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Effects of choline and exercise on resting bradycardia in rats

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EFFECTS OF CHOLINE AND EXERCISE ON RESTING BRADYCARDIA IN RATS

by

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ABSTRACT

The purpose of this study was to examine the effect of choline on bradycardia in rats when administered alone or in conjunction with a prescribed endurance training program. Subjects were 24 male Sprague-Dawley rats that were randomly assigned to four groups of six rats which ingested choline and exercised (CE), ingested choline only (CO), exercised only (EO), or neither ingested choline nor exercised (CON). Exercised rats ran 5 days per week for 8 weeks on a motor-driven treadmill. Exercise duration increased progressively until the rats ran for a 60-min period each day at 26.8 m/min, on a 10% grade, with a 30-sec sprint at 40 m/min interposed every 10 min. CE and CO rats were force fed .5 cc of choline chloride 5 days per week. Food consumption was measured daily during the final 6 weeks of the study. Average resting heart rates (HR) were determined for each rat from six physiograph readings taken every 5 min. HRs were recorded prior to treatment, after 5 weeks of treatment, and after 8 weeks of treatment. Three-way ANOVAs were performed on the variables of resting HR, body weight, and food consumption. Pretreatment resting HRs were significantly higher than mid- and posttreatment resting HRs for all groups of rats, which appears to be related to maturation of the rats or the specific laboratory conditions. The results indicate that training bradycardia was not attained by the exercising rats. Mean body weights of rats receiving choline

did not increase as drastically as mean body weights in rats not fed choline. The dietary increase in choline in this experiment did not affect mean food consumption in the rats.

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A Thesis Presented to the Faculty of
the School of Health, Physical
Education, and Recreation
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In Partial Fulfillment of the
Requirements for the Degree
Master of Science

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by
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May 1988

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Ithaca, New York

CERTIFICATE OF APPROVAL

MASTER OF SCIENCE THESIS

This is to certify that the Master of Science Thesis of

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submitted in partial fulfillment of the requirements for the degree of Master of Science in the School of Health, Physical Education, and Recreation at Ithaca College has been approved.

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Chapter 1

INTRODUCTION

Heart attacks kill more than half a million Americans every year (Keelor, 1980). In order to reduce risk factors associated with cardiovascular disease, physicians and physiologists are offering exercise prescriptions designed to improve patients' fitness levels and reduce resting heart rates (Apple & Cantwell, 1979).

Bradycardia from exercise training is an important sign of fitness (Apple & Cantwell, 1979; Brooks & Fahey, 1984). Past research has proven the value of certain exercise training programs in producing bradycardia (Lin & Horvath, 1972; Tipton & Taylor, 1965). A training program of sufficient type, intensity, and duration can consistently lower resting heart rates (HR) and increase stroke volume (Brooks & Fahey, 1984; Clarke, 1975).

A lower resting HR combined with increased stroke volume allows the heart to pump more blood per beat, elicits fewer beats to supply the body with blood, and provides greater resting time for the heart between beats (Clarke, 1975). Thus, through training, a more efficient transport of blood and oxygen delivery to the tissues is accomplished, without the expense of high HRs, which place undue stress on the heart and circulatory system.

Many sedentary people wish to attain an exercise training effect from a minimal amount of time and effort. In

order to determine the feasibility of producing a bradycardia response more rapidly than the customary training period of 8 weeks, the nutritional factor of choline was examined in this study. High levels of dietary choline have been shown to increase the production of acetylcholine (ACh), a neurotransmitter that slows HR (Cohen & Wurtman, 1976).

Scope of the Problem

The purpose of this study was to examine the effect of choline on bradycardia in rats when the choline was administered alone or in conjunction with a prescribed endurance training program. Subjects were 24 male Sprague-Dawley derived rats from Blue Spruce Farms. A 2 x 2 factorial experiment was designed to include the variables of physical activity (exercise or sedentary) and diet (choline or no choline). The rats were randomly assigned to four groups, each consisting of six rats. In the four groups respectively, the treatment required that each rat (a) ingest choline and exercise (CE), (b) ingest choline only (CO), (c) exercise only (EO), or (d) neither ingest choline nor exercise (CON).

Exercise consisted of running 5 days per week for 8 weeks on a motor driven treadmill. The duration of the exercise was progressively increased until the rats ran for a 60-min period each day at 26.8 m/min, on a 10% grade, with a 30-sec sprint interposed at a speed of 40 m/min every 10 min.

Twelve rats, the six in the CE group and the six in the

CO group, were force-fed .5 cc of choline. Food and water were administered ad libitum to all rats.

Average resting HRs were determined for each rat from six physiograph readings taken every 5 min. HRs were recorded prior to treatment, after 5 weeks of treatment, and after 8 weeks of treatment.

Statement of the Problem

The purpose of this study was to determine the effect of choline on resting HR when the choline is administered alone or in conjunction with an endurance exercise training program.

Hypothesis

A greater and faster onset of bradycardia in resting HRs will be attained by the group of rats receiving choline and an endurance exercise training program than by the control group or by the groups receiving choline alone or endurance exercise training alone.

Assumptions

1. An 8-week endurance training program produces bradycardia.

2. Nutritionally increased choline causes an increased rate of acetylcholine production.

3. Acetylcholine causes bradycardia.

4. A training effect was attained by all exercising rats following 8 weeks of treadmill running.

Definition of Terms

1. Choline. A vitamin of the B complex group that can be enzymatically converted to acetylcholine.

2. Acetylcholine (ACh). A neurotransmitter, produced enzymatically from the combination of choline and acetyl-Co A, that produces bradycardia.

