Organ and Tissue Weight Expression in a Rat Population with Controlled Nutritional Manipulations

by

Pamela A. Schedler, R.N., B.S.N.

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APPROVED:

[redacted]

Una Elizabeth Westfall R.N., Ph.D., Associate Professor, Adult Health and Illness, Research Advisor

[redacted]

Sue Bradley Davidson R.N., M.S., Associate Professor, Adult Health and Illness, Committee Member

[redacted]

Charold Baer R.N., Ph.D., FCCM, CCRN, Professor, Adult Health and Illness, Department Chairperson

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ABSTRACT

Title: Organ and Tissue Weight Expression in a Rat Population with Controlled Nutritional Manipulations

Author: Pamela A. Schedler, R.N., B.S.N.

Approved: [redacted]
Una Elizabeth Westfall R.N., Ph.D.

Purpose: The primary purpose of this study is to determine if there is a linear relationship between final total body weight and organ and tissue weights (heart, right lung, left lung, liver, pancreas, spleen, stomach, duodenum, jejunum, ileum, large intestine, kidneys, adrenals and *extensor digitorum longus* muscle) under three selected nutritional manipulations. The secondary purpose is to determine the effect of three nutritional interventions (kilocalorie level, fiber content and feeding schedule) on selected organ and tissue weights and final total body weights.

Methods: The sample included 40 healthy, Sprague-Dawley, post-pubescent, male rats. Body weights on day one ranged from 175.1-211.4 G. Feeding group means (±SE) ranged from 188 (±3.9) to 197.7 (±.4). On the last full day of the study, body weights ranged from 214.3-290.8 G. Feeding group menas (±SE) ranged from 228.9 (±2.9) to 281.1 (±4.1). A randomized block, factorial 2x2x2 design was implemented over 21 consecutive days. The three independent variables were kilocalorie level (80 or 55), fiber content (high or low) and feeding schedule (12 hr or 24 hr). Before independent variables were introduced the animals had a 7 day acclimation period. The dependent variables were weights of the following organs and tissues: heart, right lung, left lung, liver, pancreas, spleen, stomach, duodenum, jejunum, ileum, large intestine, kidneys, adrenals and *extensor digitorum longus* muscle as well as Day 20 body weight (final total body wieght). Organs were harvested within 20 minutes of sacrifice.

Results: Initial descriptive methods were used to identify the distributional charecteristics of the dependent variables. Regression analysis and corresponding residual scatterplots were directed toward the primary purpose. Standardizing organ weights by body weight appears most defensible for the liver (Pearson's r .7389 with a random residual scatterplot) while less defensible for other organs and tissue weights. Even when correlation coefficients are high, if there is a pattern in the residual plots there may be a better fitting model than an unadjusted simple linear one.

Three-way ANOVAs were directed toward the secondary puropose. Kilocalorie level was significant ($p \leq .05$) for the organ weights of the heart, liver, spleen, and kidneys and Day 20 body weight, with the group receiving more kcals having higher mean weights than the group consuming fewer kcals. There was also a significant interaction between feeding schedule and kcal level for the pancreatic weight. On the 12 hr feeding scedule, the pancreatic weights in the 80 kcal feeding group were significantly heavier than those consuming 55 kcals per day.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Relevance

Nurses as members of a scientific discipline are working to build a sound base for its activities and practice. In so doing, nurses draw from basic sciences as well as from the emerging nursing science (Bond & Heitkemper, 1987). This demands that nurses deliberatively critique the research to determine both scientific soundness and clinical readiness of reported findings. Multiple questions are involved in such critique, including careful analysis of findings (Tanner, 1987).

In one conceptualization of nursing science, nurses focus on responses derived from individual and environmental factors acting separately or together. One such area is how humans respond to environmental factors. Some responses to environmental factors are not possible to evaluate in humans but have been evaluated in rats. Organ weights are one such response variable when considering the effects of enteral feedings. There are multiple ways of expressing organ weights which often use assumptions that may not have been tested. For example, it is assumed that there is a linear relationship between body weight and organ weight. The expression of organ weights in the nutrition literature will be reported.

A gram is a unit of mass and not a unit of weight. However, it is convention that the word weight is used instead of the word mass; therefore, the word weight will be used throughout this paper.

Purpose

The purpose of this study is to determine if there is linear relationship between body and selected organ and tissue weights in groups of rats after controlled nutritional manipulations. From this determination a more informed judgement may be made regarding accurate reporting of organ weights. Therefore, the question driving this study is how to accurately represent or present organ and selected tissue weights.

Statement of the Problem

Organ weights in animal studies are reported in one of three ways: absolute or mean weight, relative weight and regressed weight. The relative weight is the most commonly reported, and regressed weight is the least commonly reported in the literature. A fourth method, that of weight corrected will be briefly discussed because it has had only limited use by one group of researchers.

Expression of Organ and Tissue Weight

Absolute weight

The phrase, absolute organ weight, means that the researcher reports the raw organ weights without any adjustment, using for example, the animal's body weight. Typically absolute weights will be reported as the mean and the standard error of the mean (SEM) for a certain group of animals (Berdanier, Johnson, Hartle & Crowell, 1992).

The disadvantage of absolute weights is that data may not be able to be compared to data reported in other experiments if animal characteristics are not similar. Most

importantly, even within the same experiment, animals size may vary greatly and make tissue weight comparisons difficult, if not misleading.

Relative weight

The phrase, relative organ weight, means that the researchers report the organ weight based on proportionality. The equation for computing relative organ weight is:

$$\text{Relative organ weight} = \frac{\text{Weight of organ}}{\text{Final body weight}} \times 100$$

This procedure enables organ weights to be normalized by body weight thereby permitting comparison of organs from animals with different body weights (Deitch, Dazhong, Specian, Qi & Berg, 1992). Final body weight is considered to be the last body weight taken closest to sacrifice. Another method of reporting relative organ weights is presenting the organ as milligrams/100 grams of body weight. This normalization has been reported by several researchers (Behne, Kyriakopoulos, Gessner, Walzog & Meinhold, 1992; Chan, Lou & Hargrove, 1993). The disadvantage of relative weight reporting is the lack of clarity as to whether or not the researcher has tested for the assumption that an organ weight has a linear relationship with the total body weight. In a classic article by Heroux and Gridgeman (1958) the argument against relative weight reporting is discussed. The assumption of linear relationship would imply that, in a pair of animals in which one animal's body weight is twice that of the other, the heart weights will exhibit the same 2:1 ratio (Heroux & Gridgeman, 1958). The assumption does not take into account the different growth phases and organ development of the animals. If the

organ is large, e.g. muscle, then assuming a linear relationship may be more appropriate; but if the organ is small, such as the heart, then the assumption is not appropriate (Heroux & Gridgeman, 1958).

Brody and Ragsdale (1922) have demonstrated that growth in warm-blooded animals occurs in three cycles, which implies that the rate of postnatal growth is inconsistent. At first the body weight increases sporadically then tapers off until maturity, and finally, either growth slowly increases or decreases during aging.

Thus, the two major criticism of reporting relative weights are: 1) the assumption of a linear relationship between body weight and organ weight and 2) the disregard for the effect of different growth phases on organ weight.

Regressed weight

The phrase, regressed organ weights, means that the researcher reports a logarithmic organ weight. The governing equation for the regressed model is $Y=cX^b$ where X=body weight, Y=organ weight and c and b are constants. Generally, the log of both sides of this equation is taken to give $\log Y=a + b\log X$, where a=logc or the y-intercept and b=the slope. A and b are estimated using the method of least squares. This means that if log Y is plotted against log X then a straight line will result (Heroux & Gridgeman, 1958). Thus, for the regressed model, as the body weight increases the organ weight increases proportionately less, whereas in the relative model, the proportion between the body weight and the organ weight is assumed to be constant. Thus, the regressed model is more consistent with the principles of body growth (Heroux & Gridgeman, 1958).

Regressed organ weights can be used to adjust the group-mean organ weights to allow for differences in group mean body weight (Heroux & Gridgeman, 1958). However, when the regression is zero, Heroux and Gridgeman (1958) suggest reporting the absolute organ weight instead of the regressed weight.

Weight corrected

A fourth method of reporting organ weight was proposed by Caster, Ponclet, Simon and Armstrong in 1956. They suggested reporting corrected weight (Wc) which is derived from the equation:

Wc = body weight - (weight of body fat + GI organs)

Organ weights are expressed as a percentage of Wc. This assumes linearity but at least attempts to account for body fat and GI organs that may rapidly change under stress (Caster et al., 1956). The way of deriving Wc is by a painstaking, time consuming, dissection-chemical approach. This method has only been reported by Caster et al. (1956).

The multiple ways of reporting organ weights demonstrate a source of confusion in the literature. Furthermore, with exploration of how values were calculated, one may question the interpretation of some of the values or other findings and conclusions based on those values. The source of confusion and possible inaccuracy of interpretation are perplexing to the practitioner and may undermine the study results. Research is needed to evaluate the accuracy of reporting organ and tissue weights. Specifically in this study, such evaluation will be done after selected nutritional manipulations in a rat population.

Significance for Nursing

The importance of accurately reporting data can not be overemphasized. Misrepresentation of data, if transferred to further research, may lead to inaccurate results in other studies. It may also lead to teaching false information to students. Clearly, these points are pertinent to nursing and ultimately to the delivery of good nutritional care by nurses.

Nurse researchers build on other sciences as they work to generate nursing's unique knowledge base. Thus, nurses are consumers of research from multiple related fields. Nurses must educate themselves as to the ways of accurately presenting data. This is a necessary step in determining scientific soundness of research, as well as determining the readiness of research findings for nursing activities, including clinical practice.

CHAPTER 2

Literature Review and Conceptual Framework

Overview

Purpose

The purpose of this study is to determine if there is a linear relationship between body and selected organ and tissue weights in groups of rats after controlled nutritional manipulations. Thus, the question driving this study is how to accurately represent or present selected organ and tissue weights.

The assumption to be tested in this study is the assumption of a linear relationship between rat organ and tissue weight and final body weight.

Introduction to selected literature

A review of organ and tissue weight presentation will be discussed. The phrase, absolute organ weight, means that the researcher reports the raw or actual organ and/or tissue weights without adjusting for the animal's body weight. The phrase, relative organ weight, means that the researcher reports the organ and/or tissue weight based on a linear relationship between final body weight and organ and/or tissue weights. The phrase, regressed organ weight, means that the researcher reports a logarithmic interpretation of organ and/or tissue weight (Heroux & Gridgeman, 1958).

The primary focus of this literature review is on selected organ and tissue weights; the secondary focus is on controlled nutritional manipulation effects on organ weights.

Journals selected

Four journals were reviewed for research studies that reported organ and tissue weights. Table 1 lists the criteria for selecting journal articles. The journals reviewed were Journal of Parenteral and Enteral Nutrition, Journal of Nutrition, Nursing Research, and Gastroenterology. The first three journals were reviewed from September 1990 to September 1993. A three year review was thought to give a representative sample of how organ weights are currently being reported.The Journal of Nutrition was limited to two years because of the large volume of articles that met the inclusion criteria.

Three of the four journals were chosen based on the large quantities of basic science research and research using animal models. The fourth journal, Nursing Research, is referred; and was chosen because of its long history of reporting nursing research. See Appendix A for a listing of the articles reviewed.

Presentation of Organ Weight Data

Twenty-nine of 68 articles reviewed (42.6%) presented organ and/or tissue weight as milligrams/100 grams of body weight. By definition, this approach is that of relative weight. Twenty-five of the 68 articles reviewed (36.7%) presented organ and/or tissue weight as absolute values in milligram units. By definition, this approach is that of absolute weight. Approximately 14.7% or 10 of 68 articles presented data as both absolute and relative. Only one article stated that transformations were performed where necessary but it is unclear if the transformation was conducted on organ weights

Table 1: Criteria for Selecting Journal Articles

Criteria	Inclusion	Exclusion
Species specific	X	
Whole organ weights	X	
Only part of organs weighed		X
Other species besides rats		X
Organs weighed outside the abdominal cavity		X

(Bates & Evans, 1992). The remaining three articles showed statistical results of organ weights but did not present organ weight values (see figure 1).

