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The influence of diet on growth rate, thyroid hormone output, and thermoregulation in laboratory white rats

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The Influence of Diet on Growth Rate, Thyroid Hormone Output, and Thermoregulation in Laboratory White Rats

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ABSTRACT

The physiological effects of the consumption of diets varying in protein and calorie content was assessed using young male Sprague-Dawley rats during their growth phase for an 8 week period at room temperature. They were then subjected to short term heat (35°C) and cold (5°C) stress to determine the effects of both diet and temperature stress on physiological function. Various quantitative and physiological parameters were measured including food intake (FI), water intake (WI), body weight (BW), rectal temperature (Tre), oxygen consumption (VO_2), hematocrit (Hct), Hemoglobin (Hb), plasma protein concentration (PP), and plasma levels of thyroxine (T_4), triiodothyronine (T_3), free thyroxine index (FT_4), and thyroid stimulating hormone (TSH). Food intake expressed per g BW was highest in the LP (5% protein) diet group, but body weight gain was the lower in the LP fed group compared to the Control group (22.5% protein). Decreases in dietary protein content were associated with of increased Hct, Hb, T_4, T_3, and heart weight along with decreased liver weight, plasma protein content, and gWI/gFI. The HP (45% protein) dietary group experienced an increased metabolic rate (MR), and gWI/gFI, and decreased T_3 when compared to Controls. Some of the trends noted during cold stress were: 1. as protein intake increased there was an increase in the loss of BW and kidney weight and 2. as protein intake decreased there was a increase in Hb, Hct, and T_3. Heat stress increased Tre with increasing protein content, and decreased FI/gBW, T_3, T_4, FT_4, and MR. Temperature stress had no effect upon

BW in the LP group. The low calorie (LC) diet produced a decreased liver weight and PP, and a faster rise in Tre under cold stress along with a lower overall Tre during heat stress. These results suggest that the percentage of protein and calories in the diet have a significant influence upon thermogenesis and many other physiological parameters.

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TABLE OF CONTENTS

TABLE OF FIGURES

Page

INTRODUCTION

Mammals belong to a group of animals known as endotherms, which have a relatively high level of metabolic heat production, enabling them to maintain a constant temperature (Tb) despite ambient temperature (Ta) variations. Thermoregulation is the general term used to identify this ability to achieve a stable Tb in the light of extreme Ta changes. There are several factors which influence the metabolic rate including body size, age, time of day, Ta, food intake and thermal insulation (Stanier et. al., 1984). The type and amount of food consumed has been shown to affect the metabolic rate of homeotherms by affecting their rate of oxygen consumption necessary for breakdown of food and ultimately heat production (Cossins et al., 1987).

There has been much research concerning how environment and nutrition either independently or synergistically affect growth rate and various physiological functions of many different animals. Dietary extremes including total protein, carbohydrate, and caloric intake are shown to be extremely detrimental to developing organisms. For example, protein deprivation has been known to impede development, impair physiological functions, affect energy balance and retard the ability of animals to thermoregulate (Balmagiya et al., 1983). On the other hand, excessive protein intake was associated with a reduction of peripheral sympathetic nervous system activity (Johnston et al., 1987). Also, large amounts of dietary

protein have been shown to increase the amount of total body fat (Donald et al., 1981) and place a demand on the body to dispose of the excess nitrogen caused by the breakdown of dietary protein (Ochs et al., 1979).

High carbohydrate diets are sometimes associated with low protein diets in order to comprise a caloric value equal to the control diet, which for rats is approximately 18-25% protein. Animals fed this type of diet tend to display a relative hyperphagia, decreased efficiency of energy utilization and increased rate of oxygen consumption which appears to be partially compensating for an inadequate protein supply in the diet. These animals overeat and then waste the excess ingested energy through increased heat production (Young et al., 1980).

There has been recent evidence that thyroid function and metabolism are directly correlated with energy intake, specifically that of carbohydrate intake (Eales, 1988). Similarly, thyroid function is known to influence physiological adjustments to stressful environments permitting an endotherm to utilize nonshivering-thermogenesis when exposed to cold, or to reduce heat production when under heat stress (Bernal et al., 1975; Rousset et al., 1975 and 1978). Few studies have studied the interelationships between energy intake, thyroid function and thermoregulation in cold and heat (Balmagiya et al., 1983; Yousef et al., 1970).

The purpose of this study is to examine the effects of diet on metabolic rate and thyroid function along with other physiological parameters and on the ability of mammals to thermoregulate under extreme thermal stresses.

More specifically the objectives are:

1. How does nutrition (level of protein and caloric intake) influence growth rate, metabolic rate and thyroid function?
2. How does nutritional status influence the ability of animals to tolerate environmental extremes in temperature?
3. What is the basic role of the thyroid gland with respect to some of these functions?

LITERATURE REVIEW

I. DIETARY EFFECTS ON GROWTH RATE AND ASSOCIATED PHYSIOLOGICAL FUNCTIONS.

A. BODY WEIGHT (BW)

1. Low Protein Diet (LP)

Several studies reported that rats fed LP (4.5-8% casein) diets for 6-12 weeks gained weight at a much slower rate than their controls (22-26% casein) (Balmagiya et al., 1983; Cox et al., 1984; Tulp et al., 1984; Villalon et al., 1987; Young et al., 1980).

2. High Protein Diet (HP)

Schreiber et al. (1955) reported that weight gains in 50% protein diets were inferior to gains observed in groups fed 25% protein. In 3, 6, 7.5, or 8 week studies it was found that there were no significant differences in weight gain when HP (45-48% casein) fed rats were compared to their controls (22-24% casein) (Harstook et al., 1963 and 1973; Hegstead et al., 1970; Lushough et al., 1960).

3. Low Calorie Diet (LC)

Various studies have reported decreased BW gain in calorically restricted animals (Johnson et al., 1966; Kibler et al., 1966 and 1967).

B. FOOD INTAKE (FI)

1. LP

Low protein groups consumed less food per rat than control groups when measured directly, but when FI was expressed in g/100g BW, LP rats actually consumed more food during an eight week period (Donald et al., 1981). Similar results were obtained in four or seven week experiments (Glass et al., 1978; Young et al., 1980).

2. HP

In 1973, Harstook et al. found no significant difference in FI between HP and control groups when animals were fed the diets for 7.5 weeks. This finding was confirmed in a 2.5 week study of female rats as well as a 4 day analysis of female mice, where HP (40% casein) fed rodents consumed about the same amount of food as their controls (20% casein) (Johnson et al., 1987; Vander Tuig et al., 1984).

3. LC

The LC diet intake determination was based upon either a percentage (70%) of what control animals consumed at room temperature (Khan et al, 1979) or based on half the ration (pair fed) of food eaten by an LP group (Glass et al., 1978), or the animals were given an allotment equal to a normal protein group which was kept at a higher ambient temperature (34°C) (Johnson et al., 1966). In each instance, the FI was always the amount given. In other words, the animals completely finished their ration of food each time.

C. WATER INTAKE (WI)

1. LP

LP groups had the lower water intake when compared to control animals, and the volume of WI depended on the type of carbohydrate (CHO) ingested. If the CHO was a simple one such as sucrose then the WI decreased along with BW. On the other hand, when the CHO was a complex CHO the WI was higher than with sucrose. When WI was expressed as g water/g BW gain per day, the amount of WI was actually the same for both types of CHO's, and higher than WI of controls (Schreiber et al., 1955). Contrary to these results, during a 28 day study of rats fed an LP (8% casein) high starch diet, the LP diet group drank less water (ml/ kg BW)

than controls (DeCastro et al., 1968).

2. HP

Animals fed HP diets (50% casein) showed increased WI (ml/day/dm^2 B.S.A) when compared to controls (25% casein) in a three week study (Schreiber et al., 1955).

3. LC

In a 12 week study completed by Quimby (1948) rats fed only 30% of the FI of their control group, had a lower total WI per animal, but when WI was expressed as a food:water ratio, WI was actually higher than controls.

D. ORGAN WEIGHTS

When expressed as a ratio of BW, kidney and liver weights were either significantly higher or lower than controls after a three week dietary period depending on the CHO type (Schreiber et al., 1955). When sucrose was the main CHO, average liver weight was greater than that of the control and it was 150%

greater than if the diet contained solely dextrin. If dextrin was the CHO used, then the liver weight was only 75% that of the control. In a different study conducted for three weeks, liver weight was lower in LP animals when compared to controls (Tyzibir et al., 1981). Kidney weight showed the same trend as liver weight, where it was higher with a sucrose diet and lower when the diet contained dextrin as the sole CHO. However, both LP/CHO diet animals had kidney weights which were lower than controls (Schreiber et al., 1955).

Heart weights expressed as g/100 g BW were not different in LP groups (Young et al., 1985). Additionally, Vander Tuig et al. (1984) reported a significant difference with heart weights after a 2.5 week LP diet, however when expressed per 100 g BW there was no difference.

2. HP

In a three week study, Schreiber and others (1955) found that kidney weights of weanling Sprague-Dawley rats fed HP (50% casein) diets were higher and liver weights were lower than controls (25% casein diet) when expressed as a ratio of organ to BW. Leathem et al. (1947) found that both liver and kidney weights increased in the HP (78% casein) diet group compared to controls (22.8% casein) in a 20 day study of adult male rats. However, no significant differences were found in kidney or heart weights after a four day diet study when compared to controls in weanling mice (Johnson et al., 1987). Moreover, there was a significant increase in

the liver weight of the HP (39% casein) diet group and controls (20% casein) but no difference was observed in the heart weights of rats (Vander Tuig et al., 1984) in a 2.5 week study. Contrasting this study, another three week experiment showed no significant difference between liver weights of male weanling Sprague-Dawley rats eating a HP (45% casein) diet and the controls (22% casein) (Tyzibir et al., 1981).

3. LC

LC diet rats (30% restricted) for a 31 day time period showed significantly decreased liver weight (Khan et al., 1979). Also, kidney and heart weights in restricted calorie groups showed no significant differences when compared to controls in a study by Johnson et al. (1987).

E. HEMATOLOGICAL CHANGES

1. LP

After feeding LP diet for 4 weeks, plasma proteins (PP) were significantly lower than controls (Villalon et al., 1987) however at eight and twelve weeks the decrease was insignificant. Moreover, in two other studies (Hishoka et al., 1974; Sagawa et al., 1978) PP and hemoglobin (Hb) were significantly lower than controls,

but there were no differences in hematocrit (Hct) in either study.

2. HP

In a HP (78% casein) dietary study for 20-27 days by Leathem et al. (1947), no difference in total PP or Hct of adult male rats was found in comparison to controls.

F. METABOLIC RATE (MR)

The MR is the sum of all the chemical reactions that occur in the body (Stanier et al., 1984). Most of these chemical reactions are heat liberating, or exothermic, and depend upon oxygen to fuel the reactions. Therefore a measurement of the rate of oxygen consumption ($\dot{V}O_2$) of an individual can be used as an indirect method to estimate the metabolic rate (Stanier, 1984).

1. LP

Young et al. (1980) reported an increased $\dot{V}O_2$ in LP fed animals as compared to controls over a 7 week period. An additional study concluded that preprandial $\dot{V}O_2$ is similar for rats fed low protein and control diets (9-10 days) and post-prandial levels were 10-13% higher for LP fed rats compared to controls

indicating thermic response to food is greater in LP/high CHO diets (Rothwell et al., 1987). In another experiment, decreased $\dot{V}O_2$ during the first week on an LP diet was observed, but a significant increase occurred after three weeks. During the weeks 2, 4, and 5 there was no difference in $\dot{V}O_2$ (Balmagiya et al., 1983).

Tulp and Krupp (1984) found that resting $\dot{V}O_2$ was higher than the controls after eight weeks of an 8% protein diet.

2. HP

Laboratory rats on HP diets ranging from 41-57% protein, for 6 or 7.5 weeks showed a significant increase in $\dot{V}O_2$ compared to animals on a control diet (Harstook et al., 1963 and 1973). Liver mitochondrial $\dot{V}O_2$ of HP animals were not different than that of the controls (Tyzibir et al., 1981). Burse et al. (1977) found that humans that had consumed an HP diet had a significantly increased resting MR (RMR).

3. LC

In one study, rats which were fed approximately 30% less food than controls for 480 days had a significantly lower $\dot{V}O_2$ than control animals. However, when the $\dot{V}O_2$ values were expressed per gram BW there was no significant difference (Johnson et al., 1964). On the contrary, Khan and Bender (1979) reported a

significant decrease in $\dot{V}O_2$ at 20 and 31 days when diet was both restricted in amount (30% less FI than controls) and protein content (5% casein).

G. THYROID FUNCTION

1. LP

When fed an LP diet for two weeks, serum triiodothyronine (T_3) was markedly higher in comparison to control rats (Young et al., 1982). However, there was no difference for serum thyroxine (T_4) or free thyroxine (FT_4) between both groups (Young et al., 1982). In yet another study for seven weeks, Young et al. (1980) found that T_3 and T_4 were significantly higher in LP diet animals than their controls. Glass et al. (1978) determined from a four week dietary study that T_3 increased in LP rats but T_4 levels showed no change. Rats fed an LP diet for 32 days showed increased T_3 levels but TSH and T_4 were similar to controls (Tulp et al., 1979).

2. HP

No significant difference in serum T_3 was found in response to feeding an HP (45% casein) diet when compared to control (22% casein) weanling Sprague-Dawley rats (Tyzibir et al., 1981). A group of weanling male Wistar rats fed a hypocaloric

(50% restricted) HP (36% casein) diet had lower serum T_3 levels than the restricted controls (18% casein). The plasma T_4 levels in this study showed no difference in either group (Glass et al., 1978).

3. LC

T_4 levels decreased significantly at sixty days post-LC feeding and remained at these low concentrations at ages 110 and 220 days (Yousef et al., 1968). Plasma T_3 levels were higher and T_4 and TSH levels remained at the normal range after 32 days of 50% calorie restriction in weanling Sprague-Dawley rats (Tulp et al., 1979). Contrasting these studies, it was determined that when caloric restrictions were 17, 22, and 48 percent of control FI, there were 8, 13, and 28 percent respective decreases in T_4 secretion rates (Turner, 1969). Another 28 day study concluded that serum T_3 and T_4 levels were no different between the LC (50% calorie restricted) weanling male Wistar rats and their controls (Glass et al., 1978). Also, Johnson et al. (1966) determined that I^{131} release rates were unaffected by LC (25% less FI than controls) diet in weanling Holtzman rats. Yet another study determined that T_3 and T_4 levels decreased significantly in LC (15% calorie restricted) when compared to controls based upon results from a seven week study using lean Zucker rats (Young et al., 1980).

H. RECTAL TEMPERATURE (T_{re})

1. LP

T_{re} was unchanged in protein-deprived young male Sprague-Dawley rats after 1 week and thereafter increased to values significantly higher than controls until week 4 and thence declined to levels higher but not significantly than the controls (Balmagiya et al., 1983).

2. LC

LC Holtzman rats had the same T_{re} as the controls at room temperature (Yousef et al., 1968). This trend was similar in two other studies using Holtzman rats where the T_{re} was not significantly higher than controls at 85, 208 and 470 days of age (Johnson et al., 1966) or when animals were calorically restricted for 200 days (Kibler et al., 1967).

II. THE EFFECTS OF THERMAL STRESS ON GROWTH RATE AND ASSOCIATED PHYSIOLOGICAL FUNCTIONS.

A. BODY WEIGHT

1. Cold Effects

Male Sprague-Dawley rats exposed to 4°C for 20 days experienced a decreased BW but not significantly when compared to the control group kept at 26°C (Scammel et al., 1981). A similar effect was also observed by Beard et al. (1988) when rats were exposed to a 10°C environment for seven days. Moreover, after five days (60 day study) exposure to 5°C, the average BW of Sprague-Dawley rats dropped an insignificant 4% (Cottle et al., 1954) and during longer exposures to 5°C (32 days) and 6°C (6 weeks) there was a significant decrease in the BW of exposed rats (Bakke et al., 1971; Jobin et al., 1975).

2. Heat Effects

Exposure to 34°C for 14 days caused significant weight loss of Holtzman rats in comparison to control animals (28°C) (Johnson et al., 1966). Holtzman rats exposed to 34°C also showed a significant depression in BW (Hamilton, 1963; Kibler et al., 1966; Yousef et al., 1968). Another study of animals fed a normal Purina rat

chow diet and exposed to 34°C showed weight losses only during the first three days of exposure; however, an increase in BW occured thereafter but at a slower rate than the controls (Horowitz, 1976). Moreover, a study conducted for seven days at 30°C resulted in no significant BW loss (Beard et al., 1988).

B. FOOD INTAKE

1. Cold Effects

Exposure to cold caused a gradual increase in FI in laboratory rats (Cottle et al., 1954). In both wild (Merriam's kangaroo rat, *Dipodomys merriami*) and laboratory rats FI increased during cold exposure (Yousef, 1979).

2. Heat Effects

Significant decreases of approximately 50% in FI were noted in rats exposed to 35°C for three weeks (Hamilton et al., 1963). A decreased FI was also noted in other studies during the first few months of exposure (Kibler et al., 1966 and 1967).

C. WATER INTAKE

1. Cold Effects

After five hours of exposure to 5°C there were no significant changes in WI of animals that acclimated to 23°C; however, when the animals were acclimated to 5°C for four months the WI was significantly lower in the 5°C acclimated animals than in the 23°C acclimated animals upon exposure to 5°C for five hours (Box et al., 1973). Diuresis is one of the responses incurred by animals when exposed to cold (Yousef, 1979).

2. Heat Effects

Sprague-Dawley rats acclimated to 24°C exhibited a three-fold increase in WI for the first 10 days of exposure to 35°C (Hamilton et al., 1963). It was noted in another study that normal rats became hyperthermic during early exposure to 40°C but they did not begin to drink until 2-3 hours later. Rats exposed to 36°C drank 5 times as much as rats at 28°C after six hours of exposure, and seven times after six hours exposure to 40°C (Hainsworth et al., 1968).

D. ORGAN WEIGHTS

1. Cold effects

Exposures to 4°C and 6°C for periods of 20 days, or four weeks or three months resulted in an increase in kidney, liver and heart weights of Sprague-Dawley rats (Heroux et al., 1958, 1963; Scammell et al., 1981). Liver weight of laboratory rats was also noted to increase after 60 days of exposure to 18°C with no change in heart weight (Herrington et al., 1942). Heroux, in 1961, found an increase in heart weight of wild rats, *Rattus norvegicus*, exposed to extreme cold (-7°C). In contrast, another experiment using Sprague-Dawley rats exposed to 10°C resulted in no difference in heart weight after seven days (Beard et al., 1988). Yousef et al. (1970) found in kangaroo rats, *D. merriami*, exposed for one and four weeks to 5°C that there was no change in liver, kidney or heart weights.

2. Heat effects

After 60 days of exposure to 35°C there was a decrease in liver and heart weights in laboratory rats (Herrington et al., 1942). Moreover, after 10 weeks exposure to 35°C there was a significant decrease in heart, liver and kidney weights (Ray et al., 1968). Another study using Sprague-Dawley rats for 7 days at 30°C resulted in no difference in heart weight (Beard et al., 1988).

E. HEMATOLOGICAL CHANGES

1. Cold Effects

Male Sprague-Dawley rats exposed to 10°C for seven days exhibited a slightly, but not significantly elevated Hct and Hb (Beard et al., 1988). Another study by Deb et al. (1956) found at 6°C an insignificant decrease in Hb and PP and no change in Hct. Kangaroo rats exposed to 5°C for 1 week resulted in no significant changes in Hct, Hb or PP (Yousef et al., 1970). Adult male Sprague-Dawley rats exposed to 4°C for 24 hours showed no significant change in Hct (Hefco et al., 1975). Yousef (1979) concluded that exposure to cold results in either no change or an increase in Hct, Hb and PP in laboratory rats and increases in all these parameters in wild rodents, *D. merriami.*

2. Heat Effects

When rats were exposed for seven days to 30°C, Hct and Hb were not affected significantly (Beard et al., 1988). Plasma proteins during heat stress were found to either increase (Hainsworth et al., 1968), or decrease (Burger et al., 1967) or remain unchanged (Frankel et al., 1972). Hct during dehydration, in heat stressed rats (40°C) rose (Hainsworth et al., 1968). Similarly during heat induced stroke (T_{re} = 41.5°C) there was a rise in Hct (Burger et al., 1967) During hyperthermia, Hb

slightly increased but not significantly (Burger et al., 1967).

F. METABOLIC RATE

1. Cold Effects

Upon exposure to cold (5°C), wild rats, *D. merriami* (Yousef, 1979) and laboratory rats increased MR (Cottle et al., 1954; Yousef et al., 1970). Also, it has been noted that even a mild cold challenge (18°C) for 90 minutes significantly increased MR (Balmagiya et al., 1983).

2. Heat Effects

When rats were exposed to 34°C for two weeks, the MR decreased to half that of control animals (Kibler et al., 1967). However, as the duration of exposure was extended to twelve and 26 weeks, the rat's MR was similar to that of the controls. When two month old rats were exposed to 34°C, MR increased by 13% over that of the controls in the first 48 hours and then it declined by the fifth day of exposure to as much as 20% of the control animals (Yousef et al., 1967).

G. THYROID FUNCTION

1. Cold Effects

Exposure to cold increased plasma T_4 after two and 24 hrs. (Beard et al., 1982; Hefco et al., 1975). However, after 24 hrs (Beard et al., 1984) and 20 days (Scammell et al., 1981) at 4°C, T_4 remained unchanged. Moreover, exposure to 10C for seven days caused a slight decrease in T_4 (Beard et al., 1988).

Exposure to 4°C, caused an increase in plasma T_3 regardless of the duration of exposure, i.e. 2 hrs., 24 hrs., or 20 days (Beard et al., 1982, 1984; Hefco et al., 1975; Scammell et al., 1981). Also, plasma T_3 increased 30% when rats were exposed to 10°C for seven days (Beard et al., 1988).

Levels of TSH have been shown to increase after 2 and 24 hrs exposure to 4°C, but then the levels returned to pre-exposure values after 48 hours (Hefco et al., 1975). Likewise, exposure to 5°C for 32 days in laboratory rats resulted in significant elevation of plasma TSH (Jobin et al., 1975). Still others found that rats adapted to 6°C for six weeks had decreased TSH levels (Bakke et al., 1971).

1. Heat Effects

Plasma T_4 in animals exposed to 34°C remained unchanged after four weeks (Johnson et al., 1966) or 5.5 weeks (Yousef et al., 1968). A Similar finding was

reported in rats exposed to 30°C for seven days (Beard et al., 1988).

Decreased plasma T_3 was observed after seven days exposure of laboratory white rats to 39°C (Beard et al., 1988). Also, a decrease in free T_3 was found in desert wood rats, *Neotoma lepida*, exposed to heat (Rousset et al., 1978).

Rats exposed for 48 hours to 39°C had no significant change in serum TSH (Bakke et al., 1971). This was also seen in desert wood rats at high temperatures (Rousset, et al., 1978).

H. RECTAL TEMPERATURE

1. Cold Effects

When 12 week old rats were exposed to a mild cold stress (18-19°C) for 90 minutes, T_{re} decreased 2.35°C for six minutes and then the animals were able to regain pre-exposure T_{re} (Balmagiya et al., 1983). After two hours of exposure to 4°C, rats were able to maintain T_{re} (Beard et al., 1982). However, T_{re} decreased in animals exposed to 1°C (Hefco et al., 1975) and after eight hours, rats were able to maintain their T_{re} to the pre-exposure levels. After 24 hours exposure to 7°C, rats increased T_{re} (Hamilton, 1963). However when rats were exposed to 4°C-5°C, no significant change befell in T_{re} (Beard et al., 1984).

2. Heat Effects

Rats exposed to 34°C for ten or 14 days, exhibited an increase in T_{re} (Horowitz, 1976; Kibler et al., 1967). Similar findings were observed during exposure to 35°C (Hamilton et al., 1963; Johnson et al., 1966; Yousef et al., 1970).

III. THE EFFECTS OF DIET AND THERMAL STRESS ON GROWTH RATE AND OTHER PHYSIOLOGICAL FUNCTIONS.

A. BODY WEIGHT (BW)

1. High Protein (HP) Diet plus Cold Exposure.

Rats fed a high protein diet lost more BW in the cold when compared to control animals (Stevenson, 1955).

2. HP Diet and Heat Exposure.

As adult male rats were fed a HP diet while exposed to 32°C for ten days, a significant decrease in BW occurred when compared to control animals at 32C (Hamilton, 1963).

3. Low Protein (LP) Diet and Cold Exposure.

A group of rats were fed an LP diet (9% casein) and exposed to a mild cold challenge at 24°C. These animals showed a similar gross efficiency when compared to those animals kept at 29°C and BW did not change (Rothwell et al., 1987). This finding confirmed an earlier study where rats maintained weight when fed a LP diet and exposed to cold (Stevenson, 1955). Schmidt et al. (1967) found that animals fed an LP diet either lost less weight in a cold environment than LP diet animals at room temperature or even gained weight.

4. LP Diet and Heat Exposure.

LP fed animals experienced a slight loss of BW at 32°C, however it was not as great a weight loss as incurred by the control diet fed animals (Hamilton, 1963).

5. Low Calorie (LC) Diet and Cold Exposure.

Rats were restricted to one-third of the food intake of control animals and showed a large decrease in BW when exposed to cold (Stevenson et al., 1957).

6. LC Diet and Heat Exposure.

Rats 23 days old were placed on LC diets and kept at 34°C for 182 days. Measurements made at 14, 83, and 182 days of exposure showed a significantly lower BW than the LC diet group kept at 28C (Kibler et al., 1967). Similar results were reported by Yousef and Johnson (1968, 1970).

B. FOOD INTAKE (FI)

Temperature regulation in the white rat can be influenced by both the quantity and the quality of the food ingested. In a cold environment there is a general increase in food consumption which must be maintained at an appropriate level to compensate for the additional heat loss which occurs during temperature regulation, activity and digestion (Hart, 1971). When exposed to a high ambient temperature the animal must dissipate as much heat as possible, therefore the caloric intake must be decreased in order to avoid the Specific Dynamic Action (SDA) of the excess calories which could be detrimental to survival (Hamilton et al.,1963).

1. HP Diet and Cold Exposure.

Giaja and Gelinco (1934) were pioneers in yielding results that survival in the cold could be changed if the sole content of the diet was either protein,carbohydrate (CHO), or fat. Results on this topic vary. One study concluded that cold exposed rats favored CHO and fat over protein (Templeton et al., 1949). However, in other experiments animals exposed to cold increased their protein and fat intake (Dugal et al., 1945). Acutely stressed rats (12 hr. fast, 10min swim at 4°C) ate 156% more than controls (Vaswani et al., 1983).

2. HP Diet and Heat Exposure.

Upon exposure to an ambient temperature of 32°C (mild heat stress) for a ten day period, HP fed rats consumed fewer calories as compared to the controls at 26.5°C (Hamilton, 1963).

