PYRIDINE NUCLEOTIDE CONCENTRATION AND RATIOS IN RAT MUSCLE, HEART, AND LIVER IN RESPONSE TO ACUTE AND CHRONIC EXERCISE

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ABSTRACT

PYRIDINE NUCLEOTIDE CONCENTRATION AND RATIOS IN RAT MUSCLE, HEART, AND LIVER IN RESPONSE TO ACUTE AND CHRONIC EXERCISE

by D. W. Edington

The purpose of this study was to investigate the concentrations and various ratios of pyridine nucleotides in trained and sedentary male albino rats in response to a severe acute-exercise stress.

Eighteen of thirty-six Sprague Dawley rats were forced to swim for two hours twice a day, five days per week, for six weeks in thirty-two degree centigrade water. A weight equivalent to up to three percent of the animal's body weight was attached to its tail during the exercise sessions.

At the end of the six-week experimental period, each animal was lightly anesthetized with ether and the left achilles tendon was severed. The distal end of the tendon was attached to a spring steel plate, upon which was mounted a strain guage, and later to a linear variable differential transformer, for static and dynamic muscle recordings respectively.

Muscle contractions were induced by direct stimulation with a current of two milliamps. Pre and post-exercise blood samples were taken for the determination of blood lactic acid. At the end of a ten-minute contraction period, the muscle was frozen between two aluminum plates, pre-cooled in liquid nitrogen. The heart and liver were removed and frozen in liquid nitrogen. Modifications of the extraction and assay procedures of Klingenberg were used to measure the levels of the four pyridine nucleotides.

The muscle of the sedentary animals performed the same absolute amount of work (2715.4 millimeter grams), with the isolated muscle preparation, as the trained animals (2645.4 millimeter grams). The muscles of the trained animals performed more work per gram of body weight (9.94 and 8.25 respectively). They also had a higher post-exercise blood lactic acid concentration (21.40 and 19.04 milligram percents). The trained animals were observed to have a higher pyridine nucleotide oxidized to reduced ratio in skeletal muscles (7.3 and 4.4), a lower total DPN to total TPN ratio in the hearts (24.1 and 35.8), and a positive correlation between muscle pyridine nucleotide oxidized to reduced ratio and liver pyridine nucleotide oxidized to reduced ratios ($r = .78$).

From the data in this study it can be concluded that the training routine did not produce a difference between the two groups in resting blood lactic acid concentration, but the ten-minute direct muscle stimulation brought about a higher blood lactic acid concentration in the trained animal. It further can be concluded that the mean, total pyridine nucleotides in skeletal muscle, heart, and liver were not different between the two treatment groups. The pyridine nucleotide oxidized to reduced ratio of the muscles suggest that total work performance may be limited by the ability of the cell to minimize the decrease in the oxidized to reduced ratio within the cell.

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By

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To Marilyn Edington whose encouragement and understanding provided a happy environment for the writing of this thesis.

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CHAPTER I

INTRODUCTION

As a social organization progresses towards a more mechanized form of living, there is a trend towards a decrease in the level of physical activity of the individual members of the society. Evidence is accumulating that persons who lead sedentary lives are more prone to diseases (45, 51, 52, 53, 61). It seems clear that this "exercise" situation is one that must be understood if the health needs of future generations are to be met. A study of the organism's adaptation to chronic exercise should allow an elucidation of these changes. An understanding of the exercise-induced adaptation eventually will permit a definition to be made of the minimal exercise level needed either to bring about the desirable changes which chronic exercise produces, or to prevent those changes associated with extreme sedentary existence.

It is well known that the trained athlete can undergo sustained exertion for a greater length of time than the untrained individual. Studies of adaptation of the cell to chronic exercise indicate the following cellular biochemical changes: (a) increased myoglobin and increased cytochrome enzymes in chronically active muscles as opposed to inactive muscles (41, 42, 48), (b) increased mitochondrial density and increased mitochondrial activity in active as opposed to relatively inactive muscles (50), and (c) increased pyruvate oxidation, total mitochondrial protein, myoglobin, cytochrome c, succinate dehydrogenase, DPNH dehydrogenase, DPNH cytochrome c reductase, succinate oxidase, aconitase, mitochondrial malate and isocitrate dehydrogenase in active muscles as opposed to inactive muscles (31). Glick has shown that acute-exercise activates the dehydrogenase steps in the liver by elevation of the intramitochondrial concentration of usable DPN (16).

These studies indicate that the changes associated with exercise may involve major changes in the oxidation-reduction capacity of the cell. The critical oxidation-reduction steps in energy metabolism suggest an important role for the pyridine nucleotides in adaptations to exercise. The availability or lack of availability of the pyridine nucleotides would effect energy metabolism through the activity of the dehydrogenases.

Pyridine nucleotides are important coenzymes in nearly every major metabolic pathway known to exist in mammalian biochemistry. It can be hypothesized that the several possible ratios of the pyridine nucleotides can regulate control mechanisms in these pathways and thus partially determine the direction of synthesis and degradation of the various metabolic products.

Evidence by Dietrich and Muniz (11) and by Deal (10) suggests that pyridine nucleotide biosynthesis is inhibited strongly by DPN, but DPNH exhibits only a weak inhibitory effect. Thus, if the DPN to DPNH ratio of the cell is decreased, then a net increased synthesis of pyridine nucleotides would be expected. The question of whether a relative short period of daily exercise will "turn on" synthesis or "turn off" inhibition cannot be answered by the current investigation. A discussion of this form of feedback control as well as of other forms of biosynthetic control by the pituitary gland, thyroxine, and adrenal secretions are presented in the chapter on Related Literature.

In this study, two extreme biological conditions were imposed upon two groups of animals. The first condition was a training routine which could be expected to produce increased DPN or alterations in the various pyridine-nucleotide ratios due to an increased energy demand. The second condition was a sedentary existence which could be expected to produce increased TPN or alterations in the various Pyridine-nucleotide ratios due to an increased reductive biosynthesis. In the former condition, an increase in muscle and heart total DPN would be expected; in the latter, an increase in the liver total TPN would be expected. The above hypotheses are based upon the generally accepted fact that the diphosphopyridine nucleotides are related to energy metabolism while the triphosphopyridine nucleotides are involved primarily with reductive biosynthesis.

Statement of the Problem

The purpose of this study was to investigate the concentrations and various ratios of pyridine nucleotides in the plantar-flexor muscles, hearts, and livers of chronically trained and sedentary male albino rats in response to a locally severe acute-exercise stress.

It was hypothesized that the chronic-exercise program which consisted of swimming the animals four hours a day, five days a week, for six consecutive weeks would: (a) result in an increased total DPN to total TPN ratio in the liver and (b) bring about an increase in the total pyridine nucleotide level in skeletal and heart muscle.

It was further hypothesized that in the trained as compared to the sedentary animals, the acute-exercise stress would result in: (a) a greater oxidized to reduced pyridine-nucleotide ratio in skeletal muscle for a given amount of work and (2) a higher blood lactic acid concentration for any given muscle oxidized to reduced ratio.

Limitations of the Study

1. The results of this study are applicable only to male, Sprague-Dawley rats of a similar age and spontaneous-activity levels.
2. The results of this study are confined to the treatment regimens and the time span used in this study.

3. A more or less strenuous acute-exercise stressor might produce dissimilar results.
4. The training program and the acute-exercise procedure were not the same type of exercise.
5. No attempt was made to measure or control food intake of the rats.

Definition of Terms

The following abbreviations have been used throughout this study: (a) DPN--the oxidized form of diphosphopyridine nucleotide, (b) DPNH--the reduced form of diphosphopyridine nucleotide, (c) TPN--the oxidized form of triphosphopyridine nucleotide, (d) TPNH--the reduced form of triphosphopyridine nucleotide, (e) total DPN--the sum of DPN plus DPNH, (f) total TPN--the sum of TPN plus TPNH, and (g) oxidized to reduced ratio--the sum of DPN and TPN divided by the sum of DPNH and TPNH.

CHAPTER II

RELATED LITERATURE

Control of Pyridine Nucleotide Biosynthesis and the Responses to Various Stimuli

Dietrich and Muniz (11, 12) have reported evidence that rat liver nicotinamide mononucleotide pyrophosphorylase is subject to strong inhibitory feedback control by DPN. DPNH was reported by these same authors to have only a weak inhibitory effect on the enzyme. Deal (10) has suggested that the same type of control may exist in yeast.

Ten minutes of tissue ischemia has been shown by Burch and Von Dippe (4) to bring about a 100 percent increase in DPNH and a twenty percent decrease in DPN. These data might suggest that the DPNH concentration is one-fifth that of DPN. But these same authors show the DPN to DPNH ratio to be between ten and twenty, not five. They also report data that indicate the TPN to TPNH ratio may be involved in the ischemic reaction. As the TPN concentration decreases by sixty percent, the TPNH concentration increases by twenty-five percent. Thus, the TPN concentration would seem to equal about half of the TPNH concentration. Other data show the TPN to TPNH ratio to approximate one. These data by Burch and Von Dippe illustrate the possible importance of the trans hydrogenases and the kinases in the intraconversions of the pyridine nucleotides. Control of these two types of enzymes could theoretically bring about shifts in the pyridine nucleotide pool and, therefore, the acute adjustments that the immediate environment requires.

Giacobina and Grasso (15) suggested that a phosphatase may be involved when they showed that stimulation of a nerve brings about a decrease of both the DPN to DPNH and TPN to TPNH ratios in the nerve. The TPN to TPNH ratio shift was shown to be the result of a decrease in TPN--suggesting a TPN to DPN conversion. The DPN to DPNH ratio shift was found to be due to a decrease in DPN and an increase in DPNH--suggesting the high-energy activity that would be expected. That is, during low-energy and high-synthesis requirements, the pyridine nucleotides could be shifted to the TPN form; in the case of high-energy and low-synthesis requirements, they could be shifted to the DPN form.

In 1956 Kaplan (38) proposed that transhydrogenase activity may function as a device for regulating the flow of electrons between the two reduced pyridine nucleotides and, therefore, for regulating the energy production and reductive biosynthesis in the cell. Thus, a relatively stable absolute number of molecules could perform in the several physiological roles involved in the cell.

Although the transhydrogenases, kinases, or phosphatases discussed above, were not investigated in the current study, it was felt that a short review would serve to illustrate the interrelationships between the pyridine nucleotides.

Indirect confirmation that pyridine nucleotides are involved in metabolically active tissues has been offered by Franz and Franz (14). They showed that all of the pyridine nucleotides decreased in the pathologically nephrotic kidney. Since exercise would require a highly active cell, it could follow from these data that a long-term exercise program would produce the opposite effects to those found by Franz and Franz.

Starvation would seem to be, in some ways, similar to prolonged exercise, in that glucose would be limited and very little reductive biosynthesis would take place. Under conditions of starvation, Glock and McLean (20, 21) and Lardy (40) showed that the liver DPN to DPNH ratio decreases while the TPN to TPNH ratio remains relatively stable or increases. Pande _et al_. (46) confirmed the work of Lardy and showed an increase in the TPN to TPNH ratio with starvation. These authors found an increase in TPN and DPNH. Refeeding experiments by Pande demonstrated a decrease in the TPN to TPNH ratio. In summary, fasting causes an increase in DPNH and TPN and no change in DPN and TPNH.

Hormonal actions almost certainly play some part in any exercise-induced effects on the pyridine nucleotide levels (13, 21, 25, 26). It has been shown by Greengard et al. (24, 25, 26) that adrenalectomy, hypophysectomy, and thyroidectomy can bring about an increase in the total DPN level. Administration of growth hormone, corticosterone, and thyroxine caused a decrease in total DPN levels in rat liver. Thus, the pituitary hormones exert a pronounced effect on DPN levels in rat liver. Lee and Lardy (43) report a decrease in DPNH with thyroxine administration, but this decrease was due to an increase in alpha-glycerophosphate dehydrogenase (alpha-GPD) which facilitates the oxidation of DPNH through the alpha-GPD cycle of Bucher and Klingenberg. The increase in alpha-GPD serves to increase the DPN to DPNH ratio by increasing the DPNH availability to the mitochondria. In part, this accounts for the increased oxygen utilization brought about by thyroxine administration. The results of Glick (16) are in agreement. He shows that acute-exercise activates the dehydrogenase steps in liver by elevating the intramitochondrial concentration of usable DPNH.

Assuming the control model of Dietrich and Muniz and of Deal, where DPN inhibits strongly but DPNH does so only minimally, if there is to be an exercise-induced change in the absolute levels of the pyridine nucleotides, the exercise stress must be of sufficient rigor and duration to push the cell into the reduced state and to hold it there long enough for the DPN inhibitory action to be removed.

Levels of Coenzymes and the Various Ratios

Table 1 gives a summary of the various tissue levels of pyridine nucleotides that have been reported in the literature. The great variations in the reported values are almost certainly due to the varying conditions of the animals at the times of analysis.

TABLE 1.--Reported Values for Pyridine Nucleotide Concentrations.

Tissue	DPN	DPNH	TPN	TPNH	DPN/DPNH	TPN/TPNH	T-DPN/T-TPN	Oxid./Red.	Units	Reference
Human RBC	198	149	115	86	1.3	1.3	1.7		mu Moles/gm. Hb.	(27)
Rat Kidney	267	108	8	51					ug/gm.	(14)
Rat Retina	191	26	5	19	7.2				ug/gm.	(23)
Dog Thyroid	56	20	6	10					mu Moles/gm.	(13)
Rat Liver					8.6	1.0				(40)
Rat Liver	300	82	248	254	3.7	.3	4.1	1.1	mu Moles/gm.	(46)
Rat Liver	543								ug/gm.	(25)
Rat Liver	582	100		233					ug/gm.	(26)
Rat Liver					725.0					(60)
Rat Liver	370	204	6	205					ug/gm.	(19)
Rat Liver	376	127	75	269				0.8	ug/gm.	(19)
Rat Liver	3.4		3.3						m Moles/kg.	(4)
Rat Heart	378	143	40	61				0.5	ug/gm.	(36)
Rat Heart	299	184	4	33					ug/gm.	(36)

	Total DPN	Total TPN	Units	Reference
Rat Heart	0.74	0.064	u Moles/gm.	(37)
Rat Liver	0.88	0.29	u Moles/gm.	(37)
Rat Muscle	0.64	0.013	u Moles/gm.	(37)

CHAPTER III

EXPERIMENTAL METHOD

Experimental Design

One hundred and five male rats of the Sprague Dawley strain, twenty-three days of age, were purchased from the Hormone Assay Company of Chicago, Illinois. Since pyridine nucleotide levels may be influenced by activity and perhaps by unknown hereditary factors, it was decided that those animals with innately high or low spontaneous activity levels should be deleted from the sample. The rats were placed into individual cages, with spontaneous-activity wheels attached, for five days. The activity wheels were 12.5 centimeters wide and 35 centimeters in diameter. The animals were allowed to run at any time, and a counter attached to each wheel recorded the revolutions run. The revolution count for each rat, over the final three days of this five-day period, was used to eliminate the extreme groups. A frequency distribution was made for each of these three days. Those animals, having revolution counts within the central-half of the distribution, were designated each day. Thirty of the required forty-eight animals were chosen at this step as each of these had revolution counts within the middle-half on all three days. The final eighteen animals had revolution counts within the middle-half of the distribution on two of the three days, and their revolution counts for the third day were not over plus (or minus) ten percent from the highest (or lowest) count in the central-half. (See Appendix A for the revolutions run by each of the chosen animals during the three days). The animals were ranked according to their three-day total revolutions run. The most active animal was assigned to group A, the next two to group B, the fourth and fifth to group A, the sixth and seventh to group B, etc. A flip of a coin decided which group would receive the physical training routine.