Within the review time period, Nursing Research had only one animal research article; however, no organ specific data were reported (Westfall & Heitkemper, 1992). Thus, the three principle ways of presenting rat organ and tissue weights can be classified as: 1) absolute or group means, 2) relative and 3) regressed or logarithmatically transformed. These findings are consistent with the search reported in 1958 (Heroux & Gridgeman, 1958). An in-depth presentation of weight expression follows.

Organs and tissues

Of the 68 articles, 16 different organs//tissues were weighed for a total of 124 organs. See table 2 for the frequency of organs weighed.

Organ weight values-selected current reports

The majority of researchers (57%) in this literature review expressed organ weights in at least relative terms (milligrams/100 grams body weight) (Behne et al, 1992; Chan et al, 1993). This approach assumes a linear relationship between organ weight and body weight. Such a relationship does not account for growth cycles and organ development of the animal (Heroux & Gridgeman, 1958).

Researchers in one study (Banwell, Howard, Kabir, Adrian, Diamond & Abramowsky, 1993) examined small intestinal growth after feeding red kidney bean phytohemagglutin lectin to male rats. The researchers began the study with an experimental group of rats weighing 185 grams $\pm$4 grams and a control group weighing 179 $\pm$ 9 grams. There was

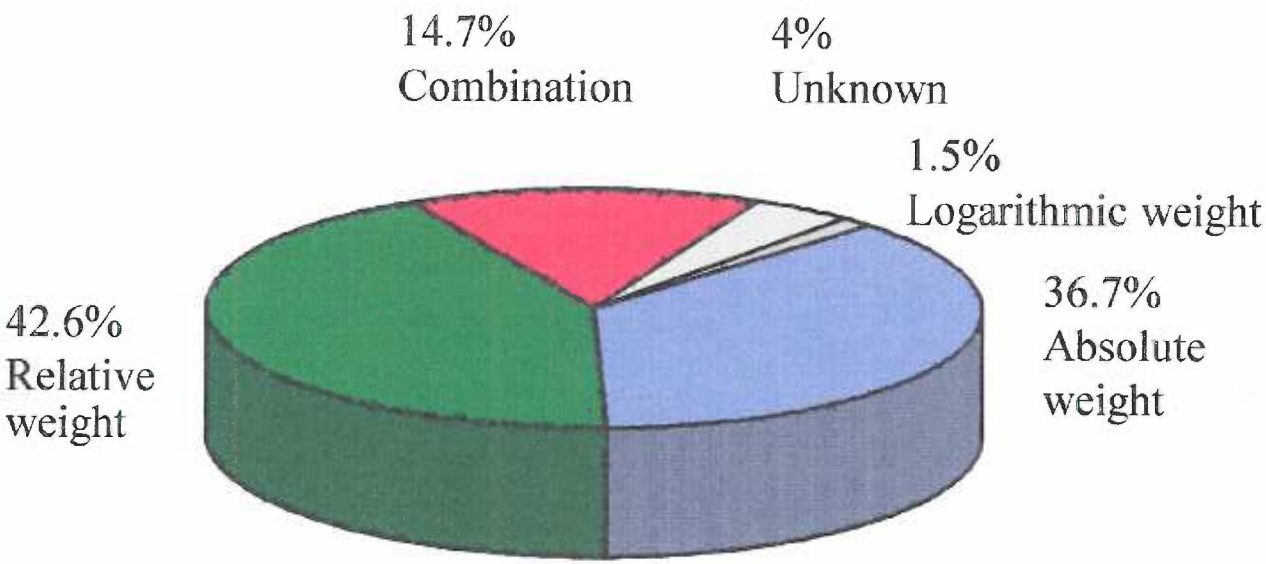


Figure 1: Literature results depicting percentage of articles and different classifications for expressing organ and tissue weights.

Table 2: Frequency of Organs Weighed

124 total organs weighed from 68 articles	
Liver	37%
Kidney	14.5%
Gastrocnemius	5.6%
Jejunum	5.6%
Duodenum	4.8%
Heart	4.8%
Ileum	4.8%
Spleen	4.8%
Large intestine	4%
Lungs	4%
Brain	2.4%
Pancreas	2.4%
Stomach	1.6%
Thyroid	1.6%
Adrenals	0.8%
Extensor digitorum longus	0.8%

Thus, depending on the duration of these studies with weanling rats, the accuracy of reporting organ weights as milligrams/100 grams of body weight may be questionable.

Diet manipulation

As one may deduce, three different ways of processing data makes it difficult for researchers to compare their data to another's data. Some researchers (Balaghi & Wagner, 1992; Marquez-Ruiz, Richter & Schneeman, 1992) have reported this difficulty but have offered no solutions.

A dependent variable of one study was pancreas weight in male Sprague-Dawley rats after folate deficiency (Balaghi & Wagner, 1992). When the animals organ weights were expressed as absolute group means, the control group pancreas weight value was heavier than the pancreas weight from the folate deficiency group ($p= 0.0001$). In contrast, when the pancreas weight was expressed as a relative weight then there was no significant difference between the control and the experimental groups. Thus, these two ways of reporting data lead to different conclusions. The researchers offer neither an explanation for this problem nor a solution.

Another study examined triglycerides and apolipoprotein B in male rats fed diets containing whole milk, skim milk, casein and whey (Marquez-Ruiz, Richter & Schneeman, 1992). Liver weight was a dependent variable. Researchers reported relative liver weight to be significantly lower in the group fed whey compared to the group fed skim milk ($p< 0.05$). However, when comparing absolute liver weight value group means, the liver weights of the whey diet group were significantly less than that of the liver weight of the

rats fed casein ($p < 0.05$). Again, the researchers offered no explanation or solution for the mixed results.

Two additional studies reported absolute organ weights and relative organ weights (VanLith, Holler, VanTinteler, Lemmens, VanZutphen & Beynen, 1992 ; Gallaher, Olson & Larntz, 1992). Again, when group means were analyzed, values that were statistically significant when weights were reported one way were not significantly different when calculated another way. No explanation was offered as to why these differences occurred.

Eisenstein and Harper (1991) presented absolute and relative organ weights with differing results. These researchers recommended that normalizing organ weights to body weights creates less divergent results (Eisenstein and Harper, 1991). Whether these normalized weights more accurately depict the actual values was not explored.

The reader of this research is faced with either two measures of the same variable with differing results or two measures and the same variable with only one interpretation. This presents difficulty in drawing conclusions from either conflicting data or incomplete data and the potential inaccuracy in comparing data from study to study.

There has been some recognition of a nonlinear relationship between organ weight and body weight. For example, authors of one study posited a nonlinear relationship of organ weight to body weight because they did not compare rats from one group that were twelve months older than rats in another group (Behne et al., 1992). However, despite this possible recognition of growth cycle effects on body weight and organ weight, the

researchers still presented values as relative organ weights. No statistical manipulations were done to prove the possibility of growth effects on organ weight.

Few researchers in the literature infer or actually report a nonlinear relationship. Bates and Evans (1992) came the closest to recognizing and acting on a nonlinear relationship between organ and body weight. In their report, these researchers indicated that logarithmic transformations were done where necessary. However, it was unclear which data were transformed.

Organ weight values-classic reports

Before the advent of such an array of assays that can be done on tissue samples, organ weights were prominent depeendent variables for studies. Two classic studies will be presented that warn against the application of absolute and relative organ and/or tissue weight reporting.

As early as 1947 Webster, Liljegren, & Zimmer conducted a study that examined spleen, liver and kidney relative weights using a wide range of total body weights. The purpose of the study was to secure additional organ weight data covering greater total body weight ranges and different experimental conditions. Animals of similar body weights were assigned to the same group. The range of body weights within each group was not greater than 10%. The sample size exceeded 500 Sprague-Dawley rats. The experimental conditions were that animals were sacrificed by exsanguination or ether. Splenectomized animals were also sacrificed using the same two experimental conditions.

Organ weights were plotted graphically, with the mean body weight in grams on the x-axis and with the mean organ weight in grams on the y-axis. The growth of organs was accounted for by using the following regression equation:
$\log Y = a + b \log X$, where X=body weight, Y=organ weight, a=y-intercept and b=slope. Interestingly when the group mean organ weight was plotted logarithmically, etherized animal mean kidney and liver weights fell on a straight line. The straight line ranged between 50 and 300 grams of body weight with a change of slope below 50 grams. The mean spleen weight fitted more accurately between the range of 75-300 grams with a change of slope occurring below 75 grams.

In another descriptive representation of the data the researchers plotted each organ weight using percentile zones of normal variation. The percentile curves illustrated a growth spurt at 50 grams to 100 grams with growth dropping off sharply between 100 to 150 grams. Additionally there was either an increase or a decrease in relative organ weight between 350 to 400 grams. Between 150 and 300 grams, growth tapered off (Webster et al., 1947). This is consistent with Brody and Ragsdale's (1922) description of the rat growth curve. Webster et al. (1947) reported a wide range of body weights with corresponding organ weight changes. Findings from their study support the contention that growth affects organ weights and illustrates that there is not a uniform linear relationship between organ weight and body weight.

Further support to this nonlinear relationship is reported by Heroux and Gridgeman (1958). They studied the effect of cold acclimation on organ and tissue weights with

emphasis on expression of results. The researchers proposed that the assumption of a linear relationship between organ weight and body weight violates the fact of differential growth rate. As a solution to this assumption, they propose the same equation as Webster et al. (1947). The researchers noted that if body weight and organ weight are not correlated, (i.e. the regression line is zero), absolute weight should be used (Heroux & Gridgeman, 1958).

In their study, the researchers analyzed the organ weight data in three forms: 1) absolute, 2) relative and 3) regressed or logarithmically transformed organ weights (Heroux & Gridgeman, 1958).

Absolute and relative weights were suggested to be unreliable because rats in the experimental (cold) group lost a considerable amount of weight due to the cold compared to the control group. Furthermore, the use of relative organ weights could lead one to interpret that the cold hypertrophied the lung, brain and genitals. However, when regressed weights were used, there was no effect of cold on the lungs, brain and genital weights.

The multiple ways of reporting organ weights demonstrate a potential source of confusion and error in the literature. Furthermore, with exploration of how values were calculated, one may question the accuracy of some of the values or other findings and conclusions based on those values. The lack of consistency and possible inaccuracy of weight expression is perplexing to the practitioner and may undermine the study results.

Selected Individual Factors Influencing Body and Organ Weights

Several individual variables may affect body and organ and tissue weight. Factors of particular interest to this study include endogenous nutritional sequence, age, species and gender.

Endogenous nutritional sequence

Cahill (1970) conceptualized starvation in three phases: 1) early starvation, 2) late starvation and 3) premorbid starvation. Characteristics of each phase will be outlined.

Early starvation commences after several hours without food intake. This phase is characterized by a decrease in blood glucose, decrease in blood insulin and an increase in blood glucagon levels. Glycolysis and gluconeogenesis are stimulated which maintain blood glucose levels within normal limits. Glycogen is mobilized from the liver and skeletal muscles. Glucose is used as fuel for the brain, white blood cells and red blood cells. An increase in free fatty acids serves as fuel for the heart, kidneys and muscle. Early starvation predominates for 5-10 days (Cahill, 1970).

Late starvation is characterized by continuing fat catabolism but with some protein conservation. The by-products of fat catabolism are ketone bodies which are used by the brain as a source of energy. The rate of loss of body weight and muscle weight are slowed. Behavioral characteristics such as the reduction in voluntary activity may be noted (Cahill, 1970).

Premorbid starvation commences when fat stores are depleted. At this stage the primary substrate for all energy needs is protein. A rapid body weight loss ensues with the

large increase in protein metabolism. Furthermore, visceral and protein stores are depleted. Thus, it would follow that organ weights would decrease in this phase. Without aggressive nutritional therapy death will ensue.

The stress response occurs parallel with the catabolic response. The stress response is characterized by sympathetic stimulation and a subsequent increase in circulated catacholamines. This increase in circulating catacholamines leads to increased catabolism and may increase the severity or progression of the starvation process (Cahill, 1970). Thus, organ weights may be affected more quickly if the stress response is activated or sustained. In animal studies, factors that may trigger the stress response are loud noises, changing the daily protocol routine and introducing a new animal handler or handling routines.