3. LP Diet and Cold Exposure.

Rats fasted for twelve hours followed by a ten minute swim in 4°C water showed a 20% increased FI when fed an LP (high CHO) diet which was less than control rats which increased FI about 56% while undergoing the same stress (Vaswani et al., 1983).

4. LP Diet and Heat Exposure.

The LP diet group (high CHO) decreased FI when exposed to 32°C as compared to LP diet group kept at 26.5°C. However, when compared to control diet rats at 32°C there was no difference in FI (Hamilton, 1963).

C. WATER INTAKE (WI)

1. HP Diet and Cold Exposure.

Rats fed a HP diet and subjected to severe cold stress did not show a significant increase in water intake compared to control animals subjected to identical stress conditions (Vaswani et al., 1983).

2. HP Diet and Heat Exposure.

HP diet animals drank more water per animal than control animals at 32°C. When the WI is expressed as WI/kcal FI this ratio was also higher in HP diet rats (Hamilton,1963).

3. LP Diet and Cold Exposure.

Rats fasted for twelve hrs, exposed to severe cold stress (4°C), and then fed an LP (high CHO) diet showed an 80% increase in WI (Vaswani et al.,1983).

4. LP Diet plus Heat Exposure.

Rats fed LP diet and kept at 32°C for ten days, drank less water (WI/animal or WI/Kcal) than the control group at 32°C, (Hamilton, 1963).

D. HEMATOLOGICAL VALUES

4. LP diet and Heat Exposure.

Female rats were fed an LP diet (1.3% casein) for 40 days, and when exposed to 50°C for 15 min, the Hct and Hb increased significantly in the control fed animals. LP diet heat exposed animals did not show any difference when compared to the unexposed protein malnourished rats, however, they had a lower Hct and Hb when compared to control animals which had experienced an increase in both parameters (Jani et al., 1977).

E. METABOLIC RATE

1. HP diet and Cold Exposure.

The $\dot{V}O_2$ of HP diet fed golden hamsters decreased with declining environmental temperature and showed a significant increase (40%) at their thermoneutral zone when compared to control hamsters (Simek, 1975).

2. HP diet and Heat Exposure.

Citrulline synthesis can be used to stimulate mitochondrial energy metabolism and is associated with O_2 uptake. It was found to be enhanced 2-3X in the liver mitochondria of HP diet animals upon exposure to 42°C (Letko et al., 1984).

3. LP diet and Cold Exposure.

Rats fed an LP diet for 6 weeks and then placed in a mild cold environment (18-19°C) for 90 min., showed a significantly greater increase in VO_2 than controls (Balmagiya, 1983).

4. LP diet and Heat Exposure.

Rats that were given an LP diet (9% casein) and housed at 29°C (mild heat stress) did not experience a change in $\dot{V}O_2$ (Rothwell et al., 1987).

5. LC diet and Cold Exposure.

Cold induced elevation of heat production was due to elevated food intake rather than the cold exposure itself in the rat (Hart, 1971). This could explain the reason that $\dot{V}O_2$ is higher in cold fed than fasted animals (Hoffman et al., 1958).

F. THYROID FUNCTION

5. LC diet and Cold Exposure.

The rate of release of radioactively tagged hormone (I^{131}) was much slower in food-restricted rats 15 days after exposure to 8°C than cold exposed rats fed ad libitum (Cottle, 1960).

6. LC diet and Heat Exposure

The thyroid function of rats raised at 34°C was similar to the thyroid function of rats raised at 28°C (Yousef et al., 1968).

G. RECTAL TEMPERATURE (T_{re})

3. LP diet and Cold Exposure.

Rats fed an LP diet (6% casein) for six weeks, and then exposed to a mild cold challenge (18°C-19°C) for 90 min., experienced a larger decrease in T_{re} than control rats and it took them longer to increase body temperature to pre-exposure levels (Balmagiya et al., 1983).

5. LC diet and Cold Exposure.

Cold acclimated rats exposed to 8°C for four hrs while FI was limited, were able to maintain their T_{re} almost as well as cold acclimated controls. Rats restricted in the amount of food available can still maintain T_{re} during cold exposure if acclimated to cold. However, warm acclimated control animals were unable to maintain their T_{re} (which dropped 14°C) (Cottle,1960).

6. LC diet and Heat Exposure.

After exposure to 34°C, the LC diet group had a significantly higher T_{re} than the LC diet group at 28°C. However, when compared to the control group at 34°C, the LC diet group exhibited no significant difference (Kibler et al.,1967). Similar results were obtained by Yousef et al. (1970) with the exception that young rats at 47 days of age on an LC diet did not experience an increase in T_{re} at 34°C.

MATERIALS AND METHODS

EXPERIMENT 1:

ANIMALS: 120 male Sprague Dawley Rats age 21 days were used in this study. Rats were housed individually in metal cages and fed normal laboratory rat chow for one week prior to the start of the experiment to allow them to adjust to their new surroundings. The room ambient temperature was approximately 24 °C and the light/dark cycles were 12 hrs. light and 12 hrs. dark.

The rats were randomly divided into 4 subgroups of 30 animals each. The Control group was fed ad lib a diet containing 22.5% protein. The high protein group (HP) was fed ad lib a diet containing 45% protein. The low protein group (LP) was fed ad lib a diet containing 5.5% protein. The low calorie group (LC) was fed the same diet as the control group but restricted to 33% less than the controls had eaten that week. All groups were allowed free access to water. The rats were fed their respective diets for a period of 8 weeks. (see Table 1 for dietary components)

MEASUREMENTS: Weekly measurements included body weight (BW), food intake (FI), water intake (WI), and rectal temperature (T_{re}). The BW was measured to the nearest 0.1 gram using an OHAUS triple beam balance, FI and WI were determined by weighing the food and water to the nearest one hundreth prior to

feeding each rat and then again weighing the remaining food and water 24 hours later using an OHAUS Galaxy 400 electronic balance. T_{re} was measured using a Model BAT-12 (Bailey Instruments Inc.) thermocouple thermometer, with a 3mm copper-constantan thermocouple. Metabolic rates (MR) were measured every 2 weeks using a closed circuit indirect calorimeter for the rate of oxygen consumption ($\dot{V}O_2$). This system is composed of a lucite metabolic chamber within which Drier-rite ($CaSO_4$, a water absorbant) and CO_2 absorbant (Barium Hydroxide Lime granules, Warren E. Collins Co.) were placed underneath the the grid which the animal laid upon. A Med Science Volume Meter (model #16, St. Louis Missouri) was connected to the metabolic chamber and then filled with O_2. The animal was then placed into the chamber and allowed to become familiar to the chamber for approximately 1/2 hr., and then continuous measurements of $\dot{V}O_2$ were recorded for approximately 30 minutes. The $\dot{V}O_2$ values were then corrected to STPD and converted to ml O_2 consumed/g BW*hr.

After 4 weeks on the diets 10 animals from each group were randomly selected. These animals were anaesthesized using Metofane and blood was drawn (2ml) via heart puncture and placed onto ice until further analysis. The blood was then analyzed for hemoglobin using a OSM 2 Hemoximeter (Radiometer/Copenhagen). Hematocrit was determined by drawing a portion of the blood sample into heparanized capillary tubes and then centrifuging the tubes in an Adams Read A Crit microcentrifuge. After centrifugation, the plasma that was separated from the packed red blood cells in the capillary tubes was then analyzed

for plasma protein concentration using a refractometer (Model # 10406 American Optical Company Buffalo NY). The remaining blood sample was then centrifuged using a Beckman Centrifuge and the plasma was decanted into microcentrifuge tubes and placed into a freezer at -10 °C for thyroid hormone determinations.

MEASUREMENT OF THYROID HORMONES: The hormones, thyroxine (T_4), free thyroxine (FT_4), triiodothyronine (T_3) and Thyroid stimulating hormone (TSH) were measured using the enzyme immunoassay technique (EIA). These measurements were made using commercially available kits (Immunotech Corp., Boston, Mass. 02134).

The quantitative determinations of plasma T_4, T_3, TSH, and FT_4 were done using EZ-Bead EIA Kits. This method utilized a highly specific monoclonal antibody which was bound to a solid support (polystyrene bead) and an enzyme-labeled analyte. The end product of the procedure was colored and its concentration was measured using a Beckman DU-65 Spectrophotometer (Beckman Instruments Inc. Fullerton Ca., 92634). Refer to Appendices 1-4 for EIA procedures.

ORGAN WEIGHTS: At the end of the 8 week period, animals from each group were bled again via heart puncture and then sacrificed by placing the anesthesized animal into a jar containing Halothane. They were then dissected and the liver, kidney and heart were removed, after removing excess fat, these organs were weighed to the nearest 0.01g on an electronic balance.

EXPERIMENT 2: Eight animals from each group were randomly chosen and placed into an environmental chamber at 5 °C during the 9th week of the study. The animals remained in the chamber for a period of 1 week. The animals continued on their respective diets with food and water ad libitum with the exception of the LC group.

MEASUREMENTS: The same measurements were conducted as in Experiment 1, except that BW was measured the day prior to introduction to the chamber and was also measured the 2nd,5th and 8th days of exposure. T_{re} measurements were recorded daily. FI and WI were measured on the third day of exposure and determined the following day after 24 hours. MR was measured on the 6th day of exposure.

EXPERIMENT 3: Eight animals from each group were randomly chosen and placed into an environmental chamber at 35°C. They had been on their respective diets for a full 9 weeks before introduction to the environmental chamber. The same measurements were collected as are discussed in Experiment 2.

STATISTICAL COMPARISONS OF DATA: One way analysis of variance was used in all the studies both between and within the each individual group, followed by multiple comparisons of groups; level of significance = $P \leq 0.05$. Linear regression analysis was performed at the level of protein and calorie intake.

Table 1. The composition of the test diets. Diets were purchased through Jones' Feed and Tack Co., Las Vegas, Nev. and were manufactured by the Ralston Purina Co., Test Diets Division, St. Louis, MO.

TABLE 1

COMPOSITION OF THE DIETS

COMPONENT	CONTROL	HIGH PROTEIN	LOW PROTEIN
CASEIN	22.5%	45.0%	5.5%
SUCROSE	15.0%	15.0%	30.6%
SOLKA FLOC	3.0%	3.0%	3.0%
VITAMIN MIX	2.0%	2.0%	2.0%
MINERAL MIX	5.0%	5.0%	5.0%
DL METHIONINE	0.2%	0.4%	0.05%
CHOLINE CHLORIDE	0.2%	0.2%	0.2%
CORN OIL	5.0%	5.0%	5.0%
LARD	5.0%	5.0%	5.0%
DEXTRIN	42.1%	19.4%	43.65%

RESULTS

I. Experiment 1

A. Body Weight

After 8 weeks on their respective diets, the HP group showed no significant difference ($P > 0.05$) in growth rate compared with Controls (Figure 1). However, the LP group had a significantly ($P < 0.001$) lower body weight gain than the Controls, HP and LC groups from the 2nd and through the 8th week of the study (Figure 1). The LC group did not exhibit a significant decrease in growth rate until after the third week of food restriction (Figure 1).

Linear regression analysis shows that there is an increase in growth rate associated with an increase in protein consumption (Figure 2a) and, although Controls had a significantly greater BW ($P < 0.001$) than the LC group, the impact of protein content of the diet on growth is greater than caloric content (Figure 2b).

B. Food Intake

When FI was expressed in grams per g BW per day the LP group ate significantly more ($P < 0.001$) than the other groups weeks 3-8 (Figure 3). Linear regression analysis showed that average FI(g)/BW(g) decreased with increased protein consumption (Figure 4).

C. Water Intake

Linear regression analysis showed that average WI(g)/FI(g) per day for an 8 week period increased with increased protein intake (Figure 5a). Although Controls consumed significantly more ($P<0.005$) WI(g)/FI(g) per day than the LC group over an 8 week period, trend analysis shows that protein content appears to have a greater impact than caloric content in the diet (Figure 5b)

D. Organ weights

Organ weight results are summarized in Figure 6 and are expressed as organ weight (g)/ 100(g) BW. The HP group animals were not available for this part of the study.

Linear regression analysis shows a decrease in heart weight with increased protein content of the diet (Figure 6a), a trend that is not affected by caloric consumption (Figure 6b).

Linear regression analysis of liver weight shows increased liver weight with increased dietary protein content (Figure 6c). The Controls had a significantly greater ($P<0.05$) liver weight than the LC group the impact of the caloric content of the diet was significantly greater than the protein content (Figure 6d).

Linear regression analysis of kidney weight shows an increased kidney weight with increased dietary protein (Figure 6e). Albeit Control kidney weight was significantly greater ($P<0.05$) than the LC group, dietary protein content has a greater effect than caloric consumption on kidney weight (Figure 6f).

E. Hematological values

The results of hematological analysis are summarized in Figure 7. Hct and Hb levels decreased with increased dietary protein content (Figures 7a and 7c), and PP increased with increased dietary protein content (Figure 7e). Whereas the Control levels were significantly greater than ($P<0.01$) the LC group for Hb, Hct, and PP, dietary protein content seems to have a greater impact than caloric content on hematological values (Figures 7b, 7d and 7f).

F. Metabolic Rate

There was no difference ($P>0.05$) between the groups after 2 weeks post-treatment. However, after 4 and 6 weeks both the HP and LP groups had a significantly ($P=0.01$, $P=0.005$) higher metabolic rate. After 8 weeks of their respective diets the only difference was an increased MR observed for the LP group ($P<0.001$) (Figure 8).

Linear regression analysis of average MR for the 8 week period shows no linear trend. However there is a significantly greater ($P<0.001$) average MR for both the HP and LP groups than the other groups.

G. Thyroid Function

The LP group had greater plasma T_4 concentrations than both HP and LC groups ($P<0.05$) and greater T_3 concentrations than all other groups ($P<0.01$). The LC group had lower FT_4 concentrations than the Controls ($P=0.005$) and the LP

group ($P<0.05$). There were no significant differences between the groups for TSH levels.

Linear regression analysis of T_4 and T_3 concentrations showed a trend for increased plasma levels as dietary protein levels decreased (Figures 9a and 9c) which is unaffected by caloric value of the diet (Figure 9b and 9d).

H. Rectal Temperature

The data on rectal temperature are presented in Figure 10. There was a significant ($P<0.05$, $P<0.001$) increase in rectal temperature in the HP and LC fed animals during week 2. There was a significant ($P<0.001$) decrease in rectal temperature of the HP group at week 3. During week 4, T_{re} was increased in Groups B, C, and D ($P<0.001$, $P<0.001$, and $P<0.05$ respectively). During week 5, the HP group had a decreased ($P<0.05$) T_{re}. and at week 7 the LP and LC groups both had an increased ($P<0.001$, $P<0.05$) T_{re} when compared to controls. There were no significant T_{re} differences during weeks 6 and 8 between treatment groups.

Linear regression analysis showed no trends between dietary protein levels and T_{re}.

II. EXPERIMENT 2

A. BODY WEIGHT

The HP and LC groups lost weight after 7 days exposure to 5°C ($P<0.05$, and $P<0.01$) (Figure 11) when compared within groups, and there was no significant difference ($P>0.05$) as to the extent of this weight loss when compared between groups. The Controls or LP group did not lose weight during cold exposure (Figure 11).

Linear regression analysis of change in BW (BW) showed that there was a trend of increased BW loss with increased dietary protein content (Figure 12a) upon cold exposure, a trend which was not greatly affected by decrease in caloric consumption (Figure 12b).

B. FOOD INTAKE

All groups significantly ($P<0.001$) increased both FI per animal and FI per g BW during exposure to cold when compared within groups (Figure 13). There was no difference ($P>0.05$) in FI between the Controls or HP groups, and the LP group ate significantly ($P<0.01$) more the other groups when expressed as FI per g BW (Figure 13).

Linear regression analysis showed an increase in FI with a decrease in dietary protein at 5°C (Figure 14).

C. WATER INTAKE

Within groups, WI per animal per day increased in Groups B and D ($P<0.005$ and $P<0.05$). WI expressed per g FI per day decreased in Controls and the HP group ($P<0.001$ and $P<0.05$) and remained unchanged ($P>0.05$) within the LP and LC groups (Figure 15).

Between groups comparisons revealed that the HP group drank more per animal ($P<0.001$) and when WI was expressed in terms of g FI, the HP and LC groups drank almost twice as much as that of the controls (Figure 15).

Regression analysis showed that there is an increased WI per g FI with an increase in dietary protein (Figure 16a) a trend slightly affected by caloric intake because when the LC group data were added to the figure the r^2 value decreased but not significantly (Figure 16b).

D. ORGAN WEIGHTS

Within groups, exposure to 5°C caused an increased kidney weight in the LC group ($P<0.001$), an increased heart weight in Controls and the LP group ($P<0.001$ and $P<0.05$), and a decreased liver weight in Controls ($P<0.05$).

The differences observed in organ weights between the animal groups exposed to 5°C was that the liver weight of the LP group was greater ($P<0.05$) than the Controls and LC group, the kidney weight was greater ($P<0.001$) in the HP group when compared to the LP and LC groups, and the LP group had a greater heart

weight than the LC group ($P<0.05$).

Trend analysis revealed that increased dietary protein was associated with an increased kidney weight (Figure 17), a trend unaffected by the caloric value of the diet.

E. HEMATOLOGICAL VALUES

Cold increased Hb in the LP and LC groups ($P<0.01$), PP in the LP group ($P<0.01$) and decreased Hct, Hb and PP in the HP group ($P<0.05$).

Between groups there were no differences ($P>0.05$) in PP levels of the different dietary groups when exposed to 5°C for one week. However, the LP group experienced a higher Hct ($P<0.001$) than the other groups, and a higher Hb concentration ($P<0.05$) than the Controls and HP groups. In the LC group the Hb concentration was greater than ($P<0.01$) the HP group but less than ($P<0.005$) the Controls. In addition, the Controls had a greater Hb concentration than the HP group.

Linear regression analysis showed a decrease in Hct and Hb concentrations when dietary protein intake was increased (Figures 18a and 18c), a trend which was still prevalent when the data pertaining to caloric restriction were added (Figures 18b and 18d). There were no trends observed for PP concentration at 5°C.

F. METABOLIC RATES

Within groups, 5°C caused an overall increase in MR ($P<0.01$) for all groups (Figure 19). Between groups, the MR of the LP and LC groups were higher than Controls ($P>0.001$) and the MR of the HP group was not significantly different ($P>0.05$) than any of the other groups (Figure 19) after 6 days exposure to 5°C.

There were no linear trends observed as a result of dietary protein intake and cold exposure.

G. THYROID FUNCTION

The exposure to cold resulted in a general decrease in plasma T_4 concentrations ($P<0.001$) within each dietary group. T_3 concentrations increased within the HP and LC groups ($P<0.001$ and $P<0.005$), and the controls showed decreased plasma FT_4 concentrations ($P<0.05$).

No significant ($P>0.05$) difference resulted in plasma T_4, FT_4 or TSH concentrations between groups. The LP group had a significantly higher ($P<0.005$) plasma T_3 concentration than the Controls and HP group.

Regression analysis revealed that increased dietary protein levels resulted in a decreased plasma T_3 concentrations (Figure 20a) and this trend is slightly affected, but not significantly, by the caloric value of the diet (Figure 20b).

H. RECTAL TEMPERATURE

Upon exposure to cold for 7 days, controls showed an increased T_{re} on day 4 and a decreased T_{re} on day 7 ($P<0.001$). The HP group increased T_{re} on days 2 ($P<0.005$), 3 and 4 ($P<0.001$) and decreased T_{re} on day 7 ($P<0.05$). The LP group increased T_{re} on days 3 and 4 ($P<0.001$) and also decreased T_{re} on day 7 ($P<0.05$). The LC group increased T_{re} on days 2, 3 ($P<0.05$), and 4 ($P<0.001$).

On day 1 of exposure to 5°C, there were no differences in T_{re} between the groups. On day 2, the LP group had a significantly lower ($P<0.001$) T_{re} than the other groups and the LC group had a significantly higher T_{re} ($P<0.05$) than controls. On Days 3-7 of exposure there were no significant differences in T_{re} between the groups (Figure 21).

III. EXPERIMENT 3

A. BODY WEIGHT

All dietary groups lost weight during heat exposure ($P<0.05$) with the exception of the LP group ($P>0.05$) (Figure 22).

Linear regression analysis disclosed that the change in BW increased with increased dietary protein intake (Figure 23a) and that caloric restriction has no significant effect on this trend (Figure 23b).

B. FOOD INTAKE

Within groups, there was a general decrease in FI per animal, and per g BW due to heat exposure ($P<0.05$) (Figure 13). Between groups comparison shows that there was no difference in the amount of FI decrease expressed per animal (Figure 13). However, when FI was expressed per g BW, the LP and LC groups ate significantly ($P<0.001$) more than Controls and the HP group (Figure 13).

Linear regression analysis showed that FI per g BW at 35°C decreased with increased dietary protein levels (Figure 14).

C. WATER INTAKE

Within dietary groups, heat exposure caused an overall increase in WI both per animal and per g FI ($P<0.005$).

All groups increased WI in the heat and there was no difference between the groups when expressed as WI per animal. When WI was expressed per g FI there was a significantly decreased WI for the LP and LC animals when compared to the Controls and HP group (Figure 15).

Linear regression analysis revealed that at 35°C WI per g FI increased with increased dietary protein (Figure 24a) and this trend is not greatly affected by the caloric value of the diet (Figure 24b).

D. ORGAN WEIGHTS

Controls, LP and LC groups experienced a decreased liver weight ($P<0.005$), Controls and the LP group had a decreased kidney weight ($P<0.001$), the LP group had a decreased heart weight ($P<0.05$), and the heart weight of the LC grouppp increased ($P<0.005$) when exposed to heat stress. All organ weights were expressed in g per 100 g BW.

A comparison within groups, revealed that the HP and LP groups had greater heart ($P<0.05$) and liver ($P<0.001$) weights than the other groups and the HP group had a greater kidney weight ($P<0.001$) than all other groups.

No linear trends were observed with liver and heart weights resultant of diet and heat stress; however, regression analysis revealed that kidney weight increased with increased dietary protein and this trend is not significantly affected by the caloric value of the diet (Figure 17).

E. HEMATOLOGICAL VALUES

Heat caused a decrease in Hct of Controls and an increased Hct in the LP group ($P<0.005$). PP levels decreased ($P<0.05$) in the HP group, and Hb increased in the LP and LC groups ($P<0.05$ and $P<0.005$).

Comparisons made between groups showed that the LP group animals had greater plasma Hct and Hb concentrations than the other groups ($P>0.001$), and Controls had a greater PP concentration than the LP and LC groups ($P<0.05$).

Linear regression analysis showed that Hct and Hb concentrations decrease as the protein content of the diet increases (Figures 25a and 25c), trends that are still significant when the caloric content of the diet is reduced (Figures 25b and 25d).

F. METABOLIC RATE

MR significantly ($P<0.001$) decreased in all dietary groups as a result of heat exposure (Figure 19). Between groups, the LC group had a greater MR ($P<0.01$) than the Controls and HP group and Controls had a higher MR than the HP group (Figure 19).

Linear regression analysis revealed that the greater the protein concentration in the diet, the lower the $\dot{V}O_2$ (Figure 26a) and this trend is not significantly affected with a reduction in caloric intake (Figure 26b).

G. THYROID FUNCTION

Heat exposure caused decreased T_4 and FT_4 levels in Controls, HP and LP groups ($P<0.001$), T_3 levels in Controls ($P<0.05$) and TSH levels in the LC group ($P<0.05$).

Between groups, the LP group had a significantly higher T3 than the other groups ($P>0.001$). The LP and LC groups had a greater plasma T_4 and FT_4 concentrations than the Controls and HP group ($P>0.01$), and the LC group had a greater TSH concentration than the HP group.

Trends observed regarding protein levels and heat stress were decreased

plasma T_3, T_4 and FT_4 with increased protein concentrations in the diet (Figures 27a, 28a, and 29a) and these trends are not significantly affected by caloric restriction (Figures 27b, 28b, and 29b).

F. RECTAL TEMPERATURE

Exposure to heat caused varied results within the diet groups. The Controls experienced an increased T_{re} on days 1-5 with the highest increase on days 1-3 ($P<0.005$, and $P<0.05$), their T_{re}'s on days 6 and 7 were at preexposure levels. The HP group experienced an increased T_{re} throughout the entire 7 day heat exposure period ($P<0.005$). The LP group had increased T_{re} on days 1 ($P<0.001$) and 7 ($P<0.05$), and had T_{re}'s similar to preexposure levels during days 2-6. The LC group had an increased T_{re} only on day 1 ($P<0.001$), on days 2-6 experienced no increase in T_{re}, and on day 7 they had experienced a decrease in T_{re} ($P<0.05$).

Between groups, on day 1 the LP group had the lowest T_{re} ($P<0.01$). On day 2 the LP and LC groups had lower T_{re}'s than controls ($P<0.005$, and $P<0.01$). On day 3 the LP and LC groups had lower T_{re}'s than controls ($P<0.005$, and $P<0.05$). On days 4 and 5 there were no significant temperature differences between the groups. Day 6 showed a lower T_{re} for the LP group ($P<0.05$), and on day 7 the T_{re} of the HP group was higher than all the other groups ($P<0.05$) (Figure 30).

Figure 1. The growth rate of white rats fed varied diets for eight weeks at room temperature. Values are mean BW which were assessed weekly. Error bars represent $\pm$ S.D.