3. Electrocardiogram (EKG). A record of changes of electrical potential occurring during the heart beat as measured by a physiograph. It was used to determine the rats' HR.

4. Treadmill. A machine that consisted of a motor-driven conveyor belt, constructed so that speed could be regulated to produce varying work loads on a 10% grade, and individual stalls for each of six exercising rats.

5. Sedentary. Groups of rats that were allowed no daily exercise.

6. Endurance trained. Groups of rats that received an exercise training program of prescribed intensity, frequency, and duration.

Delimitations

1. The subjects consisted of 24 male Sprague-Dawley derived rats.

2. Average resting HR was determined by taking an average of six physiograph readings recorded every 5 min while rats rested in individual cages with subdermal electrodes and a transmitter on their backs.

3. Exercise consisted of running on a motor-driven

treadmill using a protocol of progressive increases in exercise duration until the rats ran for 60 min per day at 26.8 m/min on a 10% grade with a 30-sec sprint every 10 min at 40 m/min.

4. Choline administration consisted of a 2.12-molar solution of choline chloride force fed through a syringe 5 days per week for an 8-week period.

Limitations

1. The results can be generalized only to the male Sprague-Dawley derived rats similar to those in this study.

2. Other procedures of recording average resting HR may yield different results.

3. Methods of exercise other than treadmill running may yield different results.

4. Other exercise protocols may yield different results.

5. Other dosages of choline may yield different results.

6. Other forms of choline ingestion may yield different results.

Chapter 2

REVIEW OF THE RELATED LITERATURE

The review of the literature includes discussion of two major topics related to the effects of choline and endurance training on bradycardia. This chapter will focus on the following areas: (a) endurance training and bradycardia, (b) choline as a precursor to acetylcholine, and (c) summary.

Endurance Training and Bradycardia

Endurance training reduces resting HR. However, bradycardia is not necessarily a sign of fitness. The reduction in resting HR with training is the important sign of fitness rather than the low HR itself (Brooks & Fahey, 1984).

It has long been established that bradycardia produced by training can be evidenced in man (Clausen, 1976; Raab, 1963; Sheuer, 1973) and in rats (DeSchryver, Mertens-Strythagen, Becsei, & Lammerant, 1969; Lin & Horvath, 1972; Tipton & Taylor, 1965).

The physiological mechanism for training bradycardia is unclear, although most researchers cite parasympaticotonia and decreased sympathetic nervous system (NS) activity as a partial explanation. However, it is not known whether the parasympaticotonia of training bradycardia is due to (a) a central change in the autonomic NS activity, (b) a peripheral change due to increased levels of ACh in the myocardium, or (c) decreased norepinephrine levels or catecholamine

susceptibility (Brooks & Fahey, 1984).

Many researchers consider ACh to be the important factor for training bradycardia (Clausen, 1977; Edington & Edgerton, 1976; Smith & El-Hage, 1982; Tipton, 1965; Tipton & Taylor, 1965; Williams, Eden, Moll, Lester, & Wallace, 1981). Tipton and Taylor (1965) noted that endurance-trained rats respond differently to atropine administration than untrained rats. Less cardiac acceleration was evident after administration of atropine to trained rats in any given time interval. "Since atropine competes with ACh for receptor sites, animals with more available ACh would be expected to demonstrate less of a cardiac response to a cholinergic inhibitor" (Tipton & Taylor, 1965, p. 482). The researchers concluded that trained populations have more nonneural ACh available in the myocardium to compete against the atropine for receptor sites and to supplement the effect of ACh released at the vagal nerve endings. Thus, bradycardia of training is related to the myocardial level of the nonneural ACh (Tipton, 1965; Tipton & Taylor, 1965).

Williams et al. (1981) also support the hypothesis "that altered neural input to the heart, rather than altered responsiveness to catecholamines, is usually the more important mechanism of training bradycardia" (p. 1236). Their cross-sectional study compared the HR responses to graded doses of isoproterenol, a vasodilating drug which increases HR, in seven elite marathon runners and seven age-

matched controls. There were no significant differences between marathon runners and controls in the dose of isoproterenol that produced a 25 beat/min increment in HR. The researchers concluded that adrenergic (epinephrine-releasing) receptor numbers or receptor affinity did not appear to change as an effect of physical training. A later study by Moore, Riedy, and Gollnick (1982) also supported this conclusion.

Smith and El-Hage (1982) evaluated the response of isolated rat atria to catecholamines following a 7-week exercise training program. The researchers found that trained atria beat at a significantly lower rate than untrained atria at all concentrations of epinephrine and norepinephrine. The chronotropic response to catecholamines was not altered by the exercise training program. Smith and El-Hage concluded that the mechanism of exercise bradycardia (a) exists at least in part at the level of the atrium, (b) could be related to the increased amount of ACh found in the exercised heart tissue, and (c) may partly be explained in terms of altered sensitivity of the pacemakers to the autonomic neurotransmitters. However, the antiadrenergic role of exercise that was consistent with the Raab (1963) results is not due to a decreased chronotropic response to catecholamines.

Because ACh appears to be an important factor in training bradycardia, the nutritional increase of ACh through

dietary choline was examined and is the next topic of discussion.

Choline as a Precursor to Acetylcholine

ACh is formed by a reversible reaction between choline and acetylcoenzyme A. The reaction is regulated by the enzyme choline acetyltransferase. Acetylation of choline by the enzyme choline acetyltransferase depends upon adequate sources of choline, acetylcoenzyme A, and energy (Barbeau, Growden, & Wurtman, 1979).

The ultimate source of choline for neuronal tissues appears to be the blood stream, because the brain does not synthesize choline (Goldberg & Hanin, 1976). According to Barbeau et al. (1979), the general view that ACh synthesis is dependent on extracellular choline seems to be valid for the central and peripheral NS.