Age

Webster et al (1947) have reported that differential growth can effect the spleen, liver and kidney.

Species

Differences in organ weights and total body weights have been reported between rat species (Hoitinga, Mathot, VanZutphen & Beynenac, 1992).

Gender

Webster et al (1947) reported that in rats the male liver and kidneys consistently weigh more than those of a female for the same body weight. Furthermore, the total body

weight for males largely exceeds that of females as the animals advance in age (Weihe, 1987). Thus, a single gender is preferred when comparing one group with another group.

Selected Environmental Factors Influencing Body and Organ Weights

Exogenous nutrients and their delivery

Kilocalorie level, fiber content and feeding schedule are three variables that can affect organ or tissue and body weights. Each of these variables will be discussed briefly in emphasizing the impact on body and organ or tissue weights.

Kilocalorie level. Sprague-Dawley rats maintained on a semistarvation diet that had 23% of kilocalories compared to a control diet showed marked body weight and organ weight losses (Young, Ramos & Harris, 1988). The semistarvation diet had the same ratio of calories from fat, carbohydrate and protein and the same essential vitamins and minerals as did the control diet. In addition to total body weight, the organs weighed were the liver, pancreas, heart and small intestine. Results were presented as absolute means and relative organ weights (i.e. milligrams/100 grams of body weight). For example, the absolute pancreas and heart weights of the semistarved group decreased compared to the control group. However, the relative pancreas and heart weights were greater in the semistarved group compared to the control group. The absolute mean and relative liver weights were decreased in the semistarvation group (Young et al., 1988). Thus, it appears that selected organ weights decreased with a reduction of kilocalories. The question remains as to how to most accurately present these data.

Findings from another study about organ weights after rats were fasted for 72 hours reported a significant decrease in final body weight, liver and intestinal relative weights (i.e. milligrams/100 grams of body weight) (Burrin, Britton & Ferrell, 1988). The kidney and stomach relative weights were unchanged. With the use of relative weights researchers assume a linear relationship between organ weight and body weight. In this study, there was no indication that this assumption was tested.

In a separate study, muscle was chosen as a variable, in part because skeletal muscle can be a sensitive indicator of malnutrition (Lopes, Russell & Whitwell, 1982). The heart, pancreas, liver, kidney, stomach and small intestine are sensitive indicators for severe or sustained malnutrition because of the presence of visceral proteins (Burrin et al., 1988; Young et al, 1988).

For the evaluation of muscle weight change, rats not receiving food for two days as well as rats fed a kilocalorie reduced diet until 25% of their initial body weight was lost, showed a significant decrease in mean *extensor digitorum longus* weight compared to control groups. Body weight also decreased significantly compared to controls (Nishio & Jeejeebhoy, 1992).

Overall rat muscle is composed of approximately 90% fast twitch fibers (Ariano, Armstrong & Edgerton, 1973). The *extensor digitorum longus* (EDL) muscle can represent this overall rat muscle fiber make up. The *EDL* is approximately 97% fast twitch fibers and 3% slow twitch fibers (Ariano et al., 1973).

Fiber content. Recently there has been increasing attention to dietary fibers, particularly guar gum, pectin, wheat bran and oat bran. Some research has been done with soy fiber, a constitute that is being incorporated into commercially available liquid feedings. The effects of soy fiber will be highlighted here.

Researchers have reported that colonic hypoplasia and/or atrophy develops when rats are fed enteral feedings (Thomas, Owen, Alexander & Williamson, 1993). With hypoplasia, it would follow that actual tissue organ weight would decline. Adding soy fiber to the diet was proposed as a way to prevent such change (Thomas et al., 1993).

Another study examining the effects of Jevity™ and Osmolite™ on the colonic structure and function in the rat demonstrated that the fiber-fed rats had a slightly greater but, statistically nonsignificant, colonic mucosal mass (Levine & Rosenthal, 1991). Proposed benefits of the prevention of colonic hypoplasia include a reduction of bacterial translocation from the gut and a decreased transit time which could contribute to maintaining the normal colonic mucosal mass (Spaeth, Berg, Specian & Deitch, 1990; Palacio, Rolandelli, Settle & Rombeau, 1990).

Another study comparing Enrich™ (containing soy fiber) and Ensure™, (containing no fiber), had slightly different results (Thomas et al., 1993). Based on crypt cell production rate, researchers reported that soy fiber in enteral feedings prevents mucosal atrophy in some colonic segments ($p<0.05$). However, the crypt cell production in the small intestine was not statistically different (Thomas et al., 1993). It may be extrapolated that increase in crypt cell production rate may affect organ weight.

The different results of the above studies may have been related to the study duration. The study by Levine et al. (1991) lasted two weeks, while the study by Thomas et al. (1993) lasted four weeks.

Osmolite HN™, a low fiber enteral formula, has been reported as maintaining gut mass when compared to Vivonex, an amino acid formula, that lowered distal gut mass when consumed (Zaloga, Ward & Prielipp, 1991).

Feeding schedule. Feeding animals on different schedules has been reported to effect glucocorticoid secretion and insulin levels (Westfall & Heitkemper, 1992) and may affect organ weights (Heitkemper, Miller & Shaver, 1989a). Researchers reported that rats maintained on a liquid diet, either *ad libitum* or with only 12 hour access to food (during rest time), demonstrated a greater proximal intestinal weight compared to distal intestinal weight in rat chow fed animals and liquid fed animals (Heitkemper et al., 1989a). However, only relative weights were reported.

Intermittent food delivery may increase intestinal mucosal cell proliferation by mechanical stimulation (Lo & Walker, 1989). With increase cell proliferation, it would follow that weight would be greater in these tissues.

Summary. Multiple nutritional manipulations can contribute to body and selected organ or tissue weight changes. Such changes may make it even more important to test for a linear relationship between organ weight and body weight.

Environmental factors: Room conditions

Weihe (1987) recommended that the room temperature and room humidity be maintained between 20^0 to 22^0 Celsius and 50 to 60%, respectively. Heroux and Gridgemen (1958) reported that rats maintained at 6^0 Celsius room temperature for four weeks had hypertrophied liver, intestine, kidney, heart and adrenals. Thus, it may be posited from these data and general knowledge about animal experiments that changes in room temperature or humidity, or both, may increase stress for an animal and thereby affect organ weight or body weight, or both.

A final environmental factor to consider is related to sacrificing and harvesting of organs. If organs sit in the body after death for any length of time then organ weights will increase as the pooled blood accumulates in the organs (Webster et al., 1947). Webster et al. (1947) notes that either decapitation or etherizing animals at sacrifice will not alter the spleen weight.

Conceptual Framework

This section will integrate the previous information from this chapter into a conceptual framework. The foundations for the framework will be discussed first.

The American Nurses Association (1980) has provided nurses with a definition of nursing "that promotes unity in nursing in a basic and common approach to practice " (p. 4) The definition focuses on nursing's responsibility for identifying actual or potential health problems and subsequently treating the responses (American Nurses Association, 1980).

Though the American Nurses Association focuses on "human responses" to illness, it is also appropriate to note the importance of animal responses in nursing research (Cunningham & Mitchell, 1982).

The first use of animals in nursing research was a published dissertation in 1930, as reported in the review by Cunningham and Mitchell (1982). Ruby Bohart evaluated killed tuberculosis antigen in guinea pigs. The general paucity of published animal research in nursing journals may be related in part to the reluctance of journal editors to publish animal research articles based on the feeling that there is too little applicability of the animal study to humans. Also, there were few nursing research journals prior to 1970 (Cunningham et al., 1982).

The results from animal testing may subsequently benefit human populations. The physiologic measurement of animal responses can be important in nursing research and in subsequent treatment of human responses to illness. For example, Mitchell studied the effect of turning on intracranial pressure in African baboons (Cunningham et al., 1982). In turn, her findings have shaped nursing protocols with selected acutely ill patient population. However, one must be cognizant that animal research does not replace human research.

With this foundation in mind, a conceptual framework is proposed. A nursing framework for individual responses to enteral feeding (Heitkemper & Shaver, 1989b) has been adapted for this study.

Components of the framework include individual and environmental factors as well as responses. In this study , individual factors of interest include species, gender, age, health status and body weight. External environmental factors of interest are diet composition, kilocalorie level, feeding schedule, as well as temperature, humidity, time cues, daily routines and tissue harvesting schedules. Individual and external environmental factors may interact with each other.

The individual and external environmental factors influence individual adaptation responses. Such responses may be physiological, pathological, experiential or behavioral in nature. For purposes of this study, the responses of interest are physiological and include both final total body weight and selected organ and tissue weights (see figure 2). Using the data from Table 2, this investigator decided to weigh organs and tissues from the thorax, abdomen and retroperitoneal space to test for a linear relationship between multiple organ and tissue weights and final body weight.

Thus, aspects of individual factors, external environmental factors and individual adaptation responses will guide this study.

Research Purposes

The primary research purposes of this nursing study using an animal model as follows:

1) To determine if there is a linear relationship between final total body weight and selected organ and tissue (wet weights of the heart, right lung, left lung, liver, spleen, pancreas, stomach, duodenum, jejunum [10cm], ileum [10cm], large intestine, kidneys, adrenals, and *extensor digitorum longus* muscle weights, under three selected nutritional

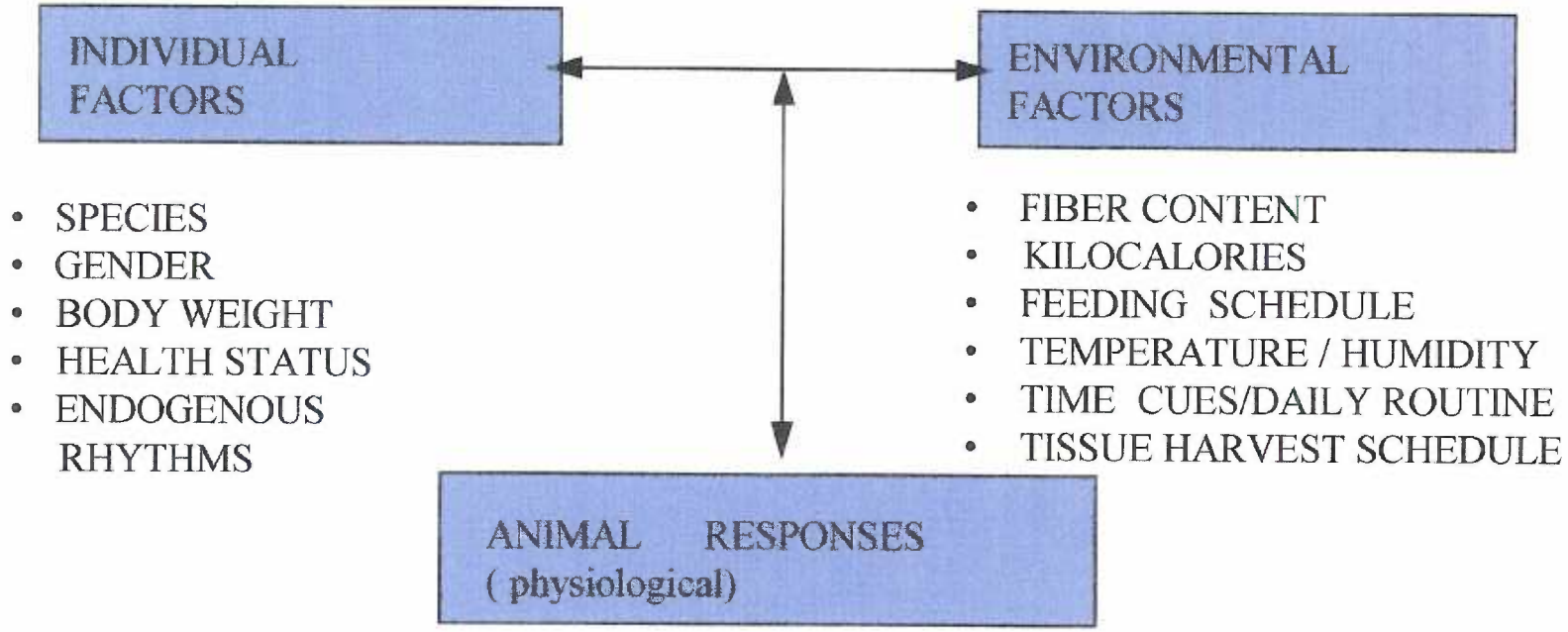


Figure 2: Conceptual framework for selected nutritional manipulations on final total body weight and selected tissue & organ weights.

manipulations.