After the groups were determined, the forty-eight animals were transferred to individual sedentary cages. These cages were arranged so that twenty-four were on each side of a single rack. Each sedentary cage measured 24 x 18 x 18 centimeters. Each animal in the sedentary group had a sheet metal plate inserted into his individual cage so that the total volume of the cage was bisected from the upper left to the lower right. This was done to restrict activity as much as possible. The twenty-four cages on each side of the rack were distributed so that there were four levels of six cages. Three experimental animals and three control animals were placed in alternate cages on each level.

Lighting in the animal room was maintained on a cycle of twelve hours of light and twelve hours of darkness. The animal room was maintained at twenty-five degrees centigrade plus (or minus) one degree. The rats were fed Wayne Laboratory Blocks _ad libitum_ and had constant access to water.

Body weight for each animal was recorded at the same hour on Thursday of each week, throughout the experimental period.

A flow chart for the experimental design is shown in Figure 1.

Training Routine

The swimming conditions were such that the experimental rats were in thirty to thirty-two degree centigrade water, from three to five P.M. and from nine to eleven P.M., five days per week. No extra weights were attached to the animals' tails during the first week of training although miniature plastic clothespins were placed on their tails during the swimming periods of the fourth and fifth days. Two percent of each animal's body weight, as determined after the first week of swimming, was added to the tail of each rat during each swimming period of the second week. For the third week of training, the weights were increased to three percent of each animal's latest body weight. In all subsequent weeks, the weights were maintained at three percent of the animals' current body weights. All of the added weights were accurate to plus (or minus) ten milligrams. Each rat was swum

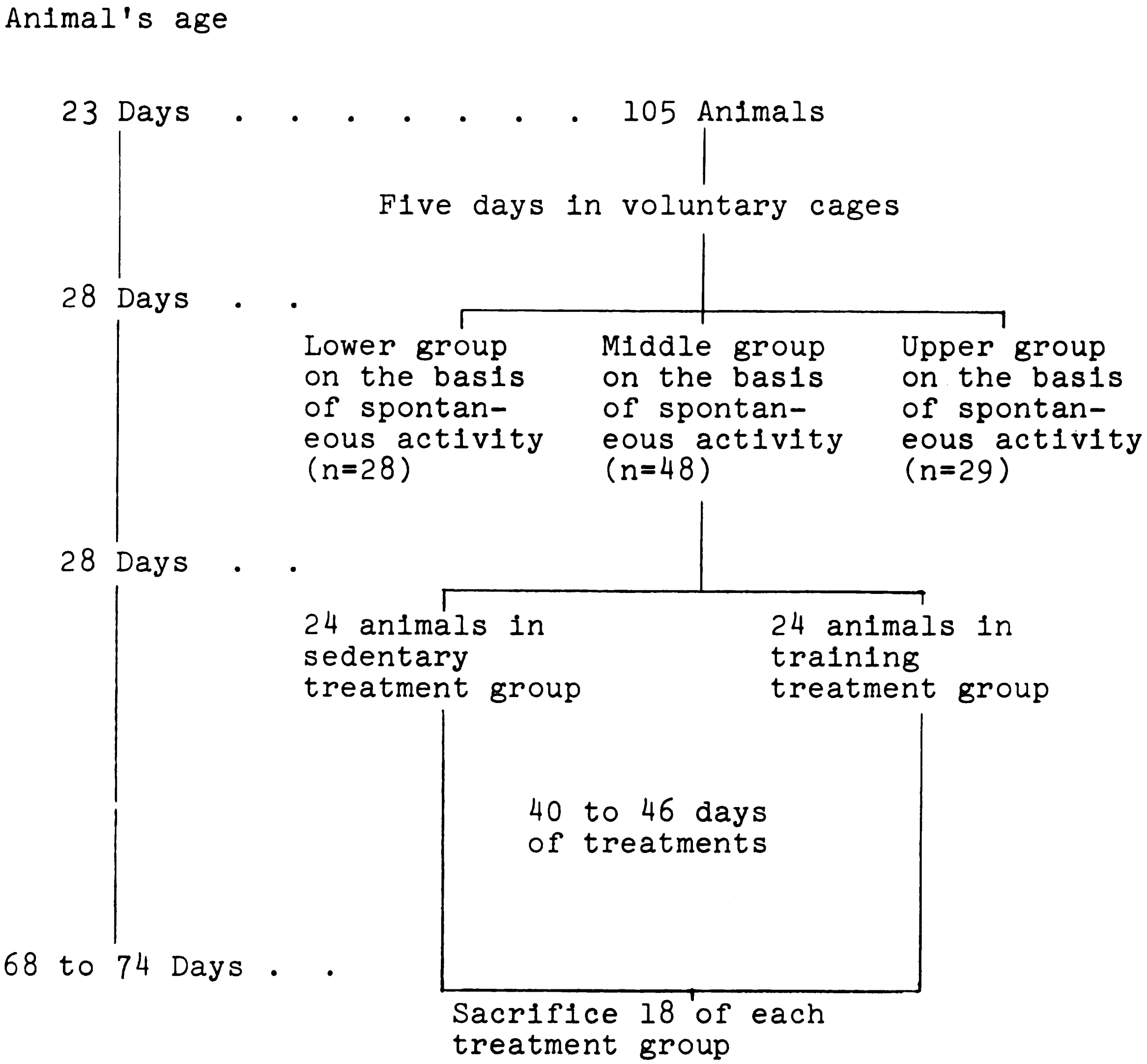

Figure 1.--Experimental Design

individually in a twenty-five centimeter diameter cylinder immersed in 1.2 meters of water. After swimming, the animals were individually dried with towels, their weights were removed, and they were returned to their cages.

Sacrifice Schedule

At the end of the experimental period, eighteen animals were chosen randomly from each group for sacrifice. The order of random selection served as the sacrifice order. This sacrifice order was established so that three animals from each group could be sacrificed on each of six consecutive days. The animals not yet sacrificed were maintained on their normal treatment schedules.

Work Performance and Methods of Taking the Tissues

At the time of sacrifice, each animal was lightly anesthetized by ether in an anesthetic chamber and then maintained in this state by the administration of ether through a nose cone. The femoral vein of the right leg was exposed and a one milliliter blood sample was taken for lactic acid determinations by the method of Barker and Summerson (2).

The soleus muscle of the right leg was removed and used for histochemical analysis not reported in this thesis.

The animal was placed on a muscle performance analyzer (44) for the measurement of the static and dynamic work performance of the gastroenemius-plantaris muscle group.[1] This muscle group was exposed in the animal's left leg and the distal end of the muscle group was cut at the achilles tendon. A clamp was attached to the cut tendon and served as the cathode for direct muscle stimulation. A hemostat attached near the proximal end of the muscle group served as the anode. A Grass stimulator, Model S4, provided a twenty-volt square wave input. A 10,000 ohm resistor, in series with the muscle preparation, insured that the current acting upon the muscle group was two milliamps. The clamp on the achilles tendon was attached to a spring steel plate and later to a linear variable differential transformer for static and dynamic contraction recordings respectively.

The force of a two-second static contraction was measured by a strain guage, mounted on the spring steel plate, and recorded on a Gilson Recorder. The stimulation frequency for the static contraction was one hundred per second with a duration of ten milliseconds. Next, ten minutes of dynamic contractions were measured by loading the muscle group with one hundred grams and measuring the distance moved per contraction (two contractions per second) by monitoring the output of the differential transformer.

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[1] The left soleus muscle was removed and used in biochemical analysis not reported in this thesis.

At the end of the ten-minute dynamic contraction cycle, a one-milliliter blood sample was taken from the left femoral vein and was used for lactic acid determination. The contracting muscle group was frozen with aluminum clamps pre-cooled in liquid nitrogen. The cooling response to this technique is shown in Figure 3. The response was measured by a thermocouple inserted into the center of the muscle group. The reference point was an ice-water bath, and the response was observed with a polaroid camera mounted on an oscilloscope which was placed in the thermocouple circuit. The quick frozen muscle tissue was removed, crushed, placed in a pyrex culture tube, recooled in liquid nitrogen, and stored at minus twenty-five degrees

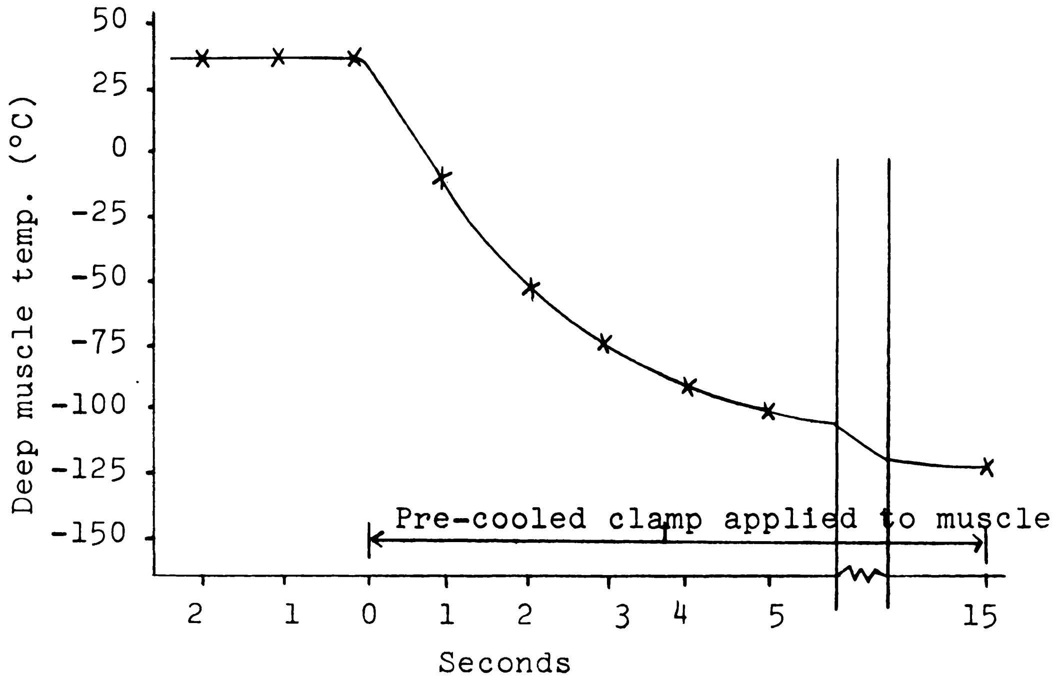

Figure 2.--Muscle Temperature Response to the Quick Freeze Technique.

centigrade. The liver and heart were removed within two minutes, cut into small sections, placed in pyrex culture tubes, quick frozen in liquid nitrogen, and stored at minus twenty-five degress centigrade. All tissues were stored for periods ranging from two days to three weeks before being assayed.

Pyridine Nucleotide Extraction Procedures

A flow chart for the extraction procedures can be seen in Figure 3. These methods are essentially those of Klingenberg (39). The tissue extractions were all performed between two days and three weeks after being frozen in liquid nitrogen.

Pyridine Nucleotide Assay Procedures

The assay methods were spectrophotometric procedures, and the reactions are depicted in Figures 4 and 5. The assays are essentially those as described by Klingenberg (39). Optical density recordings were made at a wave length of 340 millimicrons using a Bechman Model DU Spectrophotometer with a Gilford Recorder. Enzyme activity was verified at the beginning of each assay period by the addition of the specific pyridine nucleotide to be assayed at any one time. LDH and the substrates used in this study were purchased from Sigma Chemical Company. The other three enzymes were purchased from Boehringer Corp., West Germany.