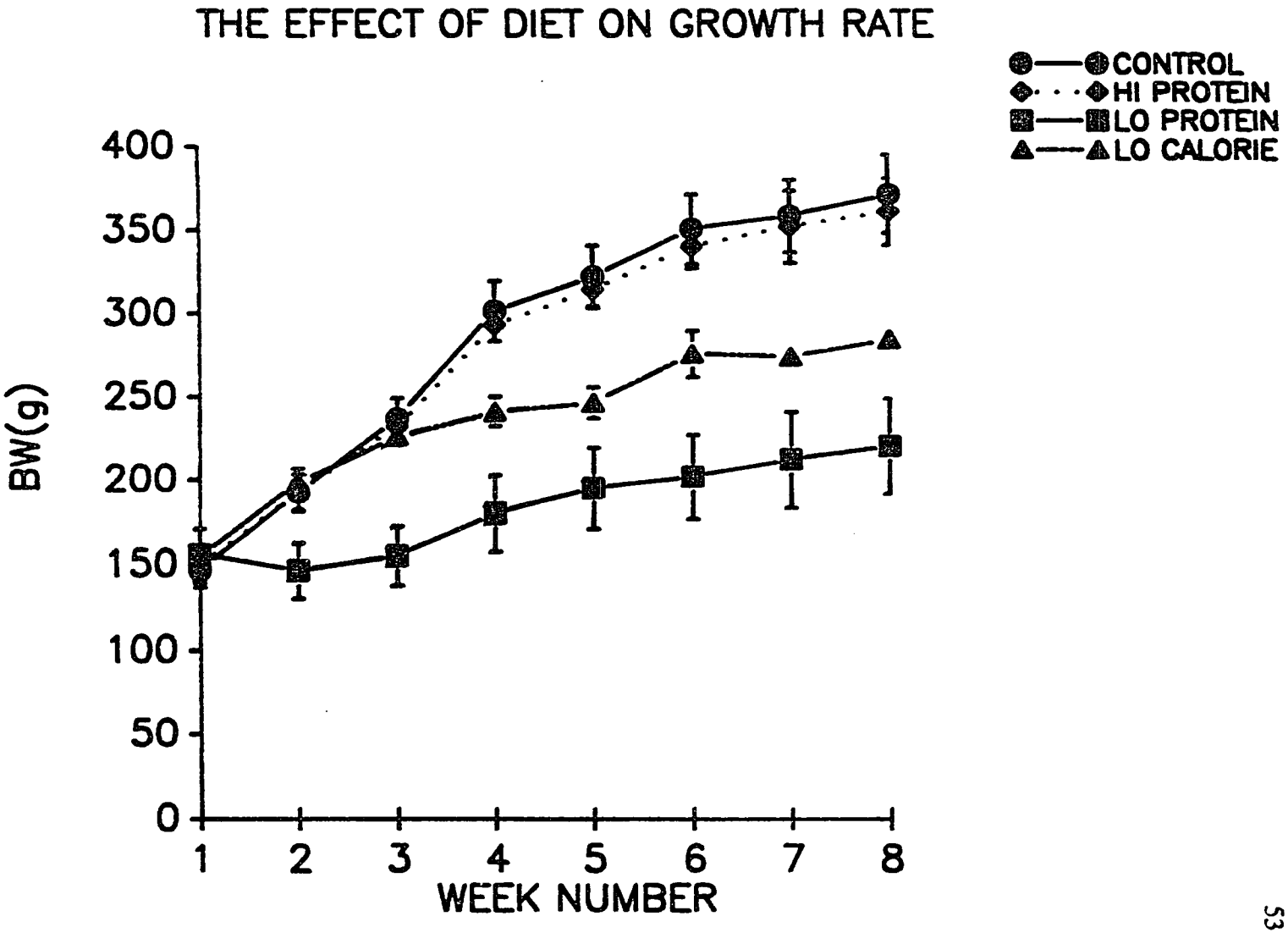

THE EFFECT OF DIET ON GROWTH RATE
CONTROL
HI PROTEIN
LO PROTEIN
LO CALORIE
BW(g)
400
350
300
250
200
150
100
50
0
1
2
3
4
5
6
7
8
WEEK NUMBER

Figure 2. Effects of dietary protein on the growth of white rats at room temperature expressed without caloric restriction (Figure 2a) and in conjunction with caloric restriction (Figure 2b). Values are BW measurements taken at week eight. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

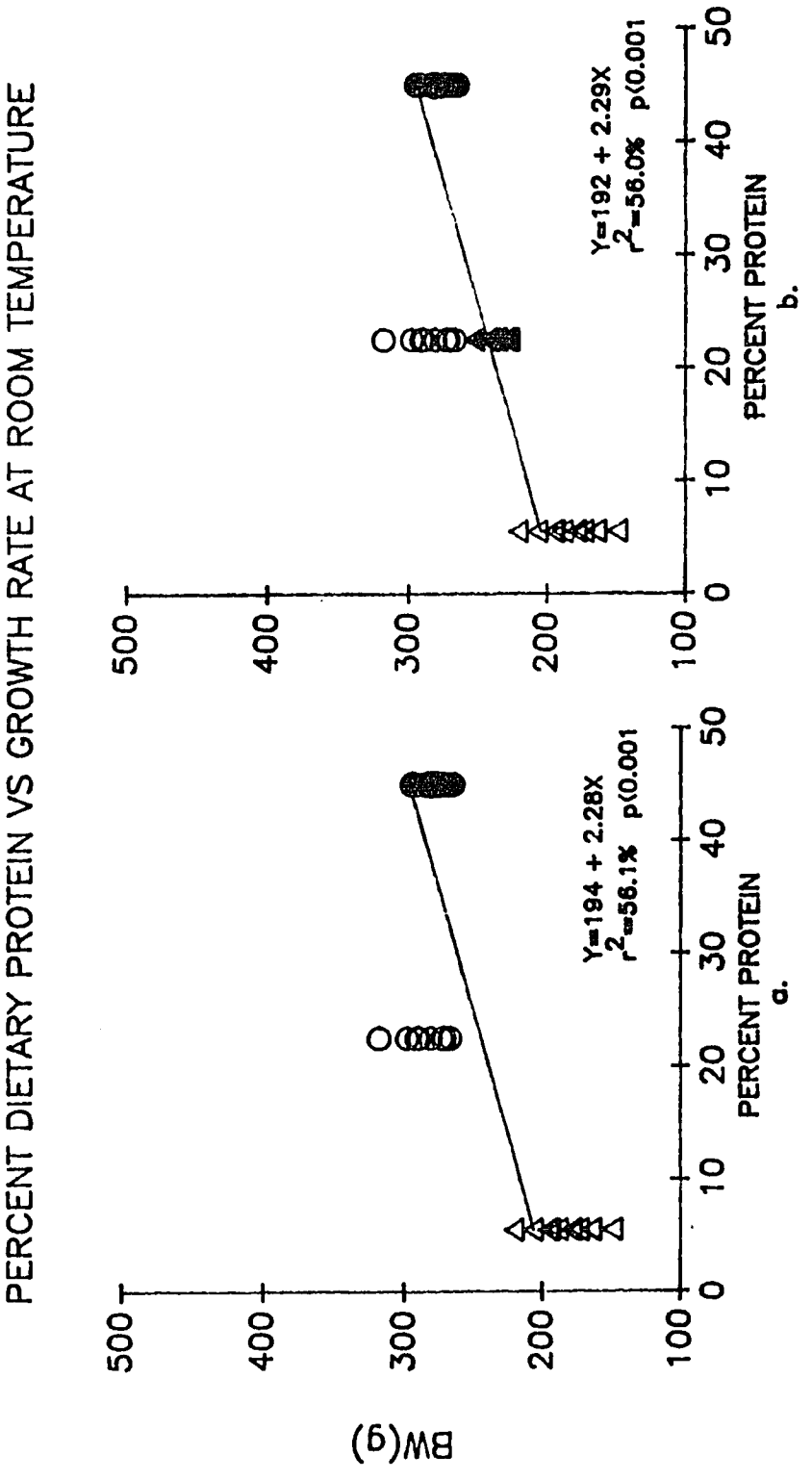

PERCENT DIETARY PROTEIN VS GROWTH RATE AT ROOM TEMPERATURE
BW(g)
500
400
300
200
100
0
10
20
30
40
50
PERCENT PROTEIN
a.
Y=194 + 2.28X
r2=56.1% p(0.001
b.
Y=192 + 2.29X
r2=56.0% p(0.001

Figure 3. Weekly mean FI, expressed per g BW per day, of white rats fed varied diets for eight weeks at room temperature. Error bars represent $\pm$ S.D.

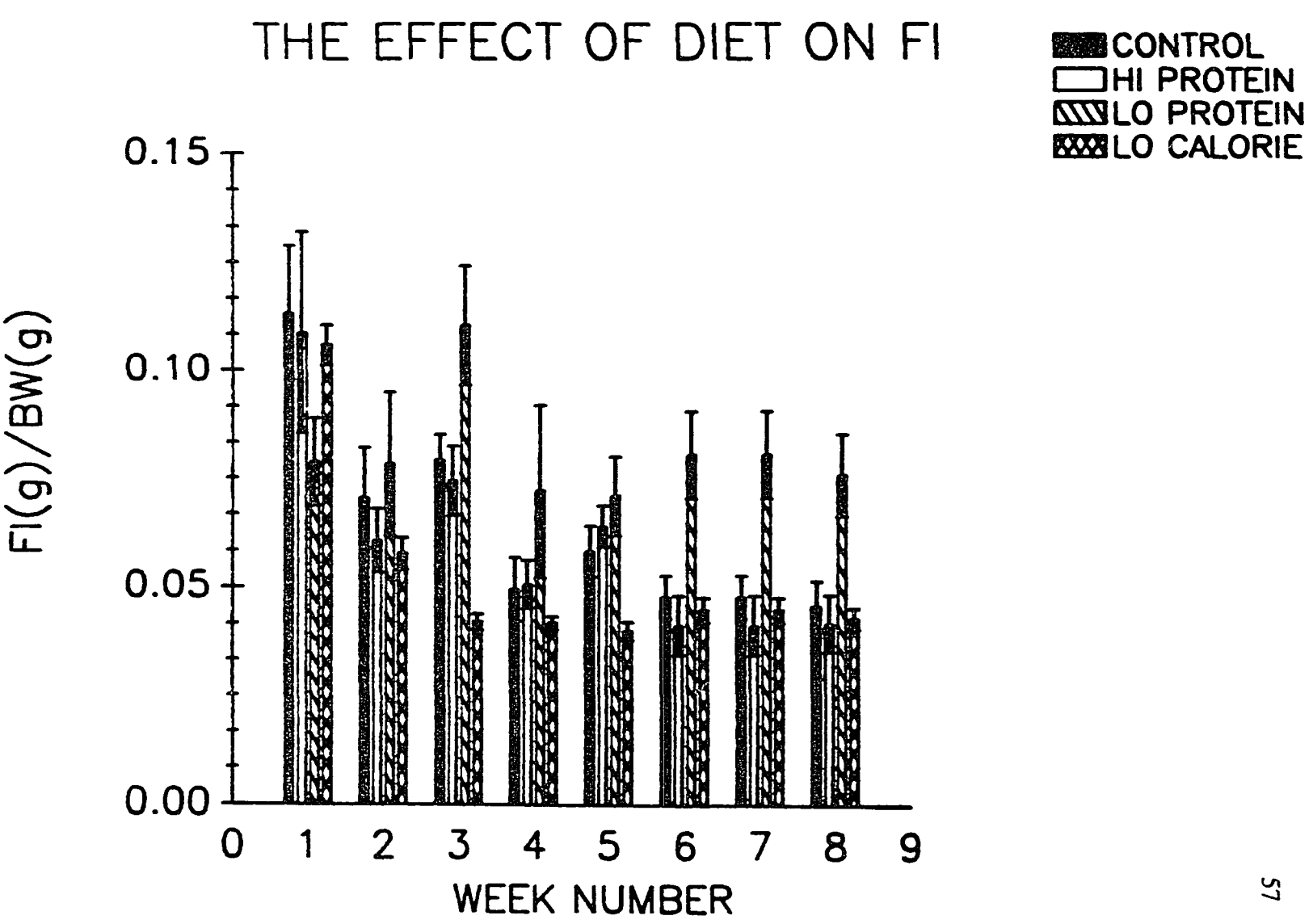

THE EFFECT OF DIET ON FI
CONTROL
HI PROTEIN
LO PROTEIN
LO CALORIE
FI(g)/BW(g)
0.15
0.10
0.05
0.00
0 1 2 3 4 5 6 7 8 9
WEEK NUMBER

Figure 4. Effects of dietary protein on average daily FI of white rats over an eight week period at room temperature. Controls=open circles, HP=filled circles, LP=open triancles, and LC=filled triangles.

DIETARY PROTEIN EFFECTS ON AVE FI AT ROOM TEMPERATURE

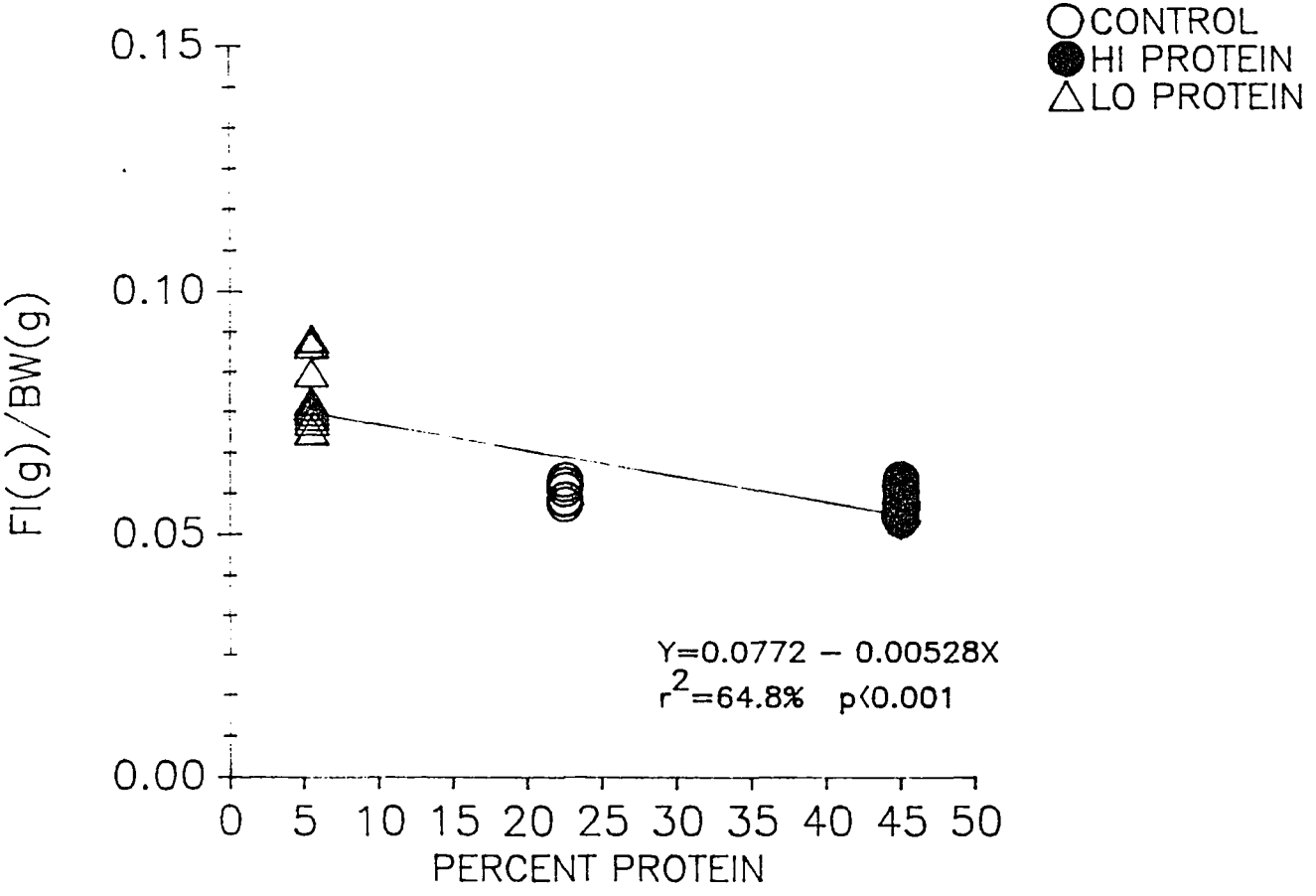


Figure 5. Effects of dietary protein on average WI, expressed per g FI per day, of white rats for an eight week period at room temperature. Figure 5a represents these effects without caloric restriction and Figure 5b shows the effects with caloric restriction. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

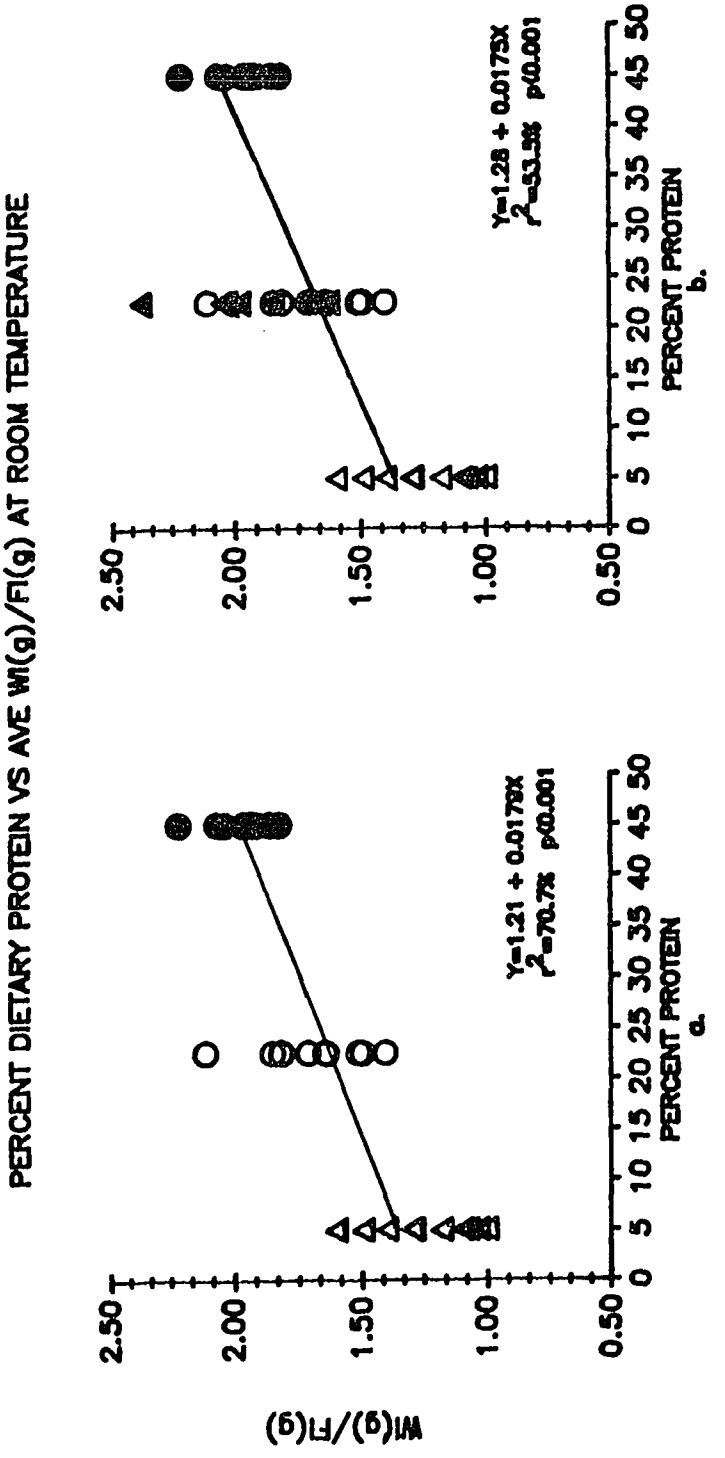

PERCENT DIETARY PROTEIN VS AVE WI(g)/FI(g) AT ROOM TEMPERATURE
WI(g)/FI(g)
2.50
2.00
1.50
1.00
0.50
0 5 10 15 20 25 30 35 40 45 50
PERCENT PROTEIN
a.
Y=1.21 + 0.017BX
r²=70.7% p<0.001
Y=1.28 + 0.017BX
r²=53.3% p<0.001
PERCENT PROTEIN
b.

Figure 6. Effects of dietary protein on organ weights, expressed per 100g BW, of white rats after eight weeks at room temperature. The upper figures represent organ weights without caloric restriction and the lower figures represent the combined effects of protein and caloric restriction. Controls = open circles, LP = filled circles, and LC = open triangles.

THE EFFECTS OF DIETARY PROTEIN LEVELS ON ORGAN WEIGHTS AT ROOM TEMPERATURE

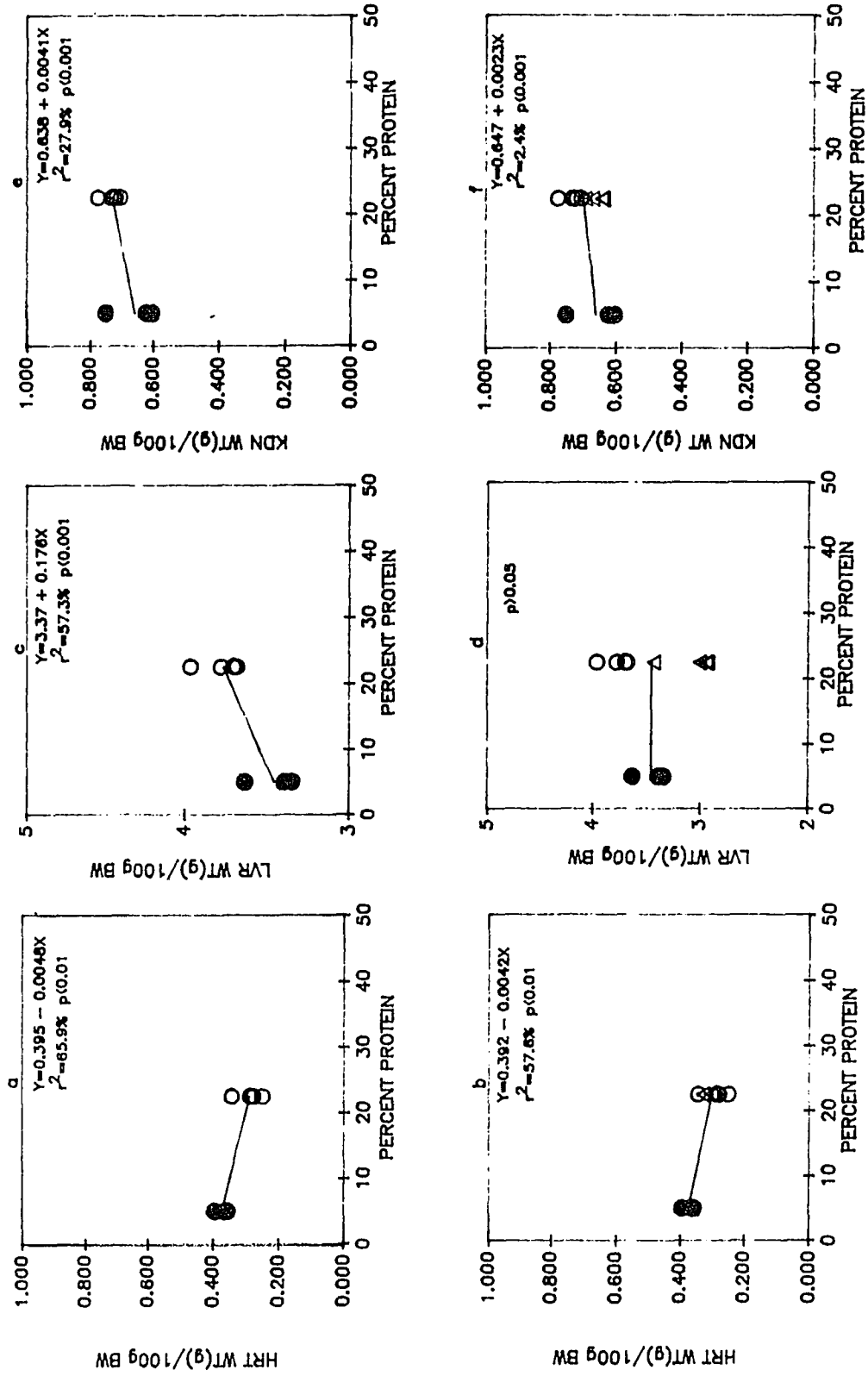


Figure 7. Effects of dietary protein on hematological values of white rats fed varied diets for eight weeks at room temp. The upper figures represent values without effects of caloric restriction and the lower figures show the combined effects of protein and calorie restriction. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

THE EFFECTS OF DIETARY PROTEIN LEVELS ON HEMATOLOGICAL VALUES AT ROOM TEMPERATURE

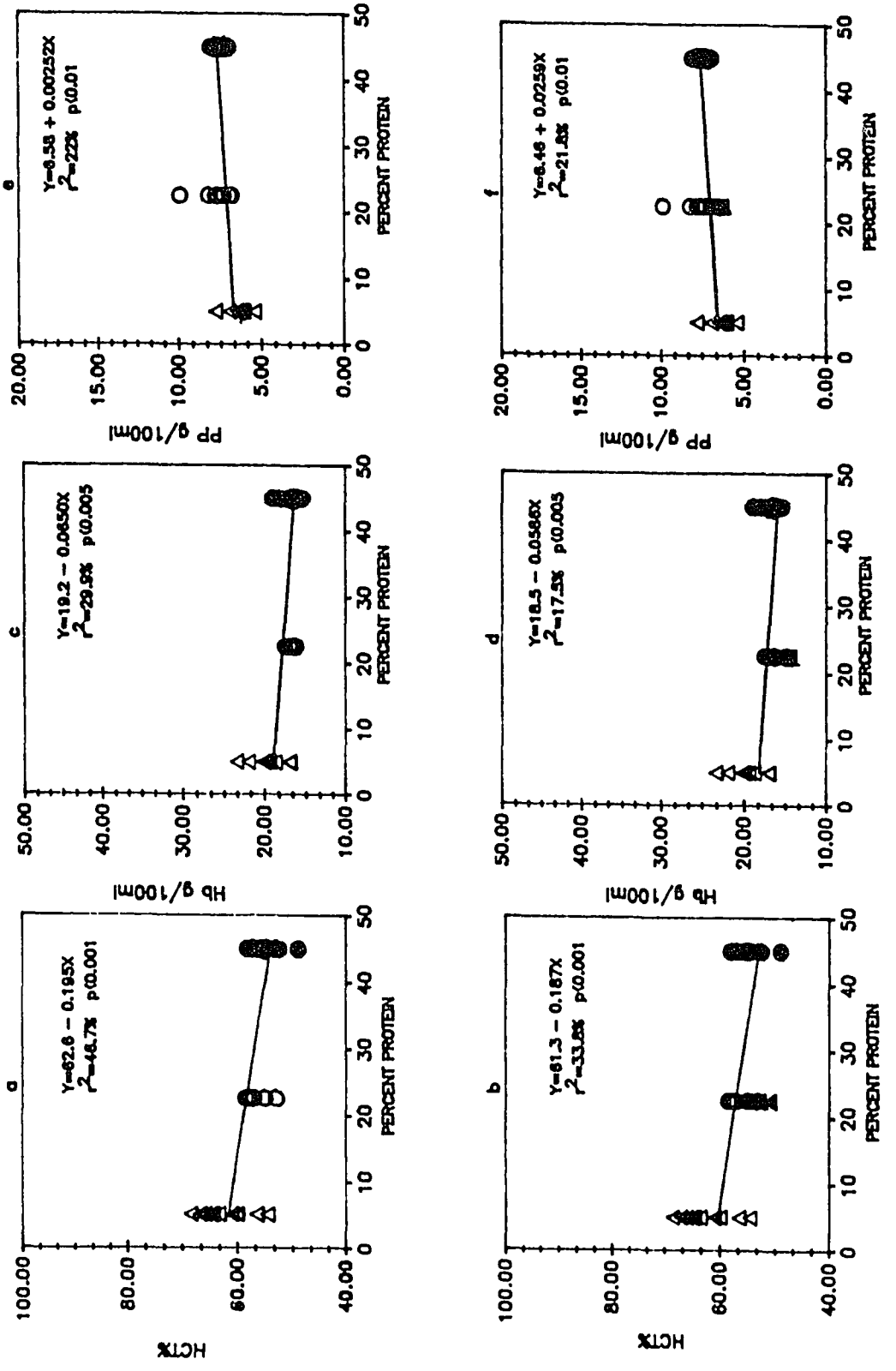


Figure 8. The mean $\dot{V}O_2$ of white rats fed varied diets for eight weeks at room temperature. Error bars represent S.D. Bars with different letters represent groups that are significantly different.