2) A secondary purpose is as follows: To determine the effect of three nutritional interventions (kilocalorie level, fiber content and feeding schedule) on selected organ and tissue weights and final total body weights.

Conclusion

The determination of a linear relationship between selected organ and tissue weights and body weight will be derived from a study using three controlled nutritional manipulations. Findings from the literature review suggest that further research is needed in the area of organ and tissue weight expression. This is a necessary step in determining the scientific soundness of research and educating

nurses to the ways of accurately presenting data.

The following chapter will describe how, when and where the controlled nutritional manipulations will be performed as well as how, when and where the dependent variables will be collected, processed and analyzed.

CHAPTER 3

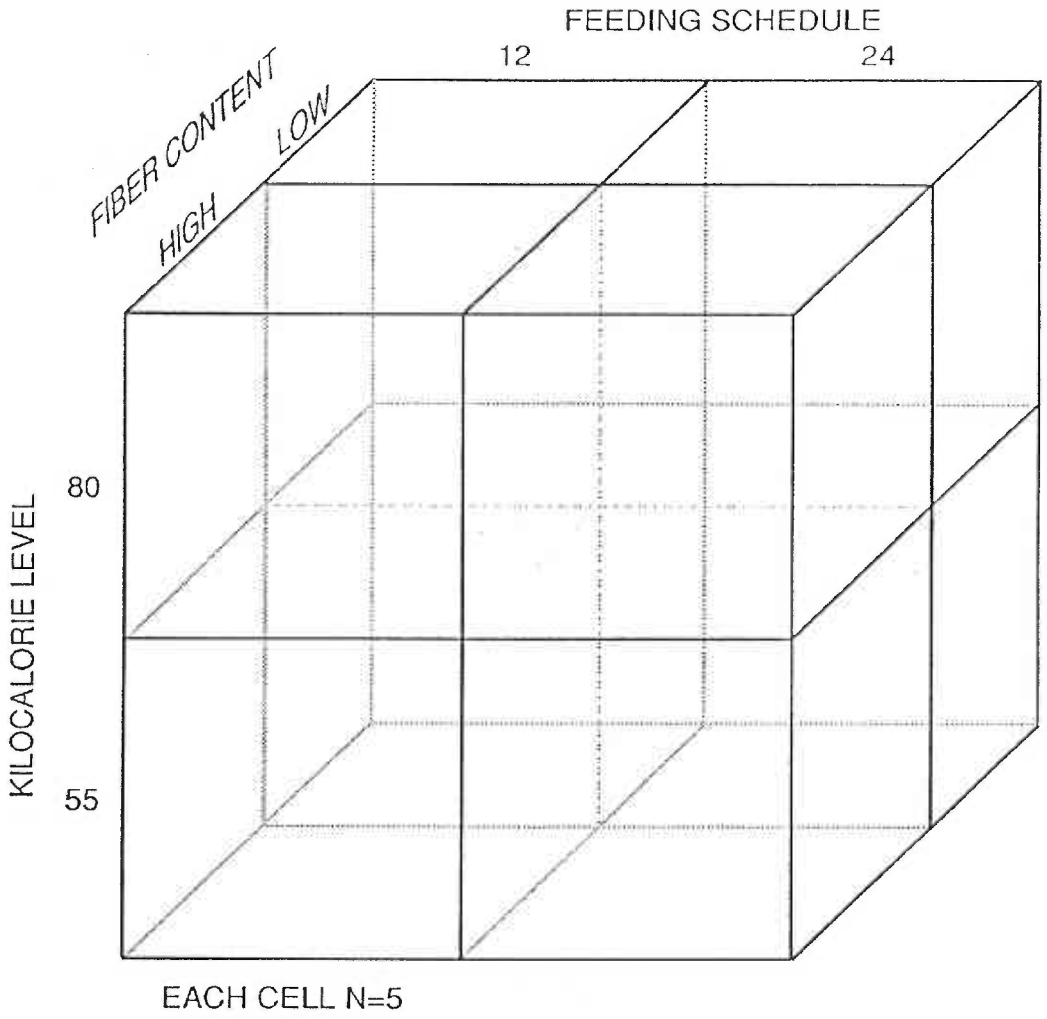
Methods

Overview

Purpose. The purpose of this study is to determine if there is linearity between body weights and selected organ and tissue weights in groups of rats after selected nutritional manipulations. From this determination, a more informed judgment may be made regarding accurate reporting of selected organ and tissue weights. Thus, the question driving this study is how to accurately present organ and selected tissue weights.

A factorial, randomized block 2x2x2 design was the chosen research method for study of a linear relationship between selected organ or tissue and final body weights. Rats were randomly assigned to one of eight nutrient manipulation cells. There were five rats per group. The independent variables were feeding schedule, kilocalorie level and fiber content. The dependent variables were organ and tissue weights (heart, right lung, left lung, liver, spleen, pancreas, stomach, duodenum, jejunum [10 cm], ileum [10 cm], large intestine, kidneys, adrenals, and *extensor digitorum longus* muscle) and final body weight. Figure 3 illustrates the study design.

For purposes of this study final total body weight will be obtained the morning of the last full day of the study. Organ weights were obtained in their immediate harvest form, (wet weight).



Kilocalorie level: Kilocalories during a 24 hour period.
Feeding schedule: 12 hours during rest period, 0600-1800.
Fiber content: High content equals 3.4 grams / 240 milliliters
Low content equals 0 grams / 240 milliliters.

Figure3 Study design with independent variables of kilocalorie level, feeding schedule, and fiber content

Sample

Forty male, disease-free Sprague-Dawley rats (Simenson Laboratories, Gilroy, CA) initially weighing 140-160 grams were requested for the study.

In order to maximize power (e.g. increase the homogeneity of the sample) several sample variables were controlled. All animals were obtained from the same vendor. All animals were male, Sprague-Dawley rats. All rats were approximately 6 weeks old.

This study was part of a larger research project. In both projects, there were 5 rats in each of the 8 nutrient manipulation cells. All cell assignments were random by using either a table of random numbers or by a random assignment method. The larger study had different dependent variables and a different purpose.

An animal model was chosen for this study because ethical and normative standards of practice prevent the use of human subjects. Rats were used because they have a gastrointestinal tract similar to humans and thus are an accepted animal model used in many gastrointestinal studies (Gallaher, 1992).

Organ weights to be sampled were as follows: heart, right lung, left lung, liver, spleen, pancreas, stomach, duodenum, jejunum (10 cm), ileum (10 cm), large intestine, kidneys, and adrenals. The selected tissue weight was the right *extensor digitorum longus* muscle.

Environment

Once animals arrived, a 7 day acclimation period began (Days -7 to -1). Initially where groups of 3-4 rats were housed together in one wire-bottom, suspended cage. Urine and stool passed through the grating onto padding which was changed daily.

Since rats are social animals, being in direct contact with others was part of this acclimation phase. A 12-hour on: 12-hour off lighting schedule was controlled by a central timer in the room. Animals were provided with rat chow and water *ad libitum*. Beginning on acclimation day 6 (Day -2) animals were given only liquid food.

All 40 rats were studied over a consecutive six week period during the summer months. Thus, seasonal variation were held constant.

Animals were housed at Oregon Health Sciences University's Animal Care Unit. The unit meets NIH standards for animal care. The room was 688.3 centimeters x 327.7 centimeters. Animals for this study shared a room with other research rats undergoing different research projects. A heavy door into the room was kept closed except when personnel entered and left the room to complete their work. Keeping the door closed minimized sporadic noises during the study.

Room temperature and humidity were monitored daily. Optimal room temperature for laboratory rats is between 19 to 23^{0}C and optimal humidity is between 45 to 65% (Weihe, 1987). Animal care personnel were notified if the temperature, humidity levels, or both were outside the optimal ranges to determine if corrections can be made. Stressing the animals by temperature/humidity fluctuations may adversely influence body weight (Weihe, 1987).

On acclimation day 6 (Day -2) rats were moved into single wire-bottom, suspended cages. Thirty individual cages fit onto one moveable rack. Steel grating was also on the front of the cage but solid steel was on the sides, top and back of the cage. Thus, animals

could not see each other. On the back wall of the cage there was a valve which dispenses water in response to pressure exerted by the animal. Rats were able to activate the water nozzle by opening the valve with their tongues. A glass graduated feeder containing the liquid food was attached to the inside front of the cage (Bioserv, Frenchtown, NJ). (see appendix B)

To open a cage, one slides the cage forward on a steel track. Animals were able to move freely in the cages. The individual cage dimensions were be 24.1 centimeters x 17.8 centimeters x 17.8 centimeters (length x width x depth).

Each animal cage had a steel plate which held the animal's study number and group designation. This hung on the outside of the cage. Despite the paraphernalia, the animals were visible through the front of the cage.

Handling

All animals were handled atleast twice daily, once shortly after the lights came on and the other time within 1 hour before the lights go off. Animals were handled gently using a flannel or terry cloth and were stroked and spoken to quietly. Each handling lasted 3-5 minutes per animal. Only 5 individuals participated in handling the animals and all used the same approach. Westfall (1993) proposed that the importance of handling animals with care during a study can contribute substantially to the discipline of nursing. See Appendix C for the daily handling and feeding protocol.

Measurement of variables

Body weight

All animals were weighed daily by the same personnel using an A & D balance, model EK-1200 (A&D Engineering, Milpitas, CA). Weights were recorded to the nearest 0.1 gram. Validity was checked with a known weight two times, once before the study began and once midway during the study. Before each use, the balance was checked for stability and rezeroed with the weighing basket in place. Animals were weighed in a plastic basket to prevent the animals from stepping off the balance.

Organ and tissue weights

Each organ or tissue was weighed immediately post-harvest using a Mettler balance, model AE 163 (Mettler Instumente, Greifensee, Switzerland). Weights were recorded to the nearest 0.001 milligram. Validity of the balance was checked with a known weight three times, once before the study begins, once midway through the studyand once after the study. Before each day's use the balance was checked for stability, left on for one hour prior to use and then calibrated according to the instructions (Mettler Instumente, 1982). A weighing boat was used for each organ or tissue. The balance was rezeroed with the boat in place before each use.

Procedures

Feeding schedule stages

The 28 day study sequence is outlined on table 3. Immediately upon the rats' arrival to the Animal Care Unit, they were placed in group housing, with 3-4 rats cage to begin

acclimatizing to a 12-hour on:12-hour off lighting schedule. They had free access to rat chow and water. On the sixth day (Day -2) rats were placed in individual wire-bottom, suspended cages and started on liquid food. The lighting schedule and the *ad libitum* water remained constant throughout the study.

On the first experimental day (Day +1) all rats had available to eat 80 kilocalories of liquid food with 24 hour access to this food. The food provided to half the rats was Osmolite HN™ (without fiber); the other half of the rats received Jevity™ (with fiber) (Ross Laboratories, Columbus, OH). Osmolite and Jevity were chosen because of their content similarities, excluding fiber. See Table 4 for a comparison of these two liquid foods.

On Day 6, half the rats from each group began a 12 hour access to food schedule; the other half remained on a 24 hour access to food schedule. On Day 11, half the rats from the 80 kilocalories (kcals)/12 hour schedule group began receiving 55 kcals/12 hour feeding schedule. Half the rats from the 80 kcal/24 hour feeding schedule began receiving 55 kcals during the 24 hour feeding schedule.