Jope (1982) reported that a high affinity transport system that is coupled to Na+ transport determines the amount of choline available to be used for ACh synthesis. Upon release of ACh from the transporting carrier, choline acetyltransferase maintains cellular equilibrium by acetylating choline. After ACh hydrolysis, choline is recycled so that sufficient choline can restore the depleted pools of ACh.

Because choline is a precursor to ACh, it is postulated that increasing dietary choline will lead to increased ACh

production. Hanin and Schuberth (1974) found that after 1 day on a choline diet, mice had a substantial amount of labelled choline in the plasma, indicating that at least 20% of the total plasma pool of choline is of dietary origin.

Cohen and Wurtman (1976) found that choline acts by increasing ACh synthesis and that ACh concentrations in rat brain and blood serum vary with dietary choline consumption. The ACh-forming enzyme does not appear to be subject to significant feedback control, therefore, increases in ACh levels are associated with parallel changes in the amounts of transmitter substance released into synapses. Furthermore, the researchers determined that the choline-induced rise in brain ACh reflected accelerated synthesis, not a decreased breakdown of the neurotransmitter.

Lindmar, Loffelholz, Weide, and Witzke (1980) measured the outputs of ACh and choline into the perfusate of isolated chicken hearts with vagus nerves attached. The researchers found that vagal stimulation caused great increases in the overflows of ACh and choline. Infusion of choline for 1 min increased the release of ACh evoked by vagal stimulation. Lindmar et al. (1980) found that

> The rate of high affinity Ch uptake reached its maximum one to two minutes after stimulation and remained activated for several minutes. Thus, the activation is not directly coupled to the nervous activity and is presumably governed by the restoration of the

equilibrium between ACh and Ch compartments affected by the release. (p. 710)

Caputi and Brezenoff (1980) examined the HR response resulting from injecting choline into the lateral cerebral ventricle of the unanesthesized rat. Transient increases in blood pressure and prolonged decreases in HR resulted. The researchers attributed the bradycardia response to a centrally mediated increase in vagal tone, probably due to choline's contribution to postsynaptic effects in the brain. The increase in plasma choline levels necessary to cause an increase in ACh levels and to enhance central cholinergic transmission in laboratory animals is well within the range of choline concentrations observed in humans eating lecithin-rich foods, such as eggs, fish, meat, cereal products, and legumes (Hirsch, Growden, & Wurtman, 1977). Magil, Zeisal, and Wurtman (1981) studied the effects of lecithins derived from eggs or soybeans to determine whether fatty acid composition of the phosphatidylcholine altered choline availability. Each form of dietary lecithin elevated blood choline, brain choline, and brain ACh significantly.

Although many studies indicate increased levels of ACh following choline administration (Caputi & Brezenoff, 1980; Cohen & Wurtman, 1976; Hirsch et al., 1977), results from other studies fail to confirm these findings (Wecker, Dettbarn, & Schmidt, 1978; Wecker & Schmidt, 1979). According to Wecker (1986),

> There is much support for the hypothesis that supplemental choline is used to enhance ACh synthesis under conditions of increased neural demand for the precursor, i.e., when the activity of central cholinergic neurons is increased. (p. 331)

However, Wecker found that choline does not appear to alter ACh levels in the brain under normal biochemical and physiological conditions.

Although Wecker (1986) does not support the hypothesis that a central increase in choline levels will alter ACh levels under normal conditions, the peripheral effects of increased choline levels in the myocardium must also be studied further. It is possible that increased levels of ACh in the myocardium results in producing bradycardia at the level of the atrium (Lindmar et al., 1980; Smith & El-Hage, 1982; Tipton & Taylor, 1965).

Summary

It is well established that bradycardia produced by endurance training can be evidenced in rats (DeSchryver et al., 1969; Lin & Horvath, 1972; Tipton & Taylor, 1965) and in man (Clausen, 1976; Sheuer, 1973).

Although the physiological mechanism for training bradycardia is unclear, most researchers cited parasympaticotonia and decreased sympathetic activity as a partial explanation. Parasympaticotonia of training bradycardia appears to be due to (a) a central change in the

autonomic NS activity, (b) a peripheral change due to increased levels of ACh in the myocardium, or (c) decreased catecholamine susceptibility (Brooks & Fahey, 1984).

Many researchers considered ACh to be the important factor for training bradycardia (Clausen, 1977; Edington & Edgerton, 1976; Smith & El-Hage, 1982; Tipton, 1965; Tipton & Taylor, 1965; Williams et al., 1981).

Choline is a precursor to ACh, and it is postulated that increasing dietary choline will lead to increased ACh production (Cohen & Wurtman, 1976; Hanin & Schuberth, 1974; Lindmar et al., 1980; Magil et al., 1981). Research by Wecker (1986) stated that a central increase in ACh through dietary choline only occurs under conditions of increased neural demands for choline. However, increasing myocardial levels of ACh through dietary choline is also tenable (Lindmar et al., 1980; Smith & El-Hage, 1982; Tipton & Taylor, 1965).

Chapter 3

METHODS AND PROCEDURES

This chapter is divided into five sections that describe the procedures for (a) treatment of subjects, (b) HR measurement, (c) choline treatment, (d) exercise, and (e) analysis of data. The analysis of data section is subdivided into sections describing statistical procedures for measurement of (a) HR, (b) body weight, and (c) food consumption.