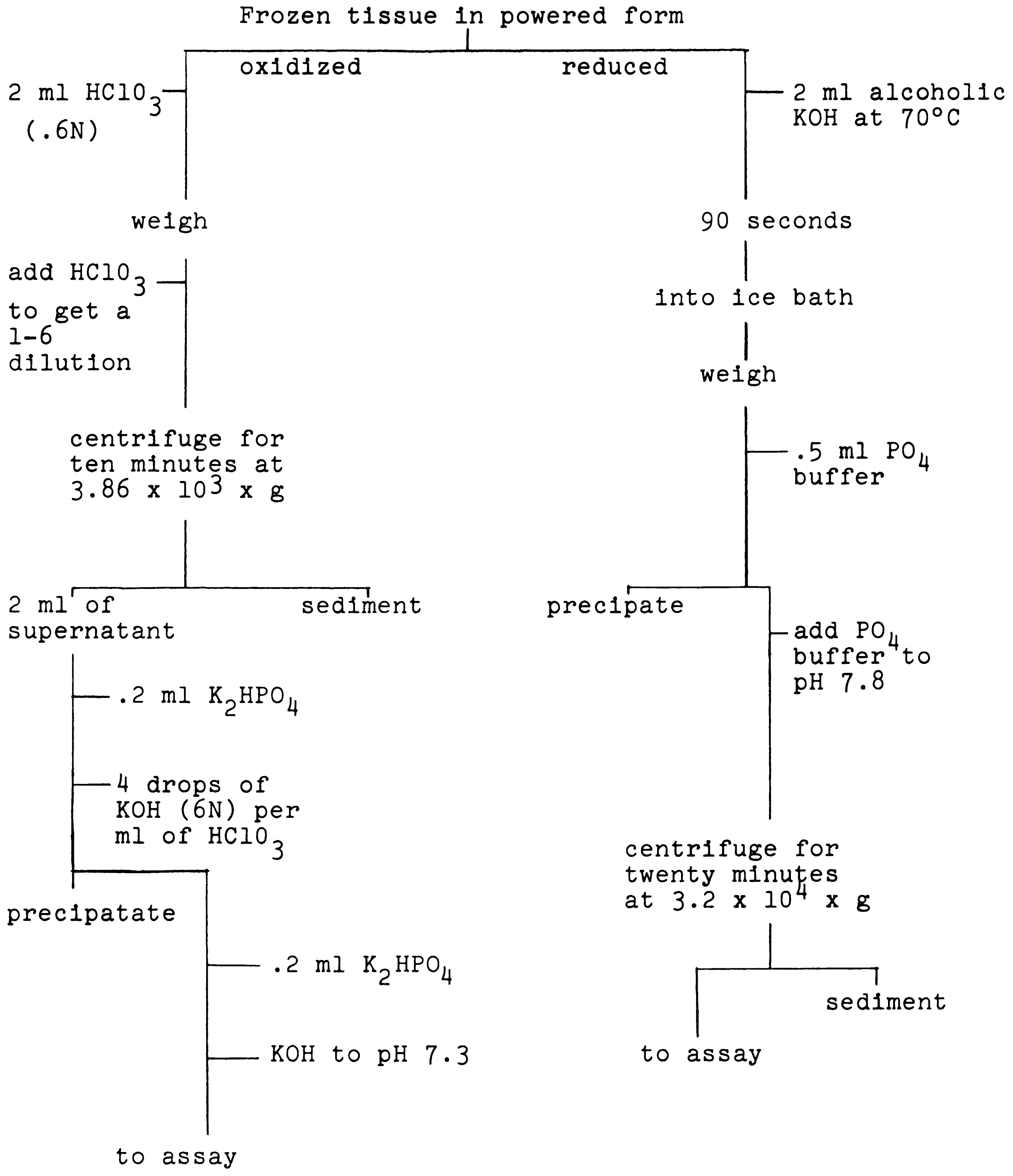

Figure 3.--Flow Chart for Tissue Extractions

$$\text{Ethanol} + \text{DPN} \xleftrightarrow[\text{Alcohol Dehydrogenase}]{} \text{Acetaldehyde} + \text{DPNH}$$

Assay procedure

1. add .15 ml extract
2. add .15 ml PO_4 buffer (.1M) with semicarbazide added
3. add .02 ml ethanol (absolute)
4. read O. D.
5. add .02 ml ADH (1.2 mg per ml)
6. after 3 minutes read O. D.

Assay characteristics

$K_7=10^{-4}$
pH 7.5
26°C
O.D. read at 340mu

$$\text{Glucose-6-Phosphate} + \text{TPN} \xleftrightarrow[\text{Glucose-6-Phosphate Dehydrogenase}]{} \text{6-Phosphogluconate} + \text{TPNH}$$

Assay procedure

1. add .3 ml extract
2. add .04 ml G-6-P (.1M)
3. read O.D.
4. add .02 ml G-6-P Dh (1 mg per ml)
5. after 3 minutes read O.D.

Assay characteristics

$K_7=10^5$
pH 7.2
26°C
O.D. read at 340 mu

Figure 4.--Assay Methods for Oxidized Pyridine Nucleotides

Calculations and Statistical Treatment of the Data

The calculational formulas used for each of the four pyridine nucleotides are those of Klingenberg (39).

$$\text{Pyruvate} + \text{DPNH} \longleftrightarrow \text{Lactate} + \text{DPN}$$

Lactate
Dehydrogenase
($K_7 = 2.9 \times 10^{-5}$)

$$\alpha\text{-Keto glutarate} + \text{NH}_4 + \text{TPNH} \longleftrightarrow \text{Glutamate} + \text{TPN}$$

Glutamate
Dehydrogenase
($K_7 = 10^{6}$)

Assay procedure

1. add .4 ml extract
2. add .005 ml pyruvate (.3M)
3. read O.D.
4. add .005 ml LDH (10 mg per ml)
5. after 3 min. read O.D.
6. add .005 ml α-keto glutarate (.5M)
7. add. 005 ml ammonium chloride (1M)
8. read O.D.
9. add .005 ml Gl-Dh (5 mg per ml)
10. after 3 minutes read O.D.

Assay characteristics

pH 7.7
O.D. read at 340 mu
26° C

Figure 5.--Assay Methods for Reduced Pyridine Nucleotides

Calculations and Statistical Treatment of the Data

The calculational formulas used for each of the four pyridine nucleotides are those of Klingenberg (39).

Statistical calculations, using the UNEQ1 routine for the one-way analyses of variance and the BASTAT routine for the correlational analyses were performed on the Michigan State University Control Data 3600 Computer.

Pilot work, completed three months prior to the experimental period of the present study, provided the basis for: (a) subjectively determining that mean differences as small as one-half of one standard deviation should be detected as statistically significant and (b) estimating the necessary and sufficient sample sizes ($n_1 = n_2 = 18$) required to hold the probability of a type I error at .05 and that of a type II error at .20. Equations employed for these determinations are found in Walker and Lev (58).

CHAPTER IV

RESULTS AND DISCUSSION

Body Weight

The mean weekly body weights for the trained and sedentary groups are shown in Figure 6. It can be seen that the trained group fell below the sedentary group and that the mean group differences were significant beginning at the end of the first week of treatments. The F-value and the probability level for each mean difference are given in Appendix C. The weekly body weights for each animal are contained in Appendix B.

The body-weight data might be very critical. Since the sedentary group grew faster, perhaps the mean differences observed in pyridine nucleotide concentrations, relative work performance, and blood lactic acid concentrations were differential growth rather than treatment effects. Van Huss (58) has observed that after forced-exercise treatments are terminated, trained rats "catch up" to sedentary rats in terms of body weights and body composition data.

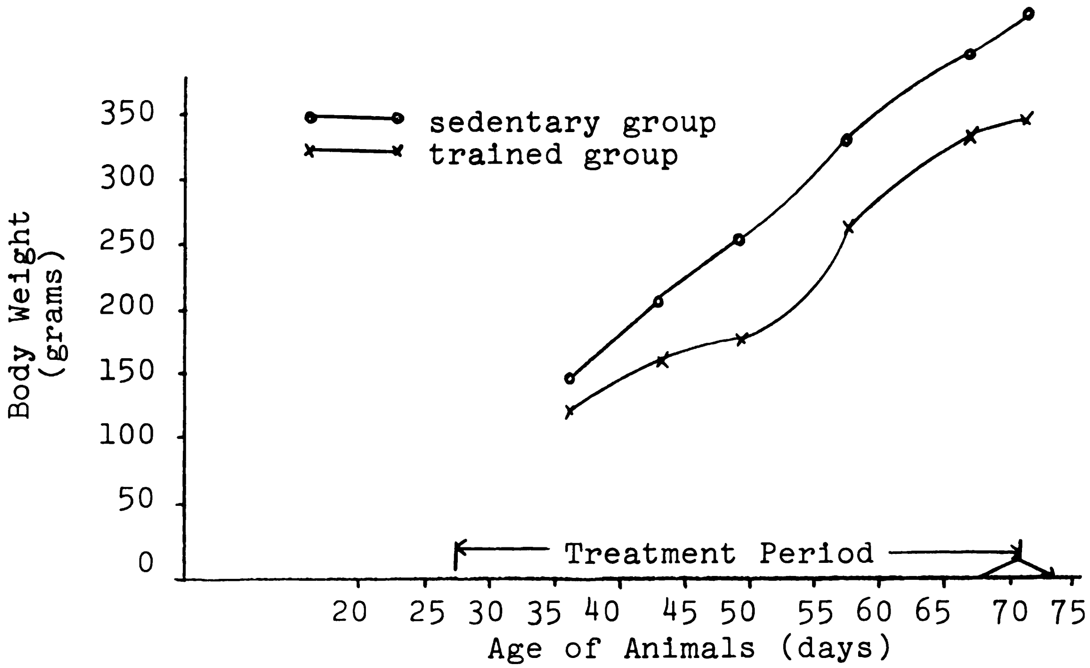

Figure 6.--Weight Profile of the Two Groups of Animals. Differences between the groups are significant ($p<.001$) beginning at the end of the first week of treatments.

Blood Lactic Acid

Eighteen[1] of the animals were measured for plasma lactic acid concentration before and after direct muscle stimulation. The chronic-exercise program did not alter the resting plasma lactic acid level. In response to the acute-exercise stress, the trained animals had a significantly greater increase in plasma lactic acid, as shown in Table 2. The F-value and the probability level for the mean differences are given in Appendix C. The post-exercise mean differences, shown in Table 2, could come

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[1] Blood samples were taken on all thirty-six animals but lactic acid was not determined until the final eighteen animals.

Table 2.--Blood Lactic Acid Concentrations Before and After Ten Minutes of Direct Muscle Stimulation. Table values are mg. % ± S.E.M.

Group	Pre-exercise Blood Lactic Acid	Post-exercise Blood Lactic Acid	Increment
Trained	14.78 ± .39	*21.40 ± .67	*6.62 ± .69
Sedentary	14.81 ± .56	19.04 ± .76	4.42 ± .77

*$p < .05$

about by three mechanisms: (a) more lactic acid might have been produced in the muscle of the trained animal (assuming the blood lactic acid was at a steady state with the muscle lactic acid concentration), (b) a greater percentage of lactic acid may have been removed from the trained muscle by the circulation, and/or (c) other tissues, chiefly the liver, could have extracted a smaller percentage of the lactic acid that was produced. Further discussion of this blood lactic acid data can be found in the section on Pyridine Nucleotides and Blood Lactic Acid as well as in Sarenac (56).

Static Strength and Dynamic Work Performance

No differences were found in the absolute static contraction strength or in the absolute total work done by the two groups. The sedentary group performed significantly more work during the first minute of the dynamic work cycle. These results are shown in Table 3. The

TABLE 3.--Static Strength and Dynamic Work Performance of the Gastrocnemius-Plantaris Muscle Group. Table values are in millimeter-grams.

Group	Static Strength	Dynamic Work (minutes)										
		1	2	3	4	5	6	7	8	9	10	Total
Trained	420.9	570.6	421.7	304.6	250.6	212.0	198.3	189.3	175.4	165.9	156.9	2645.4
	± 21.7	± 22.8	± 20.9	± 19.1	± 17.4	± 16.8	± 13.6	± 13.3	± 13.7	± 13.7	± 13.0	± 142.5
Sedentary	395.6	677.6	468.0	320.6	241.3	200.8	189.3	171.0	159.0	147.3	140.3	2715.4
	± 18.9	± 15.9	± 19.4	± 18.8	± 17.3	± 17.5	± 19.8	± 16.2	± 16.7	± 15.9	± 16.0	± 145.5
		Dynamic Work per Gram of Body Weight (minutes)										
Trained	1.60	2.13	1.59	1.15	0.95	0.79	0.75	0.72	0.67	0.63	0.59	9.94
	± .09	± .06	± .06	± .07	± .06	± .06	± .05	± .05	± .05	± .05	± .05	± .51
Sedentary	1.21	2.06	1.42	0.97	0.73	0.60	0.57	0.52	0.48	0.44	0.43	8.25
	± .06	± .04	± .06	± .05	± .05	± .49	± .06	± .05	± .05	± .05	± .05	± .41

*p < .05

F-value and the probability level for each mean difference are given in Appendix C. It can be observed that the isolated muscles of the trained animals were stronger and performed significantly more work, per gram of body weight, than the muscles of the sedentary group.[2] Further discussion of these data can be found in the section on Pyridine Nucleotides and Work Performance as well as in Lund (44).

Pyridine Nucleotides

Simultaneous determinations (extractions and assays) were carried out on twenty-three tissues. The results of the second determination, expressed as mean percents of the results of the first, can be found in Table 4. Each individual determination is recorded in Appendix E.

Table 4.--Test-Retest Determinations. Percent of pyridine nucleotides recovered during the retest procedures.

Tissue	DPN	DPNH	TPN	TPNH
Muscle n = 9	98.4	94.7	101.4	92.0
Heart n = 5	100.3	97.5	101.2	99.3
Liver n = 9	98.8	100.8	106.3	98.8

It can be seen that there was a maximum of eight percent difference between any one test-retest pyridine-nucleotide determination.

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[2] From previous work in the Human Energy Research Laboratory at Michigan State University, it can be calculated

Frozen Tissue Over Time

Replicate determinations, over time, were made on each of nine tissues. These determinations were performed two, four, fourteen, and twenty-eight days after the tissues were frozen in liquid nitrogen and stored at minus twenty-five degrees centigrade. Table 5 shows the results of the later determinations, expressed as mean percents of the two-day determinations. Appendix F shows each individual value. No more than an average of twelve percent of any one tissue pyridine nucleotide was lost in the twenty-eight-day period when the tissues were frozen.

Table 5.--Frozen Tissue Over Time. Percent of pyridine nucleotides recovered after intervals as minus 25°C.

Tissue		2 Days	4 Days	14 Days	28 Days
	DPN	100	101.0	101.3	101.0
Muscle	DPNH	100	98.3	98.3	98.4
n = 3	TPN	100	89.9	89.1	88.7
	TPNH	100	101.9	101.3	101.3
	DPN	100	99.4	98.7	98.0
Heart	DPNH	100	96.8	95.6	91.1
n = 3	TPN	100	98.9	98.1	98.7
	TPNH	100	100.7	99.5	98.4
	DPN	100	100.1	98.8	98.8
Liver	DPNH	100	98.6	97.6	95.9
n = 3	TPN	100	101.3	101.6	101.6
	TPNH	100	99.7	101.2	100.7

that the gastrochemius-plantaris muscle weight (y) and body weight (x) are correlated at $r = .89$. The regression line $y = .4885 + .0057\ x$ is significant at $p < .0005$.

Assays Over Time

Replicate assays, over time, were made on each of twenty-seven extractions. The extractions were stored at two degrees centigrade until assayed, twelve to thirty-six hours later. Table 6 shows the results of the assays, expressed as mean percents of the results of immediate assays. Appendix G shows the individual values. Less than six percent of the activity was lost during the hours when the extractions were stored.

Table 6.--Assays Over Time. Percent of pyridine nucleotides recovered after intervals at 2°C.