There was be a total of 8 groups: 1) high fiber, 80 kcals/24 hours; 2) high fiber, 55 kcals/24 hours; 3) high fiber, 80 kcals/12 hours; 4) high fiber, 55 kcals/12 hours; 5) low fiber, 80 kcals/24 hours; 6) low fiber, 55 kcals/24 hours; 7) low fiber, 80 kcals/12 hours and 8) low fiber, 55 kcals/12 hours (See figure 3).

Surgery and perioperative activities

On Day 15, 21 rats had catheters surgically placed in the carotid artery for planned

Table 3: **Study Sequence**

Day	Event	Phase
-7	Lighting: 12 hr/on, 12 hr/off Ad libitum Rat Chow Group housing	Environmental acclimation
-2	Liquid food Fiber content: Low / High Single cage housing	Nutritional acclimation
+1	Begin Experiment	
+6	Feeding Schedule: 24 hr./ 12 hr.	
+11	Kilocalories: 80 / 55	Maintenance
+15	Surgery	
+19 +20	Blood draws	
+21	Tissue harvesting	

Table 4: Comparison of Osmolite HN™ and Jevity™

Food Types

PER 8 FLUID OUNCES

Product	**Jevity**	**Osmolite HN**
Calories per ml	1.06	1.06
Osmolarity	310	300
Total cal/N ratio	150:1	150:1
Protein (G)	10.5	10.5
Carbohydrate (G)	35.9	33.4
Fat (G)	8.5	8.5
Calories	250	250
Dietary fiber	3.4	-
Water (mL)	197	199
Sodium (mg)	220	220
Potassium (mg)	370	370
Calcium (mg)	215	179
Phosphorus (mg)	179	179

Adapted from Ross Laboratories, 1993

blood draw on Days 19 and 20. Immediately prior to surgery, rats received a prophylactic intramuscular injection of 0.15 milliliters (concentration 40 mg/ 1 ml) Gentamycin (Solo Pak Laboratories: Franklin Park, IL). Rats underwent halothane general anesthetic induction. Under sterile conditions and using the same surgeon and assistant, tissue was cut down to the carotid artery and the catheter was secured in place. The tubing was tunneled under the pelt to exit suprascapularly. The surgery took approximately 20-30 minutes, with anesthesia effects lasting only a total of 35-40 minutes (B. Ogden, personal communication, May, 1992).

Rats were placed on a heated surface during surgery as temperature regulation is disrupted by anesthesia. Postoperation, lycra jackets were worn for added protection of the catheter. Despite the catheter, the rats were able to move freely about the cage. Immediately post-anesthesia, rats were placed in a semi-fowlers position and assessed for respiratory distress and pigmentation changes. Suctioning equipment and supplemental oxygen was in the operating room should an animal experience distress. Once the animal regained his capacity to move and clear his airway, a brief neurological assessment was done before returning him to his cage.

Patency of catheters was first checked 24-48 hours after surgery and every day there after. Once the catheter was checked or blood was drawn, the catheter was flushed with a combination of 1:1 50% dextrose and Heparin 1000 units/ml. When there was no blood return a set dose of streptokinase was given (M. VonDreele, personal communication, July, 1993). Catheter sites were assessed daily for signs of infection.

Twice each day, primarily during handlings, animals were assessed for well-being. Activity or behavior changes were noted because such changes may be associated with pain or illness.

On Days 19 & 20, blood samples were drawn from the carotid catheter as part of the larger study.

Final total body weight

Final total body weights were measured on Day 20.Final total body weights were determined by subtracting the amount of feeding consumed prior to being weighed from Day 20's weight (ml of feeding = 1 gram). In addition, if a protective jacket was present, the individual weight of each jacket was subtracted from the total final body weight on Day 20.

Sacrifice

On Day 21 animals were sacrificed by guillotine and trunk blood was drained. Rats were guillotined within 15 seconds of removal from their cage. One rat was sacrificed each hour to allow time for tissue harvesting from one animal before sacrificing the next animal. Animals were sacrificed sequentially rather than simultaneously to minimize blood pooling in the organs. Because animals were entered into the study in groups of 10 animals each week over 4 weeks, each week only 3-5 animals underwent surgery and 10 animals underwent sacrifice procedures on the specified protocol Day 15 and Day 21 respectively.

Harvesting of organs and tissues

All organs and tissues were harvested within 20 minutes of sacrifice. After the trunk blood was drained, the researcher of the larger study removed the gut. The carcass was then given to this investigator. The carcass was placed on an iced tile covered with saline soaked paper towels. This was done to keep the organs and tissues moist and cold.

Organs were removed and weighed in the following order: pancreas, stomach, duodenum, spleen, liver, kidneys, adrenals, heart, lungs and the right *extensor digitorum longus* muscle. The jejunum [10 cm], ileum [10 cm] and colon were weighed on the same balance but by the other researcher. See Appendix D for the harvest protocol and Appendix E for the data collection form. See Appendix F for the location of the organs and see Appendix G for the location of the tissue, *extensor digitorum longus* muscle.

Pilots

In preparation for data collection, four Sprague-Dawley female rats were obtained and used to refine the harvest protocol, including locating and cleaning of the organs and tissues.

Inter-rater reliability during the *extensor digitorum longus* muscle excision was performed with Dr. Hall, Director of Animal Care at Oregon Health Sciences University. Completeness of removing debris from the tissues was confirmed with a second investigator. Furthermore, a more experienced rat researcher was present during dissection. Criterion validity for selected organs and tissue excision was obtained by utilizing published illustrations.

Animal Care

Review process

Tissues were collected under the approved animal protocol of Una Elizabeth Westfall, Oregon Health Sciences University project number 92-055 and agency number 1R15 NR03337-01 (Appendix H).

Plan for Data Analysis

Descriptive analysis

Initially descriptive methods were used to identify the distributional charecteristics of the dependent variables. Plots, as well as mean and corresponding standard error of the mean were done for each dependent variable by: 1) the complete sample (N=40): 2) the independent variable levels-- 2 kilocalorie levels (n=20/group), 2 fiber contents (n=20/group), and 2 feeding schedules (n=20/group) and 3) the 8 individual feeding option cells (n=5/group). Pearson's r correlation coefficients were performed to examine the relationship between body weight on Day 20 and each organ or tissue weight for each independent variable (n=20) and for the overall sample (N=40).

Ninety-five percent confidence intervals for the correlation coefficients on the total sample were calculated to provide boundries for consideration of stability (Guilford & Fruchter, 1973). Since the sample size also influences the confidence interval, it was reccommended that it only be calculated only for the total sample (n=40) (B. Stewart; personal communication, April, 1994).

The sample size strongly influences the correlation coefficient value (B. Stewart; personal communication, April, 1994). For example, in a sample of 5 to 10 conclusions can be subject to wide variations and potential error. Thus only results from the total sample, and the groupings by independent variable levels were calculated.

Inferential analysis

Regression analysis, corresponding residual scatterplots and analysis of variance (ANOVA) were the inferential statistics used. The regression analysis and residual scatterplot were directed toward the primary purpose and the ANOVAs were directed toward the secondary purpose.

Linear regressions were performed between Day 20 body weight and each organ and tissue weight for each independent variable (n=20), as well as for the overall sample (N=40). Results were identical to the correlation coefficients obtained during the descriptive phase.

Residual scatterplots were graphed for each linear regression(N=40) to ascertain if assumptions of linearity, normality and homoscedasticity were met. If so, the pattern was considered "random" (Ran). The presence of residuals suggesting a violation of assumptions are designated as "some deviant pattern" (SP) or "definite pattern" (DP).

Three-way ANOVAs (kilocalorie level x fiber content x feeding schedule) were performed for each dependent variable to determine if there were any significant interactions. ANOVA allows comparison across the three independent variables within each feeding group. When there are equal numbers per cell, ANOVA is robust for

violations of niormality and homogeneity of variance. If significant three-way or two-way interaction effects were found, then a subsequent test was done with each of the 8 cells treated as a separate group. An *a posteriori* test (Tukey B) was performed to determine which group or groups differed significantly from each other. If no significant interactions were present, then main effects were interpreted. To confirm main effect findings, one-way ANOVAs were performed for each independent variable separately (B. Stewart, personal communication, April, 1994)..

A p-value $\leq$.05 (2-tailed) was the level of significance set *a priori* for this study. To meet this significance level, a multiple comparison correction was calculated for the following 5 dependent variables: Day 20 body weight, heart, left and right lung, pancreas, liver, spleen, stomach, duodenum, jejunum, ileum, large intestine, adrenals (2), kidneys (2) and *extensor digitorum longus* muscle (p=.05/15=.0033). A two-tailed test has been chosen because the investigator is unable to predict in which direction the data will fall on the distribution. It is noted that selecting a p-value $\leq$ .05 (as compared to a p $\leq$ .01)increases the possibility of detecting differences when there is not one present (i.e. Type I error) (Woods, 1988). The statistical package that was used to perform these calculations is SPSS 4.0 for the Macintosh.

CHAPTER 4

Results

Overview

The purpose of this study was to determine if there was a linear relationship between final total body weights and selected organ and tissue weights in groups of rats after three controlled nutritional manipulations. From this determination a more informed judgment may be made regarding accurate reporting of organ weights. Therefore, the question driving this study is how to accurately represent or present organ and selected tissue weights. A secondary purpose of this study was to determine the effect of the three nutritional manipulations on final total body weight and selected organ and tissue weights.

The sample consisted of 40 healthy, Sprague-Dawley, postpubescent, male rats. Rats were randomly assigned to one of eight feeding groups. All 40 animals completed the 21 day study.

At the conclusion of the study there were five animals per cell in this 2x2x2 factorial block design study. Some animal specific organs were eliminated from the statistical analysis. Organ or tissue weights that were 1.5 box-lengths from the 25th or 75th percentile, called outliers, or that were 3 box-lengths from the 25th or 75th percentile, called extremes, were examined carefully (SPSS, 1990). Some outliers and extremes values were deleted from the data because of harvest technique error. Others values were adjusted to the next highest or next lowest weight when they were a consequence of catheter insertion or surgery.

Final total body weights were measured on study Day 20. All tissues were collected on Day 21 of the study. The selected organ and tissue variables are presented by feeding group as actual weights (±SE) by grams or milligrams (mg). (Table 5) The selected organ and tissue results are organized by location within the body, i.e. thoracic, abdominal, retroperitoneal and lower extremity.

Linear regression and ANOVA procedures were conducted to examine the primary and secondary purposes, respectively. Figure 4 illustrates overall (N=40) Pearsons R and r squared. Appendix I illustrates residual scatterplots.

Body Weight

There were no body weight adjustments made, other than those described in the previous section. All 40 animal body weights were included.

The overall mean (±SE) by feeding group for body weight was 255.9 grams (±3.4). Mean (±SE) body weight by feeding group ranged from 228.9 grams (±2.9) in the low fiber, 55 kcal, 12 hour feeding schedule group to 281.1 (±4.1) in the low fiber, 80 kcal, 24 hour feeding schedule group.

Using three-way ANOVA there were no significant interactions between the three independent variables. (Table 6) There were, however, significant main effects found for kcal level ($p \leq .05$) demonstrating significant heavier body weights for the animals receiving 80 kcals per day compared to 55 kcals per day and feeding schedule ($p \leq .05$) demonstrating significant heavier body weights for those animals receiving food on a 24

hour feeding schedule. There were no significant differences ($p \leq .05$) between the two fiber content groups.

An analysis of covariance on the last full experimental day, while controlling for body weight the day after beginning the 12 hour feeding schedule (Day 7), demonstrated significant differences between the kilocalorie groups ($p \leq .05$). Not unexpectedly, those receiving more kcals had heavier weights than those receiving fewer kcals. Using a multiple comparison correction for only the three-way ANOVAs for final body weight ($p = .05/2 = .025$) , there was also a significant difference between the feeding schedule groups ($p \leq .05$), although the F ratio was reduced by almost half (8.89 from 17.89). When a multiple comparison correction was made for the 15 ANOVAs performed on final body weights and organ as well as tissue weights, there was no significant difference between the feeding schedule groups.

(Table 6)

Thoracic

Heart

There were no heart weight adjustments made. All 40 animal heart weights are included.

