thus seems to be inherent in the aging process and, as Sapolsky (9) postulated, may be a predisposing factor in some of the pathologies that accompany aging.

An apparent trend exists for an inverse relationship between serum corticosterone and ACTH concentrations with TAT activity. While serum corticosterone and ACTH concentrations increased with age, TAT activity decreased. These observations provide evidence for alterations at the cellular level. For example, since many hormones exert their effects either directly or indirectly on the genome, any aging changes in the properties of the chromatin may influence the quality and/or quantity of the induced enzyme (82). Though the present study did not examine the chromatin, ACTH, corticosterone, hepatic receptor concentration as well as hepatic TAT activity were measured which might have been indicative of chromatin response to glucocorticoids. It is of interest to note that while corticosterone and ACTH concentrations increased and the liver receptor concentration remained unchanged with age, TAT activity decreased. This may be indicative of altered binding of the receptor to the chromatin or post-translational changes, as described above. An age-associated

decrease in the entry of activated steroid-receptor complexes into the nuclei has also been reported for glucocorticoids in the rat (82). Whether this is due to changes in the nuclear membrane or in the chromatin remains unclear. Recent findings by Kalimi et al. (86) suggest that an impairment in glucocorticoid-receptor stability and activation observed in aged rats may account for the delayed or complete lack of induction by glucocorticoids of hepatic enzymes.

Other alterations which affect the pituitary-adrenal axis may also occur with age. Whereas the present study observed a nonlinear increase in serum ACTH concentration with age, Segall (83) reported that animal studies have indicated no well-defined age changes in this hormone. He suggested the possibility that qualitative rather than quantitative alterations may be occurring in ACTH as well as other pituitary hormones. For example, a possible change with age may involve amino acid sequence, whereby the pituitary decreases its rate of proteinase production, inhibiting post-translational maturation of its tropic hormones and stimulating a greater ratio of release of prohormones to active pituitary hormones. The result would be high levels of prohormones in the serum, such as is often observed with insulin. These prohormones would compete with the active hormone for receptor binding sites, preventing the hormones from performing optimally even though RIA measures, as in the present study, show high concentrations of that hormone.

As reported earlier, the current investigation found no changes in hepatic glucocorticoid receptor concentration with age, diet or exercise. Most of the literature supports these findings (15,19,84) though there are exceptions (16,18,81). Roth (82) suggested some

mechanisms for his observed decreases in receptor concentrations. One possibility is that receptors may not actually be lost in aged cells, but rather may become blocked or inactive. Since assays are dependent on functionality of the receptor, the inactive receptors may go undetected. This theory would explain why it was observed that, in those studies reporting losses in receptor concentration, no changes in binding affinity were observed. Roth's second suggestion was that there is either a decreased rate of synthesis or an increased rate of degradation of the receptor.

The age at which the receptors were measured appears to be an important factor. Sharma and Timiras (84) reported that a loss of glucocorticoid receptors does not occur in very old age but upon reaching reproductive maturity. Others have observed either no change in receptor concentration or a decrease in early adulthood (81). Since the present study only began analyzing endocrine function at 12 months of age, it is possible that early changes in receptor concentrations might have occurred.

CHAPTER VI

SUMMARY

Male weanling Wistar rats were used to investigate the effects of feed restriction and exercise on growth, feed intake and the pituitary-adrenal axis during aging. Serum ACTH and corticosterone concentrations, adrenal weight, hepatic glucocorticoid receptors and tyrosine aminotransferase (TAT) activity were measured to evaluate pituitary-adrenal function.

A training effect was verified in the exercised animals by measuring the heart weight:body weight (HW:BW) ratio. HW:BW was significantly larger in the exercisers when compared to the non-exercisers ($P<0.05$). Exercised animals ate approximately 12% more than the sedentary animals, but weighed about 7-8% less. Body weight curves of A and AE were similar, reaching maximum weights at 19-20 mo and decreasing thereafter. R and RE also had similar maximum body weights at approximately 26-33% less than A and AE.

As the animals aged, serum ACTH and corticosterone concentrations increased significantly ($p<0.05$) in all experimental groups. Additionally, adrenal weights became significantly enlarged with age, with AE showing the most dramatic increase. Glucocorticoid receptor levels remained unchanged. By contrast, TAT activity decreased with age in all groups.

Neither exercise nor feed restriction resulted in any major changes in the pituitary-adrenal axis. The only exceptions were observed when.feed restriction prevented TAT activity from

decreasing as rapidly as did ad libitum feeding ($p<0.05$). Also, the adrenal weights of AE, as mentioned above, were significantly larger than the other groups ($p<0.05$). This may indicate that the exercise regimen stressed the animals. Diet restriction may have allowed the animals to cope with the stress better, since it was observed that RE adrenals, though larger than those of sedentary animals, did not become as large as A or AE.

Relationships between the variables were analyzed. There was a quadratic relationship between ACTH and corticosterone, both rising with age, but moving in opposite directions early in life. Though not significant, there was a tendency for an inverse relationship between corticosterone concentration and TAT activity. As corticosterone increased with age, TAT activity decreased.

Aging, therefore, appears to have caused a loss of feedback inhibition in the pituitary-adrenal axis, as indicated by increased concentrations of both serum ACTH and serum corticosterone. The corresponding losses in TAT activity suggest possible changes occurring at the cellular level, either in the cell membrane, in chromatin activity or in post-translational changes. Neither feed restriction nor exercise were able to maintain the integrity of the pituitary-adrenal axis at a youthful level, though feed restriction did sustain TAT activity at a somewhat higher level.

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VITA

Madeline Dellwo was born in Spokane, Washington, where she attended Holy Names Academy for both her elementary and secondary education. In August, 1977, she graduated from the Coordinated Undergraduate Program in Dietetics at the University of Washington in Seattle, with a Bachelor of Science degree in Nutrition Science. She then joined Peace Corps where she worked in Malaysia as a clinical dietitian and a nutrition lecturer. This was followed by other experiences as both a clinical and administrative dietitian in Seattle and Saudi Arabia. In September of 1984, she began graduate work in Nutrition Science at the University of Tennessee, where she received a Master of Science degree in December, 1986. Madeline continued with her graduate studies and completed a Doctor of Philosophy degree in August, 1989. During her graduate program, she served as a graduate teaching assistant and a graduate research assistant.