Subjects

Male Sprague-Dawley derived rats (N = 24) were obtained from a commercial breeder, housed in individual wire cages (24 cm x 18 cm x 18 cm), and allowed access to food and water ad libitum. The rats were housed in an environment with a room temperature of 23°C ± 2°C, 14-hour light cycles of 20-200 footcandles beginning at 3:00 a.m., and 10-hour dark cycles beginning at 5:00 p.m. All rats received Charles River Rat and Hamster Chow (R-M-H 3000). Food consumption was measured daily during the final 6 weeks of the study.

Rats were weighed every other day after arrival from Blue Spruce Farms for a 1-month period to ensure that the animals were healthy and experienced normal patterns of weight gain. Once the experiment began, the rats were weighed weekly on an Ohaus triple beam balance scale, model 700.

The animals were exposed to brief runs on a motor-driven

treadmill for 4 days prior to the study. The runs consisted of periods of 10 min per day on a flat treadmill at a speed of 26.8 m/min. The 12 rats selected for running ability were randomly divided into choline exercise (CE) or exercise only (EO) groups of 6 rats each. The remaining 12 rats were sedentary animals assigned to control (CON) or choline only (CO) groups of 6 rats each.

Heart Rate Measurement

The average resting HR of each rat was taken at three different periods during the experiment: (a) prior to the experimental treatment for a baseline HR, (b) after 5 weeks of treatment, and (c) after 8 weeks of treatment. Electrocardiograms (EKG) were recorded using an MK111 #1735 model preamplifier (E & M Instrument Co. Inc., Houston, Texas) connected to a PMP-4A type physiograph, model V5 KG (E & M Instrument Co. Inc., Houston, Texas). A HR transmitter (designed by K. Cyzernicki, Cornell University Poultry Science Department, Ithaca, New York) was attached to a velcro strap around the thorax of the rat. Alligator clips attached two subdermal electrodes to the HR transmitter.

At least 24 hours prior to recording HR, rats were anesthesized, and subdermal electrodes were placed bilaterally on the dorsal aspect of the thorax. The electrodes consisted of stainless steel 1-in. safety pins. Prior to recording pretreatment resting HR, the 24 rats were injected interperitoneally with 42 mg pentobarbital sodium

(Butler Co., Columbus, Ohio) per kg of body weight. However, ether (lot #KMTK, stock #0804, Mallinckrodt Co.) replaced sodium pentobarbital as the method for anesthesizing rats prior to recording HR after 5 and 8 weeks of treatment. The change in anesthetic was necessary because of delay in licensing of the laboratory by the New York State Narcotic Bureau.

At all data collection periods, the rats rested in the testing laboratory in individual plastic cages (25 x 19 x 15.5 cm) for a 30-min adaptation period before obtaining resting HR. During the HR recording sessions, individual HRs of all rats were taken over a 4-day period between the hours of 8:00 a.m. and 4:00 p.m. The pretreatment resting HRs of the 24 rats were recorded in random order. The order in which resting HRs were recorded was then rotated during subsequent measurements so that the HRs of all rats were recorded in different time slots over the three HR recording sessions.

The physiograph paper speed was set at 1 cm/sec, and a 10-sec record of the HR was obtained every 5 min. The HRs were recorded for a 30-min period or until six readings were obtained.

The individual readings were converted into minute values, averaged, and expressed as mean resting HRs. EKG readings that exhibited signs of movement by the rat were not used in obtaining the mean resting HR. Instead, additional

readings were taken until six records that showed no signs of extraneous movements were obtained.

Two groups of choline-treated rats were force fed a 2.12-molar solution of choline chloride (lot #86C-0170, stock #C-1879, Sigma Chemical Co.). For 5 days per week, CO and CE rats received a .5-cc dosage administered per subject. The dosage was administered by a sterile tuberculin syringe fitted with a 5-cm intubation tube.

Exercise Treatment

Exercise consisted of an 8-week running program on a motor-driven treadmill. The initial running program began at an intensity of 26.8 m/min on a 10% grade for 15 min. The running time was progressively increased until the rats were running 60 min per day at 26.8 m/min on a 10% grade. When 30 min of continuous running was achieved, 30-sec sprints (40 m/min) were interspersed every 10 min during each running session. The rats were observed continuously, and gentle prodding was used to stimulate running. Following each running session, betadine solution (lot #98G; stock #Ndc 003-4210080, Purdue Frederick Co.) was applied to the animals' feet to guard against blisters and abrasions.

Analysis of the Data

Statistical methods were used to analyze data gathered on the rats: (a) HR, (b) body weight, and (c) food consumption. The level of significance for all tests was set at .05.

Heart Rate

A three-way ANOVA (running by choline by trials) with repeated measures on the third factor of resting HR trials was run using a 2 x 2 x 3 factorial design on a BMD.P2V program to determine if any significant differences existed in resting HR. Kirk's (1968, pp. 65-67) procedures were followed in the case of significant interaction. Following no significant three-way interaction of trials by running by choline, the possibility of two-way interactions was examined prior to examining main effects. A Tukey test was performed following a significant F for main effects.

Body Weight

A three-way ANOVA with repeated measures on the trials factor was run on a BMD.P2V program to determine if any significant differences existed in body weight throughout the 8 weeks of training. A 2 x 2 x 8 factorial design was used. Following a significant F, a Tukey test was performed on the mean body weights to find where the differences occurred.

Food Consumption

Food consumption was measured daily during the final 6 weeks of the study. Mean weekly food consumption was determined for each treatment group. A repeated measures three-way ANOVA of running conditioning by choline conditioning by weeks was run on a BMD.P2V program. Following a significant F, a Tukey test was run on the cell means to determine where the differences among weeks

occurred.

Summary

The subjects of this study were 24 male Sprague-Dawley derived rats. The 12 rats selected for running ability were randomly divided into CO or CE groups of 6 rats each. The remaining 12 rats were sedentary animals randomly assigned to CON or CO groups of 6 rats each.