The overall mean (±SE) by feeding group for heart weight was 1,006.7 mg (±15.9). Mean (±SE) heart weight by feeding group ranged from 905.4 mg (±21.5) in the low fiber, 55 kcal, 12 hour feeding schedule group to 1,143.4 mg (±48.4) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 5 Pearson R and r^2 of heart weight values, correlated with Day 20 body weight, for each independent variable. The overall heart weight Pearson R is .8110 with corresponding 95% confidence interval ranged from .895 to .665. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values.

Using a three-way ANOVA, there were no interactions between the independent variables of fiber content, kilocalorie level and feeding schedule. (Table 7) There were, however, significant main effects for kcal level ($p \leq .05$), with the group receiving more kcals (80 kcals/24 hours) demonstrating significantly heavier hearts than those receiving less kcals (55 kcals/24 hours). One-way ANOVAs were performed for each of the three independent variables. Because each variable had two levels, there were 20 animals in each group. One-way ANOVAs showed no significant effects ($p \leq .05$) for fiber content and feeding schedule. One-way ANOVA did confirm significant effects ($p \leq .05$) for kcal level. (Table 8)

Right lung

The right lung weight for animal #432 was an outlier. This right lung weight was subsequently adjusted from 985.7 mg to 823.4 mg. This animal had had a carotid artery catheter placed. Subsequent behavior of the animal led the investigator to conclude that the animal may well have experienced complications from the insertion or maintenance of the catheter.

The overall mean ($\pm$SE) by feeding group for right lung weight was 753.3 mg ($\pm$11.7 mg). Mean ($\pm$SE) right lung weight by feeding group ranged from 708.1 mg

(±38.6) in the low fiber, 55 kcal, 12 hour feeding schedule group to 823.4 mg (±24.9) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 6 for Pearson R and r^2 of right lung weight values, correlated with Day 20 body weight, for each independent variable. The overall right lung Pearson R is .5393 with corresponding 95% confidence interval ranged from .730-.275. (Figure 4) The residual plots have definite patterns for each independent variable and overall values.

Using a three-way ANOVA, there were no interactions between the independent variables of fiber content, kilocalorie level and feeding schedule and there were no significant main effects. (Table 9) Even when a multiple comparison correction was not done, there were no statistically significant results. One-way ANOVAs were performed for each of the three independent variables. Because each variable had two levels, there were 20 animals in each group. One-way ANOVAs showed no significant effects ($p \leq .05$) for each of the independent variables. (Table 10) Using unadjusted values, these results remained unchanged.

Left lung

The left lung weight for animal #428 was neither an outlier nor extreme according to the box-plot. However, when a scatterplot of left lung weights to total final body weights was viewed, it showed that this lung weight was much greater than other left lung weights. Thus, animal #428's left lung weight was adjusted from 524.4 mg to 488.8mg. This weight was likely a possible consequence of the insertion of the arterial catheter.

The overall mean (±SE) by feeding group for left lung weight was 419.4 mg (±6.2 mg). Mean (±SE) left lung weight by feeding group ranged from 395.5 mg (±8.7)

in the low fiber, 55 kcal, 24 hour feeding schedule group to 454.1 mg (±10.3) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 7 for Pearson R and r^2 of left lung weight values, correlated with Day 20 body weight, for each independent variable. The overall left lung Pearson R is .5776 with corresponding 95% confidence interval ranged from .755 to .325. (Figure 4) The residual plots had definite patterns for each independent variable and overall values except for the residual plots for the 24 hour feeding schedule group. This group has somewhat of a pattern to the residual plot.

Using a three-way ANOVA, there were no interactions between the independent variables of fiber content, kilocalorie level and feeding schedule and there were no significant main effects. (Table 11) When a multiple comparison correction was not done, feeding schedule was the only significant variable ($p \leq .05$). One-way ANOVAs were performed for each of the three independent variables. Because each variable had two levels, there were 20 animals in each group. One-way ANOVAs with multiple comparison correction showed no significant effects ($p \leq .05$) for each of the independent variables. (Table 12) Using unadjusted values, these results remained unchanged.

Abdominal

Liver

There were no liver weight adjustments made. All 40 animal liver weights are included.

The overall mean (±SE) by feeding group for liver weight was 10,374 mg (±190.9mg). Mean (±SE) liver weight by feeding group ranged from 9391.6 mg (±422.1) in the high fiber, 55 kcal, 12 hour feeding schedule group to 12233 mg (±198.8) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 8 for Pearson R and r^2 of liver weight values, correlated with Day 20 body weight, for each independent variable. The overall liver Pearson R is .7389 with corresponding 95% confidence interval ranged from .855 to .555. (Figure 4) The residual plots had definite patterns for each independent variable except for the high fiber group residual plot has somewhat of a pattern. The overall, high fiber and 12 hour feeding schedule residual plots have no pattern.

Using a three-way ANOVA, there were no significant interactions between the independent variables of fiber content, kilocalorie level and feeding schedule; however, there was a significant main effect for kilocalorie level ($p \leq .05$). (Table 13) When a multiple comparison correction was not done, fiber content, kilocalorie level and feeding schedule had significant main effects ($p \leq .05$).

One-way ANOVAs were performed for each of the three independent variables. Because each variable had two levels, there were 20 animals in each group. One-way ANOVAs showed no significant effects ($p \leq .05$) for each of the independent variables, though a trend was evident in the kcal group.

(Table 14)

Spleen

The spleen weight for animal #421 was adjusted from 912.9 to 757.1 mg because animal #421 was an outlier. This high weight was a likely sequela of the arterial catheter placed on Day 15. One spleen weight was missing from the data. Thus, 39 animals were included in this analysis.

The overall mean (±SE) by feeding group for spleen weight was 580.2 mg (±13.6mg). Mean (±SE) spleen weight by feeding group ranged from 506.9 mg (±28.3) in the high fiber, 55 kcal, 12 hour feeding schedule group to 638.7 mg (±32.5) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 9 for Pearson R and r^2 of spleen weight values, correlated with Day 20 body weight, for each independent variable. The overall spleen Pearson R is .5256 with corresponding 95% confidence interval ranged from .725 to .255. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values except for the high fiber group. This residual plot has somewhat of a pattern.

Using a three-way ANOVA, there were no interactions between the independent variables of fiber content, kilocalorie level and feeding schedule. There was a significant main effect for kilocalorie level ($p \leq .05$). (Table 15) One-way ANOVAs were performed for each of the three independent variables. One-way ANOVAs showed kilocalorie level to be a significant effect ($p \leq .05$). Thus, the animals receiving 80 kcals daily had heavier spleens than those receiving 55 kcals daily. (Table 16) Using unadjusted values, these results remained unchanged.

Pancreas

Midway through the study this investigator noted that the pancreas was being weighed with the mesentery attached. Thus, the sample size for the pancreas without mesentery was 25. The pancreas without mesentery attached was believed to be a more accurate depiction of pancreatic weight. In addition, animal #426 was an outlier. Therefore, this pancreatic weight was adjusted from 2,272.3 mg to 1,494.9 mg.

The overall mean (±SE) by feeding group for pancreatic weight was 1,105.8 mg (±61.2mg). Mean (±SE) pancreatic weight by feeding group ranged from 732.8 (±72.2) in the high fiber, 55 kcal, 12 hour feeding schedule group to 1,776.7 mg (±32.5) in the high fiber, 80 kcal, 12 hour feeding schedule group.

See Figure 10 for Pearson R and r^2 of pancreas weight values, correlated with Day 20 body weight, for each independent variable. The overall pancreas Pearson R is .7842 with corresponding 95% confidence interval ranged from .900 to .565. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values except the high fiber group.

Using a three-way ANOVA, there were significant interactions between feeding schedule and kilocalorie level. (Table 17)

A posteriori test (Tukey B) was performed to determine which groups differed significantly from each other. Tukey B shows that the animals receiving the 12 hour feeding schedule and 80 kcals had significantly heavier pancreas' than animals receiving 12 hour feeding schedule and 55 kcals.

Stomach

All stomachs were emptied of contents before weighing and were patted dry. No stomach weight adjustments were made. All 40 animal stomach weights were represented.

The overall mean (±SE) by feeding group for stomach weight was 1,315.2mg (±21.9 mg). Mean (±SE) stomach weight by feeding group ranged from 1,253.4 mg (±43) in the low fiber, 55 kcal, 12 hour feeding schedule group to 1,422.1 mg (±94.5) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 11 for Pearson R and r^2 of stomach weight values, correlated with Day 20 body weight, for each independent variable. The overall stomach Pearson R is .5812 with corresponding 95% confidence interval ranged from .755 to .325. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values.

Using a three-way ANOVA, there were no significant 2-way or 3-way interactions. There were also no significant main effects. One-way ANOVA was performed on each of the independent variables and showed that there were no significant effects. (Tables 18 & 19, respectivley)

Standardized duodenum

All duodenal segments were flushed of contents with normal saline and were patted dry before weighing. Duodenal lengths varied from 4-11 centimeters. All duodenal weights were calculated for a standardized 10 centimeter length. These calculated 10 cm weights

were used in subsequent analysis for duodenal weights. All 40 animal duodenal weights were included.

The overall mean (±SE) by feeding group for standardized duodenal weights was 815.5mg (±31.5 mg). Mean (±SE) standardized duodenal weight by feeding group ranged from 651.8 mg (±80.6) in the low fiber, 55 kcal, 12 hour feeding schedule group to 998.2 mg (±154.3) in the high fiber, 55 kcal, 12 hour feeding schedule group.

See Figure 12 for Pearson R and r^2 of standardized duodenum weight values, correlated with Day 20 body weight, for each independent variable. The overall standardized duodenum Pearson R is .1959 with corresponding 95% confidence interval ranged from .480 to -.125 (Figure 4) The residual plots have a definite pattern for each independent variable and overall values.

Using a three-way ANOVA, there were no significant 2-way or 3-way interactions. There were also no significant main effects. One-way ANOVA was performed on each of the independent variables and showed that there were no significant effects. (Tables 20 & 21, respectively)

Jejunum

Beginning 3 cm after the Ligament of Treitz, a 10 cm segment of jejunum was obtained. There were no jejunum weight adjustments made. All 40 animals were included. The segment was harvested and weighed by a second investigator. All jejunums were flushed of contents with normal saline and patted dry before weighing.

The overall mean (±SE) by feeding group for jejunal weights was 354.2mg (±11mg). Mean (±SE) jejunal weight by feeding group ranged from 338.9 mg (±36.5) in the low

fiber, 55 kcal, 12 hour feeding schedule group to 362.9 mg (±16.6) in the high fiber, 80 kcal, 12 hour feeding schedule group.

See Figure 13 for Pearson R and r^2 of jejunum weight values, correlated with Day 20 body weight, for each independent variable. The overall jejunum Pearson R is .2561 with corresponding 95% confidence interval ranged from .525 to -.060. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values except for the high fiber and 24 hour group. These residual plots have somewhat of a pattern.

Using a three-way ANOVA, there were no significant 2-way or 3-way interactions. There were also no significant main effects. One-way ANOVA was performed on each of the independent variables and showed that there were no significant effects. (Tables 22 & 23, respectively)

Ileum

A 10 cm segment of the ileum was obtained beginning 2 cm proximal to the cecum entrance. Animal # 427 had his ileal weight trimmed from 627.8 to 481 because of a variation in harvesting technique. This ileum was an outlier. All 40 animals were included in this analysis. This segment was harvested and weighed by a second investigator. All ileums were flushed of contents with normal saline and patted dry before weighing.

The overall mean (±SE) by feeding group for ileal weights was 362.6 mg (±11.6 mg). Mean (±SE) ileal weight by feeding group ranged from 323 mg (±38.1) in the high fiber, 55 kcal, 24 hour feeding schedule group to 411.8 mg (±32.5) in the low fiber, 55 kcal, 24 hour feeding schedule group.

See Figure 14 for Pearson R and r^2 of ileumweight values, correlated with Day 20 body weight, for each independent variable. The overall ileum Pearson R is .3369 with corresponding 95% confidence interval ranged from .585 to .025. (Figure 4) The residual plots have a definite pattern for each independent variable except for the 12 hour group, high fiber and 55 kcal residual plots. The 12 hour group residual plot has somewhat of a pattern while the high fiber and 55 kcal residual plots have no pattern. The overall residual plot has no pattern and was classified as random.