Tissue		0 Hours	12 Hours	24 Hours	36 Hours
Muscle n = 9	DPN	100	95.0	95.9	95.3
	DPNH	100	95.6	96.3	95.7
	TPN	100	95.5	95.9	95.4
	TPNH	100	95.6	95.4	94.9
Heart n = 9	DPN	100	96.4	97.3	95.0
	DPNH	100	94.8	95.6	94.8
	TPN	100	95.7	96.6	95.6
	TPNH	100	95.5	96.1	95.5
Liver n = 9	DPN	100	95.6	96.6	95.8
	DPNH	100	96.1	96.9	95.6
	TPN	100	94.6	96.1	95.4
	TPNH	100	96.1	97.2	95.7

Percent Recovery

A known amount of the specific pyridine nucleotide was added to each appropriate extraction buffer solution at the first step in the extraction procedure in order to determine the percent recovery. Table 7 shows that only TPNH lost any appreciable amount of detectable activity. 213.4 micro moles of each specific pyridine nucleotide was added to the actual tissue extractions and the resulting percent recoveries were essentially the same as the test-retest recovery data. These data can be found in Appendix E.

Table 7.--Percent Recovery. Percent of pyridine nucleotides recovered after the addition of a known amount to the specific buffer solution.

	DPN	DPNH	TPN	TPNH
micro moles added	213.4	213.4	213.4	213.4
mirco moles recovered	210.8	208.9	211.1	199.0
percent recovered	98.7	97.8	98.9	93.2

From the previous discussion of errors of technique, it can be seen in Table 8 that if the maximum variability is allowed for each of the possible sources of variation, there exists between 8.1 and 21.8 percent variability in the data.

Tissue Concentrations in Trained and Sedentary Animals Following Acute-Exercise

Pyridine nucleotide concentrations in muscle, heart, and liver were determined for trained and sedentary animals

Table 8.--Maximum Percent Error Due to Random Sampling Techniques.

Tissue	DPN	DPNH	TPN	TPNH
Muscle	9.2	13.6	18.4	21.8
Heart	8.6	17.8	8.6	13.8
Liver	8.1	11.5	14.4	13.5

following acute-exercise. Group mean values are shown in Table 9. The F-value and the probability level for each mean difference are given in Appendix C. The individual concentrations for each animal are recorded in Appendix D.

The three statistically significant mean differences in muscle pyridine nucleotides can be attributed to the difference in DPNH levels. Since DPNH is the predominate reduced pyridine nucleotide in muscle, the total reduced pyridine nucleotide level was decreased in the muscles of the trained animals. This decreased reduced level was responsible for the higher pyridine nucleotide oxidized to reduced ratio which was observed. The higher oxidized to reduced ratio in the muscles of the trained rats indicates an increased capacity of the mitochondria to keep the cell in a more oxidized state; or, this higher ratio may indicate a more efficiently operating cell in terms of oxygen transport and/or energy utilization. The capability of the muscle of the trained animal to maintain a higher oxidized to reduced ratio suggests that there

TABLE 9.--Pyradine Nucleotide Concentrations. Table values are in micro moles per kilogram of tissue.

	Muscle		Heart		Liver	
	Sedentary	Trained	Sedentary	Trained	Sedentary	Trained
DPN	970.8 ± 75.4	1027.3 ± 90.0	949.8 ± 119.3	1015.3 ± 77.6	429.0 ± 60.2	636.0 ± 131.3
DPNH	262.8 ± 51.5*	124.9 ± 19.4	404.9 ± 78.2	367.0 ± 43.5	161.0 ± 40.5	157.0 ± 26.8
TPN	8.1 ± 1.5	10.2 ± 3.1	23.1 ± 3.2	27.1 ± 3.8	42.3 ± 10.9	38.4 ± 5.4
TPNH	106.4 ± 30.9	66.9 ± 15.9	30.3 ± 7.3	48.9 ± 7.4	545.5 ± 96.3	401.5 ± 87.2
DPN/DPNH	7.2 ± 1.7	10.2 ± 1.3	3.9 ± .8	4.7 ± 1.3	5.2 ± 1.2	6.6 ± 1.9
TPN/TPNH	.25± .07	.43± .23	1.8 ± .60	.8 ± .16	.12± .03	.19± .05
Total DPN	1233.6 ± 94.8	1152.3 ± 94.8	1354.8 ± 131.6	1382.4 ± 82.1	590.5 ± 63.6	794.6 ± 130.2
Total TPN	114.5 ± 30.8	77.1 ± 16.3	53.4 ± 9.4*	76.1 ± 9.2	587.8 ± 100.4	439.9 ± 90.1
Total DPN/Total TPN	23.2 ± 4.2	41.6 ± 12.2	35.8 ± 4.7*	24.1 ± 3.9	1.9 ± .4	3.7 ± 1.3
Total oxidized	978.9 ± 76.2	1037.5 ± 90.1	972.9 ± 121.9	1042.4 ± 76.9	471.3 ± 58.3	675.1 ± 133.2
Total reduced	369.2 ± 77.6*	191.8 ± 30.2	435.3 ± 79.5	416.1 ± 44.6	706.9 ± 98.5	559.5 ± 98.9
Oxidized/Reduced	4.4 ± .7*	7.3 ± 1.2	3.5 ± .7	3.5 ± .7	1.0 ± .2	2.3 ± 1.0
Total pyridine nucleotide	1348.1 ± 109.4	1229.3 ± 94.2	1408.2 ± 136.7	1458.5 ± 83.8	1178.3 ± 101.0	1234.6 ± 169.2

* = $p < .05$

exists a less dependence upon glycolysis for energy supplies. These results support the data showing an increased reliance upon fatty-acid oxidation as a source of energy for trained muscles (5, 35, 49, 62).

It was previously noted that blood lactic acid was elevated in the trained animals and that the trained animals did more work per unit of body weight and per unit of muscle weight. Therefore, it seems logical to conclude that the removal of lactic acid through blood transport was much more efficient in the trained animals than in the sedentary animals.

The treatment for the trained animals was a program that stressed their hearts to a much greater degree than the acute direct muscle stimulation, which was imposed immediately prior to sacrifice. In fact, it is somewhat questionable that the bout of acute-exercise was sufficiently intense to force any of the animals' hearts into marked exercise metabolism. If that was the case, the pyridine-nucleotide determinations that were made on the hearts, would reflect essentially resting myocardial metabolism.

Upon sacrifice, it was found that the total TPN was increased, and thus the total DPN to total TPN ratio was decreased, in the hearts of the trained animals. Although no satisfactory explanation can be offered to explain this data, three related possibilities can be enumerated: (a) there was an increased capacity for reductive biosynthesis within the hearts of these animals, which could be used for energy storage or structural components, (b) there was an increase in the TPN dependent enzymes, and/or (c) there was an increase in the pentose shunt activity.

There were no significant treatment effects on the mean total pyridine nucleotides in the gastrocnemius-plantaris, heart, or liver. Thus, one of the initial hypothesis of this study was not realized and the hypothesis of no treatment-induced group differences (beta $\leq .20$) can be accepted.

Although not statistically significant, it is interesting to note that the mean differences in liver total DPN and total TPN appear to be in the direction that was originally hypothesized. The smaller average DPN to TPN ratio in the livers of the sedentary rats indicate a mobilization for fat synthesis. The higher ratio in the livers of the trained rats indicate a greater capacity for energy production. The F-value is such that no definite statistical decision can be formulated.

In muscle and heart, the data show that DPN is the primary pyridine nucleotide. In liver, the TPNH concentration is approximately equal to the DPN concentration. In muscle and heart, the pyridine nucleotides are primarily of the oxidized type. In liver, the ratio of oxidized to reduced is close to one. These results are to be expected in light of previously reported data and the biological roles of these tissues.

Interrelationships

Pyridine Nucleotides and Blood Lactic Acid

During strenuous work performance, it is well documented that blood lactic acid increases (8, 30, 54). Blood lactic acid can increase, as a result of muscular activity, in three general ways: (a) there can be an increased muscular production of lactic acid, (b) the blood can transport a greater percentage of that which is produced, and/or (c) other tissues, chiefly the liver, can extract a smaller percentage of the lactic acid that is transported.

In this investigation, the trained animals had significantly higher post-exercise blood lactic acid concentrations than the sedentary animals. A discussion of the most probable causal mechanism(s) for this observed group difference is warranted.

As lactic acid production increases (assuming an aerobic to anaerobic metabolism transition), the oxidized to reduced ratio of the muscle cell is lowered due to increased DPNH (28, 32, 33, 34). It has been shown that, in this study, the trained group had the higher muscle oxidized to reduced ratio. This result suggests that less lactic acid was formed in the muscles of the trained animals. (No attempt was made to determine muscle lactic acid directly.)

The work-performance data support this hypothesis, of less lactic acid production in the trained muscles, and are in accord with several classical theories of the limiting factors of muscular work (29, 32, 34, 54, 57). The gastrocnemius and plantaris muscles of the sedentary animals were capable of less total work per unit of muscle weight than were the corresponding muscles of the trained animals. Assuming that the lower mean oxidized to reduced ratio in the muscles of the sedentary animals represented a greater production of lactic acid, it might be deduced that the increased lactic acid concentration in these muscles was one of the factors which impaired work performance in the sedentary animals.

If increased lactic acid production in the muscles was not a causal mechanism for the higher blood lactic acid concentrations observed in the trained animals, then blood transport of lactic acid must have been more efficient and/or liver extraction of lactic acid from the blood must have been less efficient.

It can be seen from the lactic acid to pyridine nucleotide correlations presented in Table 10 that there was a correlation of r = .83 between the change in blood lactic acid (post minus pre-exercise blood lactic acid concentrations) and the oxidized to reduced ratio of the muscle of the trained rats. That is, the lower the muscle oxidized to reduced ratio the less the change in the blood lactic acid level. This correlation indicates a direct muscle to blood relationship in the amount of lactic acid transported. Note that a similar relationship was not found in the sedentary animals ($r = .09$).

The ability of the blood of the trained animal to reflect the oxidized to reduced ratio of the cell could be an indication of an adaptation to exercise. The cellular changes involved in this increased ability cannot be identified from the data of this study, but two possibilities exist: (a) an alteration in the cellular membrane transport system for lactic acid, and (b) an alteration of the equilibrium constant and/or the concentration of lactate dehydrogenase (LDH) within the cell. Data concerning the concentration of LDH as a function of exercise appear to be quite confusing (1, 22). Structural changes of the enzymes with changing ratios of metabolites may be the more logical explanation. Unpublished data from Deal's Laboratory at Michigan State University indicate that enzymes (especially those of the glycolytic pathway) are altered in vitro by varying the concentrations of the cellular constituents (9).

The possibility of a difference between groups, in the efficiency of the livers to extract lactic acid from the blood, also must be examined. One might suspect that

TABLE 10.--Correlation Coefficients Between Blood Lactic Acid and Pyridine Nucleotides.

	Oxidized/Reduced		DPN/DPNH		DPN/TPN		Total pyr. Nucleotides		Total DPN		Total TPN	
	Sed.	Train.	Sed.	Train.	Sed.	Train.	Sed.	Train.	Sed.	Train.	Sed.	Train
						Muscle						
Pre-blood Lactate	-.45	-.41	-.45	-.49	-.09	.12	-.07	.27	-.07	.22	-.03	.21
Post-blood Lactate	-.29	.61	-.25	.40	-.02	.72*	.12	.48	.11	.62	.07	-.50
Change-blood Lactate	.09	.83*	.08	.66*	.04	.63	.17	.31	.16	.48	.09	-.61
						Heart						
Pre-blood Lactate	-.41	-.53	-.45	-.50	.33	.40	-.19	-.33				
Post-blood Lactate	-.28	-.06	-.28	-.15	.31	.41	-.38	.08				
Change-blood Lactate	.01	.24	.05	.13	.06	.13	-.23	.27				
						Liver						
Pre-blood Lactate	.00	-.56	.24	-.26	.18	-.72*	.14	-.13				
Post-blood Lactate	-.21	.48	-.28	.42	-.22	.37	.66	.47				
Change-blood Lactate	-.21	.79*	-.45	.55	-.35	.77*	.55*	.53				

* = p < .05

since the trained animals were smaller than the sedentary animals, the livers of the trained animals would extract a lessor absolute amount of lactic acid from the blood.[3] However, the correlation of $r = .79$, between the change in blood lactic acid and the oxidized to reduced ratio within the livers of the trained rats, indicates that the livers of these animals were able to convert the increased lactic acid to pyruvate and thus were forming large amounts of DPN. The non significant correlation of $r = .21$, for the sedentary animals, indicates that the livers of these animals were not adapted to decrease the exercise-induced blood lactic acid to the same extent as were the livers of the trained animals. As was the case with muscle, membrane permeability, enzyme structural alterations, and enzyme concentration may have been involved in this ability to increase the oxidized to reduced ratio in response to an increase in blood lactic acid.

A positive correlation of $r = .77$, between the change in blood lactic acid and the total DPN to total TPN ratio, was found for the livers of the trained rats. A non significant negative correlation of $r = .35$ was found for the livers of the sedentary rats. The contrast between these two correlation coefficients is another indication

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[3] From previous work in the Human Energy Research Laboratory at Michigan State University, it can be calculated that the body weight (x) and liver (y) weights are correlated at $r = .81$. The regression line $y = .9698 + .0283\ x$ is significant at $p < .005$.

of the differences which existed in the energy metabolism of the trained as opposed to the sedentary rats. The liver DPN to TPN ratio has been discussed earlier. In this context, it indicates that an increase in the blood lactic acid concentration can be handled better by the liver of the trained rat than by that of the sedentary rat.

One other possibility, which is specific to this investigation, should be mentioned. The lower blood lactic acid concentrations in the larger sedentary animals might be explained partially by a greater dilution of the lactic acid in the blood of those animals--assuming that a larger animal has a greater volume of blood than a smaller animal. No attempt was made to check this possibility; however, the muscle and liver results would indicate that a blood dilution did not account for much, if any, of the difference observed in blood lactic acid concentrations.

In summary, it appears that, in the trained animals as compared to the sedentary animals: (a) there was less muscular production of lactic acid, (b) the blood transport of lactic acid was much more efficient, and (c) the liver extraction of lactic acid from the blood was more efficient.

Pyridine Nucleotides and Work Performance

It can be seen from the regression lines relating the oxidized to reduced ratio (x) and the total work divided by body weight (y), that for any given work performance level, the muscle of the trained animal ($x = 80.73 - 7.38y$) had a higher oxidized to reduced ratio than the muscle of the sedentary animal ($x = 63.21 - 7.13y$). Although the slopes of the regression lines were statistically non sifnificant these data tend to support one of the initial hypotheses of this study, that for any given amount of work, the muscle of the trained animal will have a greater ozidized to reduced level than that of the sedentary animal. Thus, to do more work, the cell must be capable of maintaining a more oxidized environment or be able to tolerate a more reduced state. The critical oxidized to reduced ratio is a function of the muscle cell; and from other data presented previously, it appears that this ratio can be altered by training.