Choline-treated rats were each force fed .5 cc of a 2.12-molar solution of choline chloride 5 days per week. Exercised rats followed an 8-week progressive running program on a motor-driven treadmill 5 days per week.

Data collection consisted of recording weekly body weights, measuring daily food consumption during the final 6 weeks of the study, and recording average resting HRs at pre-, mid-, and posttreatment periods.

Six 10-sec EKG recordings for each rat were converted into minute values, averaged, and expressed as mean resting HRs. Those EKG readings that exhibited signs of movement by the rat were not used in obtaining the mean resting HR. If necessary, additional readings were taken until six records that showed no signs of extraneous movement were obtained. Average resting HRs were taken at pre-, mid-, and posttreatment intervals throughout the study.

Three-way ANOVAs were performed on the variables of resting HR, body weight, and food consumption, with Kirk's (1968, pp. 65-67) procedures followed in the case of

significant interaction. Following a significant F, a Tukey test was performed.

Chapter 4

ANALYSIS OF DATA

Methods of statistical analysis and their results are presented in this chapter. This chapter is subdivided into sections that describe measurements of the rats' (a) resting HR, (b) body weight, and (c) food consumption.

Resting Heart Rate

Resting HR was measured at three points throughout the study. Pretreatment HRs were recorded before the experiment began, midtreatment HRs were recorded after 5 weeks of treatment, and posttreatment HRs were recorded following 8 weeks of treatment.

Although the group sizes were small, three-way ANOVAs were performed on HRs and subsequent variables because no suitable nonparametric test was available (Borg & Gall, 1971). Kirk's (1968, pp. 65-67) procedures were followed in case of significant interaction for HR and subsequent variables.

A three-way ANOVA with repeated measures on the factor of resting HR trials was run using a 2 x 2 x 3 factorial design on a BMD.P2V program. Following no significant three-way interaction of trials by running by choline, the possibility of two-way interactions was examined. As indicated in Table 1, no significant interaction was found, although the F value for trials by choline was large, $F(2, 38) = 2.42$.

Table 1

ANOVA Summary Table for Resting Heart Rates

Source of Variation	SS	df	MS	F
Runners	89.81	1	89.81	.11
Choline	1054.89	1	1054.89	1.29
Choline by Runners	585.56	1	585.56	.72
Error	15552.18	19	818.54	
Trials	16764.70	2	8382.35	30.22*
Trials by Runners	738.16	2	369.08	1.33
Trials by Choline	1341.34	2	670.67	2.42
Trials by Runners by Choline	997.08	2	498.54	1.80
Error	10541.69	38	277.41	

*$p < .05$.

The main effects of running, choline, and trials can be interpreted directly, because there were no significant two-way interactions. The analysis of trials indicated a significant difference. A Tukey test was performed to determine where the significant difference across trials occurred. An HSD of 11.95 indicated that resting HR at the beginning of the experiment was significantly higher than at Week 5 or Week 8 for all rats.

Table 2 displays the mean resting HR of the four different treatment groups for pre-, mid-, and posttreatment trials. Although the interaction of choline by trials was not statistically significant, it may be of interest to note that a different pattern among means appears in the groups of choline rats. In both groups of rats receiving choline, the pattern of highest HRs for the pretreatment trial to the lowest HRs for the midtreatment trial, with midrange HRs for the posttreatment trial, differs from the pattern of HRs for rats not receiving choline. Rats not receiving choline exhibit a pattern of decreasing resting HRs across the pre-, mid-, and posttreatment sessions.

Body Weight

A three-way ANOVA with repeated measures on the third factor of weeks was run on a BMD.P2V program. A 2 x 2 x 8 factorial design was used, with Kirk's (1968, pp. 65-67) procedures followed in case of significant interaction. No significant three-way interaction was found, therefore, two-

Table 2

Mean Resting Heart Rates for the Treatment Groups

Group	Pretreatment	Midtreatment	Posttreatment
Choline Runners	360.8	316.4	328.4
Runners	383.3	350.0	321.7
Choline Sedentary	361.7	324.7	327.0
Sedentary	363.3	336.7	336.0

Note. All data represent HR in beats/min.

way interactions were studied. There was no significant two-way interaction of runners by choline or weeks by runners (see Table 3). Because the factor of runners was not involved in a significant interaction, the main effects of runners versus sedentary could be interpreted directly. No significant difference was found, $F(1, 18) = .24$, $p > .05$.

A value of $F(1, 126) = 3.18$, $p < .05$ indicated a significant interaction of weeks by choline, as shown in Table 3. Because of this interaction, direct interpretation of the main effects for weeks and choline would be misleading. Therefore, the simple main effects of weeks by runners and weeks by sedentary were investigated separately. The simple main effects that represent the comparison of choline and sedentary groups at each of the 8 weeks were also analyzed. The results of the tests of simple main effects can be found in Table 4.

Following a significant F for the weeks factor for both the sedentary and the runners, as indicated in Table 4, Tukey tests were performed on the means (see Table 5) to find where the differences occurred. An HSD of 15.56, $p < .05$, indicated the following results for rats receiving choline:

1. The only increase in body weights from one week to the next that was significant occurred from Week 5 to Week 6.

2. All body weight data gathered at least 2 weeks apart showed significant increases with the exception of Week 8, which was not significantly different from Week 6.