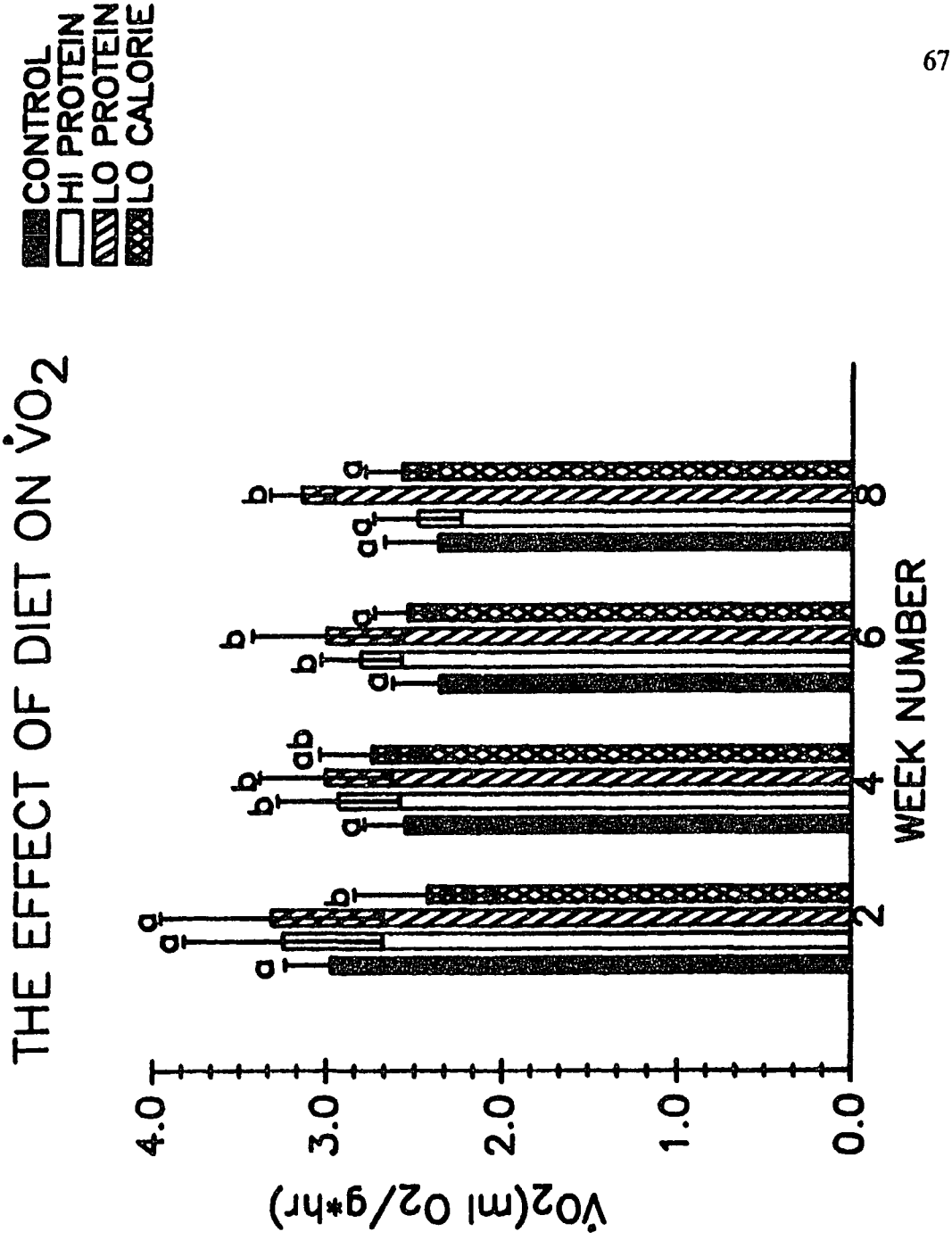

CONTROL
HI PROTEIN
LO PROTEIN
LO CALORIE
THE EFFECT OF DIET ON $\dot{V}O_2$
$\dot{V}O_2$(ml O2/g*hr)
4.0
3.0
2.0
1.0
0.0
2
4
6
8
WEEK NUMBER

Figure 9. Effects of dietary protein on thyroid function of white rats fed varied diets for eight weeks at room temperature. Upper figures represent effects due to dietary protein levels alone and lower figures represent effects of both protein and caloric content. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

THE EFFECTS OF DIETARY PROTEIN LEVELS ON PLASMA THYROID HORMONES

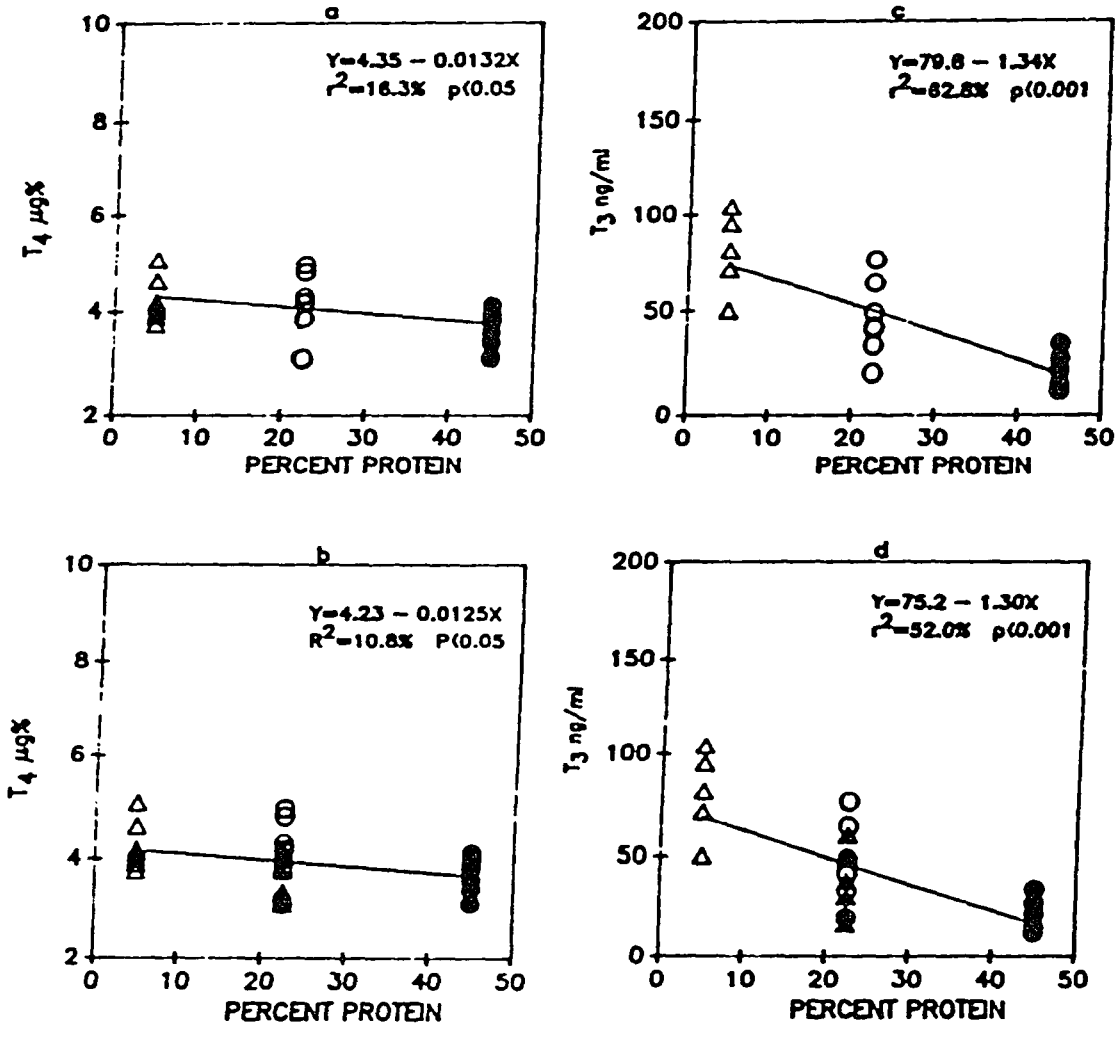


Figure 10. The mean weekly rectal temperatures of white rats fed varied diets for eight weeks at room temperature. Error bars represent S.D.

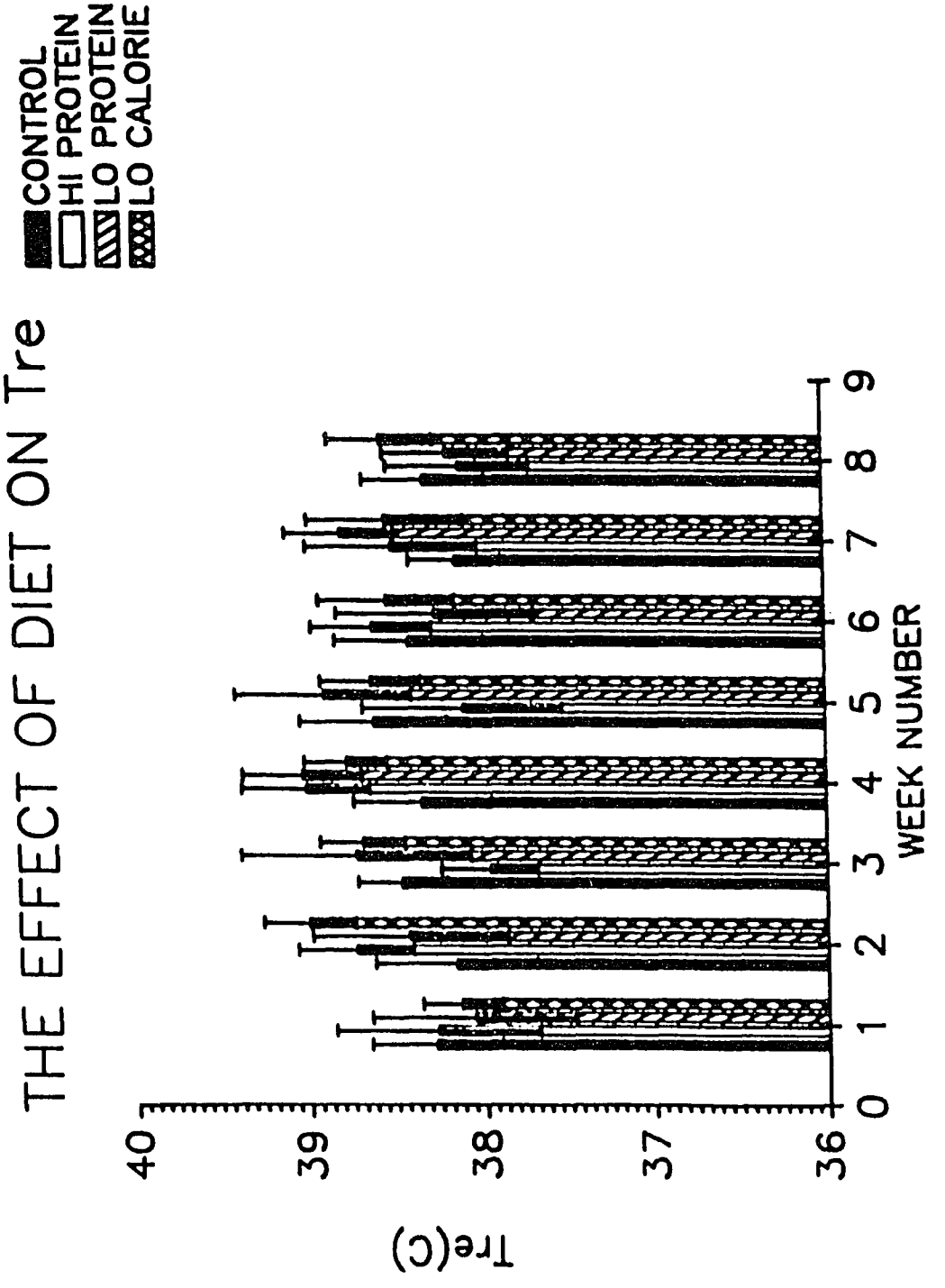

THE EFFECT OF DIET ON Tre
CONTROL
HI PROTEIN
LO PROTEIN
LO CALORIE
Tre(C)
40
39
38
37
36
0
1
2
3
4
5
6
7
8
9
WEEK NUMBER

Figure 11. The BW of white rats fed varied diets and kept at 5°C for seven days. Values are mean BW assessed at days 1, 5 and 7. Error bars represent $\pm$ S.D. Bars with different letters represent groups that are significantly different.

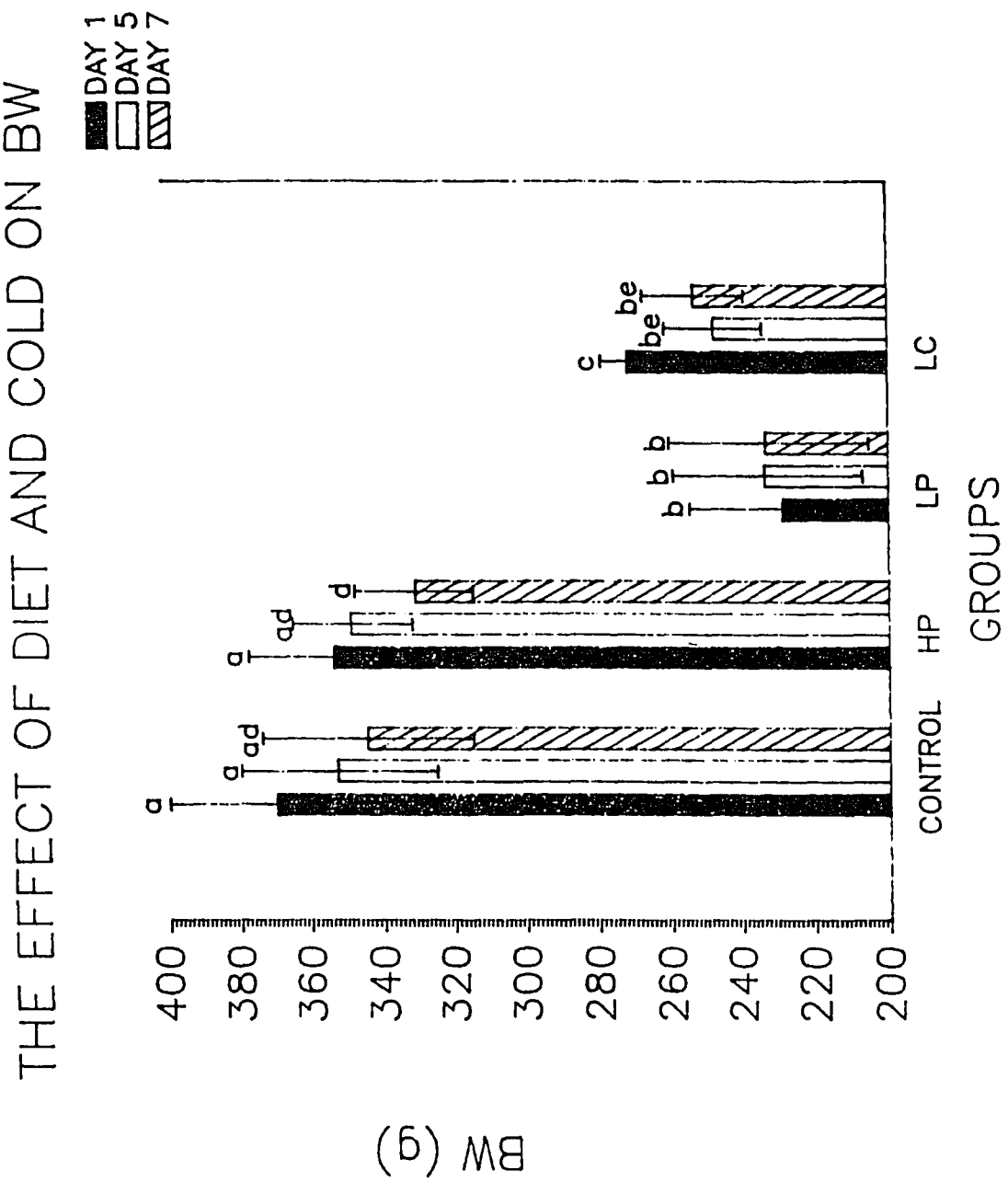

THE EFFECT OF DIET AND COLD ON BW
DAY 1
DAY 5
DAY 7
BW (g)
400
380
360
340
320
300
280
260
240
220
200
a
a
ad
ad
a
d
d
b
b
b
c
be
be
CONTROL
HP
LP
LC
GROUPS

Figure 12. Effects of dietary protein on the growth of white rats at 5°C expressed without caloric restriction (Figure 12a) and in conjunction with caloric restriction (Figure 12b). Values represent the difference in BW from day one to day seven of cold exposure. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

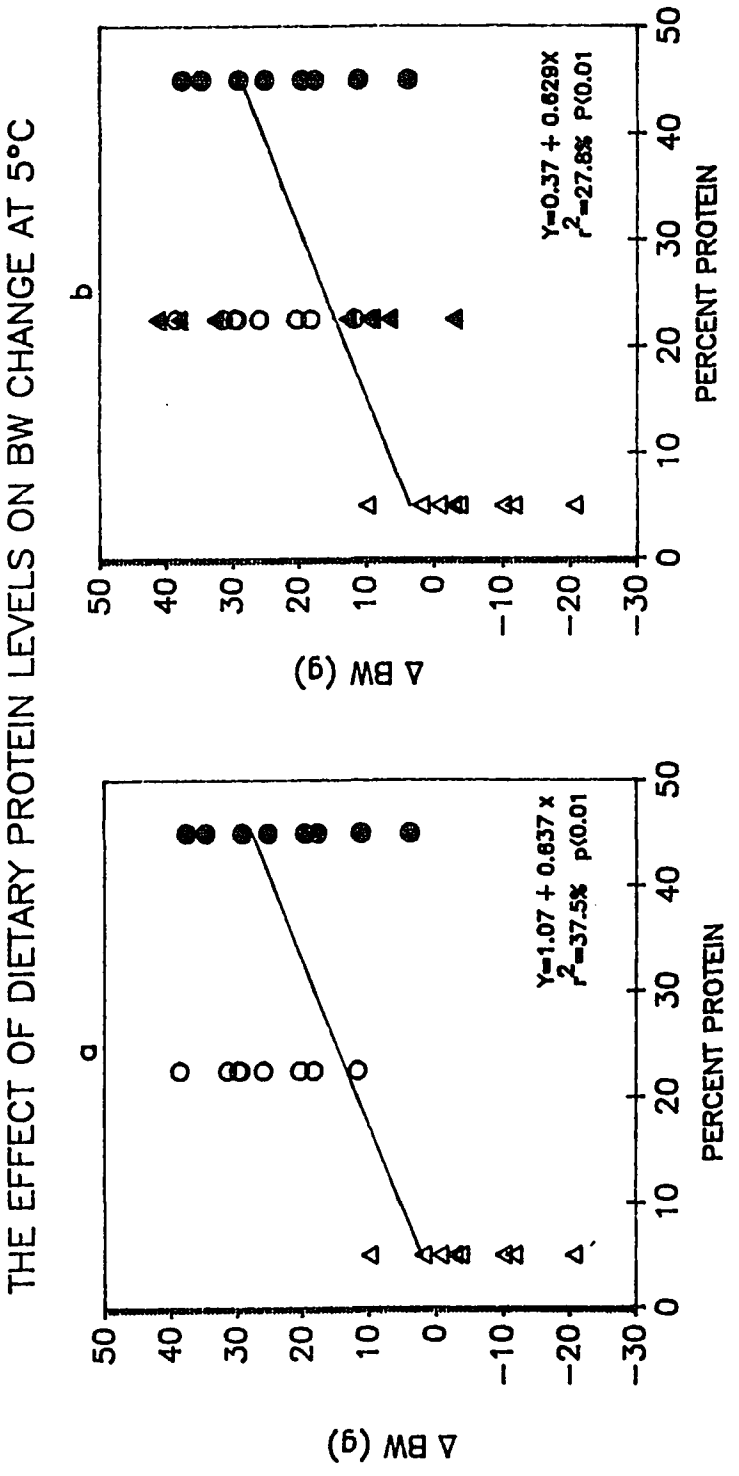

THE EFFECT OF DIETARY PROTEIN LEVELS ON BW CHANGE AT 5°C
a
b
Y=1.07 + 0.837 x
r²=37.5% p<0.01
Y=0.37 + 0.629X
r²=27.8% P<0.01
Δ BW (g)
PERCENT PROTEIN
50
40
30
20
10
0
−10
−20
−30
0
10
20
30
40
50

Figure 13. Effects of ambient temperature on FI, expressed per g BW per day, of white rats fed varied diets. Error bars represent $\pm$ S.D.

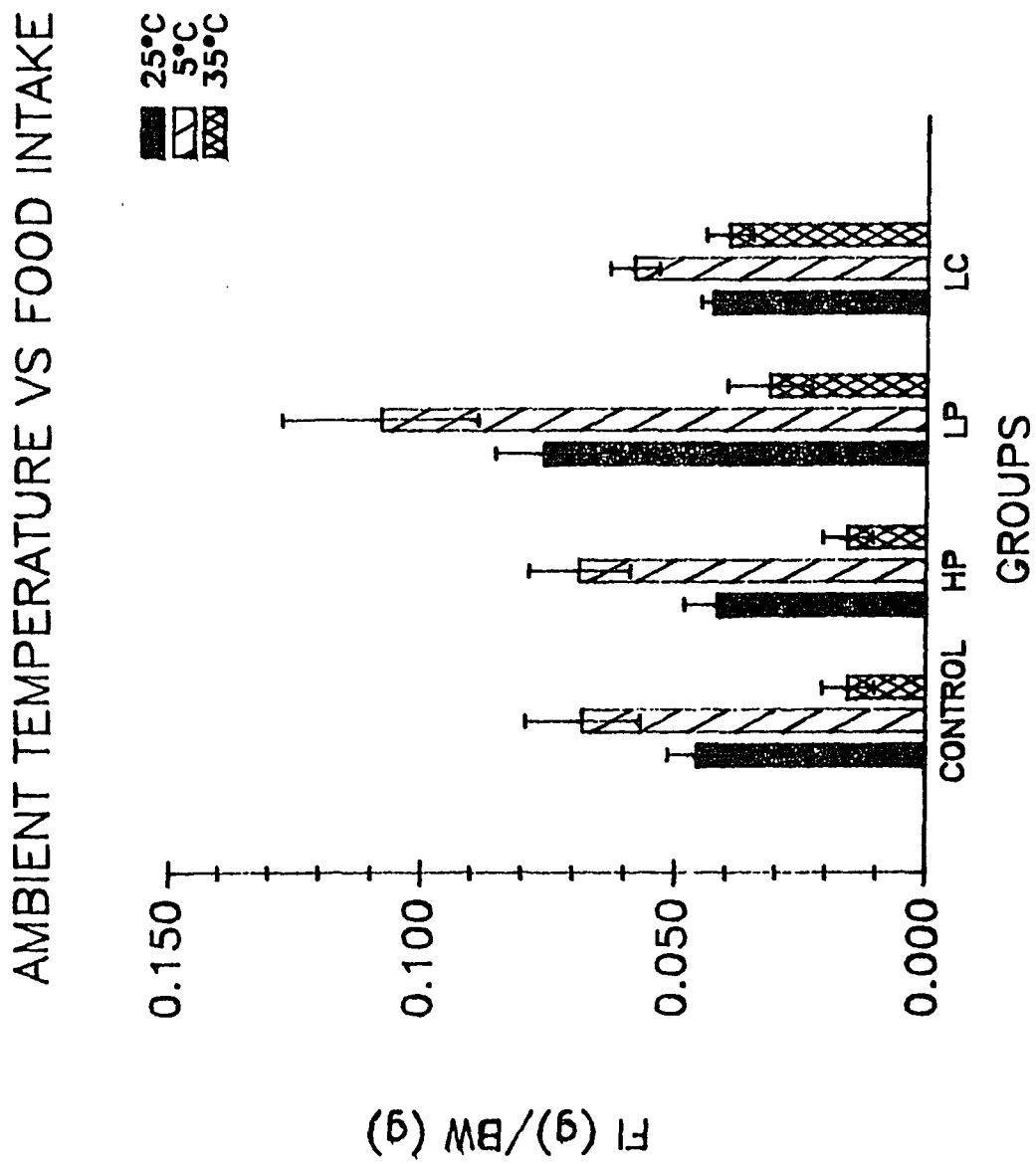

AMBIENT TEMPERATURE VS FOOD INTAKE
25°C
5°C
35°C
0.150
0.100
0.050
0.000
FI (g)/BW (g)
CONTROL
HP
LP
LC
GROUPS

Figure 14. Effects of dietary protein and temperature on FI, expressed per g BW per day, of white rats for one week at 5°C or 35°C. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

THE EFFECT OF DIETARY PROTEIN LEVELS AND TEMPERATURE ON FOOD INTAKE

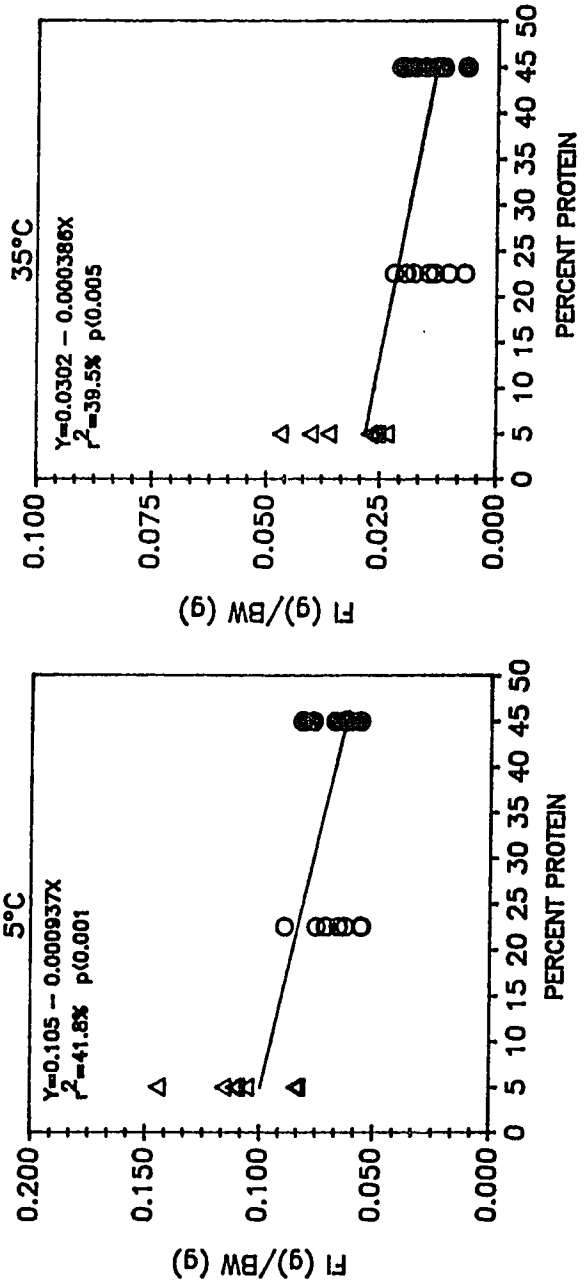


Figure 15. Effects of ambient temperature on mean WI, expressed per g FI per day, of white rats. Error bars represent $\pm$ S.D.