Within each treatment group no statistically significant correlations were found between the muscle pyridine nucleotides and any of the work parameters.

Intercorrelations Between Pyridine Nucleotides and Body Weight

By examining Tables 11 and 12, it can be observed that, within the sedentary group, body weight had a distinct relationship to total pyridine nucleotides in the muscle and heart. The trained group did not show this relationship. These results could be expected, as it may be possible to think of the body weights of the trained group as being controlled by the external stress of the four-hour daily swim. Thus, food intake in the trained animal was converted to energy storage, and then to energy utilization, during the training sessions. The body weights of the sedentary animals were controlled from within, as food intake was used only for energy storage and the normal body functions.

In the sedentary animals, heart total pyridine nucleotides correlated with muscle total pyridine nucleotides ($r = .52$) and with body weight ($r = -.52$). The negative correlation with body weight suggests that the total pyridine nucleotides (especially in the heart) become diluted within the tissues in these sedentary animals. That is, as the animal increased in body weight, the tissue concentration decreases. This could lead, possibly, to a pathological condition if the dilution becomes critical. These relationships were not observed in the trained animals.

The trained animals showed simultaneous changes between muscle and liver pyridine nucleotides. Alterations in the oxidized to reduced ratio, the DPN to DPNH ratio, and the total DPN to total TPN ratio were in the same direction ($r > .59$). As the muscle worked, and thus became more reduced, the liver also became more reduced. The mechanism for this increased reduced state (decrease in the oxidized to reduced ratio) in the liver of the trained animals was not investigated by the present study. No hypothesis can be offered to explain this

TABLE 11.--Correlation Coefficients Between Pyridine Nucleotides and Body Weight of the Sedentary Group.

Muscle	DPN/DPNH	1.00														
	Total DPN	-.20	1.00													
	Total TPN	-.26	.34	1.00												
	Oxidized/Reduced	.83*	-.32	-.55*	1.00											
	Total DPN/Total TPN	-.09	.08	-.65*	.25	1.00										
	Total pyr. neu.	-.25	.96*	.58*	-.43	-.12	1.00									
Heart	DPN/DPNH	-.07	-.15	-.09	-.07	-.09	-.15	1.00								
	Oxidized/Reduced	-.09	-.12	-.06	-.09	-.09	-.12	.97*	1.00							
	Total DPN/Total TPN	-.41	-.02	.04	-.42	-.27	-.01	.05	.16	1.00						
	Total pyr. nuc.	.25	.49*	.33	-.09	-.20	.52*	.15	.20	-.15	1.00					
Liver	DPN/DPNH	-.12	-.17	-.25	-.05	.08	-.22	.16	.07	-.20	-.21	1.00				
	Oxidized/Reduced	-.15	-.26	-.18	-.13	.07	-.27	.34	.19	-.45	-.04	.53*	1.00			
	Total DPN/Total TPN	-.13	-.33	-.19	-.02	.14	-.34	.17	.13	-.25	.06	.03	.71*	1.00		
	Total pyr. nuc.	.13	-.07	-.12	.25	.06	-.10	.07	.10	-.12	-.31	.26	-.24	-.40	1.00	
	Body Weight	-.41	-.48*	-.12	-.22	.16	-.45	-.06	-.06	.26	-.52*	.37	.08	.12	.03	1.00

* = $p < .05$

TABLE 12.--Correlation Coefficients Between Pyridine Nucleotides and Body Weight of the Trained Group.

Muscle	DPN/DPNH	1.00														
	Total DPN	.41	1.00													
	Total TPN	-.44	-.13	1.00												
	Oxidized/Reduced	.91*	.47*	[illegible]	1.00											
	Total DPN/Total TPN	.61*	.10	-.69*	.78*	1.00										
	Total pyr. nuc.	.34	.98*	.04	.36	-.00	1.00									
Heart	DPN/DPNH	.35	.17	-.24	.31	-.02	.13	1.00								
	Oxidized/Reduced	.42	.21	-.31	.41	.05	.15	.98*	1.00							
	Total DPN/Total TPN	.12	.34	-.17	.16	-.01	.31	-.18	-.13	1.00						
	Total pyr. nuc.	.00	.08	.10	-.21	-.44	.10	-.09	-.11	.07	1.00					
Liver	DPN/DPNH	.59*	.10	-.35	.71*	.68*	.04	.14	.26	-.16	-.60*	1.00				
	Oxidized/Reduced	.68*	.16	-.33	.78*	.84*	.11	.07	.16	-.10	-.55*	.89*	1.00			
	Total DPN/Total TPN	.70*	.20	-.34	.79*	.81*	.14	.12	.20	-.09	-.42	.79*	.96*	1.00		
	Total pyr. nuc.	.05	-.04	-.39	.21	.24	-.11	.30	.40	-.08	-.29	.44	.24	.13	1.00	
	Body Weight	.20	.02	.03	.16	-.08	.02	.41	.42	-.06	.14	.05	.03	.12	-.11	100

* = $p < .05$

phenomenon but one or more of the following events could account for this change: (a) the first step in the extraction of lactic acid from the blood, the conversion to pyruvate, (b) oxidation of fatty acids, and/or (c) an ischemic environment in the liver cell.

Since parallel changes were not observed in the sedentary animals, perhaps these rats were not "adapted" to exercise. That is, their systems seemed to operate more independently of one another. The coordination of metabolic systems of different tissues may be a crucial factor in any "adaptation" to training. This concept merits further investigation.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purpose of this study was to examine the pyridine nucleotide concentrations in skeletal muscle, heart, and liver of trained and sedentary male albino rats in response to a ten-minute acute in-situ, exercise stress. It was also the purpose of this investigation to assess the total pyridine nucleotide concentrations of the two groups as a result of the treatments applied over the six-week experimental period. The general concept underlying the experimental hypothesis was that the diphosphopyridine nucleotides (DPN) are involved in energy metabolism, while the triphosphopyridine nucleotides (TPN) are utilized, primarily, in reductive biosynthesis.

Two groups of eighteen young male albino rats (Sprague-Dawley strain) were assigned randomly to treatments: (a) sedentary housing, and (b) sedentary housing with two two-hour swim sessions per day, five days per week. The animal that was forced to swim had an additional weight of up to three percent of its body weight attached to the tip of its tail.

The cage housings for the trained group were standard 24 x 18 x 18 centimeter small-animal cages. The sedentary animals lived in this same type of cage but a sheet metal plate was inserted to diagonally bisect the total volume of the cage.

During the six-week experimental period, the trained animals were forced to swim from three P.M. to five P.M. and from nine P.M. to eleven P.M. five days per week.

Body weights for all the animals were recorded on Thursday mornings of each week.

At the end of the six-week training period, six rats per day were lightly anesthetized with ether, the right femoral vein exposed and a blood sample taken for the pre-exercise blood lactic acid determination. The distal end of the gastrocnemius-plantaris muscle group was exposed by severing the achilles tendon. A clamp attached to the cut tendons served as the cathode for direct muscle stimulation. The clamp was connected to a spring steel plate, upon which was mounted a strain guage, for muscle static work recordings. Following the static recordings the clamp was connected to a linear variable differential transformer for dynamic work recordings. The stimulation frequency was two per second and the current was two milliamps.

At the end of the ten-minute stimulation period a blood sample was taken from the exposed left femoral vein and used for the post-exercise blood lactic acid determinations. The muscle was than clamped between two aluminum plates, pre-cooled in liquid nitrogen. The heart and liver were removed within two minutes and frozen in liquid nitrogen. These tissues were stored at minus twenty-five degrees until the pyridine nucleotides were extracted. Extraction procedures and assays are essentially as those described by Klingenberg (39).

Significant differences between the trained and sedentary groups were found in muscle DPNH (124.9 and 262.8 micro moles per kilogram of muscle), muscle pyridine nucleotide oxidized to reduced ratio (7.3 and 4.4), and heart DPN to TPN ratio (24.1 and 35.8 respectively). Significant correlations were found in the relationship between: (a) body weight and muscle DPN in the sedentary animals ($r = -.48$), (b) body weight and heart total pyridine nucleotides in the sedentary animals ($r = -.52$), (c) elevation in blood lactic acid and muscle oxidized to reduced ratio in the trained animals ($r = .83$), (d) elevation in blood lactic acid and liver oxidized to reduced ratio in the trained animals ($r = .79$), and (e) oxidized to reduced ratio in muscle to the oxidized to reduced ratio in the liver of the trained animal ($r = .78$).

Conclusions

1. The six-week training stress was severe enough to result in lower body weights in the trained animals.

2. The training routine did not produce a difference between the two groups in resting blood lactic acid concentration.
3. A ten-minute direct muscle stimulation brought about a higher blood lactic acid concentration in the trained animal.
4. The mean work performance was not different between the two experimental groups; but work per unit of body weight was greater for the trained group.
5. Correlations of work to blood lactic acid concentration and work to oxidized to reduced ratio of the muscle suggest that total work performance may be limited by the ability of the cell to minimize the decrease in the oxidized to reduced ratio within the cell. The ten-minute work stress resulted in a less reduced state in the muscle of the trained as compared to the sedentary animal.
6. Mean, total pyridine nucleotides in skeletal muscle, heart, and liver were not different between the two treatment groups.
7. In the trained animals there existed a significant correlation (r = .83) between the change in blood lactic acid and the muscle oxidized to reduced ratio.
8. The decrease in muscle oxidized to reduced ratio following an acute-exercise stress was accompanied by a decrease in the liver oxidized to reduced ratio in the trained animals.

9. Heart total TPN was greater and thus the total DPN to TPN ratio was lower in the trained group.

Recommendations

A considerable amount of research, at the cellular level, is needed in order to establish a workable model for the acute and long term animal responses to the various types of exercise stressors. This future research should include the following recommendations.

1. Regulation of diet should be a major criteria in any experimental design.
2. The size of the individual muscle fiber in relation to the various muscle sizes should be established.
3. Not only enzyme concentrations, but more importantly the specific activities of each enzyme in response to the various exercise stressors should be examined.
4. Pyridine nucleotide concentrations should be separated as to their concentrations within the various cellular compartments in relation to the several oxidized to reduced ratios.
5. The relationship should be established between muscle lactic acid and blood lactic acid and observations made of the alteration, if any, of this function with training.
6. Correlative studies should be carried out to link observable changes in the various exercise related parameters.

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APPENDICES

APPENDIX A.--Revolutions Run by Each Animal During the Pre-experimental Period.

Animal Number	First Day	Second Day	Third Day	Total
1	1059	903	1034	2996
2	906	991	1085	2982
3	1151	1026	783	2960
4	1249	747	946	2937
5	818	871	1232	2921
6	520	997	1393	2910
7	788	902	1032	2722
8	598	641	1452	2691
9	606	986	1096	2688
10	1188	539	959	2686
11	1380	572	727	2679
12	1267	598	781	2646
13	957	909	743	2609
14	933	920	745	2598
15	835	549	1210	2594
16	638	740	1105	2483
17	625	654	1181	2460
18	916	936	603	2455
19	831	843	761	2435
20	954	825	611	2390
21	834	707	789	2330
22	910	734	660	2304
23	795	774	709	2278
24	771	556	948	2275
25	823	552	895	2270
26	763	626	878	2267
27	1303	505	446	2254
28	801	876	545	2222
29	781	591	779	2151
30	757	540	753	2050
31	851	774	422	2047
32	687	876	472	2035
33	462	659	893	2014
34	496	709	767	1972
35	442	582	846	1870
36	406	606	827	1839
37	610	549	656	1815
38	645	565	545	1755
39	495	539	595	1629
40	551	585	466	1602
41	816	322	460	1598
42	540	511	525	1576
43	645	533	346	1524
44	337	511	620	1468
45	343	489	633	1465
46	528	471	431	1430
47	409	425	510	1344
48	548	355	422	1324

APPENDIX B.--Body Weight of Each Animal for Each Week.

Animal Number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Animal Number	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
2	130	174	208	246	292	322	1	108	152	160	200	242	244
6	150	207	228	294	333	353	5	138	160	147	222	268	284
11	136	189	232	265	304	325	8	120	175	175	230	278	245
14	148	192	235	280	306	329	9	120	171	185	250	286	296
15	141	188	235	272	314	328	12	122	157	180	214	246	264
18	134	186	231	280	312	349	13	120	178	174	254	278	309
19	134	187	173	268	306	288	16	126	183	186	256	294	304
22	140	194	237	278	316	342	17	129	142	157	202	232	248
23	144	194	243	298	348	333	20	150	190	147	242	282	304
26	150	211	225	288	342	373	24	147	184	187	258	294	304
27	138	193	240	282	320	304	25	131	183	195	276	318	296
30	139	187	222	280	322	353	29	112	161	186	216	252	229
31	138	185	230	278	312	338	32	125	167	176	228	250	234
35	137	168	208	268	322	340	36	120	142	166	210	248	258
38	122	170	207	264	280	321	37	117	153	151	208	252	279
39	131	171	207	254	282	295	44	118	146	167	222	257	270
42	119	163	179	244	266	294	45	115	136	148	205	246	257
47	125	181	199	260	307	323	48	123	160	190	219	257	234
$\bar{X}$	136	186	219	272	310	347	$\bar{X}$	124	163	171	228	266	267

APPENDIX C.--One Way Analysis of Variance With the Exercise Group as the Category Variable.