Using a three-way ANOVA, there were no significant 2-way or 3-way interactions. There were also no significant main effects. One-way ANOVA was performed on each of the independent variables and showed that there were no significant effects. (Tables 24 & 25, respectivley)

Standardized large intestine

Some large intestinal lengths were less than 10 cm. All large intestinal weights were calculated for 10 centimeters. These calculated weights were used in subsequent analysis for large intestinal weights. All 40 animals were included. This organ was harvested and weighed by a second investigator. All large intestines were flushed of contents with normal saline and patted dry before weighing.

The overall mean (±SE) by feeding group for standardized large intestinal weights was 531.9 mg (±10.5mg). Mean (±SE) standardized large intestinal weight by feeding group ranged from 479.4 (±37.1) in the low fiber ,55 kcal, 12 hour feeding schedule group to 595.6 mg (±33.5) in the high fiber, 80 kcal, 12 hour feeding schedule group.

See Figure 15 for Pearson R and r^2 of standardized large intestine weight values, correlated with Day 20 body weight, for each independent variable. The overall standardized large intestine Pearson R is .2925 with corresponding 95% confidence interval ranged from .555 to -.020. (Figure 4) The residual plots have a definite pattern for each independent variable except for animals receiving high fiber and 12 hour feeding schedule. The high fiber residual plot has somewhat of a pattern and the 12 hour residual plot has no pattern. The overall residual plot has somewhat of a pattern.

Using a three-way ANOVA, there were no significant 2-way or 3-way interactions. There were also no significant main effects. One-way ANOVA was performed on each of the independent variables and showed that there were no significant effects. Tables 26 & 27, respectivley)

Retroperitoneal

Kidneys, total

The right and left kidney weights were combined to produce a total kidney weight because these organs are not anatomically or physiologically different from each other. There were no kidney weight adjustments made. All 40 animals were included.

The overall mean (±SE) by feeding group for total kidney weights was 2162.8 mg (±26.4). Mean (±SE) total kidney weight by feeding group ranged from 2,038 mg (±43.71) in the low fiber ,55 kcal, 12 hour feeding schedule group to 2,379 mg (±29) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 16 for Pearson R and r^2 of total kidney weight values, correlated with Day 20 body weight, for each independent variable. The overall total kidney Pearson R is

.6998 with corresponding 95% confidence interval ranged from .830 to .495. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values except for the 55 kcal, 12 hour and 24 hour residual plots. The 55 kcal residual plot and the hour residual plots have somewhat of a pattern while the 24 hour residual plot has no pattern.

Using a three-way ANOVA, there were no significant 2-way or 3-way interactions; however, significant main effects were present for kilocalorie level ($p \leq .05$). (Table 28) Animals receiving 80 kilocalories had significantly heavier kidneys than those receiving 55 kilocalories.

One-way ANOVA showed that kilocalorie level remained significant ($p \leq .05$). (Table 29)

Adrenals, total

The right and left adrenal weights were combined to produce a total adrenal weight because these organs are not anatomically or physiologically different from each other. The right and left adrenal from animals # 404 and # 409 and right adrenal from animal # 413 were omitted from further analysis. They were outliers that were inadvertently dissected through the cortex to the medulla during harvesting. Thus, an accurate weight could not be obtained. The right adrenal from animal # 406 was adjusted from 18.5 to 14.8 mg because it was an outlier, most likely related to harvesting procedure. The left adrenal for animal # 406 was harvested with good technique and was not trimmed or omitted from data analysis. Thus, 37 total adrenal weights were included in this analysis.

The overall mean (±SE) by feeding group for total adrenal weights was 48.4 mg (±0.8). Mean (±SE) total adrenal weight by feeding group ranged from 45.2 mg (±1.9) in the high fiber, 80 kcal, 12 hour feeding schedule group to 51.9 mg (±2.4) in the high fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 17 for Pearson R and r^2 of total adrenal weight values, correlated with Day 20 body weight, for each independent variable. The overall total adrenal Pearson R is -.0909 with corresponding 95% confidence interval ranged from .335 to -.326. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values except for the 12 hour group. This residual plot has somewhat of a pattern.

Using a three-way ANOVA, there were no significant 2-way, 3-way interactions or main effects. (Table 30) Using one-way ANOVAs there were also no significant effects of each independent variable on total adrenal weight. (Table 31).

Tissue

Extensor digitorum longus muscle

There were no *extensor digitorum longus* muscle weight adjustments made. All 40 animal *extensor digitorum longus* muscle weights were included.

The overall mean (±SE) by feeding group for *extensor digitorum longus* muscle weights was 118 mg (±1.6). Mean (±SE) *extensor digitorum longus* muscle weight by feeding group ranged from 111.8 mg (±2.4) in the high fiber, 55 kcal, 12 hour feeding schedule group to 127.1 mg (±3.1) in the low fiber, 80 kcal, 24 hour feeding schedule group.

See Figure 18 for Pearson R and r^2 of *extensor digitorum longus* muscle weight values, correlated with Day 20 body weight, for each independent variable. The overall *extensor digitorum longus* muscle Pearson R is.7495 with corresponding 95% confidence interval ranged from .810 to .575. (Figure 4) The residual plots have a definite pattern for each independent variable and overall values.

Using a three-way ANOVA, there were no significant 2-way, 3-way interactions or main effects were . Using one-way ANOVAs there were also no significant effects of each independent variable on *extensor digitorum longus* muscle weight. (Tables 32 & 33, respectively)

To summarize the findings, there was support for a strong linear relationship between liver weight and Day 20 body weight. Detectable patterns in residual plots weaken the support for linearity of other organ weights that had correlation coeffients in the 0.5-0.8 range. Additionally, six tissue weights had correlation coefficinets less than 0.4.

Significant differences in weight have been detected between kcal levles for Day 20 body weight as well as for the following organ weights: heart, liver, spleen and kidneys. In addition pancreatic weights were significantly different in the 12 hour (rest period) fed groups, with the groups receiving 80 kcals being heavier than the group receiving 55 kcals.

Table 5:

Weight Mean and Stardard Error of the Mean Values in Eight Different Feeding Groups of Rats

Variable	Feeding Groups							
	80 kcal; hi fib 24 hr sched Mean±SE	55 kcal; hi fib 24 hr sched Mean±SE	80 kcal; hi fib 12 hr sched Mean±SE	55 kcal; hi fib 12 hr sched Mean±SE	80 kcal; lo fib 24 hr sched Mean±SE	55 kcal; lo fib 24 hr sched Mean±SE	80 kcal; lo fib 12 hr sched Mean±SE	55 kcal; lo fib 12 hr sched Mean±SE
Body Weight (Gm)	272.0 ±6.7 (5)	259.8 ±4.6 (5)	268.0 ±5.6 (5)	232.7 ±3.8 (5)	281.1 ±4.1 (5)	249.8 ±5.2 (5)	254.9 ±13.3 (5)	228.9 ±2.9 (5)
Thorax								
Heart (mg)	1028.8 ±36.6 (5)	974.5 ±21.3 (5)	1023.5 ±33.8 (5)	957.2 ±9.5 (5)	1143.4 ±48.4 (5)	967.7 ±10.9 (5)	1052.8 ±59.6 (5)	905.4 ±21.5 (5)
Lung, Right (mg)	795.8 ±33.0 (5)	762.5 ±24.5 (5)	733.9 ±28.7 (5)	760.9 ±45.9 (5)	823.4 ±24.9 (5)	716.6 ±13.8 (5)	725.1 ±30.3 (5)	708.1 ±38.6 (5)
Lung, Left (mg)	444.4 ±18.1 (5)	437.2 ±12.2 (5)	417.0 ±20.5 (5)	401.1 ±17.1 (5)	454.1 ±10.3 (5)	395.5 ±8.7 (5)	398.1 ±17.9 (5)	408.1 ±20.0 (5)
Abdomen								
Pancreas (mg)	1105.5 ±105.6 (3)	1218.5 ±169.6 (3)	1776.7 ±248.6 (3)	732.8 ±72.2 (3)	1165.2 ±132.3 (4)	959.3 ±106.0 (4)	1493.7 ±34.6 (2)	812.2 ±4.1 (3)
Stomach (mg)	1339.4 ±96.3 (5)	1322.5 ±46.6 (5)	1304.7 ±50.9 (5)	1300.5 ±37.2 (5)	1422.1 ±94.5 (5)	1323.2 ±41.0 (5)	1255.6 ±66.6 (5)	1253.4 ±43.0 (5)

( ) = Number/Group

Table (con't)

Variable	Feeding Groups							
	80 kcal; hi fib 24 hr sched Mean±SE	55 kcal; hi fib 24 hr sched Mean±SE	80 kcal; hi fib 12 hr sched Mean±SE	55 kcal; hi fib 12 hr sched Mean±SE	80 kcal; lo fib 24 hr sched Mean±SE	55 kcal; lo fib 24 hr sched Mean±SE	80 kcal; lo fib 12 hr sched Mean±SE	55 kcal; lo fib 12 hr sched Mean±SE
Duodenum• (mg)	838.1 ±46.6 (5)	747.6 ±79.2 (5)	840.5 ±89.2 (5)	998.2 ±154.3 (5)	873.7 ±87.2 (5)	774.9 ±25.6 (5)	799.2 ±67.1 (5)	651.8 ±80.6 (5)
Jejunum, (mg)	362.9 ±16.7 (5)	349.3 ±48.5 (5)	350.3 ±47.7 (5)	370.4 ±27.5 (5)	371.6 ±23.0 (5)	340.7 ±25.6 (5)	349.7 ±32.5 (5)	338.9 ±36.5 (5)
Ileum (mg)	390.2 ±28.2 (5)	323.0 ±38.1 (5)	355.2 ±19.3 (5)	369.0 ±17.3 (5)	381.7 ±21.1 (5)	411.8 ±32.5 (5)	343.8 ±50.6 (5)	325.8 ±41.9 (5)
Large Intestine• (mg)	521.7 ±25.5 (5)	523.2 ±36.5 (5)	595.6 ±33.5 (5)	527.5 ±22.7 (5)	547.3 ±26.7 (5)	547.1 ±26.4 (5)	513.3 ±14.2 (5)	479.4 ±37.1 (5)
Liver (Gm)	10.296 ±.387 (5)	10.066 ±.302 (5)	10.391 ±.260 (5)	9.392 ±.422 (5)	12.233 ±.199 (5)	10.489 ±.236 (5)	10.666 ±.777 (5)	9.461 ±.543 (5)
Spleen (mg)	621.0 ±39.3 (4)	589.5 ±45.3 (5)	608.6 ±33.5 (5)	506.9 ±28.3 (5)	638.7 ±32.5 (5)	524.1 ±29.1 (5)	633.0 ±34.2 (5)	528.1 ±28.8 (5)
Retroperitoneum								
Kidneys, R&L (mg)	2123.1 ±66.1 (5)	2073.8 ±38.9 (5)	2205.9 ±57.1 (5)	2048.4 ±43.8 (5)	2379.3 ±29.0 (5)	2142.2 ±29.8 (5)	2291.1 ±116.8 (5)	2038.4 ±43.7 (5)
Adrenals, R&L (mg)	51.9 ±2.5 (5)	49.1 ±1.7 (5)	45.2 ±1.9 (5)	49.4 ±3.1 (5)	46.8 ±2.3 (4)	49.2 ±2.3 (5)	47.2 ±1.9 (5)	47.5 ±2.4 (3)

( ) = Number/Group • = standardized to 10cm R&L = right and left organ weights combined

Table (con't)

Variable	Feeding Groups							
	80 kcal; hi fib 24 hr sched Mean±SE	55 kcal; hi fib 24 hr sched Mean±SE	80 kcal; hi fib 12 hr sched Mean±SE	55 kcal; hi fib 12 hr sched Mean±SE	80 kcal; lo fib 24 hr sched Mean±SE	55 kcal; lo fib 24 hr sched Mean±SE	80 kcal; lo fib 12 hr sched Mean±SE	55 kcal; lo fib 12 hr sched Mean±SE
Tissue								
Muscle (mg) *Extensor Digitorum Longus*	119.6 ±3.8 (5)	115.8 ±1.5 (5)	123.9 ±5.4 (5)	111.8 ±2.4 (5)	127.1 ±3.1 (5)	115.8 ±4.2 (5)	114.0 ±8.9 (5)	115.7 ±3.15 (5)