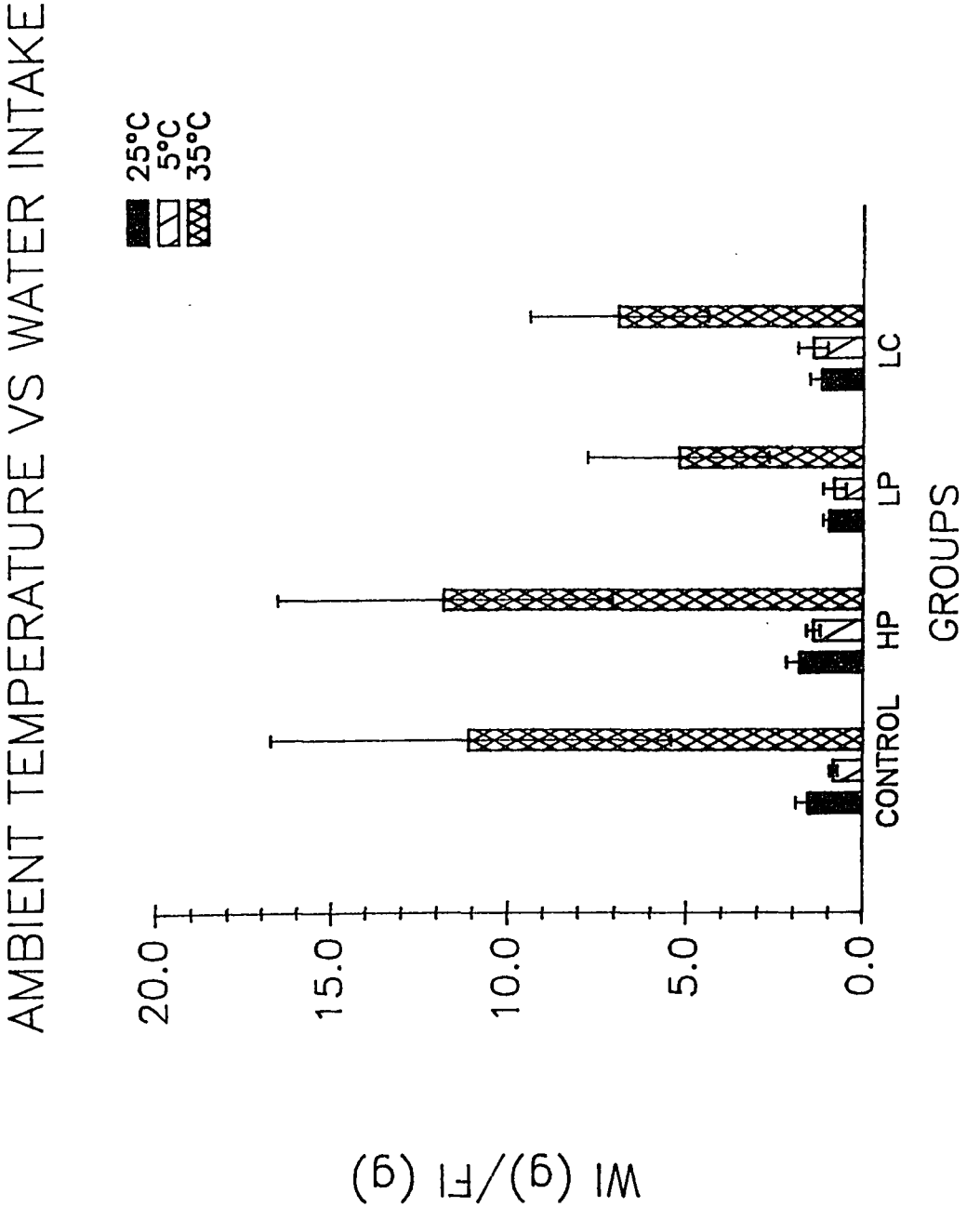

AMBIENT TEMPERATURE VS WATER INTAKE
25°C
5°C
35°C
20.0
15.0
10.0
5.0
0.0
CONTROL
HP
LP
LC
GROUPS
WI (g)/FI (g)

Figure 16. Effects of dietary protein and cold on WI, expressed per g FI per day, of white rats for one week at 5°C. Figure 16a represents the effects without caloric restriction and Figure 16b shows the effects with caloric restriction. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

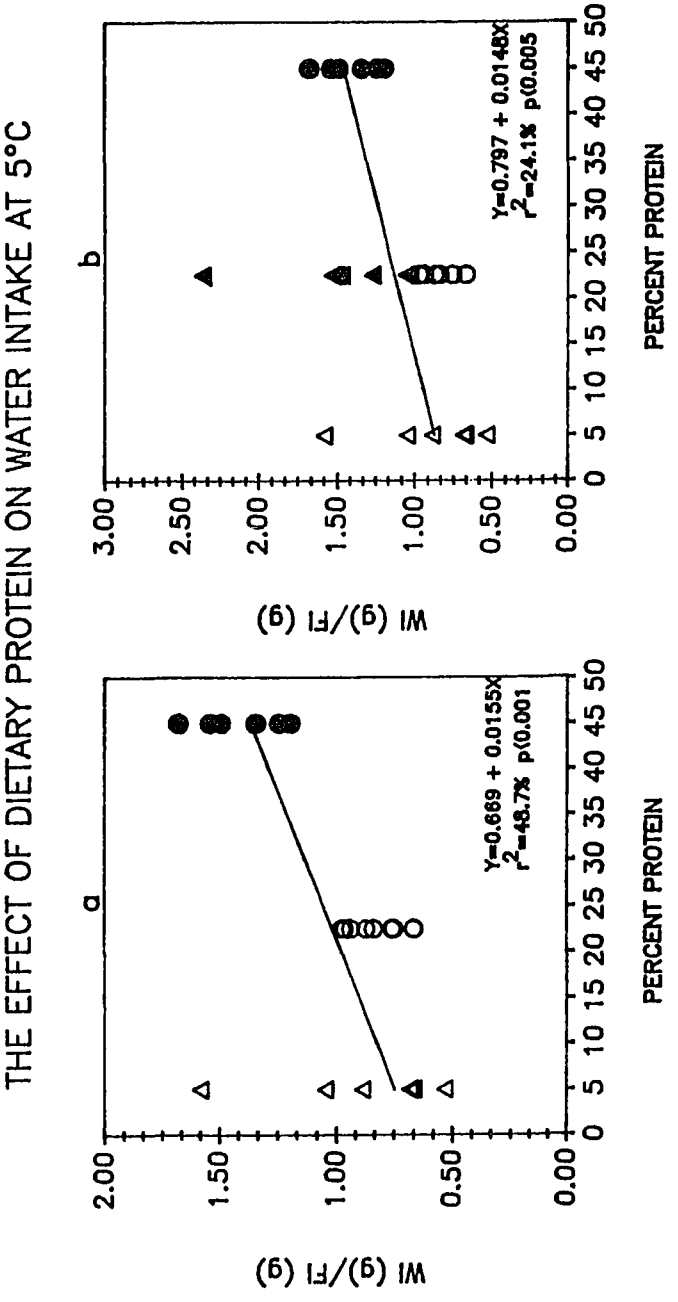

THE EFFECT OF DIETARY PROTEIN ON WATER INTAKE AT 5°C
a
b
WI (g)/FI (g)
PERCENT PROTEIN
Y=0.669 + 0.0155X
r²=48.7% p<0.001
Y=0.797 + 0.0148X
r²=24.1% p<0.005
0.00
0.50
1.00
1.50
2.00
2.50
3.00
0 5 10 15 20 25 30 35 40 45 50

Figure 17. Effects of dietary protein and ambient temperature on kidney weight, expressed per 100g BW, of white rats for one week at 5°C or 35°C. Upper figures represent kidney weights without caloric restriction and the lower figures represent the combined effects of protein and caloric restriction. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

THE EFFECT OF DIETARY PROTEIN AND TEMPERATURE ON KIDNEY WEIGHT

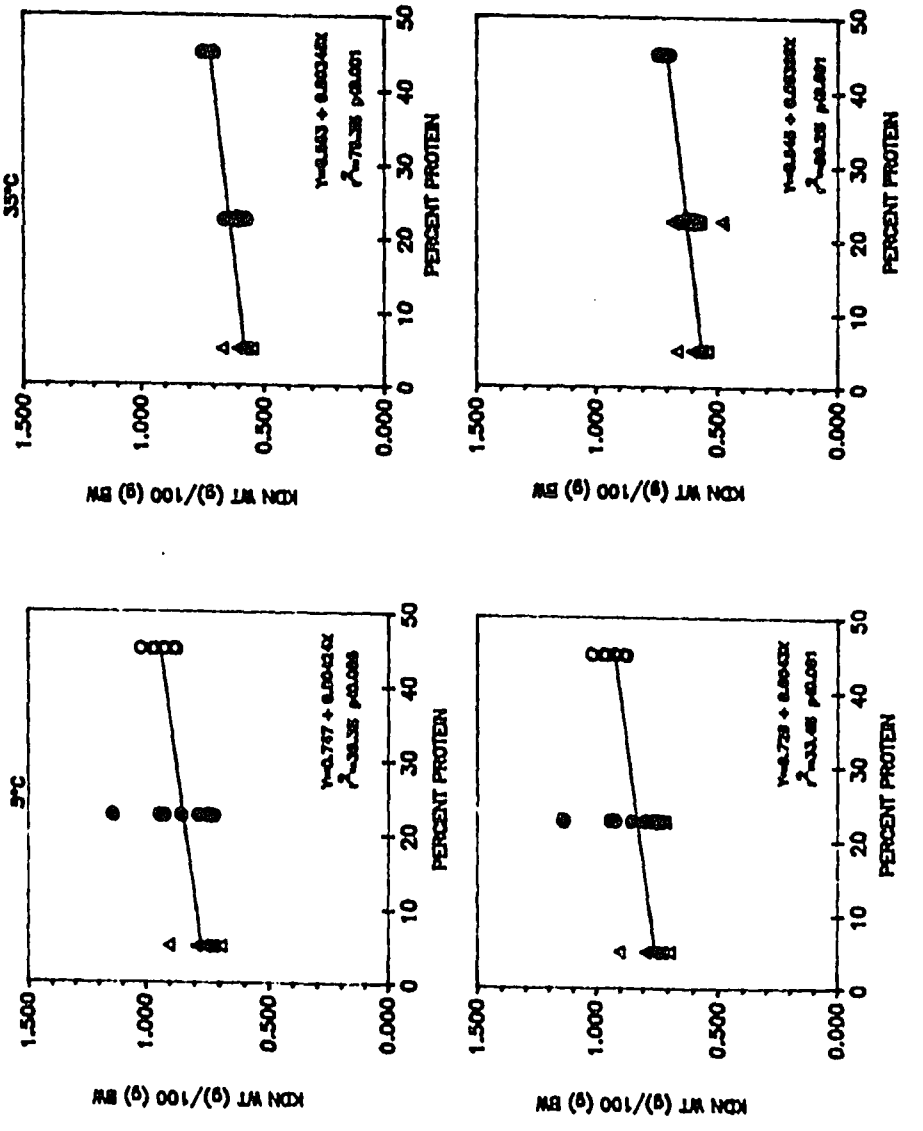


Figure 18. Effects of dietary protein and cold on hematological values of white rats fed varied diets for one week at 5°C. The upper figures represent values without effects of caloric restriction and the lower figures show the combined effects of protein and calorie restriction. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

THE EFFECT OF DIETARY PROTEIN AND COLD ON HEMATOLOGICAL VALUES

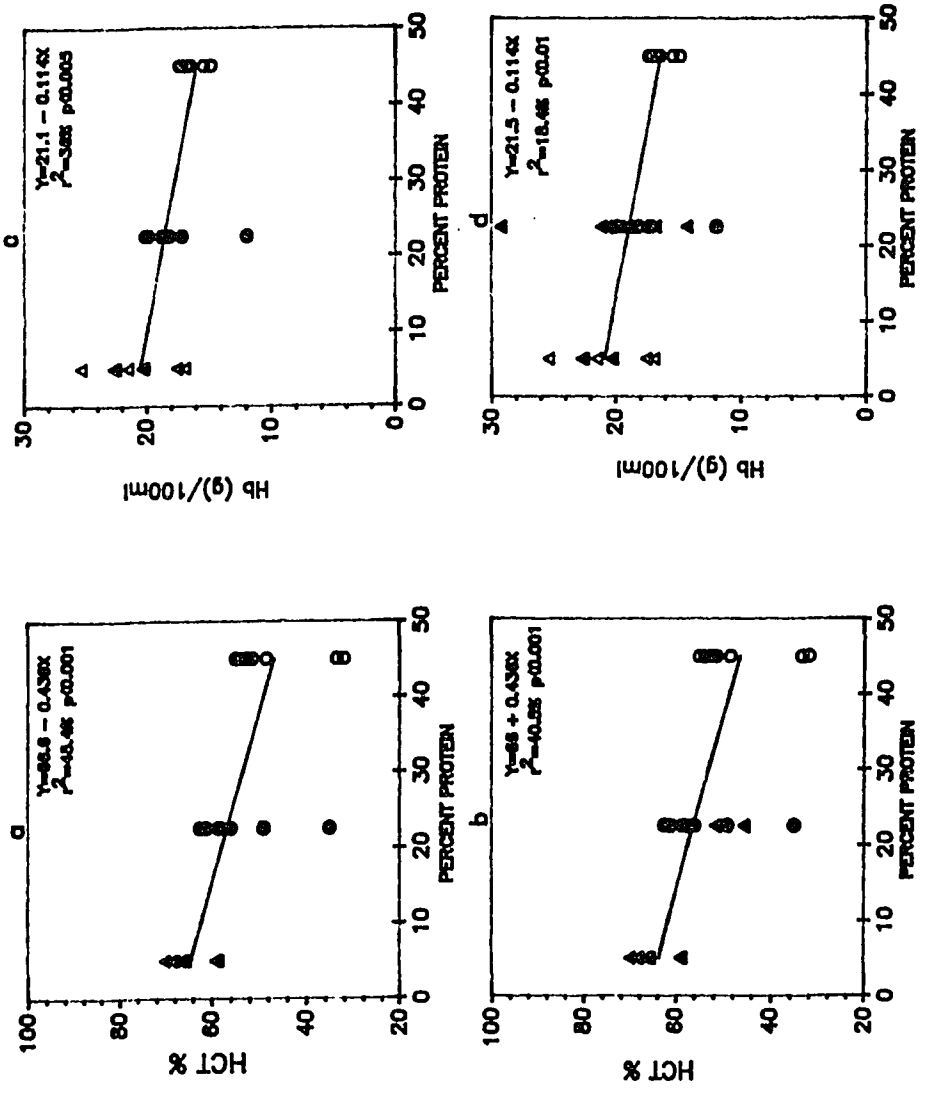


Figure 19. Effects of diet and ambient temperature on mean $\dot{V}O_2$ of white rats fed varied diets. Error bars represent $\pm$ S.D. Bars with different letters represent groups that are significantly different.

THE EFFECT OF DIET AND TEMPERATURE ON $\dot{V}O_2$

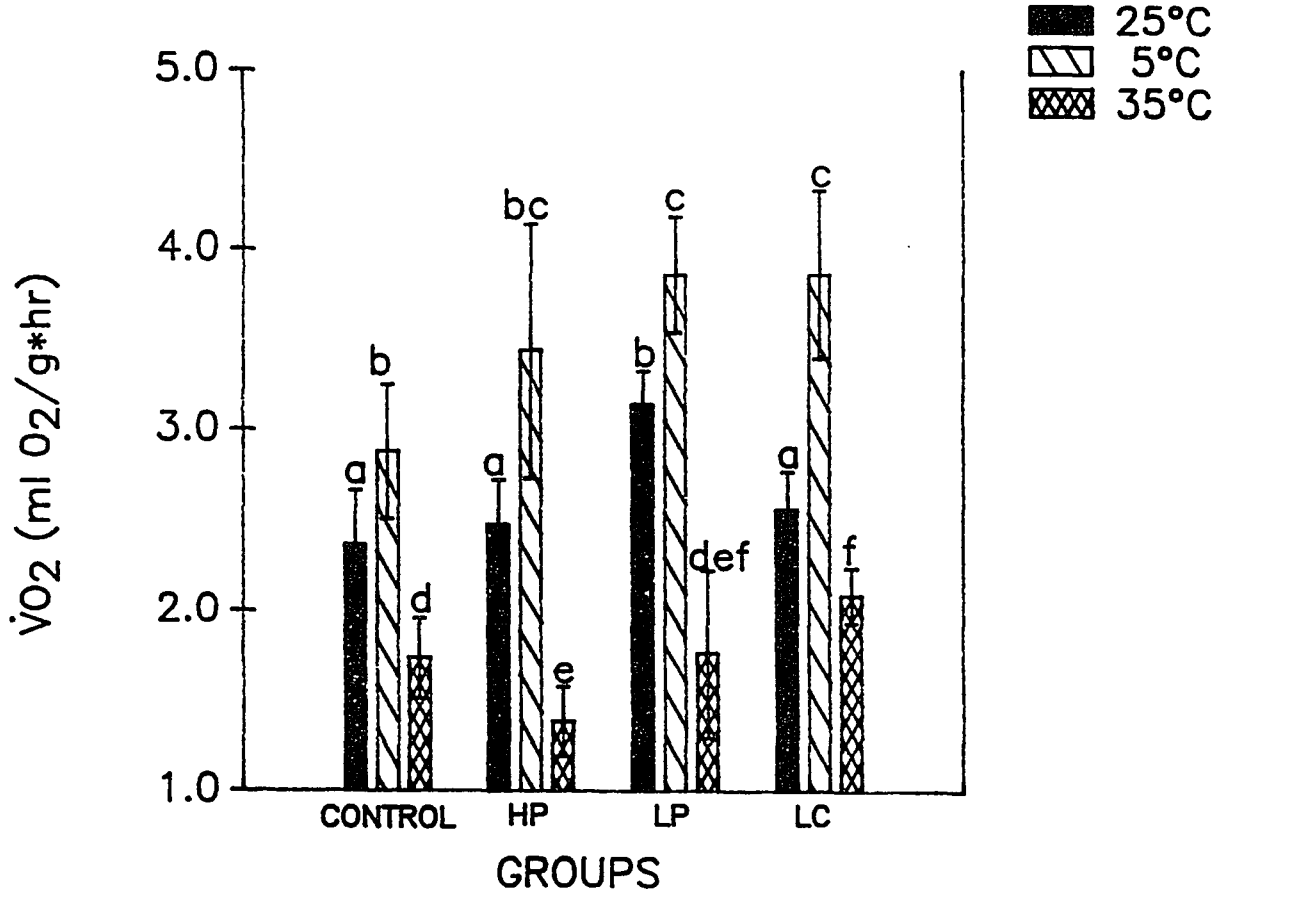


Figure 20. Effects of diet and cold on plasma T_3 of white rats for one week at 5°C. Figure 20a represents T_3 concentrations without caloric restriction and Figure 20b represents the combined effects of protein levels and caloric restriction on T_3 concentrations. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

THE EFFECTS OF DIETARY PROTEIN AND COLD ON PLASMA T_3 LEVELS

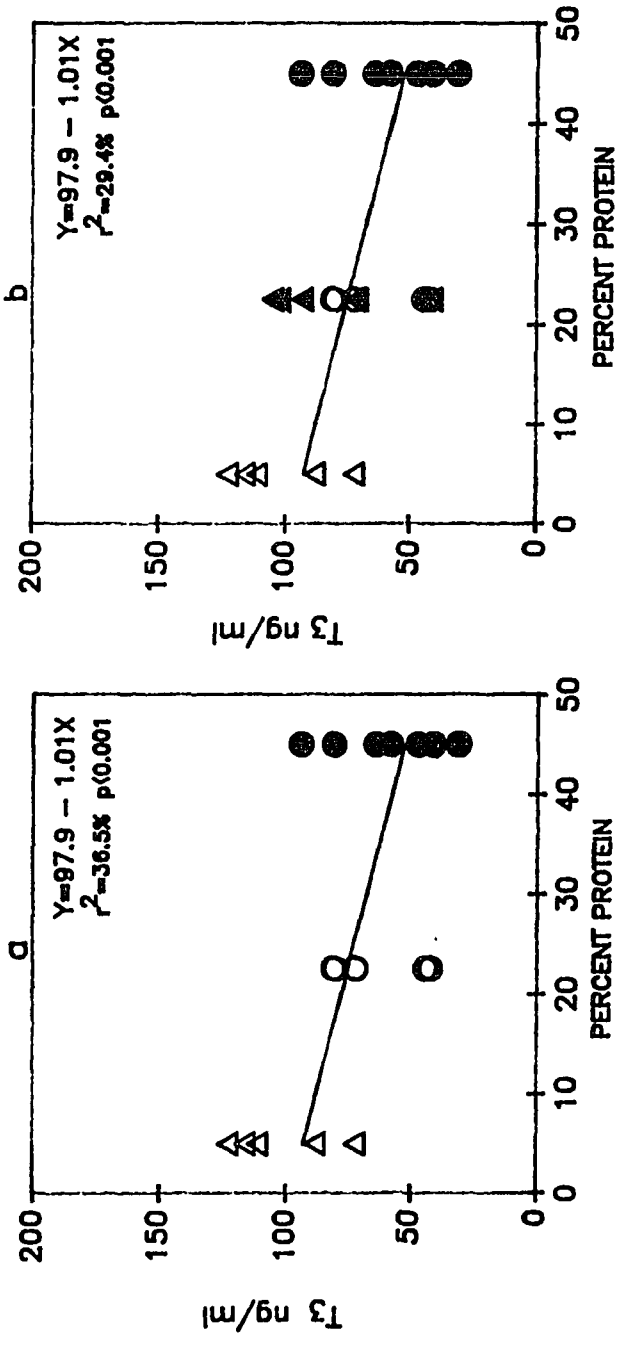


Figure 21. The mean daily rectal temperatures of white rats fed varied diets for one week at 5°C. Error bars represent $\pm$ S.D.

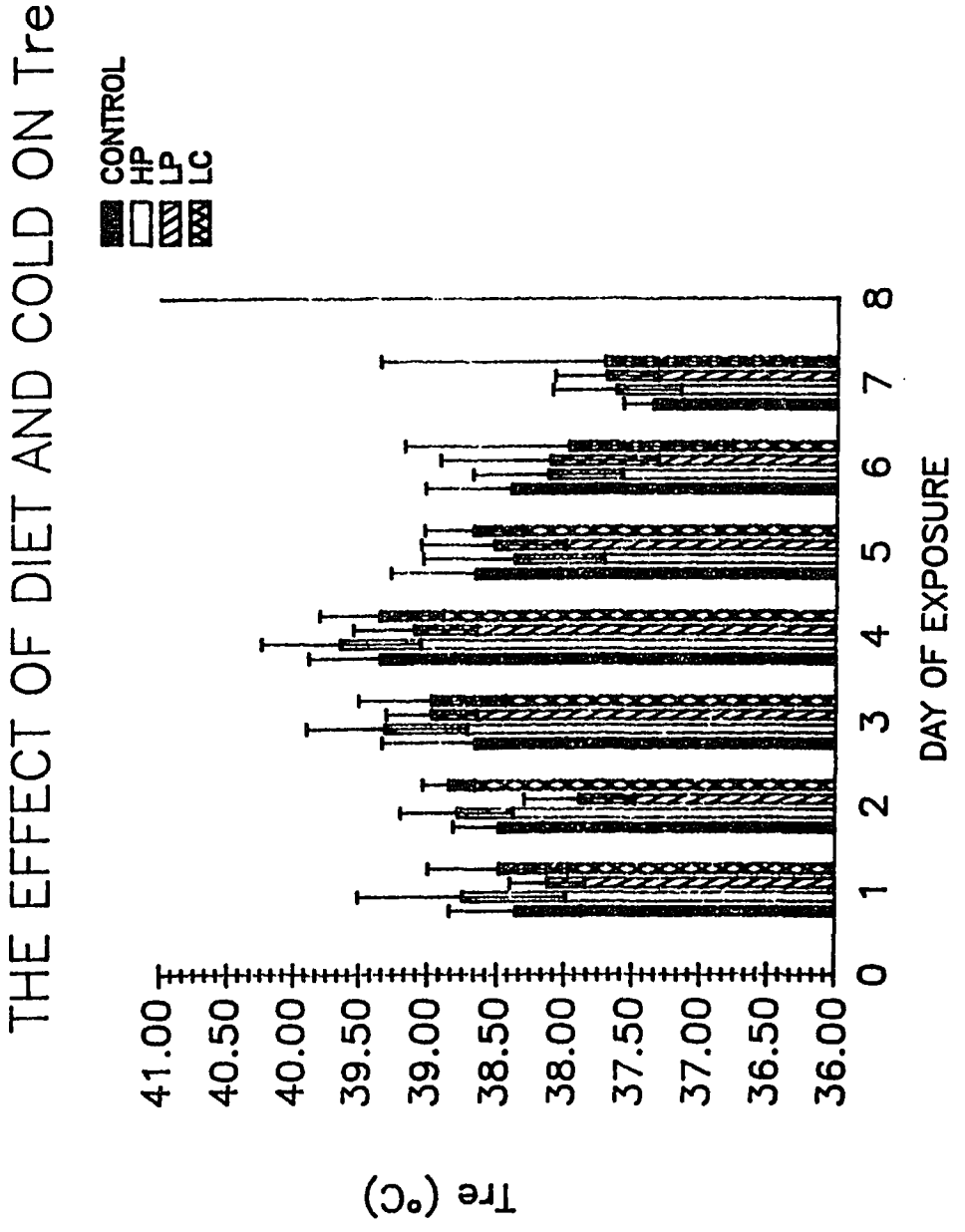

THE EFFECT OF DIET AND COLD ON Tre
CONTROL
HP
LP
LC
41.00
40.50
40.00
39.50
39.00
38.50
38.00
37.50
37.00
36.50
36.00
Tre (°C)
0
1
2
3
4
5
6
7
8
DAY OF EXPOSURE

Figure 22. The BW of white rats fed varied diets and kept at 35°C for seven days. Values are mean BW assessed at days 1, 5 and 7. Error bars represent $\pm$ S.D. Bars with different letters represent groups that are significantly different.