Dependent Variable	F-Value	Probability	Dependent Variable	F-Value	Probability
		Pyridine Nucleotides			
MDPN	.23	.63	Total H--reduced	.04	.83
MDPNH	6.25	.02	Total oxid/reduced	.00	.98
MTPN	.37	.54	LDPN/LDPNH	.36	.55
MTPNH	1.29	.26	LTPN/LTPNH	1.51	.23
HDPN	.21	.65	Total LDPN	1.98	.17
HDPNH	.18	.67	Total LTPN	1.20	.28
HTPN	.63	.43	Total LDPN/LTPN	1.75	.19
HTPNH	3.21	.08	Total L-pyr. nuc.	.08	.78
LDPN	2.06	.16	Total L-oxidized	1.96	.17
LDPNH	.00	.94	Total L-reduced	1.11	.30
LTPN	.10	.75	Total oxid./reduced	1.62	.21
LTPNH	1.23	.28	Total Muscle wt.	49.35	<.0005
MDPN/MDPNH	1.88	.18	DPN in Muscle	.66	.42
MTPN/MTPNH	.59	.45	DPNH in Muscle	8.19	.007
Total MDPN	.37	.55	TPN in Muscle	.11	.75
Total MTPN	1.15	.29	TPNH in Muscle	2.04	.16
Total MDPN/MTPN	2.01	.16	Total DPN in Muscle	4.08	.05
Total M pyr. nuc.	.68	.42	Total TPN in Muscle	1.95	.17
Total M-oxidized	.25	.62	Total pyr. nuc. muscle	4.85	.03
Total M-reduced	4.54	.04	Total oxid. in muscle	.64	.43
Total oxid./reduced	4.53	.04	Total reduc in Muscle	6.13	.02
HDPN/HDPNH	.25	.62			
HTPN/HTPNH	2.57	.12			
Total HDPN	.03	.86			
Total HTPN	2.98	.09			
Total HDPN/HTPN	3.61	.07			
Total N pyr. nuc.	.10	.76			
Total H-oxidized	.23	.63			
		Body Weight			
Week 1	12.69	<.001	Week 5	37.34	<.0005
Week 2	20.45	<.0005	Week 6	49.35	<.0005
Week 3	61.20	<.0005	Final	52.51	<.0005
Week 4	47.16	<.0005			
		Blood Lactate			
Pre-lactate	0.00	.97	Δ-lactate	5.34	.03
Post-lactate	5.44	.03			
		Work			
Static	.78	.38	One/body wt.	1.23	.27
One	14.84	<.0005	Two/body wt.	3.98	.05
Two	2.63	.11	Three/body wt.	4.41	.04
Three	.36	.55	Four/body wt.	7.60	.009
Four	.14	.71	Five/body wt.	6.14	.02
Five	.21	.65	Six/body wt.	5.10	.03
Six	.14	.71	Seven/body wt.	8.18	.007
Seven	.76	.39	Eight/body wt.	6.32	.02
Eight	.57	.45	Nine/body wt.	6.77	.01
Nine	.81	.37	Ten/body wt.	5.76	.02
Ten	.64	.43			
First three min.	5.27	.028			
Last seven min.	.45	.51			
Total work	.11	.73			
Static/body wt.	12.66	.001			

APPENDIX D.--Pyridine Nucleotide Concentrations for Each Animal. Values are in Micro-moles per Kilogram of Tissue.

Animal Number	Final Body Weight	Muscle				Heart				Liver			
		DPN	DPNH	TPN	TPNH	DPN	DPNH	TPN	TPNH	DPN	DPNH	TPN	TPNH
		Sedentary Group											
2	322	807.4	142.9	5.5	72.0	1031.4	79.6	28.8	40.7	778.4	63.4	36.3	174.5
6	353	622.2	79.3	5.4	18.2	633.3	97.3	16.1	5.6	560.9	72.0	78.3	1179.0
11	325	1156.0	454.9	5.0	274.6	684.0	1488.8	23.1	14.9	50.1	124.4	34.3	128.8
14	329	1325.9	566.6	3.6	49.6	1707.1	217.7	19.2	12.4	88.8	104.0	8.1	887.9
15	328	706.0	94.3	11.5	12.2	265.7	249.4	11.1	5.0	427.3	134.8	18.6	278.7
18	349	588.3	881.6	6.9	489.4	742.6	479.1	23.7	47.9	431.3	96.8	192.5	915.6
19	288	1242.5	40.6	11.8	87.2	1717.2	446.5	56.0	95.8	15.9	77.2	78.5	1145.9
22	342	872.3	60.1	5.4	24.7	863.5	216.9	14.8	15.1	283.0	766.6	22.8	285.4
23	333	1147.2	261.4	2.3	16.9	941.1	346.2	20.9	95.8	372.2	180.4	55.1	134.3
26	373	474.4	179.6	.9	38.6	611.9	102.4	2.7	5.8	481.7	42.2	17.3	769.2
27	304	1452.4	164.0	11.3	138.3	586.8	349.8	18.6	5.2	312.7	237.2	68.2	272.8
30	353	525.9	172.3	6.5	18.8	966.1	403.3	25.2	16.6	527.7	144.0	.8	85.3
31	338	824.2	335.2	1.7	212.9	385.8	488.6	5.5	8.5	221.9	70.4	67.2	665.2
35	340	1215.9	189.6	5.5	30.9	1062.7	332.5	25.2	59.7	716.4	43.2	.8	551.1
38	321	815.9	42.7	6.2	81.6	1206.8	613.9	37.9	65.8	722.4	76.0	.7	365.7
39	295	1022.5	449.2	11.3	302.5	2262.3	230.2	51.5	4.6	421.9	266.4	48.2	139.6
42	294	1183.8	232.7	16.4	29.2	815.9	29.5	19.4	5.9	358.9	340.0	29.2	1353.5
47	323	1491.4	383.1	22.6	23.3	612.7	851.7	16.0	40.4	950.8	67.0	4.5	486.4
		Trained Group											
1	244	1152.5	186.7	29.8	18.4	846.2	308.8	44.1	66.3	502.6	52.2	49.0	281.7
48	257	1554.4	140.7	4.6	50.7	855.4	332.7	9.4	6.6	766.1	141.4	59.7	471.8
5	284	1246.3	85.4	4.6	17.8	1282.6	292.4	13.8	46.5	566.0	63.6	38.0	580.6
8	245	1143.8	122.2	.8	45.5	1440.3	274.2	19.2	39.8	554.6	292.8	76.3	1318.3
9	296	757.5	74.1	.8	11.0	1252.3	483.1	19.1	16.9	354.6	319.2	5.8	159.5
12	264	1285.5	52.4	.9	5.2	543.4	110.9	24.6	11.0	1591.6	48.4	32.6	36.4
13	309	887.7	86.4	42.9	92.8	1164.6	569.6	22.9	23.4	19.7	82.4	28.7	144.9
16	304	851.0	120.8	18.8	98.4	1180.4	311.5	33.8	55.9	469.4	147.4	20.5	63.3
17	248	1791.7	97.3	6.4	39.8	1851.2	375.3	6.0	84.0	759.4	144.4	.9	144.2
20	304	1064.3	139.4	2.4	80.0	969.4	375.3	33.5	59.8	1082.0	98.0	12.5	327.0
24	304	1327.4	88.0	.8	54.3	1299.6	56.1	58.2	54.1	315.1	394.2	51.1	146.4
25	296	958.3	83.4	34.0	8.3	950.0	62.2	16.4	32.0	2303.2	121.4	82.9	484.3
29	229	151.0	25.8	.6	144.7	941.1	343.8	23.1	87.5	296.1	45.0	34.7	501.1
32	234	405.4	174.6	.9	5.8	838.9	357.7	26.3	124.3	827.4	350.9	58.8	1235.5
36	258	720.5	61.0	.9	176.9	811.8	778.4	69.0	70.0	364.4	94.8	31.5	510.8
37	229	1074.8	219.9	20.1	93.6	588.4	570.5	28.6	10.0	252.9	269.4	61.1	158.2
44	270	1095.1	390.2	12.0	249.4	752.7	487.9	19.5	52.8	147.6	110.0	18.3	591.9
45	245	1024.4	95.8	1.8	11.3	707.7	517.6	20.5	33.4	286.8	68.8	33.6	71.5

APPENDIX E.--Test-retest and Recovery of Pyridine Nucleotides. Values are in Micro-moles per

Animal Number	First Extraction	Second Extraction	With 213.4 M added	First Extraction	Second Extraction	With 213.4 M added
		Muscle DPN			Muscle TPN	
2	807.4	811.0	1001.4	5.6	4.6	209.4
18	588.3	594.2	804.5	7.0	7.3	216.3
22	872.3	843.1	1110.4	5.5	5.7	205.8
35	1215.9	1166.5	1421.4	5.5	5.9	208.1
8	1143.8	1193.7	1328.8	.9	1.8	211.1
36	720.5	643.5	903.1	.9	1.3	203.4
27	1452.4	1328.4	1593.6	11.3	9.8	213.7
47	1491.4	1582.4	1683.8	27.7	28.5	228.6
29	151.0	143.2	357.4	.6	1.1	207.8
		Heart DPN			Heart TPN	
6	633.3	566.2		16.2	15.7	
19	1717.2		1921.1	56.1		266.7
38	1206.8	1299.7		38.0	37.0	
8	1440.3		1643.8	19.2		225.1
20	969.4	1029.2		33.5	30.1	
37	588.4		801.1	28.6		221.8
26	611.9	628.1		2.7	3.4	
35	1062.7		1261.4	25.2		227.7
16	1180.4	1091.3		33.8	34.4	
		Liver DPN			Liver TPN	
15	427.3	384.2	627.1	18.6	16.9	230.1
22	283.0	270.9	481.4	22.9	18.7	209.8
31	221.9	262.4	426.1	67.2	73.9	268.4
48	766.1	759.9	968.4	54.8	61.4	261.6
24	315.1	347.3	530.8	51.2	56.8	247.3
9	354.6	410.6	548.7	5.8	6.3	208.6
25	2303.2	2200.1	2465.0	82.9	89.3	300.1
26	481.7	434.9	701.8	17.3	19.7	221.8
9	354.6	370.5	557.6	5.8	4.9	215.3

Kilogram of Tissue.

Animal Number	First Extraction	Second Extraction	With 213.4 µ M added	First Extraction	Second Extraction	With 213.4 µ M added
		Muscle DPNH			Muscle TPNH	
14	566.6	489.5	770.1	49.6	44.9	238.1
30	172.3	200.9	371.5	18.8	17.4	201.9
42	232.7	223.6	431.8	29.2	28.1	225.3
13	86.4	80.3	296.5	92.8	93.7	287.1
29	25.8	33.3	245.6	144.7	154.2	331.6
44	390.2	351.2	607.4	249.4	193.0	447.8
6	79.3	84.1	283.4	18.2	19.8	207.1
24	88.0	93.1	301.4	54.3	51.1	251.4
9	74.1	69.4	272.4	11.0	12.9	198.8
		Heart DPNH			Heart TPNH	
14	217.7	192.0		12.4	13.3	
27	349.8		547.8	5.2		206.8
12	110.9	115.6		11.0	10.3	
24	56.1		261.1	54.1		241.1
25	62.2	67.6		32.0	37.1	
45	517.6		714.9	33.4		228.8
31.	488.6	436.4		8.5	7.8	
9	483.1		696.4	16.9		210.4
29	343.8	380.4		87.5	82.1	
		Liver DPNH			Liver TPNH	
22	766.6	846.2	908.8	285.4	254.8	472.4
23	180.4	171.4	376.7	134.3	127.6	341.7
42	340.0	297.9	523.8	1353.5	1261.9	1384.9
12	48.4	42.8	260.8	36.4	32.1	234.8
29	45.0	53.1	240.3	501.1	540.2	700.4
36	94.8	108.2	297.5	510.8	544.7	691.6
18	96.8	89.4	301.4	915.6	839.5	1170.2
27	237.2	208.7	426.1	272.8	294.5	450.3
44	110.0	117.5	313.9	591.9	650.1	774.3

APPENDIX F.--Extractions After Days Frozen at -25°C. Values are in Micro-moles per Kilogram of Tissue.

Animal Number	Days frozen at -25°C							
	2 days	4 days	14 days	28 days	2 days	4 days	14 days	28 days
	DPN				TPN			
1 M	1147.6	1152.5	1168.8	1152.5	30.1	29.8	29.8	30.1
31 M	816.4	830.1	816.4	824.2	1.7	1.7	1.7	1.7
25 M	958.3	969.4	975.3	975.3	34.9	34.1	34.1	34.1
2 H	1050.0	1031.4	1031.4	1021.2	30.2	28.9	28.9	28.9
23 H	941.1	960.0	951.4	930.0	20.9	20.9	20.9	20.9
1 H	862.3	846.2	834.2	846.2	52.0	50.0	48.8	44.1
8 L	542.2	542.2	554.6	557.2	78.0	78.0	76.3	74.4
2 L	793.3	778.4	763.2	763.2	37.2	36.4	36.4	35.5
1 L	502.6	520.1	498.4	498.4	50.6	49.1	49.1	49.1
	DPNH				TPNH			
1 M	186.7	190.3	183.4	182.1	18.4	18.9	18.9	18.9
31 M	341.1	335.2	335.2	335.2	210.4	214.4	212.9	212.9
25 M	83.4	83.4	84.8	83.4	8.3	8.3	8.3	8.3
2 H	81.1	79.6	79.6	77.4	40.7	41.2	40.0	40.0
23 H	352.1	340.1	340.1	346.2	97.0	97.1	95.8	95.8
1 H	308.8	314.2	308.8	308.8	66.3	67.2	67.2	65.0
8 L	287.2	290.4	292.8	292.8	1295.6	1308.6	1334.6	1318.3
2 L	62.5	64.7	63.4	63.4	178.0	170.0	170.0	174.5
1 L	52.2	52.2	52.2	52.2	268.8	279.4	279.4	281.7

APPENDIX G.--Assay After Sample was Stored at 2°C. Values are in Micro-moles per Kilogram

Hours sample remained at 2°C.