Table 3

ANOVA Summary Table for Mean Weekly Body Weights

Source of Variation	SS	df	MS	F
Runners	87.70	1	87.70	.01
Choline	16188.56	1	16188.56	1.35
Error	216646.29	18	12035.91	
Weeks by Runners	1398.16	7	199.74	1.39
Weeks by Choline	3207.69	7	458.24	3.18*
Weeks by Choline by Runners	334.84	7	47.83	.33
Error	18161.69	126	144.14	

*$p < .05$.

Table 4

Tests of Simple Main Effects for Body Weight Following Significant Week By Choline Interaction

Source of Variation	SS	df	MS	F
Ch across 8 weeks	79260.9	7	11323.0	84.00*
No Ch across 8 weeks	145935.0	7	20847.9	148.84*
Error	18629.3	133	140.1	
Ch vs No Ch at Week 1	105.0	1	105.0	.08
Ch vs No Ch at Week 2	611.2	1	611.2	.05
Ch vs No Ch at Week 3	1040.3	1	1040.3	.80
Ch vs No Ch at Week 4	1668.1	1	1668.1	1.28
Ch vs No Ch at Week 5	2545.6	1	2545.6	1.95
Ch vs No Ch at Week 6	3499.2	1	3499.2	2.68
Ch vs No Ch at Week 7	5508.9	1	5508.9	4.23
Ch vs No Ch at Week 8	5573.9	1	5573.9	4.28
Error	24762.7	19	1303.3	

Note. Ch refers to choline.

*$p < .05$.

Table 5

Mean Body Weights

Week	Choline	No Choline
1	325.47	329.97
2	340.18	350.78
3	354.85	368.90
4	369.59	386.87
5	378.51	400.43
6	401.28	426.28
7	408.43	439.80
8	410.60	442.34

Note. All weights are in g.

An HSD of 14.89, $p < .05$, indicated that for rats receiving no choline, body weights increased from week to week across all 8 weeks. These differences were statistically significant in all cases with the following exceptions:

1. Week 6 did not differ significantly from Week 7.
2. Week 7 did not differ significantly from Week 8.

When the choline groups were compared to the no choline groups at each week, the F values increased consistently from Week 2 through Week 8 (see Table 4). The F values at Weeks 7 and 8 are very close to the critical value of $F(1, 19) = 4.38$.

Food Consumption

Food consumption was measured daily during the final 6 weeks of the study. Mean weekly food consumption was determined for each treatment group, and data for all 6 weeks are presented in Table 6.

A three-way ANOVA of running by choline by weeks was run on a BMD.P2V program. The results shown in the ANOVA summary table in Table 6 indicated no significant three-way interactions, $p > .05$. No significant interaction of choline by weeks was found, but the runners by week interaction was significant. Because choline was not involved in a significant interaction, the test of main effects is valid; this test indicates no significant differences between choline and no choline in food consumption.

Table 6

ANOVA Summary Table for Food Consumption

Source of Variation	SS	df	MS	F
Runners	132.84	1	132.84	2.73
Choline	15.29	1	15.29	.31
Runners by Choline	118.54	1	118.54	2.43
Error	926.18	19	48.74	
Weeks	321.42	5	64.29	18.65*
Weeks by Runners	56.67	5	11.33	3.29*
Weeks by Choline	4.55	5	.91	.26
Weeks by Runners by Choline	6.98	5	1.40	.40
Error	327.52	95	3.45	

*$p < .05$.

Because weeks was a factor involved in a significant interaction, the significance indicated for weeks ($F[5, 95] = 18.65$, $p < .05$) could be misleading. The pattern of the change over weeks is not the same for the runners and the sedentary group, as can be seen by the means presented in Table 7.

Because of the significance of the running by week interaction, tests for the simple main effects were conducted. Results are seen in Table 8. There was a significant difference between the running and the sedentary group at Week 1, with the runners eating significantly more than the sedentary rats.

Differences among weeks were significant for both running and sedentary groups of rats. To identify where these differences occurred, a Tukey test was run on the cell means seen in Table 7. The HSD for these Tukey tests was 9.37. In comparing the means across weeks for runners, no two means were significantly different. The largest difference was seen between Week 1 and Week 6 and was only 6.13 g. The same phenomenon was seen for the sedentary group, in which the largest difference between means was 4.02 g between Week 1 and Week 5.

Summary

Resting Heart Rate

A three-way ANOVA with repeated measures on the third factor of resting HR trials indicated no significant three-

Table 7

Weekly Means for Food Consumption

	Weeks					
Group	1	2	3	4	5	6
Runners	32.82	28.40	28.25	27.37	27.24	26.69
Sedentary	28.50	26.68	26.22	25.60	24.48	26.78

Note. All data are in g.

Table 8

Tests of Simple Main Effects for Food Consumption Following Significant Training by Week Interaction

Source of Variation	SS	df	MS	F
Runners at Week 1	100.90	1	100.09	9.18*
Runners at Week 2	.03	1	.03	.00
Runners at Week 3	23.61	1	23.61	2.15
Runners at Week 4	15.37	1	15.37	1.40
Runners at Week 5	17.14	1	17.14	1.56
Runners at Week 6	15.00	1	15.00	1.36
Error	1254.00	114	11.00	
Weeks at Runners	49470.99	10	4947.10	101.49*
Weeks at Sedentary	45847.77	11	4167.98	85.50*
Error	920.74	19	48.46	

*$p < .05$.

way or two-way interactions, although a high F value for trials by choline was noted.

A significant difference among trials was found for resting HR trials ($p < .05$). A Tukey test was performed. The HSD of 11.95 indicated that resting HR at the beginning of the experiment was significantly higher than at Week 5 or Week 8 for all rats.