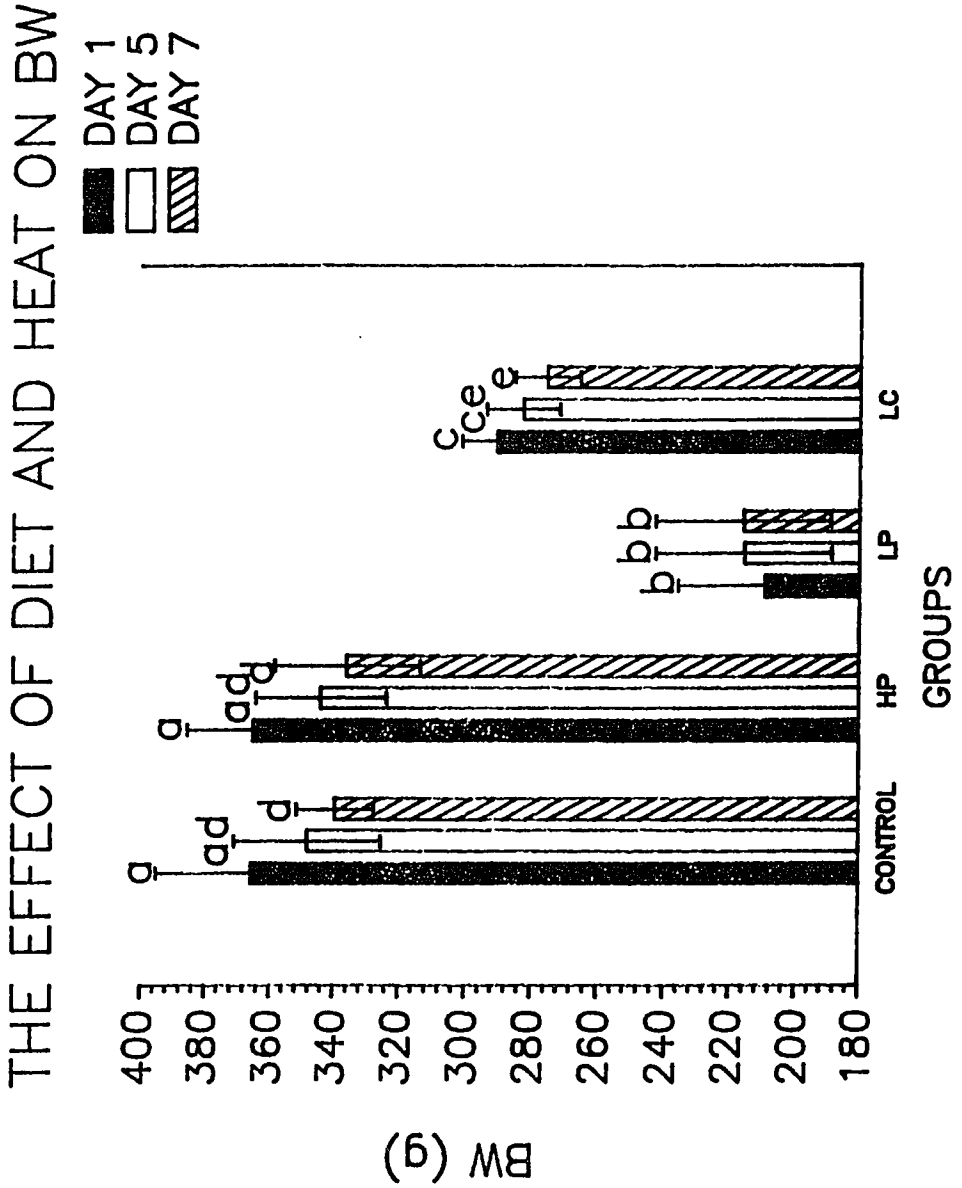

THE EFFECT OF DIET AND HEAT ON BW
DAY 1
DAY 5
DAY 7
BW (g)
400
380
360
340
320
300
280
260
240
220
200
180
a
ad
d
a
ad
d
b
b
b
c
ce
e
CONTROL
HP
LP
LC
GROUPS

Figure 23. Effects of dietary protein on the growth of white rats at 35°C expressed without caloric restriction (Figure 23a) and in conjunction with caloric restriction (Figure 23b). Values represent the difference in BW from day one to day seven of cold exposure. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

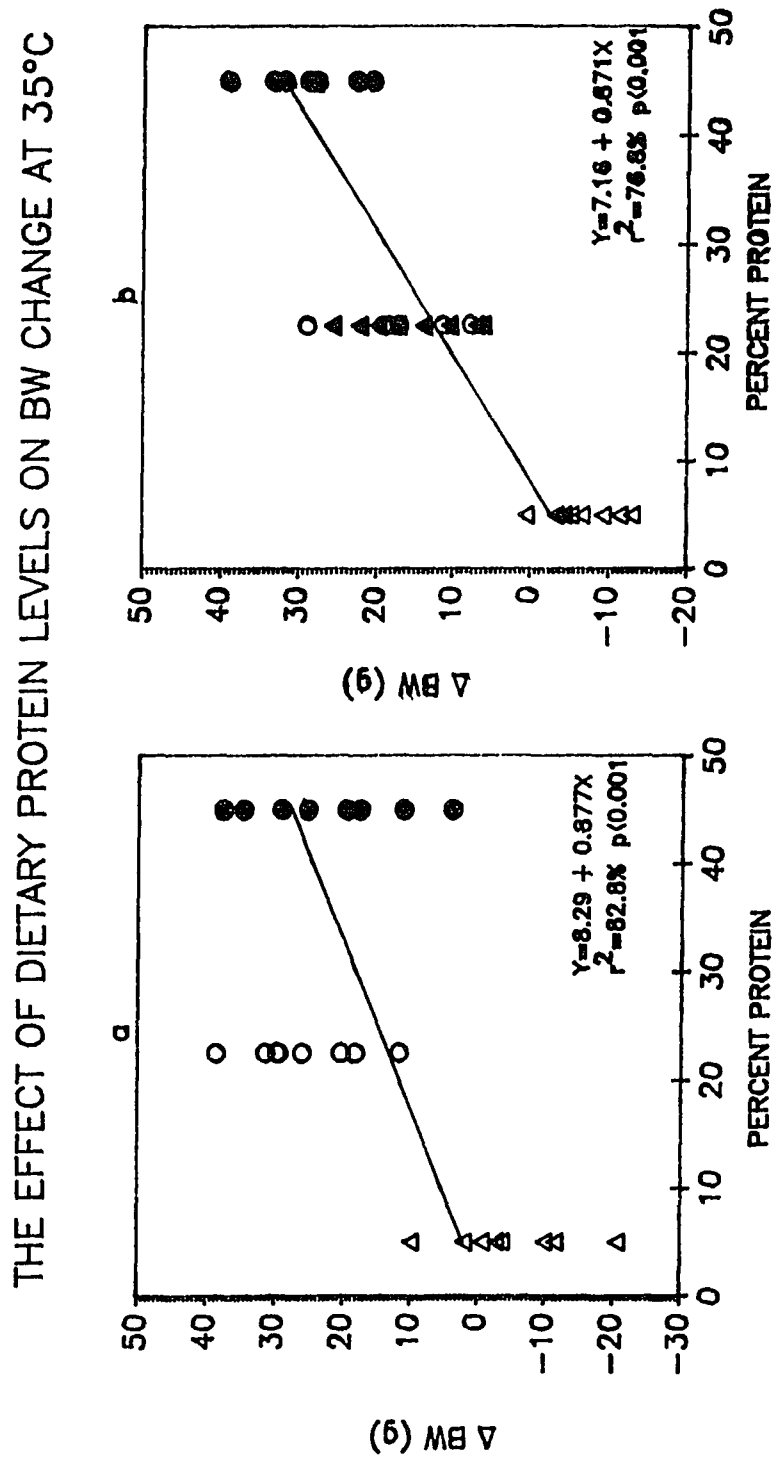

THE EFFECT OF DIETARY PROTEIN LEVELS ON BW CHANGE AT 35°C
a
b
Y=8.29 + 0.877X
r²=82.8% p<0.001
Y=7.16 + 0.871X
r²=76.8% p<0.001
Δ BW (g)
PERCENT PROTEIN

Figure 24. Effects of dietary protein and heat on WI, expressed per g FI per day, of white rats for one week at 35°C. Figure 24a represents the effects without caloric restriction and Figure 24b shows the effects with caloric restriction. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

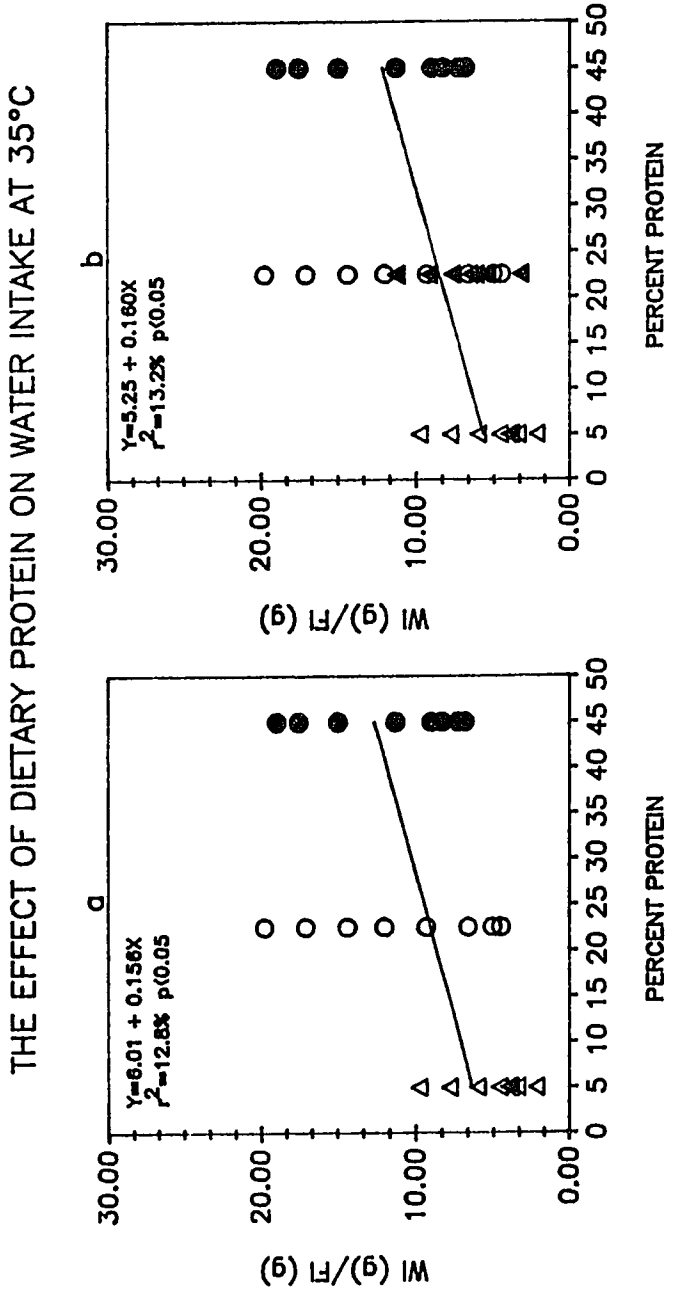

THE EFFECT OF DIETARY PROTEIN ON WATER INTAKE AT 35°C
a
Y=6.01 + 0.156X
r²=12.8% p<0.05
b
Y=5.25 + 0.160X
r²=13.2% p<0.05
WI (g)/FI (g)
30.00
20.00
10.00
0.00
0 5 10 15 20 25 30 35 40 45 50
PERCENT PROTEIN

Figure 25. Effects of dietary protein and heat on hematological values of white rats fed varied diets for one week at 35°C. The upper figures represent values without effects of caloric restriction and the lower figures show the combined effects of protein and calorie restriction. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

THE EFFECT OF DIETARY PROTEIN AND HEAT ON HEMATOLOGICAL VALUES

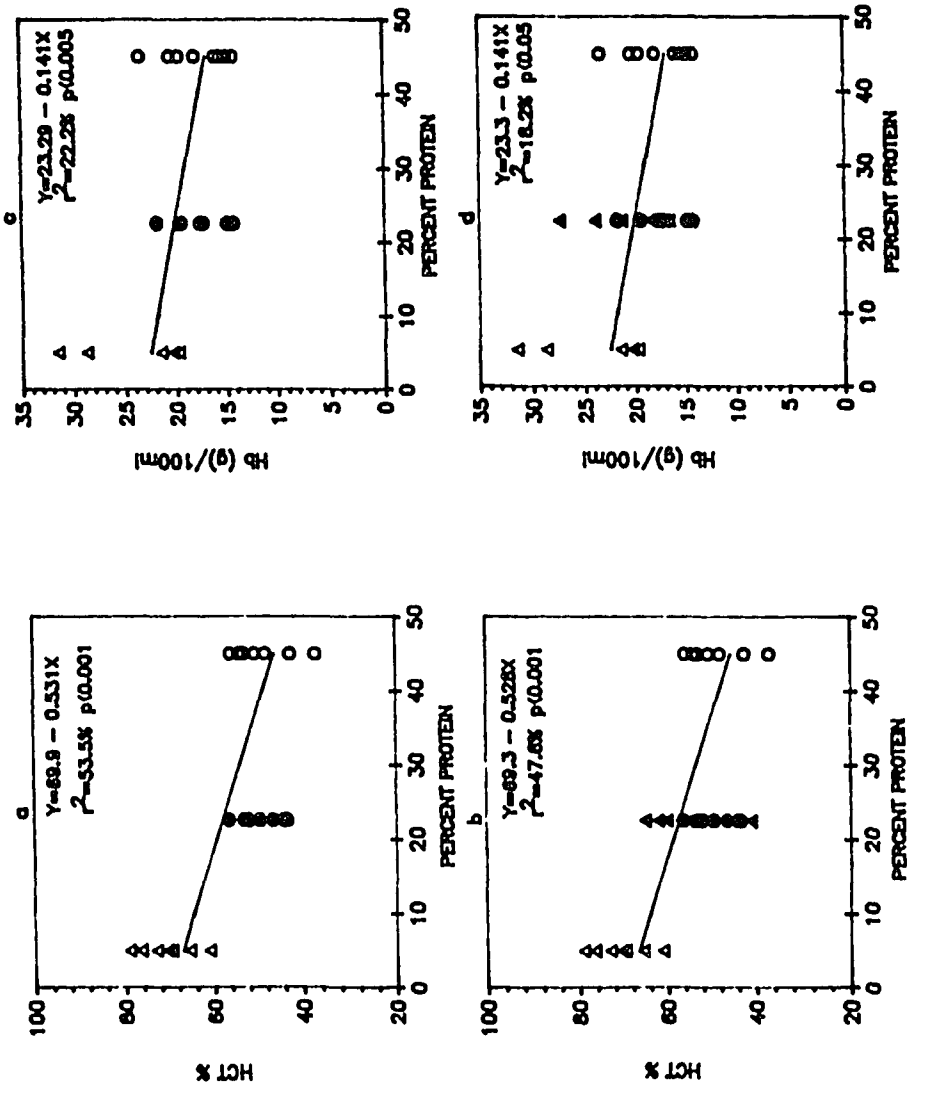


Figure 26. Effects of dietary protein and heat on $\dot{V}O_2$ of white rats fed varied diets for one week at 35°C. Figure 26a represents the effects without caloric restriction and Figure 26b shows the effects with caloric restriction. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

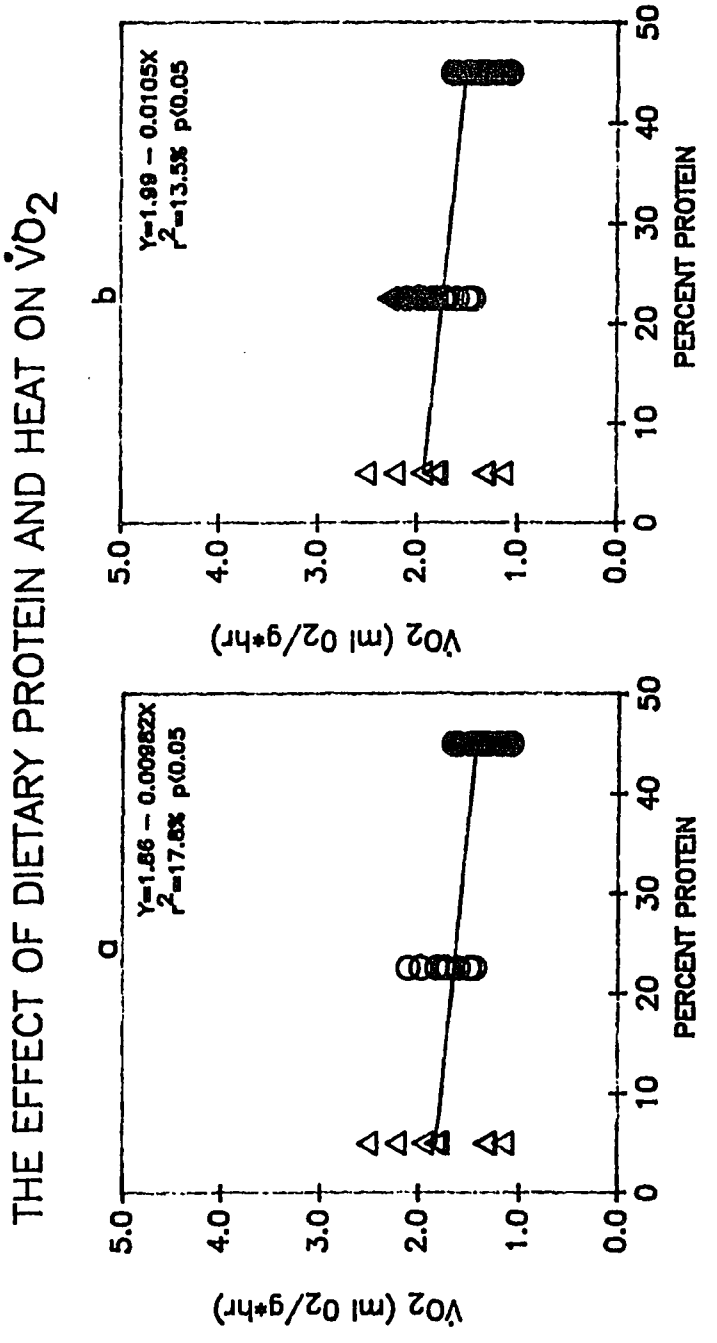

THE EFFECT OF DIETARY PROTEIN AND HEAT ON $\dot{V}O_2$
a
Y=1.86 – 0.00982X
r^2=17.8% p<0.05
b
Y=1.99 – 0.0105X
r^2=13.5% p<0.05
$\dot{V}O_2$ (ml O2/g*hr)
PERCENT PROTEIN
0.0
1.0
2.0
3.0
4.0
5.0
0
10
20
30
40
50

Figure 27. Effects of diet and heat on plasma T_3 of white rats for one week at 35°C. Figure 27a represents T_3 concentrations without caloric restriction and Figure 27b represents the combined effects of protein levels and caloric restriction on T_3 concentrations. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

THE EFFECTS OF DIETARY PROTEIN AND HEAT ON PLASMA T_3 LEVELS

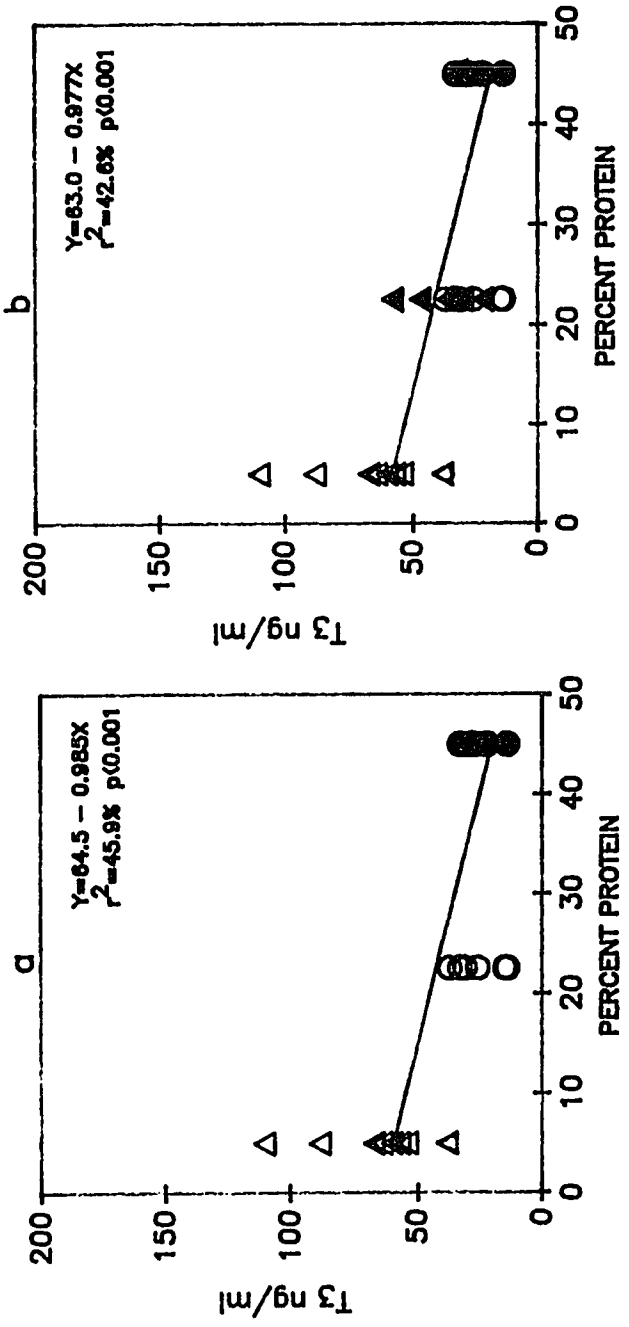


Figure 28. Effects of diet and heat on plasma T_4 of white rats for one week at 35°C. Figure 28a represents T_4 concentrations without caloric restriction and Figure 28b represents the combined effects of protein levels and caloric restriction on T_4 concentrations. Controls = open circles, HP = filled circles, LP = open triangles, and LC = filled triangles.

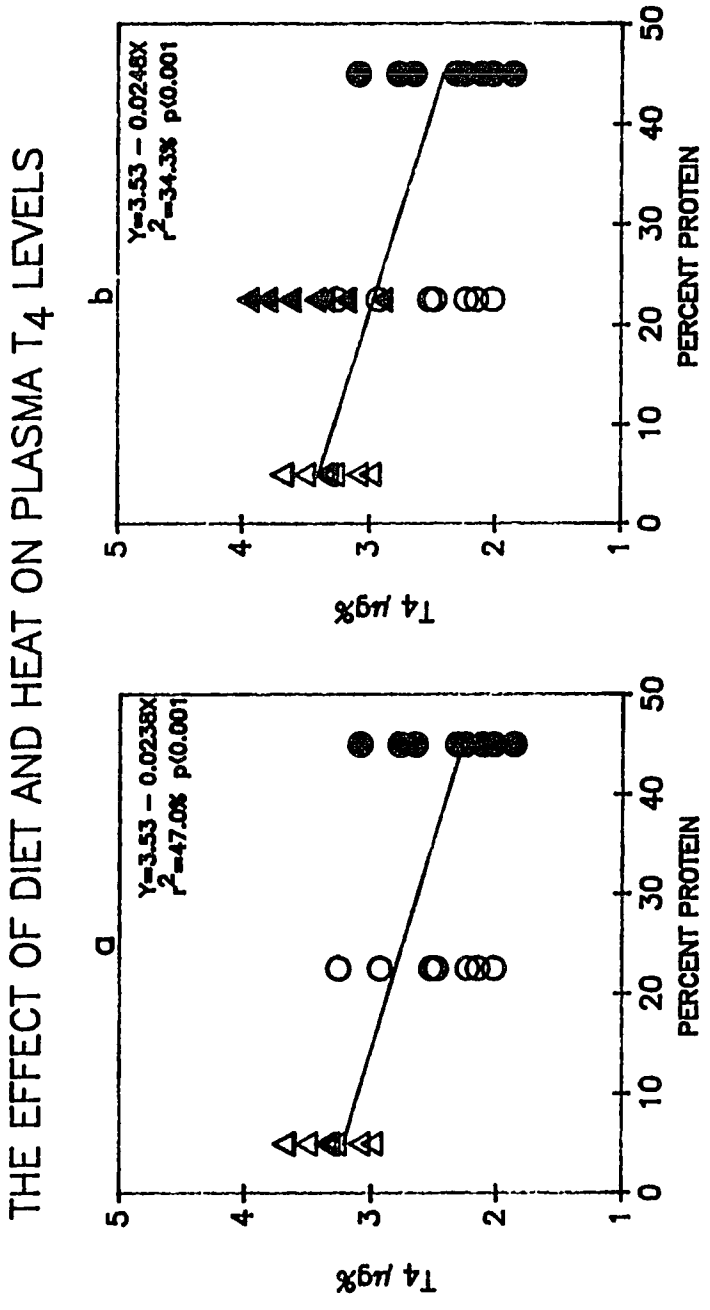

THE EFFECT OF DIET AND HEAT ON PLASMA T4 LEVELS
a
Y=3.53 – 0.0238X
r²=47.0% p<0.001
b
Y=3.53 – 0.0248X
r²=34.3% p<0.001
T4 µg%
PERCENT PROTEIN
1
2
3
4
5
0
10
20
30
40
50

Figure 29. Effects of diet and heat on plasma FT_4 of white rats for one week at 35°C. Figure 29a represents FT_4 concentrations without caloric restriction and Figure 29b represents the combined effects of protein levels and caloric restriction on FT_4 concentrations. Controls=open circles, HP=filled circles, LP=open triangles, and LC=filled triangles.