Animal Number	0	12	24	36	0	12	24	36
	Muscle DPN				Muscle DPNH			
31	865.3	821.2	838.4	824.2	351.3	334.2	338.8	335.2
45	1077.8	1017.3	1024.4	1011.8	99.8	94.8	95.8	93.7
39	1072.1	1025.7	1036.4	1022.5	470.1	449.2	451.8	450.6
22	913.8	870.6	881.5	872.3	63.3	60.1	60.8	60.0
12	1351.9	1292.1	1299.8	1285.5	5.5	5.2	5.3	5.2
6	651.4	622.2	618.7	635.5	83.1	80.1	79.0	79.3
29	158.2	151.0	145.0	149.1	27.1	25.8	26.0	25.5
1	1207.1	1139.4	1159.9	1152.5	191.4	185.5	186.7	186.6
24	1388.4	1312.4	1328.8	1327.4	92.1	88.0	89.2	88.3
	Heart DPN				Heart DPNH			
12	570.8	543.4	547.7	541.4	116.8	110.9	111.4	111.2
5	1349.5	1380.5	1397.4	1282.6	307.5	292.4	295.5	292.8
47	643.4	612.7	619.3	615.6	901.8	860.2	870.1	851.7
22	907.8	862.1	871.8	863.5	227.2	216.9	221.3	216.9
37	616.4	584.4	588.4	588.4	600.0	570.5	570.5	570.5
25	1007.4	958.4	950.0	950.0	65.8	62.2	63.0	62.8
11	718.4	690.4	695.0	684.0	1561.1	1480.0	1488.8	1476.4
9	1320.1	1252.3	1277.4	1252.3	510.1	475.5	483.1	480.4
45	742.0	707.7	717.7	704.8	543.1	514.1	517.6	517.6
	Liver DPN				Liver DPNH			
1	528.3	502.6	511.3	504.6	53.0	53.0	52.2	52.2
16	486.1	469.4	469.4	469.4	153.2	147.4	149.0	147.4
32	865.1	827.4	845.1	827.4	367.2	358.8	358.8	350.4
24	330.8	315.1	324.4	315.1	413.2	392.1	399.8	394.2
47	988.4	950.8	950.8	958.8	69.8	66.1	67.0	66.8
18	460.1	431.3	439.9	431.3	99.1	95.4	96.8	94.4
17	794.1	761.1	771.8	759.4	150.1	143.8	144.4	143.8
29	308.8	293.8	298.8	296.1	47.0	45.0	46.0	45.0
20	1131.6	1082.0	1082.0	1082.0	103.1	98.0	98.0	98.0

of Tissue.

0	12	24	36	0	12	24	36
Muscle TPN				Muscle TPNH			
1.9	1.8	1.7	1.7	225.3	213.6	212.9	211.5
1.9	1.8	1.8	1.8	11.7	11.3	11.5	11.4
11.9	11.3	11.4	11.3	322.2	309.8	307.5	306.5
5.8	5.5	5.6	5.5	25.6	24.4	24.8	24.7
.9	.9	1.0	.9	5.4	5.2	5.3	5.2
5.6	5.4	5.4	5.4	18.8	18.2	18.3	18.2
.6	.6	.5	.7	151.1	144.7	144.7	143.3
31.3	29.8	30.0	29.8	19.3	18.4	18.4	18.4
.8	.8	.8	.8	56.7	54.1	54.3	54.3
Heart TPN				Heart TPNH			
25.9	24.5	24.7	24.4	11.5	11.1	11.1	11.0
14.4	13.8	13.9	13.9	48.7	46.5	46.5	46.5
16.8	16.1	16.1	16.1	42.5	40.8	40.5	40.4
15.5	14.8	14.8	14.8	15.8	15.1	15.3	15.1
29.6	28.6	28.6	28.6	10.5	10.0	10.0	10.0
17.5	16.8	16.4	16.8	33.6	32.0	33.0	32.4
24.3	23.3	23.8	23.1	15.6	14.9	14.9	14.9
20.1	19.0	19.8	19.2	17.7	16.9	17.2	16.9
21.5	20.8	21.2	20.5	35.1	33.4	33.4	33.4
Liver TPN				Liver TPNH			
51.6	49.1	49.9	49.1	292.3	281.7	287.1	281.7
21.3	20.9	20.5	20.5	65.3	64.0	63.3	63.3
64.0	57.7	58.9	58.9	1291.7	1248.8	1264.8	1235.5
53.3	50.8	51.2	51.9	153.6	146.8	150.4	146.4
4.8	4.6	4.6	4.6	502.1	487.7	493.3	486.4
201.8	192.5	196.4	193.7	963.3	915.6	925.8	918.8
0.9	0.9	0.9	0.9	148.8	142.3	144.2	141.7
37.1	34.7	35.4	35.0	526.6	506.6	506.6	501.1
13.1	12.6	12.6	12.6	342.2	327.0	330.2	327.0

APPENDIX H.--Correlation Coefficients for the Trained Group.

	BODY WEIGHT	MDPN	MDPNH	MTPN	MTPNH	HDPN	HDPNH	HTPN	HTPNH	LDPN	LDPNH	LTPN	LTPNH	STATIC	ONE	TWO	THREE	FOUR	FIVE	SIX	SEVEN	EIGHT
1	1.00																					
2	0.05	1.00																				
3	-0.17	-.14	1.00																			
4	0.28	0.01	0.17	1.00																		
5	-0.02	-0.24	0.45	0.02	1.00																	
6	0.27	0.32	-0.27	-0.05	-0.18	1.00																
7	-0.19	-0.26	0.19	0.01	0.50	-0.17	1.00															
8	0.15	-0.24	-0.08	-0.05	0.28	-0.22	0.17	1.00														
9	-0.23	-0.39	0.03	-0.23	0.16	0.20	0.06	0.26	1.00													
10	0.11	0.15	-0.23	0.08	-0.45	-0.11	-0.61	-0.22	-0.09	1.00												
11	0.01	-0.03	0.10	-0.25	-0.20	0.26	-0.14	0.13	0.07	-0.14	1.00											
12	-0.30	-0.06	0.02	0.20	-0.28	-0.24	-0.38	0.04	-0.11	0.33	0.27	1.00										
13	-0.38	-0.29	0.21	-0.24	0.03	0.07	-0.04	-0.11	0.42	0.06	0.31	0.50	1.00									
14	-0.10	0.25	0.20	0.03	0.05	0.21	-0.47	-0.09	-0.02	0.22	0.08	0.27	0.29	1.00								
15	0.64	0.03	0.00	0.14	-0.08	0.28	-0.24	0.15	-0.06	0.07	0.19	-0.34	-0.19	0.42	1.00							
16	0.57	-0.16	-0.10	0.30	-0.13	-0.01	-0.01	0.45	-0.21	-0.05	0.27	-0.12	-0.34	0.03	0.73	1.00						
17	0.37	-0.24	-0.05	0.26	-0.08	-0.04	0.16	0.54	-0.03	-0.15	0.34	-0.05	-0.25	-0.13	0.49	0.91	1.00					
18	0.27	-0.17	0.08	0.14	0.02	-0.12	0.27	0.64	0.14	-0.17	0.25	-0.16	-0.25	-0.15	0.40	0.75	0.90	1.00				
19	0.27	-0.04	0.19	0.16	0.05	-0.13	0.19	0.61	0.07	-0.18	0.15	-0.26	-0.37	-0.07	0.47	0.78	0.85	0.93	1.00			
20	0.09	0.04	0.26	0.10	-0.02	-0.17	0.17	0.48	0.01	-0.11	0.21	-0.15	-0.29	0.02	0.38	0.69	0.80	0.90	0.95	1.00		
21	-0.03	0.00	0.22	0.01	0.04	-0.26	0.33	0.54	0.07	-0.21	0.12	-0.18	-0.30	-0.11	0.19	0.56	0.71	0.87	0.90	0.95	1.00	
22	0.02	0.03	0.11	0.14	0.00	-0.20	0.17	0.62	0.06	-0.10	0.05	-0.07	-0.34	0.02	0.26	0.62	0.75	0.87	0.92	0.94	0.93	1.00
23	0.12	-0.06	-0.00	0.08	0.01	-0.25	0.14	0.71	0.02	-0.10	0.05	-0.08	-0.37	-0.02	0.29	0.69	0.79	0.88	0.90	0.90	0.92	0.96
24	0.20	-0.12	-0.10	0.17	-0.05	-0.26	0.19	0.55	-0.11	-0.17	0.02	-0.09	-0.43	-0.13	0.28	0.73	0.80	0.83	0.82	0.83	0.87	0.86
25	-0.49	0.20	0.23	-0.08	0.04	0.09	-0.32	-0.16	0.07	0.13	0.04	-0.35	0.42	0.90	0.10	-0.19	-0.25	-0.24	-0.18	-0.02	-0.09	0.01
26	0.01	0.02	0.14	-0.03	-0.10	0.15	-0.18	0.07	0.14	0.02	0.20	-0.17	0.09	0.65	0.76	0.45	0.30	0.28	0.37	0.40	0.26	0.31
27	0.20	0.55	-0.57	-0.20	-0.40	0.24	-0.36	-0.06	-0.32	0.38	-0.18	-0.16	-0.40	0.01	0.09	-0.11	-0.31	-0.35	-0.27	-0.31	-0.30	-0.22
28	0.01	0.97	0.34	0.04	-0.13	0.25	-0.21	-0.24	-0.37	0.10	-0.00	-0.06	-0.22	0.28	0.03	-0.17	-0.24	-0.14	-0.00	0.10	0.05	0.06
29	0.02	-0.23	0.48	0.21	0.98	-0.18	0.49	0.26	0.11	-0.43	-0.25	-0.23	-0.01	0.05	-0.05	-0.07	-0.02	0.05	0.08	-0.00	0.04	0.02
30	0.16	0.58	-0.41	-0.14	-0.61	0.11	-0.51	-0.28	-0.42	0.53	-0.14	-0.04	-0.34	0.06	0.12	-0.09	-0.34	-0.40	-0.27	-0.26	-0.29	-0.24
31	-0.08	0.16	-0.25	-0.33	-0.55	-0.26	-0.33	-0.20	-0.20	0.40	-0.05	-0.04	-0.12	-0.10	0.06	-0.09	-0.28	-0.30	-0.20	-0.18	-0.15	-0.23
32	0.02	0.94	0.42	0.08	0.02	0.22	-0.12	-0.20	-0.35	0.02	-0.05	-0.10	-0.22	0.29	0.02	-0.18	-0.24	-0.13	0.01	0.10	0.05	0.06
33	0.41	0.23	-0.23	0.03	-0.24	0.29	-0.71	0.23	-0.03	0.35	0.39	0.36	-0.08	0.31	0.26	0.17	0.08	-0.02	-0.02	-0.08	-0.22	-0.02
34	0.42	0.28	-0.28	0.06	-0.33	0.32	-0.79	0.12	-0.09	0.47	0.33	0.38	-0.06	0.37	0.28	0.15	0.01	-0.11	-0.10	-0.14	-0.30	-0.09
35	-0.05	0.36	-0.04	0.03	-0.18	0.10	0.10	-0.51	-0.65	-0.06	0.08	0.02	-0.09	0.15	0.02	-0.02	-0.05	-0.14	-0.23	-0.08	-0.13	-0.18
36	0.14	0.11	-0.15	-0.06	0.11	0.84	0.37	-0.04	0.32	-0.44	0.18	-0.43	0.07	-0.05	0.14	0.00	0.06	0.07	0.00	-0.04	-0.03	-0.06
37	0.05	0.16	-0.29	0.00	-0.35	-0.32	-0.53	-0.11	-0.21	0.79	-0.46	0.09	-0.19	0.09	0.03	-0.08	-0.27	-0.29	-0.16	-0.17	-0.20	-0.10
38	0.03	0.23	-0.26	-0.11	-0.31	-0.32	-0.42	-0.08	-0.30	0.57	-0.30	-0.06	-0.31	0.02	0.09	-0.02	-0.24	-0.27	-0.12	-0.13	-0.11	-0.11
39	0.12	0.27	-0.28	-0.14	-0.32	-0.18	-0.42	-0.06	-0.30	0.49	-0.18	-0.18	-0.43	0.01	0.20	0.08	-0.14	-0.17	-0.02	-0.03	-0.02	-0.04
40	-0.11	-0.03	-0.05	-0.09	-0.38	-0.01	-0.53	-0.20	0.15	0.79	0.21	0.60	0.63	0.35	-0.02	-0.17	-0.19	-0.23	-0.32	-0.21	-0.30	-0.25
41	-0.05	-0.05	0.27	0.13	0.04	-0.25	0.27	0.58	0.13	-0.22	0.13	-0.12	-0.26	-0.05	0.25	0.60	0.74	0.87	0.93	0.94	0.93	0.94
42	0.34	-0.08	0.05	0.18	-0.03	-0.09	0.13	0.59	-0.01	-0.11	0.21	-0.18	-0.35	0.00	0.57	0.88	0.92	0.94	0.96	0.93	0.86	0.89
43	-0.15	-0.10	0.17	0.10	-0.03	-0.26	0.24	0.54	0.08	-0.17	0.20	-0.00	-0.17	0.04	0.25	0.63	0.77	0.85	0.87	0.93	0.92	0.93
44	0.60	-0.12	-0.05	0.25	-0.11	0.10	-0.04	0.41	-0.11	-0.04	0.29	-0.20	-0.29	0.13	0.84	0.97	0.87	0.75	0.77	0.68	0.53	0.59
45	0.15	-0.05	0.11	0.12	0.01	-0.20	0.22	0.62	0.04	-0.14	0.13	-0.15	-0.35	-0.07	0.35	0.72	0.84	0.94	0.96	0.97	0.96	0.96
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22

NINE	TEN	STATIC/BODY WEIGHT	ONE/BODY WEIGHT	MDPN/MDPNH	TOTAL MDPN	TOTAL MTPN	M-OXID/REDUCED	TOTAL MDPN/MTPN	TOTAL M-PYR. NUC.	HDPN/HDPNH	H-OXID/REDUCED	TOTAL HDPN/HTPN	TOTAL H-PYR. NUC.	LDPN/LDPNH	L-OXID/REDUCED	TOTAL LDPN/LTPN	TOTAL L-PYR. NUC.	FIVE/BODY WT.	TOTAL WORK	TOTAL WK./BD. WT.	FIRST THREE WORK	LAST SEVEN WORK
1.00																						
0.93	1.00																					
-0.07	-0.20	1.00																				
0.27	0.18	0.57	1.00																			
-0.17	-0.15	-0.07	-0.03	1.00																		
-0.06	-0.14	0.24	0.04	0.41	1.00																	
0.03	-0.02	0.03	-0.10	-0.43	-0.12	1.00																
-0.22	-0.18	-0.02	0.04	0.91	0.46	-0.62	1.00															
-0.17	-0.10	-0.07	0.16	0.60	0.09	-0.60	0.77	1.00														
-0.05	-0.14	0.25	0.03	0.33	0.98	0.04	0.36	-0.00	1.00													
0.01	-0.09	0.07	0.01	0.34	0.17	-0.24	0.30	-0.01	0.13	1.00												
-0.05	-0.13	0.12	0.03	0.42	0.20	-0.30	0.41	0.05	0.15	0.96	1.00											
-0.20	-0.08	0.19	0.05	0.12	0.34	-0.17	0.16	-0.01	0.31	-0.17	-0.13	1.00										
-0.12	-0.13	-0.07	0.06	0.00	0.07	0.10	-0.20	-0.44	0.09	-0.09	-0.10	0.07	1.00									
-0.06	-0.04	0.05	0.02	0.59	0.09	-0.32	0.71	0.68	0.04	0.14	0.26	-0.16	-0.60	1.00								
-0.03	0.03	-0.00	0.10	0.68	0.16	-0.32	0.77	0.84	0.10	0.06	0.16	-0.10	-0.55	0.89	1.00							
0.04	0.13	-0.05	0.15	0.70	0.19	-0.34	0.77	0.80	0.14	0.11	0.20	-0.08	-0.42	0.78	0.96	1.00						
-0.27	-0.28	0.34	0.09	0.05	-0.03	-0.38	0.20	0.23	-0.10	0.30	0.40	-0.08	-0.28	0.44	0.23	0.12	1.00					
0.88	0.77	-0.03	0.36	-0.35	0.00	0.07	-0.32	-0.18	0.02	-0.16	-0.24	-0.23	-0.05	-0.18	-0.14	-0.07	-0.29	1.00				
0.91	0.88	-0.13	0.44	-0.23	-0.06	0.00	-0.23	-0.18	-0.07	0.03	-0.02	-0.11	0.00	-0.15	-0.10	0.00	-0.24	0.87	1.00			
0.89	0.82	0.10	0.44	-0.35	-0.06	-0.01	-0.32	-0.15	-0.06	-0.17	-0.23	-0.09	-0.08	-0.18	-0.13	-0.07	-0.19	0.95	0.87	1.00		
0.64	0.66	-0.11	0.58	-0.11	-0.13	-0.05	-0.10	-0.10	-0.14	0.20	0.17	-0.01	0.08	-0.11	-0.05	0.06	-0.14	0.58	0.87	0.60	1.00	
0.97	0.91	-0.12	0.31	-0.27	-0.02	0.03	-0.28	-0.20	-0.01	-0.06	-0.13	-0.16	-0.04	-0.16	-0.12	-0.02	-0.28	0.94	0.95	0.92	0.70	1.00
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45