Although the interaction of choline by trials was not significant, a different pattern among means for the rats receiving choline was noted. Rats receiving choline exhibited highest HRs for the first trial, lowest HRs for the midtreatment trial, and midrange HRs for the posttreatment trial. Rats not receiving choline exhibited highest HRs for the pretreatment trials, midrange HRs for the midtreatment trial, and lowest HRs for the posttreatment trial.

Body Weight

A three-way ANOVA with repeated measures on the third factor of weeks was run on a BMD.P2V program. No significant three-way or two-way interactions were found ($p > .05$). No significant difference in body weight was found between runners and sedentary rats.

A value of $F(1, 126) = 3.18$, $p < .05$ indicated a significant interaction of weeks by choline. Therefore, the simple main effects of weeks by runners and weeks by sedentary were investigated separately. The Tukey test indicated the following results for rats receiving choline:

1. The only change in body weights from one week to the next that were statistically significant occurred from Week 5 to Week 6.

2. All body weights gathered at least 2 weeks apart showed statistically significant increases with the exception of Week 8, which was not significantly different from Week 6.

Other findings indicated that for rats receiving no choline, statistically significant differences in body weight occurred across all 8 weeks, with the following exceptions:

1. Week 6 did not differ significantly from Week 7.

2. Week 7 did not differ significantly from Week 8.

When the choline groups were compared to the no choline groups at each week, the *F* values increased consistently from Week 2 through Week 8. Although the body weight increases were not significant, a high *F* value for Weeks 7 and 8 was noted.

Food Consumption

A three-way ANOVA of running by choline by trials indicated no significant difference ($p > .05$) in food consumption for rats receiving choline and rats not receiving choline. A significant interaction for the factor of weeks by running occurred, therefore, tests for the simple main effects were conducted. A significant difference between the running and sedentary groups at Week 1 occurred, with the runners eating significantly more than the sedentary group.

Differences in food consumption among weeks were

significant for both running and sedentary groups of rats. A Tukey test was run on the cell means. In comparing the means across weeks for runners, no two means were significantly different. The largest difference was seen between Week 1 and Week 6. Similarly, in the sedentary groups the largest difference between means was only 4.02 between Week 1 and Week 5.

Chapter 5

DISCUSSION OF RESULTS

This chapter presents a discussion of the results reported in chapter 4. Topics examined include discussion of the rats' (a) resting HR, (b) body weight, and (c) food consumption.

Resting Heart Rate

Resting HR at the beginning of the experiment was significantly higher than at Week 5 or Week 8 for all rats. This could be due to the factor of maturation of the rats. Lin and Horvath (1972), taking resting HRs in rats in a similar study, noticed in their control and exercise-trained groups of rats an initial rapid decrease in HR, which they attributed to an increase in age and handling.

The decrease in resting HRs for all groups of rats may also be due to specific testing conditions that might have made the rats more excited for the first trial of HR measurement compared to later trials. For example, college classes were in session outside the laboratory during pretreatment HR recordings, but laboratory conditions were quieter at the time of mid- and posttreatment trials because classes were recessed. An added factor could be that additional rest time was allowed during midtreatment HR recordings because the HR transmitter required minor repairs during the recording session.

In light of the fact that all groups of rats experienced

decreases in resting HR, it is highly probable that a training effect of bradycardia did not occur after the 8 weeks of treadmill running. Perhaps extending the running program beyond the customary 8-week training period might have produced bradycardia. It is also possible that another exercise protocol for endurance training of the rats would be more successful in producing bradycardia.

In both groups of rats receiving choline, the pattern of highest HRs for the pretreatment trial to lowest HRs for the midtreatment trials to midrange HRs for the posttreatment trial differs from the pattern of HRs for rats not receiving choline. Rats not receiving choline exhibit a pattern of high to medium to low resting HRs for pre-, mid-, and posttreatments.

Because of the difficulty in obtaining resting HRs without the interference of extraneous movements of rats, a more reliable method of obtaining resting HRs of the rat is advised.

Body Weight

Data gathered on the rats' body weights indicated no significant difference in body weights between runners and sedentary rats. Even though the body weights showed no significant difference between runners and sedentary rats, it is possible that body weight in runners was distributed in a more ideal manner. Although body composition was not examined in this study, changes such as greater muscle mass

and bone density in the exercising rat were probably realized.

For rats receiving choline, body weights gathered 2 weeks apart showed significant changes, with the exception of Week 8, which was not significantly different from Week 6. Rats not receiving choline significantly increased their body weights weekly, with a few exceptions as noted in chapter 4. The steady increases in body weight over the weeks is probably due to the normal process of maturation, because the steady increases were also apparent in the control group.

Mean body weights of rats receiving choline did not increase as drastically over the 8 weeks as did the weights of rats not receiving choline. Table 3 in chapter 4 seems to indicate that the normal maturational increases in body weights are apparently depressed by choline. Because large doses of choline depress food consumption (Cohen & Wurtman, 1976), slower weight gains in choline-fed rats might be expected.

When the choline groups were compared to the no choline groups at each week, the F values increased consistently from Week 2 through Week 8. The F values at Weeks 7 and 8 are very close to the critical value of $F(1, 19) = 4.38$. It is possible that a practical difference in weekly body weights exists even if it is not statistically significant. Therefore, if the experimental period were to be extended, perhaps a significant change would be seen. It is not known

whether differences in body weight changes between the choline and no choline groups reflect only maturational differences. There may also be body composition differences, which in turn may have either positive or negative effects on the rats. Certainly these areas deserve more study because no indications were found in the literature to support one view or the other.