THE EFFECT OF DIETARY PROTEIN AND HEAT ON PLASMA FT_4 LEVELS

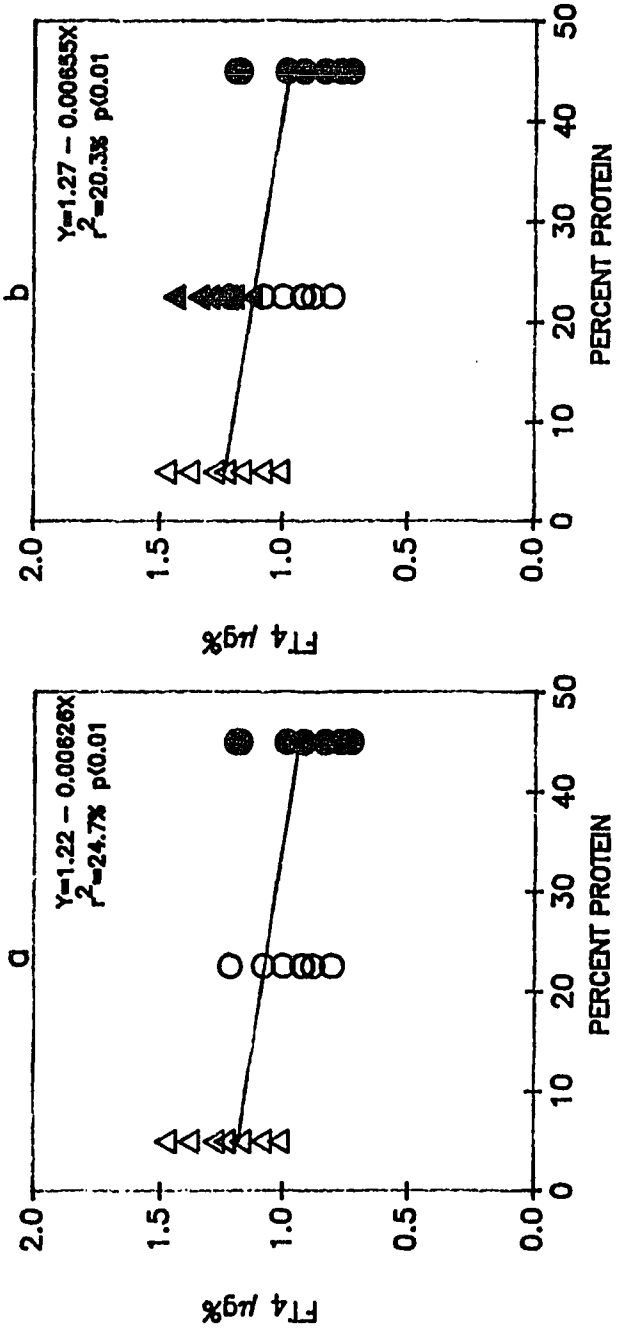
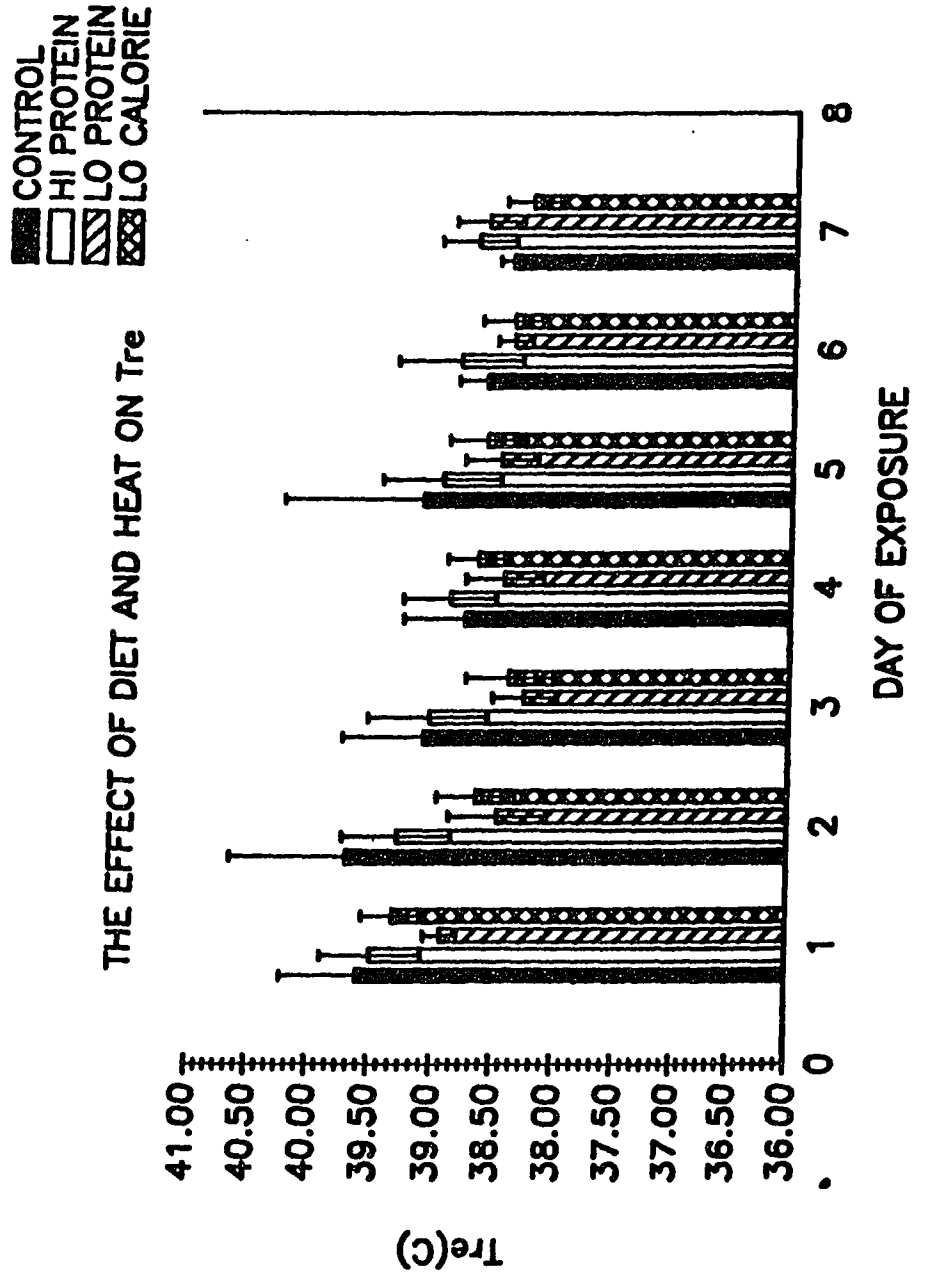


Figure 30. The mean daily rectal temperatures of white rats fed varied diets for one week at 35°C. Error bars represent $\pm$ S.D.


CONTROL
HI PROTEIN
LO PROTEIN
LO CALORIE
THE EFFECT OF DIET AND HEAT ON Tre
Tre(C)
41.00
40.50
40.00
39.50
39.00
38.50
38.00
37.50
37.00
36.50
36.00
0
1
2
3
4
5
6
7
8
DAY OF EXPOSURE

DISCUSSION

A. BODY WEIGHT

In this study, rats fed an HP diet for eight weeks experienced no change in BW. These results confirm a three week study (Hegsted et al., 1970) and a six week study (Harstook et al., 1973). However, these results contradict a 3 week study by Schreiber et al. (1955) that showed a significant increase in weight, as did an 8 week study by Lushbaugh and others (1960).

The fact that all the studies above used diets containing 45%-50% protein suggests that the discrepancy in experimental results might be attributed to: 1) the level of dietary fat consumed; 2) the duration of the study; 3) the type of CHO source; or 4) any combination of all the above.

In this study, a combination of sucrose and dextrin was used for CHO, with fat (lard/corn oil) comprising approximately 10% of the diet. Hegsted et al., (1970) used similar fat percentages with starch as the sole source of CHO. Harstook et al., (1973) also used approximately 10% fat but used glucose as the CHO source.

In a three week study, Schreiber et al. (1955) noted that loss of BW occurred when the CHO was sucrose in combination with 5% fat. This mixture resulted in a higher CHO:fat ratio and a lower protein:CHO ratio; however, the 8 week study by Lushbough et al. (1960) noted weight gain using the same high CHO:fat ratio. The only major difference between these studies was their duration.

Studies using LP diets reported a significant decrease in BW in laboratory rats

over an 8 week period (Villalon et al., 1987; Tulp et al., 1983; Balmagiyia et al., 1983; and Young et al., 1980). When compared to Controls, the LP group of the present study showed a decrease in BW and an increased FI per gram BW as reported in other studies (Donald et al., 1980; Grass et al., 1978; and Young et al., 1980). The rats fed the LP diet seemed to compensate for the low level of protein in their diet by over-eating. Moreover, Young et al. (1980) concluded that the LP diet group developed an "adaptive thermogenesis" which caused the excess non-protein energy to be dissipated through heat production.

In this study, an LC diet was provided for a seven week period and resulted in reduced weight gain from the second week until the end of the experiment; the animals grew at a slower rate (86 gm.) than the control group (200 gm.) during the period of study. Other studies restricting diets to 20%-30% of control group diet reported similar results (Johnson et al., 1970; Kibler et al. 1966 and 1967).

COLD

Exposure to cold caused a significant effect on BW in two of the groups in this study as compared to animals kept at room temperature. HP and LC groups lost weight when exposed to 5°C. This finding supports studies by Bakke et al. (1971) and Jobin et al. (1975). The largest weight loss occurred within the HP group, followed by the LC group. Controls and the LP group experienced no significant weight loss. Similar results in BW loss in rats fed HP and LP diets in cold environments were reported by Stevenson (1955) and Stevenson et al. (1957),

respectively.

Maintenance of BW upon exposure to 5°C environments in LP groups was also noted in other studies (Rothwell et al., 1987; Schmidt et al., 1967; and Stevenson, 1955). This seemed to occur due to the aforementioned inefficiencies in fuel cycling as discussed for the LP diets at room temperature. The process enables animals to maintain body temperatures without resorting to "calorically expensive" methods of thermogenesis which would otherwise require excess food consumption. Therefore, it appears that the LP group does not rely on the body's energy stores for heat production. BW maintenance in this group could be the result of the use of brown fat thermogenesis, thought to be enhanced in malnourished rats (Rothwell et al., 1987).

The HP, LC and Control groups required excessive caloric consumption to support their increased heat production. However, the increased FI was insufficient because a loss of BW occurred in these groups despite high caloric consumption.

HEAT

The facts that heat stress caused a decreased BW within Controls, HP, and LC groups but not LP groups in this study, the HP group lost more weight ($P<0.01$) and than the other groups and there were no differences in the extent of these losses between the Controls and LC, shows that there is a direct relationship between protein intake and BW loss during heat stress.

Conflicting results concerning BW change during heat exposure studies have been reported for animals on a normal laboratory diet or Rat Chow. Results of this study confirmed the findings of previous research (Hamilton, 1963; Johnson et al, 1966; Kibler et al., 1966; and Yousef et al., 1968). However, research by Beard et al. (1988) showed no significant decrease in BW at 30°C a temperature within the TNZ of white rats but selected because white rats behaviorally thermoregulate to temperatures below their TNZ's (30°C may actually pose a heat stress to the animal)(Hart, 1971). The difference in Beard's results and those reported in this study may be due to the fact that this study used 35°C as the heat stress temperature, while Beard et al. (1988) used 30°C which must not have effected enough stress to cause BW loss. Moreover, in another study BW decreased only during the first 3 days at 35°C, followed by an increase in BW, but at a slower rate than the controls (Horowitz, 1976). The white rats used in the study by Horowitz in 1976 weighed about 200g less than the rats used in the present study and were of the zabar strain. Perhaps the smaller body size enabled them to better withstand the heat due to a larger surface area to body size ratio, as was seen in the LP and LC groups of this study.

HP diet group weight loss reported by Hamilton (1963) and LC group weight loss (Kibler et al., 1967; Yousef et al., 1968 and 1970) support the results of this study. When animals are exposed to heat, one of the methods used to help maintain a tolerable body temperature is to decrease metabolic rate (MR) which may be associated with decreased food consumption, hence a loss of BW. Therefore, less

food consumption means less heat production.

The results of this LP diet study are supported by Hamilton (1963) showing that a decrease in FI did not cause a loss of BW at 32°C. Perhaps inefficient methods of fuel cycling are somehow shut off during heat stress, enabling the animals to maintain their BW. One notable observation is a 44% decreased MR with increased heat exposure compared to the 33% reduction in MR seen in the Control group which did experience weight loss. Another possibility is that the 60% reduction in FI/BW was not as great as the 75% reduction observed in the Controls and HP groups.

B. WATER INTAKE

The HP group drank more water per gram FI(WI/gFI) than controls but only significantly during weeks 2,3,and 6. In the present study there is a definite trend of increased WI/gFI with increased protein consumption. These results support a three week study by Schreiber et al. (1955) who found that WI was significantly higher in animals fed a 50% protein diet. The elevated level of protein intake is coupled with increased need for the metabolism of amino acids which in turn causes an elevation of nitrogen(N) in the blood. The excess N must be excreted as urea via the kidneys resulting in an increased need for water to eliminate the urea molecules. Therefore WI must be increased in animals consuming a HP diet.

The decreased (WI/gFI) results seen in the LP group are similar to those reported by DeCastro et al. (1968) in a four week study with rats fed an 8% protein

diet. Schreiber et al. (1955) concluded that water consumption was diminished on high sucrose-low protein diet when compared to a high dextrin-low protein diet and suggested that sucrose as a source for CHO may have contributed to the metabolic water pool more than other CHO's or protein. The LP diet in the present study contained two times as much sucrose as the control diet. Perhaps this is why the water need is decreased on an LP diet.

COLD

Studies have shown that cold acclimated animals drink less than controls at room temperature (Box et al.,1973). These results are in agreement with the present results showing that gWI/gFI in the cold had significantly decreased in comparison to room temperature values.

Within group comparisons revealed the decreased WI/gFI for both Controls and the HP group. Therefore, although FI and WI increased per animal, the water intake did not increase at the same rate as the FI. The LP and LC groups showed no difference within their groups for WI/gFI. However, in comparisons between groups, the HP and LC groups drank almost 2 times the water as the controls when expressed as WI/gFI. Therefore, the HP diet resulted in an increased need for water when compared to Controls, but overall water intake is decreased by cold temperatures. Hamilton (1963) suggested that heat causes an increased excitability of the drinking center thereby masking the desire for food consumption. Perhaps cold stress could have caused the reverse of this situation because of the needed

increase in FI to supply energy for an enhanced MR. The increased amount of food intake could also have contributed to the metabolic water pool especially because of the increased metabolic rate and therefore less WI was required.

HEAT

The current results of the heat related increase in WI/g FI have been confirmed by a previous study by Hamilton et al. (1963) that showed as much as a 3-fold increase in the WI of animals exposed to 35°C for 10 days.

In the present study the WI/FI clearly depicts a trend of increased WI with increasing dietary protein intake a trend seemingly unaffected by caloric intake. The reason the LP group had a decreased WI could be due to the increased metabolic water production due to the high percentage of sucrose in their diets. Another possible explanation could be that the LP diet produces fewer urea molecules, therefore less water is required to eliminate them via the kidney.

C. ORGAN WEIGHTS

Unfortunately in this study the organ weights for the HP group at room temperature were not measured. It has been reported from other studies that animals fed HP diets (78%, 39%, and 50%) had increased kidney and liver weights (Leathem et al.,1947; Schreiber et al.,1955; Vander Tuig et al., 1984). Other studies have concluded that there was no difference between liver weights for a 45% protein

diet after 28 days or the kidney weights for a 40% protein diet after 4 days (Johnson et al., 1987; Tyzibir et al., 1981). There were no differences noted for heart weights between HP groups and their controls at either 39% protein or 40% protein (Johnson et al.,1987; Vander Tuig et al.,1984).

The kidney weight increase noted by Leathem et al. (1947), could be due to the increased load placed upon the kidney to eliminate the excess N resulting from amino acid metabolism. Liver protein content has been reported to increase as a result of a HP (78%) diet thus resulting in an increased liver weight/gBW (Leathem, 1947).

The organ weight results of the LP group in the present study were also noted by DeCastro et al. (1968) following a four week study. They have suggested that the protein calorie deprivation caused a tendency for increased liver water content. This could have been caused by an increased glycogen storage by the liver which stores 3-5g water per g glycogen stored (Schmidt-Nielsen, 1983). Contrary to these findings were the increased liver and decreased kidney weights observed in a study by Schreiber et al. (1955). The increased heart weight of the present study along with that of DeCastro et al. (1968) could be due to the hypertrophy of the heart muscle to support an increased MR.

Supporting this study's results on the liver weight in calorically restricted animals is a study by Khan and Bender (1979) who found that liver weight decreased when expressed per 100g BW. According to this study, although percentage of total protein had increased in the livers of the restricted animals when compared to

controls, the total liver protein was less than on ad libitum diets because smaller size of the calorically restricted animals.

Support of this study's results of kidney and heart weight in the LC group was also provided in a study by Johnson et al. (1987) that reported no difference between the kidney and heart weights of calorically restricted animals and those of the controls. These results further support the trends of decreased heart and increased kidney weights as a function of an increasing protein content of the diet, and that caloric value of the diet is of no consequence to these parameters.

COLD

Previous studies concerning the effects of cold exposure on organ weights have shown conflicting results. Long term studies have determined that kidney, liver and heart weights increased during exposures to 4°C and 6°C (Scammell et al., 1981, and Heroux et al., 1958, 1963). A seven day study reported no changes in any of the weights of the above mentioned organs upon exposure to 5°C in kangaroo rats (Yousef et al.,1970). The Controls in the present study had an increased heart and kidney weight when exposed to 5°C which supports the aforementioned studies regarding laboratory white rats. The Controls also had a decreased liver weight which was not seen in any of the previous studies noted in this report. These data are confusing and are suggestive of a hypotrophy of the liver within this group that may be associated with the decreased FT_4 levels along with the stress associated with thermoregulation in the cold without a significant increase in plasma T_3

concentrations. It has been well documented that the liver is one of the body tissues affected by the actions of the thyroid hormones, and that the thyroid hormones are indirectly involved in MR regulation (Tepperman, 1981). Without the proper levels of thyroid hormones, the liver cells would be less metabolically active and since the size of the cell is related to its activity, these cells would become smaller without a sufficient amount of thyroid hormone to support their activity.

Within groups, 5°C cold stress resulted in an increased kidney weight, and no change in liver weight in the LP and LC groups, and an increased heart weight in the LC group with no change in heart weight in the LP group. Due to the alotted food amount increase in the LC group at 5°C the WI must have also increased; therefore, amount of water excreted via the kidneys must have increased also. This may have lead to a hypertrophy of the kidney due to the increased activity of elimination. According to Koushanpour et al. (1986) any factor that increases the amount of sodium filtered such as increase in glomerular filtration rate will cause a parallel increase in Na-K-ATPase activity and amplification of the basolateral membrane. Also, a increased T_3 concentration would increase Na-K-ATPase activity at the level of the kidney since the kidney is another organ affected by thyroid hormone concentrations (Tepperman, 1981). Increasing MR would increase the load upon the heart to pump faster in order to deliver more oxygen to the tissues, therefore causing hypertrophy in the heart muscle cells which occurred in Controls and the LC group.

The only differences that existed between the dietary groups was a significantly greater liver weight in the LP compared to the Controls and LC group, and an increased kidney weight in the HP group when compared to the LP and LC groups. The fact that the Controls had lost liver weight due to cold exposure and the LP group did not when both groups were compared to their room temperature controls, suggests that a low protein diet must have some sort of hypertrophic effect on the liver. The aforementioned effect could be indirectly caused by the action of T_3 which is always elevated in the LP diet fed rats, or directly through elevated enzymatic activities such as ∝-glycerophosphate oxidase which is an indicator of thyroid hormone action at the cellular level (Young et al.,1980). Also the increased sucrose intake of the LP group would effect more glucose uptake by the hepatocytes and thus more glycogen along with its associated water would be stored in the liver thus increasing its weight.

HEAT

Long term studies (9-10 weeks) on the effect of heat stress on organ weights depicted decreases in liver, heart, and kidney weights (Ray et al., 1967; Herrington et al.,1942). The results are in agreement with the decrease noted for the liver and kidney weights of the Control group; however, the Controls experienced no change in heart weight. Perhaps the time period necessary to induce a decrease in heart weight via heat stress is longer than seven days.

Heat stress produced a decrease in kidney, liver, and heart weights within the LP group supporting the above mentioned studies. The Controls and LP group also experienced a decrease in all thyroid hormone levels in the heat. These hormones indirectly affect the liver and kidney cells and therefore hormone decrease could cause cellular hypotrophy.

The unchanged kidney and increased heart weights in the LC group are difficult to explain. However, the plasma levels of T_3, FT_4, and T_4 also remained unchanged in the calorically restricted animals when the Control group had notably decreased all of these parameters within their groups. These data coupled with the fact that Hb significantly increased along with an increase in Hct (however not significant) possibly placed an added load upon the heart muscle causing a subsequent hypertrophy.

Although the LP group experienced a decrease in heart weight within their group, this effect is insignificant when compared between the groups because heart weight is still proportionally higher than controls. The decreased heart weight in the LP group may be due to the decreased MR in the heat, thereby a slower heart rate could cause a hypotrophy of the heart muscle mass.

The higher organ weights of the HP group when compared between groups, was possibly due to the fact that HP diets result in an increased protein mass in the tissues (Leathem, 1947) or, in the case of the liver, hypertrophy has occurred due to an excessive citrulline synthesis seen in HP diets (Letko et al.,1984). Therefore, if their organ weights were higher at room temperature, then even if a loss in any of

the organ weights had occurred, they would probably still be heavier than controls.

D. HEMATOLOGICAL VALUES

The HP diet caused no significant effects upon the Hct, Hb or PP. These results are supported by a study conducted by Leathem (1947) which determined that a 78% protein diet had no effect on Hct or PP. The author is unaware of studies regarding to Hb changes and HP diets.

The LP group sustained a decrease in PP and increases in Hct and Hb. The decrease in PP seen in the present study is supported by three prior studies (Hishoaka et al., 1974; Sagawa et al., 1978; Villalon et al., 1987). This effect could be the result of the lower rate of protein synthesis at the level of the hepatocytes as an adaptive response to lower protein intake (Villalon et al.,1987). However the increases incurred in Hb and Hct values are not confirmed by Hishaoka et al., (1974) nor Sagawa et al., (1978). Both of these studies reported that there was no change in these parameters. Perhaps this is due to the shorter time length of the previous studies (35-40 days).

An increase in Hct and Hb makes sense because the LP diet animals had an increased MR therefore an increased need for tissue oxygenation. Tissue oxygenation is primarily maintained by the red blood cells (rbc's). Therefore, an increase in the number of rbc's and amount of Hb would serve to support this increased oxygen demand. This may be an adaptation to a Low Protein diet.

Caloric restriction caused no effect on Hb or Hct. However, these animals experienced a significant decrease in PP. The restriction of calories also causes a protein deficiency because these animals are actually receiving a third less protein than the controls. The LC group also experienced a significant decrease in liver weight which, coupled with decreased PP, were the same results seen by Villalon et al. (1987) for a LP diet. These authors felt that lower PP concentrations resulted from a lower rate of protein synthesis at the level of the hepatocytes. In the present study because of the decreased availability of protein coupled with a decrease in total calories, the animals must have utilized more of their dietary amino acids to support their metabolism and, therefore, there were less amino acids available to synthesize plasma proteins, an effect similar to that seen by Villalon et al. (1987) in LP diets.

COLD

Past studies indicate that cold stress has no significant effect on Hct, Hb or PP values (Beard et al.,1988, and Deb et al., 1956). These results are in agreement with the present study which concluded that the Controls experienced no changes in these values due to cold stress.

Cold had a significant effect upon the plasma total protein concentration with an HP diet as was seen within their diet group, but it also had more of an effect on HP fed animals than the other dietary groups as was noted in between group

comparisons. The decrease in both the PP values suggests that there may have been a slight increase in the blood plasma volume. Deb and Hart (1956) reported an increase in blood and plasma volume in rats exposed to 6°C for 1 week. They suggested that the physiological significance of the increased blood volume in the cold is probably due to increased vascularity of the tissues reflecting an increased metabolic capability. The resultant increase in blood fluid volume would tend to cause a dilution of the PP values making it seem that PP values had actually decreased. This increase in blood plasma volume would also affect the Hct.

HEAT

The effects noted by other authors upon exposure of animals to heat stress have been controversial. Dehydration has been known to increase PP and Hct (Hainsworth et al.,1968), whereby hyperthermia alone increased either Hct or Hb (Jani et al.,1967), increased Hct (Burger et al., 1967) or caused no change in Hct and Hb levels (Beard et al., 1988; Burger et al., 1967).

The present study has shown a decreased Hct in control animals exposed to heat for 1 week, with no significant changes in Hb or PP when compared to room temperature conditions. Perhaps the decline in MR due to heat exposure caused a subsequent decline in Hct because less oxygen was needed at the level of the tissues. The fact the Hb levels remained unchanged as Hct declined is perplexing and could indicate hyperthermia induced red cell fragility causing hemolysis (Burger et al., 1967)

The HP group displayed a decrease in Hct and PP levels within groups, however between groups the HP diet caused no significant difference in these blood parameters.

Heat stress consequences on blood values for the LP group in the current study disagree with Jani et al., (1977) who had determined that short term heat stress caused no significant effects on Hct and Hb.

Exposure to 35°C did not change PP levels within the LP and LC groups, however when compared between groups, these diet groups had a significantly lower PP than controls. This effect was not due to heat but in fact to the diet as was mentioned earlier.

E. METABOLIC RATE

There were significantly higher MR's of the HP group than the Control group when the MR values were averaged over an eight week period. These results confirmed two other studies by Harstook et al., (1963 and 1973) who determined that an increase in $\dot{V}O_2$ did occur in HP fed animals when protein intake was 41-57% of the dietary intake. This increase in RMR was also noted in a human study conducted for 14 days (Burse et al., 1977). Harstook et al. (1973) believed that the excess heat production associated with the HP diet is due to the Specific Dynamic Action (SDA) of the high protein level in the diets. The MR increase experienced by the LP diet group in this study was supported by Young et al. (1980) who reported an increase in MR after 6 weeks. In another study, MR rose after 3 weeks

but not during weeks 2,4 and 5 (Balmagiya et al., 1983). During the first week, MR was significantly lower than controls but in the 4th and 5th weeks it was higher, but not significantly, than controls. They suggested that the animals adjusted their energy expenditure to the decrease in protein consumption in an attempt to unload excess calories by increased $\dot{V}O_2$. Tulp and Krupp (1980) had also studied resting $\dot{V}O_2$ of white rats fed a protein deficient diet and after eight weeks had noted an increase. They suggested that this was due to a "futile cycling" of substrates that appear to be thyroidally mediated.

Calorically restricted animals (33%) exhibited no difference in MR when compared to the controls in this study. These results confirmed those by Johnson et al. (1964) who found no difference in MR in a 480 day study on animals whose FI was restricted by 30%. However, Khan et al. (1979) had determined that the MR decreased in adult rats fed a restricted diet consisting of only 5% protein. This difference in MR was probably due to the decrease in caloric consumption in combination with a decreased protein consumption.