Correlation Coefficients for the Sedentary Group

	BODY WEIGHT	MDPN	MDPNH	MTPN	MTPNH	HDPN	HDPNH	HTPN	HTPNH	LDPN	LDPNH	LTPN	LTPNH	STATIC	ONE	TWO	THREE	FOUR	FIVE	SIX	SEVEN	EIGHT
1	1.00																					
2	-0.66	1.00																				
3	0.08	0.08	1.00																			
4	-0.52	0.50	0.02	1.00																		
5	-0.09	-0.10	0.75	-0.09	1.00																	
6	-0.47	0.25	0.12	-0.00	0.14	1.00																
7	-0.14	0.32	0.29	0.22	0.34	-0.16	1.00															
8	-0.64	0.22	-0.03	0.17	0.24	0.81	0.07	1.00														
9	-0.17	0.22	-0.08	-0.00	-0.05	0.26	0.12	0.49	1.00													
10	0.23	-0.15	-0.16	0.33	-0.25	-0.18	-0.16	-0.08	0.10	1.00												
11	-0.14	0.07	-0.16	0.05	-0.08	0.05	-0.15	-0.05	-0.24	-0.25	1.00											
12	0.00	-0.19	0.51	-0.10	0.72	-0.07	-0.02	0.11	0.14	-0.25	-0.07	1.00										
13	-0.05	0.01	0.09	0.11	-0.02	0.00	-0.23	-0.04	-0.00	-0.19	-0.15	0.33	1.00									
14	-0.11	0.15	-0.03	0.02	-0.10	-0.12	0.09	-0.26	-0.43	-0.13	0.21	-0.34	-0.42	1.00								
15	0.37	-0.19	-0.00	-0.42	0.03	-0.32	-0.11	-0.48	-0.16	-0.17	-0.05	0.04	-0.17	0.06	1.00							
16	0.37	-0.06	-0.11	-0.26	0.04	-0.47	0.20	-0.49	-0.22	0.17	0.02	-0.14	-0.40	0.11	0.62	1.00						
17	0.43	-0.07	0.14	-0.29	0.19	-0.35	0.15	-0.46	-0.10	0.17	0.09	0.00	-0.14	-0.09	0.46	0.85	1.00					
18	0.54	-0.46	0.19	-0.36	0.25	-0.35	0.11	-0.43	-0.13	0.15	0.04	0.13	0.05	-0.16	0.42	0.60	0.79	1.00				
19	0.55	-0.52	0.24	-0.35	0.22	-0.29	0.14	-0.40	-0.11	0.16	0.03	0.06	0.00	-0.10	0.38	0.44	0.63	0.95	1.00			
20	0.31	-0.36	0.19	-0.25	0.27	-0.21	0.27	-0.20	-0.06	0.15	-0.04	0.04	-0.13	-0.16	0.42	0.48	0.60	0.84	0.88	1.00		
21	0.37	-0.37	0.31	-0.26	0.36	-0.21	0.24	-0.27	-0.11	0.10	-0.00	0.17	-0.03	-0.18	0.44	0.48	0.64	0.90	0.92	0.96	1.00	
22	0.25	-0.27	0.25	-0.21	0.36	-0.12	0.30	-0.12	0.05	0.11	-0.01	0.18	-0.03	-0.23	0.40	0.46	0.63	0.87	0.87	0.94	0.97	1.00
23	0.14	-0.16	0.21	-0.16	0.37	-0.06	0.36	-0.01	0.13	0.13	-0.09	0.13	0.00	-0.23	0.33	0.44	0.57	0.79	0.78	0.86	0.90	0.96
24	0.02	-0.07	0.04	-0.14	0.21	0.00	0.34	0.09	0.24	0.23	-0.19	0.03	-0.05	-0.13	0.21	0.36	0.43	0.63	0.64	0.77	0.76	0.86
25	-0.40	0.32	-0.03	0.18	-0.05	0.02	0.12	-0.05	-0.37	-0.18	0.23	-0.31	-0.37	0.95	-0.04	-0.00	-0.22	-0.31	-0.26	-0.24	-0.28	-0.29
26	-0.34	0.38	-0.07	-0.05	0.10	0.00	-0.01	-0.02	-0.04	-0.34	0.04	0.05	-0.13	0.12	0.74	0.36	0.15	0.01	-0.04	0.19	0.15	0.20
27	-0.41	0.15	-0.60	0.10	-0.26	0.26	-0.03	0.53	0.51	-0.17	0.13	-0.03	0.19	-0.27	-0.12	-0.22	-0.24	-0.23	-0.27	-0.13	-0.20	-0.05
28	09.48	0.84	0.61	0.41	0.32	0.27	0.42	0.16	0.13	-0.21	-0.03	0.12	0.05	0.10	-0.15	-0.11	0.01	-0.26	-0.28	-0.18	-0.12	-0.07
29	-0.11	-0.08	0.75	-0.04	0.99	0.14	0.35	0.25	-0.05	-0.24	-0.08	0.72	-0.02	-0.10	0.01	0.03	0.17	0.23	0.20	0.26	0.35	0.36
30	-0.22	0.11	-0.75	0.14	-0.55	0.01	-0.22	0.20	0.30	-0.02	0.42	-0.21	0.12	-0.12	-0.22	-0.15	-0.16	-0.24	-0.30	-0.26	-0.32	-0.23
31	0.16	0.23	-0.20	0.04	-0.65	-0.10	-0.21	-0.26	0.30	0.15	0.13	-0.33	-0.04	-0.05	-0.15	-0.12	-0.06	-0.17	-0.14	-0.27	-0.27	-0.30
32	-0.45	0.70	0.74	0.34	0.56	0.27	0.46	0.21	0.09	-0.25	-0.05	0.31	0.04	0.05	-0.13	-0.09	0.06	-0.16	-0.19	-0.08	-0.00	0.03
33	-0.06	-0.14	-0.06	-0.22	-0.07	0.55	-0.58	0.28	-0.10	0.13	-0.00	-0.07	-0.00	0.15	-0.10	-0.34	-0.36	-0.25	-0.19	-0.24	-0.20	-0.19
34	-0.06	-0.13	-0.01	-0.23	-0.05	0.62	-0.59	0.29	-0.20	0.06	0.04	-0.08	0.05	0.09	-0.11	-0.32	-0.32	-0.21	-0.17	-0.22	-0.16	-0.17
35	0.26	-0.13	0.15	-0.25	0.05	-0.13	0.03	-0.49	-0.72	-0.38	0.03	-0.20	0.12	0.31	0.50	0.33	0.24	0.29	0.28	0.17	0.25	0.15
36	-0.52	0.43	0.27	0.12	0.32	0.81	0.43	0.80	0.37	-0.24	-0.06	-0.06	-0.13	-0.08	-0.37	-0.31	-0.23	-0.25	-0.19	-0.03	-0.06	0.06
37	0.37	-0.11	-0.15	0.11	-0.25	-0.18	-0.09	-0.15	0.18	0.85	-0.48	-0.27	-0.02	-0.17	-0.05	0.24	0.27	0.18	0.14	0.06	0.04	0.08
38	0.07	-0.19	-0.19	0.09	-0.18	0.00	-0.11	0.14	0.19	0.72	-0.25	-0.27	-0.57	0.18	-0.09	-0.01	-0.20	-0.16	-0.04	0.02	-0.09	-0.07
39	0.11	-0.26	-0.22	0.00	-0.19	0.13	-0.10	-.16	-0.02	0.34	0.23	-0.34	-0.69	0.23	-0.11	-0.02	-0.23	-0.19	-0.06	-0.04	-0.15	-0.18
40	0.03	-0.07	-0.02	0.32	-0.13	-0.09	-0.38	-0.10	-0.02	0.27	0.09	0.23	0.80	-0.43	-0.38	-0.28	0.00	0.17	0.12	-0.04	0.05	0.05
41	0.41	-0.44	0.24	-0.26	0.24	-0.23	0.19	-0.32	-0.09	0.16	0.06	0.04	-0.00	-0.07	0.31	0.40	0.61	0.93	0.98	0.90	0.93	0.89
42	0.41	-0.30	-0.17	-0.32	0.27	-0.29	0.24	-0.34	-0.06	0.15	-0.02	0.08	-0.11	-0.12	0.56	0.69	0.80	0.93	0.89	0.92	0.94	0.95
43	0.13	-0.12	0.15	-0.18	0.31	-0.17	0.31	-0.17	-0.03	0.11	0.02	0.06	-0.13	-0.09	0.47	0.65	0.74	0.83	0.79	0.91	0.91	0.94
44	0.44	-0.12	0.00	-0.36	0.10	-0.44	0.10	-0.54	-0.18	0.08	0.03	-0.04	-0.28	0.03	0.77	0.95	0.89	0.70	0.55	0.57	0.59	0.58
45	0.34	-0.34	0.22	-0.26	0.31	-0.19	0.27	-0.21	-0.00	0.16	-0.04	0.12	-0.03	-0.18	0.40	0.50	0.66	0.91	0.92	0.96	0.98	0.98
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22

NINE	TEN	STATIC/BODY WEIGHT	ONE/BODY WEIGHT	MDPN/MDPNH	TOTAL MDPN	TOTAL MTPN	M-OXID/REDUCED	TOTAL MDPN/MTPN	TOTAL M-PYR. NUC.	HDPN/HDPNH	H-OXID/REDUCED	TOTAL HDPN/HTPN	TOTAL H-PYR. NUC.	LDPN/LDPNH	L-OXID/REDUCED	TOTAL LDPN/LTPN	TOTAL L-PYR. NUC.	FIVE/BODY WT.	TOTAL WORK	TOTAL WK./BL. WT.	FIRST THREE WORK	LAST SEVEN WORK
1.00																						
0.94	1.00																					
-0.26	-0.13	1.00																				
0.21	0.18	0.22	1.00																			
0.01	0.12	-0.15	0.18	1.00																		
-0.01	-0.03	0.23	0.18	-0.20	1.00																	
0.36	0.20	-0.04	0.09	-0.25	0.34	1.00																
-0.21	-0.11	-0.07	-0.05	0.83	-0.32	-0.54	1.00															
-0.36	-0.30	-0.11	-0.27	-0.09	0.07	-0.65	0.24	1.00														
0.09	0.02	0.19	0.18	-0.25	0.96	0.58	-0.43	-0.11	1.00													
-0.11	0.02	0.18	-0.07	-0.06	-0.14	-0.09	-0.07	-0.09	-0.15	1.00												
-0.11	-0.02	0.13	-0.07	-0.09	-0.11	-0.06	-0.09	-0.09	-0.11	0.97	1.00											
0.10	-0.02	0.22	0.29	-0.41	-0.02	0.04	-0.42	-0.27	-0.00	0.05	0.16	1.00										
0.16	0.21	0.07	-0.01	0.25	0.48	0.33	-0.09	-0.20	0.51	0.15	0.20	-0.15	1.00									
0.15	0.25	-0.26	-0.33	-0.12	-0.17	-0.25	-0.05	0.08	-0.22	0.15	0.07	-0.20	-0.21	1.00								
-0.04	0.14	0.14	-0.15	-0.15	-0.25	-0.18	-0.12	0.07	-0.27	0.34	0.19	-0.44	-0.04	0.53	1.00							
-0.25	-0.19	0.17	-0.19	-0.13	-0.32	-0.19	-0.02	0.14	-0.34	0.16	0.12	-0.25	0.06	0.03	0.70	1.00						
0.07	0.01	-0.40	-0.30	0.13	-0.07	-0.12	0.25	0.06	-0.09	0.06	0.09	-0.11	-0.30	0.26	-0.24	-0.40	1.00					
0.81	0.70	-0.19	-0.01	-0.24	-0.21	0.22	-0.29	-0.16	-0.12	-0.19	-0.17	0.24	-0.10	0.09	-0.03	-0.06	0.12	1.00				
0.89	0.78	-0.24	0.25	-0.16	-0.15	0.26	-0.26	-0.25	-0.05	-0.24	-0.22	0.27	-0.13	0.16	-0.06	-0.17	-0.01	0.99	1.00			
0.92	0.84	-0.13	0.37	-0.07	-0.01	0.31	-0.22	-0.31	0.07	-0.24	-0.23	0.20	0.02	0.07	-0.07	-0.18	-0.04	0.83	0.95	1.00		
0.52	0.39	-0.10	0.45	-0.22	-0.09	0.08	-0.20	-0.12	-0.05	-0.32	-0.29	0.40	-0.34	0.18	-0.11	-0.14	-0.21	0.51	0.78	0.71	1.00	
0.94	0.85	-0.27	0.14	-0.12	-0.15	0.30	-0.26	-0.27	-0.04	-0.18	-0.17	0.19	-0.02	0.13	-0.03	--.16	0.06	0.94	0.96	0.93	0.60	1.00
23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45