Food Consumption

Although Cohen and Wurtman (1976) have reported depressed food intake following large doses of choline, no significant differences in food consumption were found in this study between the choline and no choline groups. There was a significant difference between the running and sedentary groups at Week 1, with the runners eating significantly more than the sedentary group. Initially, the greater caloric demands caused by the running program appear to have been met by increased food consumption. As seen in Table 7, mean food consumption throughout the 8 weeks of the study appears to have remained relatively consistent for both running and sedentary groups of rats. Perhaps the sedentary groups of rats were following the pattern explained by Astrand and Rodahl (1986), in which sedentary animals that are confined to small cages are apt to overeat and become obese. This may explain the lack of significant difference in food intake between running and sedentary rats in Weeks 2 through 8 of the study.

Summary

Resting HR at the beginning of the experiment was significantly higher than at Week 5 or Week 8 for all rats. Because all groups of rats experienced decreases in resting HR, it appears that maturational changes have occurred and not training bradycardia. Perhaps another exercise protocol or an extension of the experiment's duration would prove more effective in producing bradycardia. The decrease in resting HRs for all groups of rats may also result from specific testing conditions that might have made the rats more excited for the first trial of HR measurement as compared to later trials. A quieter laboratory environment and greater time periods between HR recordings could have affected the significant decrease in resting HRs noted for all groups of rats. A different pattern among mean resting HRs exists for all rats receiving choline than for rats not receiving choline.

During the 8 weeks, the rats fed no choline experienced steady increases in body weight, which was probably due to normal maturation. However, mean body weights of choline-fed rats did not increase as drastically over the 8 weeks as rats not fed choline, and significant differences occurred biweekly. The normal maturational increases in body weights are apparently depressed by choline. It is not known whether differences in body weight changes between choline and no choline groups reflect only maturational differences and what

positive or negative effects such weight changes bear. More research on body weight and composition of choline-treated rats is indicated.

Although Cohen and Wurtman (1976) have reported depressed food consumption following large doses of choline, no significant differences in food consumption were found in this study between the groups of choline and no choline rats. Mean food consumption throughout the 8 weeks of the study appears to have remained relatively constant for both running and sedentary groups of rats.

Chapter 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to determine the effect of choline on bradycardia in rats when administered alone or in conjunction with an endurance exercise training program.

Subjects were 24 male Sprague-Dawley derived rats that were randomly assigned to four groups, each consisting of 6 rats, which ingested choline and exercised (CE), ingested choline only (CO), exercised only (EO), or neither ingested choline nor exercised (CON).

Exercise consisted of running 5 days per week for 8 weeks on a motor-driven treadmill. The duration of the exercise was progressively increased until the rats ran for a 60-min period each day at 26.8 m/min, on a 10% grade, with a 30-sec sprint at 40 m/min interposed every 10 min.

Twelve rats, the six in the CE group and the six in the CO group, were force fed .5 cc of choline 5 days per week. Food and water were administered ad libitum to all rats. Food consumption was measured daily during the final 6 weeks of study.

Average resting HRs were determined for each rat from six physiograph readings taken every 5 min. HRs were recorded prior to treatment, after 5 weeks of treatment, and after 8 weeks of treatment.

Three-way ANOVAs were performed on the variables of

resting HR, body weight, and food consumption. Kirk's (1968, pp. 65-67) procedures were followed in case of significant interaction. A Tukey test was performed following a significant F for main effects.

Resting HR at the beginning of the experiment was significantly higher than at Week 5 or Week 8 for all rats. Although a different pattern of mean resting HR existed for rats not receiving choline than for rats receiving choline, the differences were not statistically significant.

Analysis of body weight data indicated no significant differences in body weights of running and sedentary rats. Because of a significant interaction of weeks by choline, the simple main effects of weeks by runners and weeks by sedentary were investigated separately. For rats receiving choline, the only change in body weights from one week to the next that was statistically significantly different occurred from Week 5 to Week 6, and all body weight data gathered at least 2 weeks apart showed significant changes, except for Week 8, which was not significantly different from Week 6.

Body weights of rats receiving no choline increased from week to week across all 8 weeks. These differences were statistically significant in all cases, except the following:

1. Week 6 did not differ significantly from Week 7.
2. Week 7 did not differ significantly from Week 8.

Data gathered on food consumption showed a significant training by week interaction. The only significant

difference among weeks for running versus sedentary rats occurred at Week 1, with runners eating significantly more than sedentary rats.

Conclusions

Considering the scope of this study, the following conclusions were made:

1. Pretreatment resting HR was significantly higher than mid- and posttreatment resting HRs for all groups of rats. Resting bradycardia that normally accompanies training did not occur. The HR decreases in all rats are probably due to maturation of the rats or the specific laboratory testing conditions.

2. Mean body weights of rats receiving choline did not increase as drastically as mean body weights in rats receiving no choline during the experiment. The normal maturational increases in body weights of rats are apparently depressed by choline.

3. No significant differences in food consumption were found in this study between rats fed choline and rats not fed choline. The dietary intake of choline in this experiment does not affect mean food consumption in the rat.

Recommendations

1. In similar studies, a more accurate method of recording resting HR in the rats is needed for a more reliable measure of a rat's true resting HR.

2. Another method of exercising the rats may prove more

reliable, because it is difficult to keep rats from sliding instead of running on the treadmill.

3. Collection of data should occur after rats have reached maturity, to eliminate the maturational decreases in resting HR from the results.

4. A study of longer duration is recommended in order for the rats to experience training bradycardia and realize greater choline effects on the body weight changes.

5. Another protocol for exercising the rats may prove more effective in achieving training bradycardia. Varying the intensity of exercise, duration of the training program, or method of exercise may yield more positive results.

6. Further studies are needed to confirm the finding that normal maturational increases in the body weights of rats are depressed by choline.

7. Further studies investigating the effects of choline on body weight and body composition in rats are recommended.

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