COLD

Generally, the MR of both wild and laboratory rats is known to increase during extreme cold stress as well as temperatures just outside the lower critical temperature (LCT) of their TNZ (Balmagiya et al., 1983; Cottle et al., 1954; Yousef et al., 1970; and Yousef, 1979). This study confirmed these previous findings in that the controls as well as all the dietary groups did experience an increased $\dot{V}O_2$ when

air temperature decreased below the TNZ. This increase was due to an increased heat requirement which is met in the form of shivering and non-shivering thermogenesis in order to maintain body temperature. All animals were 12 weeks of age when placed in the cold and all had increased food intake considerably to meet the increased caloric output. The result was the ability of all the diet groups to thermoregulate during the seven day exposure to 5°C whether it be via shivering or non-shivering thermogenesis.

Comparisons between the groups demonstrated a greater MR increase in both LP and LC groups upon cold exposure when compared to the Control group. The LP diet results are supported by the findings of Balmagiya et al. (1983) who concluded that at a mild cold challenge, LP diet animals increased $\dot{V}O_2$ significantly more than in the Controls. However, in their study, they concluded that the LP diet group had an impaired thermoregulatory response to a cold challenge which is in disagreement with the present study. The LP fed animals had eaten more and did not lose BW as did the other groups. These results suggest that perhaps another method of thermogenesis is prevalent in protein malnourished animals. One possibility is enhanced brown adipose tissue (BAT) thermogenesis, possibly present in all groups due to cold exposure but already well established in the LP group. Sudies have shown that increased BAT lipogenesis occurs in sucrose overfeeding as well as with cold temperature (Granneman et al., 1983). BAT lipogenesis taken into account plus the 20% increase in caloric intake (approximately 1200 kcal) with only a 450 kcal increase in body fat, suggests that BAT may play an important role

in diet-induced thermogenesis (DIT). A few similarities between DIT and non-shivering thermogenesis (NST) are increased energy intake and expenditure, reduced efficiency of energy utilization and raised plasma T_3 (Girardier et al., 1983). These similarities are seen only in the LP group at room temperature suggesting that they exhibit a DIT.

The higher $\dot{V}O_2$ in the LC group could reflect the increased FI at 5°C or the increase in T_3 that they experienced compared to their room temperature controls. Moreover, the LP and LC groups were smaller than the controls giving them a larger surface area per volume for heat loss, therefore they had to compensate for this heat loss by increasing their MR more than the Controls did. It has been stated that animals on LC diets are more efficient in their caloric utilization (Khan et al. 1979; Kibler et al., 1966). Perhaps this quality enables the LC group to raise $\dot{V}O_2$ levels higher than the Control group in the cold.

The HP group had a slightly higher MR than the Control group and showed increased plasma T_3 levels. In golden hamsters MR increased about 40% due to the SDA of the HP diet and this increased MR resulted in increasing the LCT of their TNZ (Simek, 1975). In this study, the increased plasma T_3 of the HP diet group, which was normally below that of the Controls, suggests that the cold stress had a greater effect on HP fed animals and they relied more upon non-shivering thermogenesis to maintain their body temperature perhaps due to an increased LCT or to a decreased insulation (white adipose tissue). It is important to note that the HP fed animals had approximately the same body surface area as the Controls and

consumed about as much food, but they behaved similarly to the LC group as far as thyroid output is concerned. Also, citrulline synthesis has been noted to stimulate mitochondrial energy metabolism and has been linearly associated with ambient temperature. For example, at lower ambient temperatures citrulline synthesis is decreased (Letko et al., 1984). Since citrulline synthesis is part of the urea cycle and the urea cycle is enhanced in HP diet, this may add to the increased $\dot{V}O_2$ experienced in the animals at room temperature and could be the reason for their reliance upon increased T_3 levels during cold exposure.

HEAT

Heat exposure generally results in a marked decrease in $\dot{V}O_2$ when compared to controls at room temperature (Haghani, 1979; Kibler et al., 1967; Yousef et al., 1967). This finding was confirmed by the present study whereby all dietary groups did experience a significant decrease in MR during exposure to 35°C. Actually it seems that a decrease in $\dot{V}O_2$ is seen in the heat because it is lower than the $\dot{V}O_2$ at room temperature. However, the $\dot{V}O2$ at room temperature (25°C) is not reflective of the $\dot{V}O_2$ seen within the animals TNZ, which is 29-31°C (Hart, 1971), but is actually a higher $\dot{V}O_2$. Therefore the MR seen in the heat is also higher than the MR which would be seen within the animals TNZ because 35°C is above the upper critical temperature (UCT) of the white rat.

The HP group experienced a significantly greater decrease in MR than the Controls. This is in disagreement with the citrulline synthesis study in liver O_2

uptake with HP animals which noted a 2-3 fold enhancement in O_2 uptake upon exposure to 42°C (Letko et al, 1984). Perhaps this is due to the higher temperature which was used in the latter study (7°C higher that the present study). It was noted in the present study that the T_{re} was increased within the HP group during the entire heat exposure period, whereas the Controls were able to reduce their T_{re} on days 6 and 7. The Control and HP groups were both very inactive during heat exposure. The present study noted that although MR decreased in the LP group, when compared to their room temperature controls, it did not decrease as much as in the Control group. It is interesting to note that T_3, $T_{4,\text{ and}}$ FT_4 levels during exposure to heat in the LP group were also higher than in the Controls.

F. THYROID OUTPUT

The plasma levels of T_4, FT_4, and TSH were no different than the control levels in the HP diet group. However the T_3 levels were significantly lower than those of the control animals. These results confirm a study by Glass et al. (1978) who reported that rats fed a hypocaloric diet of HP value had a lower T_3 than the controls which had received the same caloric intake but a decreased level of protein. Also, Tyzibir et al. (1981) had fed a 45% protein diet to a group of rats and found that the T_3 values were lower than the controls (19% protein) but not significantly. In their study they found a significantly lower (27%) liver mitochondrial alpha glycerophosphate dehydrogenase (M-α-GPD) enzyme activity in HP diet animals. The concentrations of α-GPD according to their results seemed to be inversely

proportional to levels of dietary protein. These authors suggest that diet composition is a regulator of hepatic intermediary metabolism mediated by the thyroid hormone. This could be the reason for the decreased levels of T_3 noted in the HP diet of the present study.

The animals fed the LP diet had similar T_4, FT_4 and TSH levels as in the controls. Nonetheless, plasma T_3 levels were significantly higher than the controls. These results support a two week study by Young et al. (1982) who found no difference in serum T_4 or FT_4 but a significantly higher plasma T_3 level when compared to controls. Also, in a 32 day study by Danforth et al. (1978), they concluded that the pooled plasma T_3 concentrations of protein malnourished rats increased after four days and remained higher for the duration of the experiment. The authors concluded that peripheral conversion of T_4 to T_3 increased the T_3 levels in the plasma. When an animal consumes more energy than required for daily maintenance, as in the LP diet animals, the excess energy could be dissipated as heat through increased thermogenesis. Tyzibir et al. (1981) noted an increase in liver M α-GPD activity in rats fed an LP diet along with an increased serum T_3, and decreased liver succinate dehydrogenase and cytochrome oxidase activities. They stated that the diet-induced thermogenesis as a function of T_3 concentration may result from the use of the α-glycerophosphate shuttle mediated by the thyroid-stimulated increase in M α-GPD activity and the coupling of the oxidation of reducing equivalents with phosphorylation of ADP at lower potential energy levels in the electron transport chain. These authors also noted that animals fed a low

protein diet may use a much greater portion of their obtainable potential for oxygen utilization because they have adapted to the increased use of reducing equivalents that are produced in the cytoplasm and transported into the mitochondrion via the diet-induced, thyroid mediated, α-glycerophosphate shuttle system.

Young et al. (1982) suggested that increased $\dot{V}O_2$ is not due to an increased serum T_3 because the FT_3 levels were normal in LP fed animals and believed that the increase in $\dot{V}O_2$ could be due to stimulation of the sympathetic nervous system (SNS). Rothwell and Stock (1980) suggested that there was an enchanced tissue sensitivity to catecholamines in the LP diet animals.

Plasma T_4, T_3 and TSH were unchanged in the LC group. However the FT_4 levels were significantly lower than the controls. The FT_4 index is a better index of thyroid function status than total T_4 (Tepperman, 1981). Therefore the decreased FT_4 must indicate a decrease in thyroid function in the calorically restricted animals. These results support the findings of Young et al. (1980) who found that animals that consumed a third less food than controls had a lower serum T_4 levels and the T_3 levels remained unchanged. Other studies noted a decreased T_4 level when determining the thyroid secretion rate via concentration of plasma protein bound iodine (Turner, 1968; Yousef et al.,1968). Additional studies established that there were no changes in serum T_3, T_4 and TSH in calorically deprived animals (Glass et al., 1978; Tulp et al., 1979).

Data from this study suggest that calorically deprived animals must have decreased thyroid output in order to adjust to their depleted caloric intake.

COLD

Plasma thyroid hormone levels in cold exposed rats have been reported to be variable. For example, plasma T_4 has been demonstrated to increase due to short term exposures (24 hrs.)(Beard et al., 1982; Hefco et al., 1975). On the contrary, seven day exposures to 10°C have resulted in decreased plasma T_4 levels (Beard et al., 1988). Moreover, 20 day exposures to 4°C have shown unchanged plasma T_4 levels (Scammell et al., 1981). Plasma T_3 levels have been demonstrated to increase regardless of duration of exposure (Beard et al., 1984, 1982; Hefco et al., 1973). Plasma TSH levels have either decreased in cold acclimated rats at 6°C (Bakke et al., 1971) or have increased after 32 days of exposure to 5°C (Jobin et al., 1975).

Several reports have concluded that T_3 enhances mitochondrial function as well as oxygen consumption (Beard et al., 1982). These data support the present findings on T_3 levels in cold exposed HP and LC groups, however, VO_2 increased in all dietary and the T_3 levels remained unchanged in Controls and LP rats. It is easy to see that the LP group already had a much higher T_3 concentration than the other groups at room temperature and their cold exposure levels were still significantly higher than the other groups. Moreover, the Controls still had the same T_3 levels as the LC and HP groups in the cold. Some authors have suggested an increased potency of T_3 (compared to T_4) in calorigenesis since there is a peripheral conversion of T_4 to T_3 in the liver (Scammell et al., 1981) to account for the large rise in serum T_3 and a decrease in serum T_4 in all of the dietary groups exposed to 5°C. Another

positive relationship between thyroid function and α-GPD in the liver is that α-GPD is associated with an increased serum T_3 (Bernal et al., 1975). Decreased T_4 levels could be due to an inhibition of TBG synthesis in the liver due to the cold temperature, which can lead to low serum total T_4 values, and that these values do not reflect upon the serum FT_4 values which clearly represent the true thyroid function status (Tepperman, 1981). The FT_4 values for most of the dietary groups remained unchanged in the cold with the exception of the controls. Therefore, this study suggests that the control animals experienced an actual decline in thyroid gland activity when exposed to 5°C for 1 week. Although increased heat production is a necessary requirement for cold survival perhaps when cold-adaptation is finally achieved the thyroid hormone reduction is necessary to conserve caloric reserves (Bakke and Lawrence, 1971). The reason that the controls had not shown a significant increase in T_3 and had actually ungergone a decrease in FT_4 may be due to their ability to increase heat production by shivering alone or that they became cold adapted faster than the other groups and experienced a decline in FT_4 values prior to the seventh day of cold exposure when the blood was drawn for analysis. Perhaps the cold stress combined with the stress imposed by each individual diet caused the significant increases in T_3 which were observed in the HP and LC diets.

The HP group had a significantly lower plasma T_3 when at room temperature. However when exposed to cold they showed a 3-fold increase in plasma T_3 levels. Nonetheless, this increase brought their T_3 levels only to the vicinity of the control

group, but was probably more detrimental to the HP diet group because the combination of T_3 and a decrease in temperature may have caused a breakdown of body protein (Templeton et al., 1949). This catabolism of body protein coupled with a high level of protein in the diet, must have placed an extreme load on the kidneys which ultimately caused significant hypertrophy of the kidneys. The calorigenesis which resulted from increased plasma T_3 levels was probably necessary for survival of the HP group because of the previously suggested increase in the lower TNZ as a result of the adaptation to an HP diet.

The plasma T_3 levels in the LP group indicates that cold exposure has no significant effect on the thyroid output in protein restricted animals. However the T_3 levels were still significantly higher than the cold-exposed Control group. Therefore, conclusions can be made as to the significant effect that decreased dietary protein levels have upon plasma T_3 values. It is suggested that although the plasma T_3 levels remain elevated during a LP diet, the plasma FT_3 levels were not, and that the increased thermogenesis is always associated with FT_3 levels (Cox et al., 1984). In their study they stated that protein malnourished animals did increase their plasma T_3 levels, but also increased was the binding capacity of T_3 so that FT_3 is not increased. This increased binding may be due to the presence of a thyroid-binding globulin found in LP diets but not seen in well fed rats (Young et al., 1982). If these findings are valid it would lead one to speculate that increased thermogenesis seen in the LP fed animals was due to sympathetic nervous system activity and BAT thermogenesis, each of which was not measured in the present study. Rothwell and

Stock (1987) suggested that the effects of an LP diet on energy balance may be related to the alterations in circulating levels of certain amino acids, such as tryptophan. Tryptophan's ratio to the large neutral amino acids in the blood has been known to modify food intake and may also affect the expenditure of energy (Rothwell et al.,1987). In a study by Wurtman (1987) it was noted that a reduction in plasma tryptophan occurred due to an excess amount of insulin (resulting from a high CHO diet) which caused the other amino acids to move out of the bloodstream and into skeletal muscle. This caused an elevated concentration of tryptophan in the brain which in turn caused an increase in brain serotonin levels known to control such factors as sleep, mood and appetite.

The calorically restricted animals exhibited a significant increase in plasma T_3 levels as a result of cold exposure. Although they had less to eat they were able to increase MR and maintain T_{re}. However they had lost more BW than the other groups possibly due to a wasting of muscle tissue to support their increased thermogenesis. Cottle (1960) asserted that rats can maintain their rectal temperature when food is restricted. He noted that at 8°C calorically restricted animals released T_4 at a rate which was much slower than controls. However Cottle did not have the means to measure T_3 levels and, therefore, the assumption that he made suggesting enhanced thermogenesis is not related to circulating thyroid levels cannot be valid today since T_3 is directly responsible for enhanced thermogenesis.

HEAT

Thyroid function has been noted to be altered by high ambient temperatures. Plasma T_3 levels and FT_3 levels have been shown to decrease upon heat exposure (Beard et al., 1988; Rousset et al., 1978) and unaltered T_4 levels (Beard et al., 1988; Yousef et al., 1968; Johnson et al., 1966) and unaltered TSH levels (Rousset et al., 1978; Bakke et al., 1971) have also been observed. These previous results support the data on the controls of the present study with the exception of T_4 and FT_4 which had significantly decreased as a result of exposure to 35°C for seven days. The differences in the former studies seem to be due to either a longer time of exposure (4 or 5.5 weeks) which would allow the animals to adjust better to the environmental conditions, or to a lower temperature (30°C) which is within their TNZ and closer to room temperature than 35°C. The present study maintains that the decrease in T_4 and FT_4 levels is a result of the heat stress placed upon the animals which may have "turned off" the hypothalamo-pituitary-thyroid system to further reduce calorigenesis.

The HP group reacted much in the same way as did the Control group. However, since the HP group already had low T_3 levels at room temperature, further physiological reduction was unnecessary. Moreover, the larger size of the Controls and the HP fed animals corresponds to a reduction in body surface area and therefore decreased capacity to lose heat via conduction, convection or evaporation. HP animals experienced the most difficulty in thermoregulating as was seen in the

maintainance of a significantly higher T_{re} during the entire 7 day period. Also, as was previously mentioned, citrulline synthesis is linearly related to temperature and, therefore, could have been enhanced even though they did decrease their food consumption because they still ate as much as the controls and their protein intake was almost twice as much as the controls. The HP intake in conjunction with an increased citrulline synthesis was more than they could effectively dissipate hence a greater associated heat stress with the HP diets.

The LP group had an unchanged plasma T_3 and TSH and experienced decreases in T_4 and FT_4 as a result of heat stress. The plasma T_3 level was of concern because it remained unchanged throughout all three of the temperature exposures which suggests that diet is the controlling factor of plasma T_3 levels in the LP diet group. Also, this group had no problem thermoregulating in the heat. Their T_{re}'s were high only during two of the seven exposure days. These results suggest that the heat production in Group C is not solely affected by the plasma T_3 levels because they were able to reduce their MR in light of unchanged plasma T_3 levels. Therefore, the reduction in FI must have caused a reduction their MR, however, in this case it can be seen that DIT could not be due to their thyroid hormone levels. The LP diet group also had an higher body surface area relative to body volume, because of their smaller size, enabling them to more effectively increase heat loss.

The LC group was able to handle the heat stress much better than the other groups. Their T_{re} only showed an increase on the first day of exposure and throughout the remainder of the period there was unchanged or even decreased

T_{re}'s. The plasma T_4 levels in the LC group were higher than the controls in the heat, however they had unchanged hormonal levels when compared to the LC diet group at room temperature. Therefore, T_4, FT_4, and T_3 levels were no different due to heat exposure. Perhaps this was due to the greater surface area of these animals enabling them to better lose excess body heat. These results support a study by Yousef et al. (1968) which concluded that thyroid function of rats at 34°C was similar to control animals at room temperature and that the food restriction seen at high temperatures may determine thyroid activity.

CONCLUSION

The effects of varying levels of dietary protein and caloric intake on the growth rate, metabolic rate, thyroid function and various other physiological factors were studied using male Sprague-Dawley rats. The variables were measured on rats acclimated to their diets at room temperature (25°C) for eight weeks and thereafter for one week exposure to 5°C and 35°C. The purpose of this study was to determine whether the physiological parameters were most affected by the protein content of the diet, the ambient temperature, or the caloric content.

Experiment 1: Room temperature acclimation to the specific diets.

There were many trends associated with the altered protein content of the diets. Growth rate increased as dietary protein increased but only to about the level of the control protein, thereafter the growth rate remained unchanged. Food intake (FI) decreased and water intake (WI) increased with an increased dietary protein intake. Organ weights were affected: liver and kidney weights increased while heart weight decreased with increasing dietary protein levels. Hematological values were affected: hematocrit (Hct) and hemoglobin (Hb) concentration decreased whereas plasma protein (PP) concentrations increased as dietary protein levels increased. The metabolic rate was increased by both high and low protein diets. All plasma thyroid hormone concentrations decreased with increasing dietary protein

intake with the exception of thyroid stimulating hormone (TSH) which remained unchanged.

Experiment 2: Upon exposure to 5°C, most of the trends seen at room temperature remained the same.

With increasing protein levels, body weight (BW) changes (losses) increased. The FI increased and WI decreased due to cold exposure in all groups but the trends which were seen at room temperature were unaffected by the cold. No liver weight trend was observed in the cold. There was an overall increase in MR due to cold exposure. A general decrease was found in plasma thyroxine (T_4) and only some groups (high protein and low calorie) increased plasma levels of triiodothyronine (T_3). Plasma T_4 levels followed no trend in the cold as was noted at room temperature, however, plasma T_3 levels followed the same trend as at room temperature.

Experiment 3: Upon exposure to 35°C, a number of the same trends were noted as at room temperature.

There was a general BW decrease due to heat exposure. The amount of BW loss was directly proportional to the protein content of the diet. All groups decreased FI and increased WI following the same room temperature trends. No

liver weight trend was observed in the heat. There was a general decrease in MR in all groups. In the heat MR was inversely proportional to dietary protein content. The thyroid hormone trends remained the same with an added decrease in free thyroxine (FT_4) as the protein content of the diet increased.

The calorically restricted (LC) animals were customarily not greatly affected by their restricted calorie intake. They were well able to adjust to their diet at room temperature and in the heat. The main effects of calorie restriction in white rats are decreased blood Hct, Hb and PP concentrations, kidney and liver weights, and plasma FT_4 concentrations. These were probably due to their small size and their more efficient MR. The only other great difference was their ability to thermoregulate in the cold, for on the seventh day of cold exposure many of the animals were starting to experience a state of hypothermia as was seen in greatly reduced rectal temperatures.

In conclusion, it has been determined from this study that the levels of dietary protein do greatly effect many physiological parameters and should be further studied in greater detail.

APPENDIX 1

The T_4 reagents provided in this kit were as follows:

1. T_4 serum standard, 0 μg%
2. T_4 serum standards 1.0,4.0,12.0 and 30.0 μg%
3. T_4 antibody-coated beads
4. T_4 Enzyme Conjugate
5. T_4 Assay Buffer
6. Substrate Reagent
7. Stopping Reagent
8. Controls

The controls were reconstituted by adding distilled water.

The T_4 EIA procedure:

1. 12 x 75 mm glass tubes were labeled for each standard, control, animal plasma and blank.
2. 100 μl of T_4 standards were added into the appropriate tubes.
3. 100 μl of control and animal plasma were added to the appropriate tubes.
4. 500 μl of working T_4 enzyme conjugate was added to each tube.
5. 1 antibody-coated bead was added to each tube after touching the bead to absorbant paper to blot excess liquid.

6. the test tube rack was then shaken gently and the samples were incubated for 30 min. at room temp.
7. Following the incubation period, 3 ml of distilled water was added to each tube. Then the bead decanting rack top was placed over the tubes and the bead rack inverted to drain the tubes. The beads were then washed 3 more times draining them thouroughly each time.
8. 1.0 ml of the Substrate Reagent was then added to all the tubes, including the blank tube.
9. The samples were then incubated for 60 minutes at room temperature.
10. 1.0 ml of Stopping Reagent was then added to each test tube and the reagents mixed by gently shaking the test tube rack.
11. The spectrophotometer (Beckmann DU-65) was then set to zero using the blank tube. The absorbance of all tubes was read at 492nm within one hour.
12. A standard curve was then determined using the absorbances determined from the serum standards and the sample T_4 concentrations were extrapolated from the standard curve.

APPENDIX 2

The T_3 EIA Reagents provided in the kit:

1. T_3 Serum Standard 0 ng/ml.
2. T_3 Serum Standards, 0.5, 1.0, 3.0, and 8.0 ng/ml.
3. T_3 Antibody-Coated Beads.
4. T_3 Enzyme Conjugate.
5. T_3 Assay Buffer.
6. Substrate Reagent.
7. Stopping Reagent.

The T_3 Assay Procedure:

1. 12 x 75 mm glass tubes were labeled for each standard, control, and animal sample and a Blank.
2. 50 μl of the T_3 standards, controls or animal sample were pipetted into the appropriate tubes.
3. 200 μl of Assay Buffer was then pipetted into each tube, and the reagents were mixed by gently shaking the tube rack.
4. Using plastic forceps, 1 antibody-coated bead was added to each tube after touching the bead to absorbant paper to blot excess antibody.

5. The reagents were mixed gy gently shaking the tube rack and then incubated at room temp. for 60 min.
6. 100 μl of T_3-Enzyme Conjugate was pipetted into each tube, and again the reagents were mixed by gently shaking the tube rack.
7. The reagents were again incubated for 15 min. at room temp.
8. 3 ml of distilled water was then added to each tube, and then decanted by placing the top onto the bead rack. The washing procedure was repeated again 3 more times.
9. 1.0 ml of the Substrate Reagent was then added to all tubes including the Blank. The tube rack was then shaken gently to mix the ingredients.
10. The reagents were then incubated for 60 min.
11. 1.0 ml of Stopping Reagent was then added to all tubes.
12. The reagents were again mixed gently and the absorbance of all tubes read at 492 nm.
13. A standard curve was then constructed, and the concentration of T_3 from the animal plasma was extrapolated from the standard curve.

APPENDIX 3

The TSH Reagents provided in the kit:

1. The TSH standard 0 μIU/ml.
2. TSH standards 0.5, 1, 20, 50 μIU/ml.
3. TSH Antibody Beads.
4. TSH Enzyme Conjugate.
5. OPD Substrate Diluent.
6. OPD Substrate Tablets.
7. OPD Stopping Reagent.

The TSH Assay Procedure:

1. 1.0 ml of animal plasma, control and each of the standards were pipetted into the labeled 12 x 75 mm glass tubes.
2. 0.1 ml of enxyme conjugate was added to all tubes and mixed gently.
3. Using plastic forceps, one antibody coated bead was added to each tube. The tube rack was then shaken gently to mix.
4. All tubes were incubated at 37 C for 60 min. in a water bath.
5. After 50 min. the OPD substrate solution was prepared.
6. 3ml of distilled water was added to each tube then decanted. The washing procedure was repeated 3x.
7. 0.5 ml of fresh substrate solution was then added to each tube.

8. All tubes were then incubated in the dark at room temp for 15 min.
9. 2.0 ml of Stopping reagent was then added to each tube, and then mixed thoroughly by shaking the rack.
10. The absorbance of all tubes were read at 492 nm against the blank tube.
11. The results were determined by making a standard curve and extrapolated from the standard curve.

APPENDIX 4

The Thyroid Uptake (TU) Reagents provided in the kit :

1. euthroid serum calibrator.
2. hypothyroid and hyperthyroid serum controls.
3. ANtibody coated beads
4. T-Uptake Enzyme Conjugate
5. Substrate Solution
6. Stopping Reagent

The TU Assay Procedure:

1. 12 x 75 mm glass tubes were labeled for the Euthyroid Calibrator, controls, patient samples and blank.
2. 50 μl of plasma sample were pipetted into the appropriate tubes.
3. 200 μl of Enzyme Conjugate was pipetted into each tube.
4. One antibody coated bead was then added to each tube, touching the bead to absorbent paper before adding.
5. The test tube rack was shaken gently to mix the reagents and incubated at room temp. for 30 min.
6. 3ml of water was then added to each tube, then the dacanting rack was placed over the test tube rack and the tubes decanted. The washing procedure was then repeated 3 more times.

7. 1.0 ml of Substrate Reagent was then added to all tubes.
8. The tubes were then incubated at room temp for 30 min.
9. 1.0 ml of Stopping Reagent was then added to all tubes and mixed by shaking the tube rack.
10. The spectrophotometer was then zeroed using the blank and the absorbance of all tubes read at 492 nm.
11. The % uptake for each sample was calculated using the formula:

$$\% \text{ Uptake} = \frac{(\text{Abs Eu}) \times (\text{TU ref})}{\text{Abs x}}$$

Where:

Abs Eu = Average absorbance for Euthyroid Calibrator

Abs x = Absorbance or the control or patient sample

TU ref = Percent uptake of Euthyroid Calibrator

12. The Free T_4 Index was determined using the following equation;

$$\text{FTI} = \frac{\text{Total } T_4(\mu g\%) \times (\% \text{ Uptake})}{100}$$

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