( ) = Number/Group

	Pearson's R	r squared	Confidence	Intervals
heart	0.811	0.6577	0.895-	0.665
left lung	0.5776	0.3336	0.755-	0.325
right lung	0.5393	0.2908	0.73-	0.275
liver	0.7389	0.5460	0.855-	0.555
spleen	0.5256	0.2763	0.725-	0.255
pancreas	0.7842	0.6150	0.9-	0.565
stomach	0.5812	0.3378	0.755-	0.325
duodenum	0.1959	0.0384	0.48-	(-)0.125
jejunum	0.2561	0.0656	0.525-	(-)0.6
ileum	0.3369	0.1135	0.585-	0.025
large intestine	0.2925	0.0856	0.555-	(-)0.02
total adrenal	-0.0909	0.0083	0.335-	(-)0.326
total kidney	0.6988	0.4883	0.83-	0.495
muscle	0.7495	0.5618	0.81-	0.575

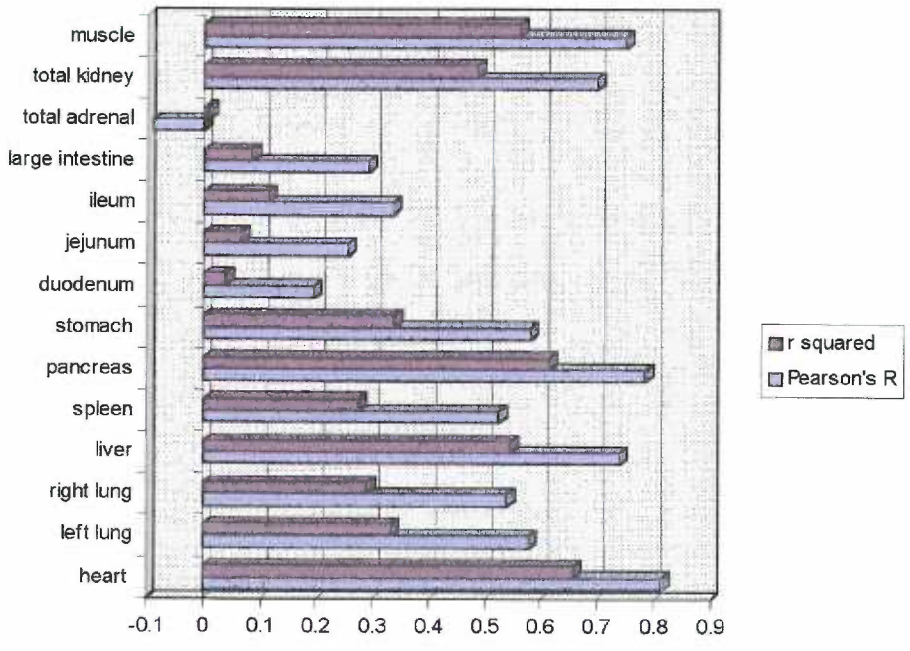


Figure 4: Pearson's R and r squared for entire sample.

Table 6:

Effect of Different Kilocalorie Levels, Feeding Schedules and Fiber Contents on Body Weight in Rats

Body Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	3628.30	3	16.97	0.000
Kcal Level	6859.16	1	32.08	0.000*
Feeding Schedule	3825.94	1	17.90	0.000*
Fiber Content	199.81	1	0.94	0.341
Two-Way Interaction	137.93	3	0.65	0.592
Sch x Kcal	195.36	1	0.91	0.346
Sch x Fib	158.40	1	0.74	0.396
Kcal x Fib	60.03	1	0.28	0.600
Three-Way Interaction				
Sch x Kcal x Fib	504.10	1	2.36	0.134
Residual	213.80	32		
Covariate (Weight Day 7)	3870.12	1	26.12	0.000*
Main Effects	3224.98	3	21.77	0.000
Kcal Level	7934.50	1	53.55	0.000*
Feeding Schedule	1330.04		8.98	0.005
Fiber Content	34.73	1	0.23	0.632
Two-Way Interaction	74.05	3	0.50	0.685
Sch x Kcal	173.73	1	1.17	0.287
Sch x Fib	47.26	1	0.32	0.576
Kcal x Fib	1.07	1	0.01	0.933
Three-Way Interaction				
Sch x Kcal x Fib	288.92	1	1.95	0.172
Residual	148.16	31		

* Multiple Comparison Correction $p \leq .05/ 15 = .0033$

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.7785	0.6214	0.8776	0.7655	0.8376	0.8223
r squared	0.606	0.3861	0.7702	0.586	0.7016	0.6778
n=	20	20	20	20	20	20

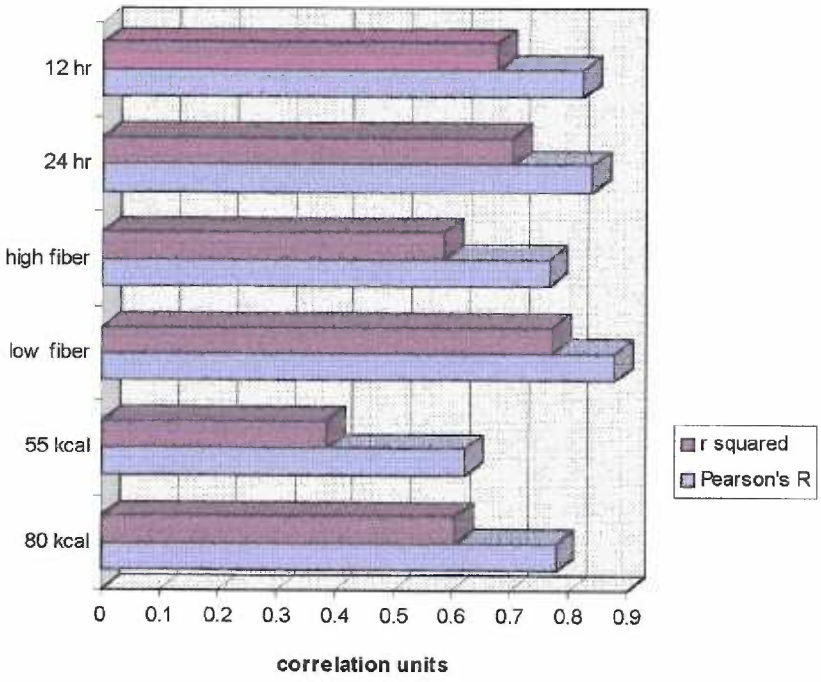


Figure 5: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule:heart weight to body weight Day 20.

Table 7:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Heart Weight in Rats: 3-Way ANOVA

Variable: Heart Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Overall Model	48957.51	3	8.241	0.000
Main Effects				
Feeding Schedule	19254.54	1	3.241	0.081
Kilocalorie Level	123076.84	1	20.718	0.000*
Fiber Content	4541.16	1	0.764	0.388
Two-Way Interaction	12138.97	3	2.043	0.127
FS x KL	164.03	1	0.028	0.869
FS x FC	10588.52	1	1.782	0.191
KL x FC	25664.36	1	4.320	0.046
Three-Way Interaction				
FS x KL x FC	1014.06	1	0.171	0.682
Residual	5940.48	32		

* Multiple Comparison Correction $p \leq 0.05/ 15 = .0033$

Table 8:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Heart Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance			
	Mean±SE (N)	MS	df	F Ratio	P Value
Kcal Level					
80 kcal	1,062.13 mg ±23.73 (20)				
		BG 123076.84	1	18.609	.0001*
		WG 6613.74	38		
55 kcal	951.19 mg ±9.91 (20)				
Fiber Content					
Low	1,017.31 mg ±27.64 (20)				
		BG 4541.16	1	0.467	NS
		WG 6613.74	38		
High	996.00 mg ±14.47 (20)				
Feeding Schedule					
24 Hour	1,028.60 mg ±22.01 (20)				
		BG 19254.54	1	2.060	NS
		WG 9345.91	38		
12 Hour	984.72 mg ±21.22 (20)				

* Multiple Comparison Correction p .05/15 =.0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.7271	0.2659	0.7502	0.2528	0.75	0.315
r squared	0.5287	0.0707	0.5641	0.0639	0.5625	0.0992
n=	20	20	20	20	20	20

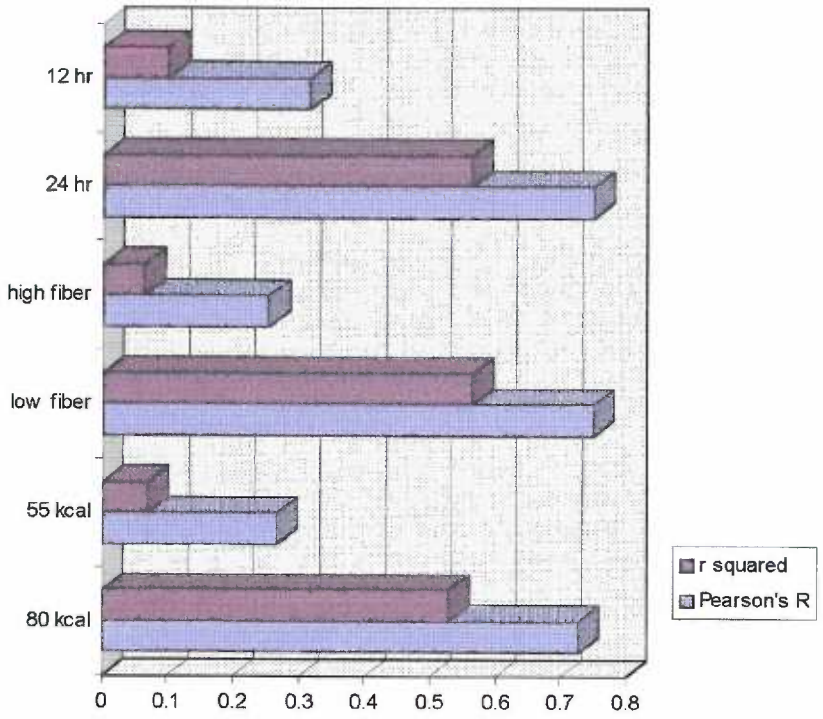


Figure 6: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule:right lung weight to body weight Day 20.

Table 9:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Right Lung Weight in Rats: 3-Way ANOVA

Variable: Right Lung Weight Three Way Analysis of Variance

	MS	df	F Ratio	P Value
Main Effects	10902.86	3	2.228	0.104
Feeding Schedule	18130.56	1	3.705	0.063
Kilocalorie Level	10582.01	1	2.162	0.151
Fiber Content	3996.00	1	0.817	0.373
Two-Way Interaction	7951.80	3	1.625	0.203
FS x KL	14055.00	1	2.872	0.100
FS x FC	1168.56	1	0.239	0.628
KL x FC	8631.84	1	1.764	0.194
Three-Way Interaction				
FS x KL x FC	541.70	1	0.111	0.742
Residual	4893.45	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 10:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Right Lung Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
80 kcal	769.55 mg ±16.48 (20)					
		BG	10582.01	1	1.980	NS
		WG	5345.11	38		
55 kcal	737.02 mg ±16.22 (20)					
Fiber Content						
Low	743.29 mg ±16.84 (20)					
		BG	3996.00	1	0.724	NS
		WG	5518.43	38		
High	763.28 mg ±16.38 (20)					
Feeding Schedule						
24 Hour	774.57 mg ±14.65 (20)					
		BG	18130.56	1	3.523	NS
		WG	5146.46	38		
12 Hour	731.99 mg ±17.32 (20)					

Multiple Comparison Correction p .05/15 = .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.6754	0.4011	0.5839	0.5671	0.7148	0.3739
r squared	0.4562	0.1609	0.3409	0.3216	0.5109	0.1398
n=	20	20	20	20	20	20

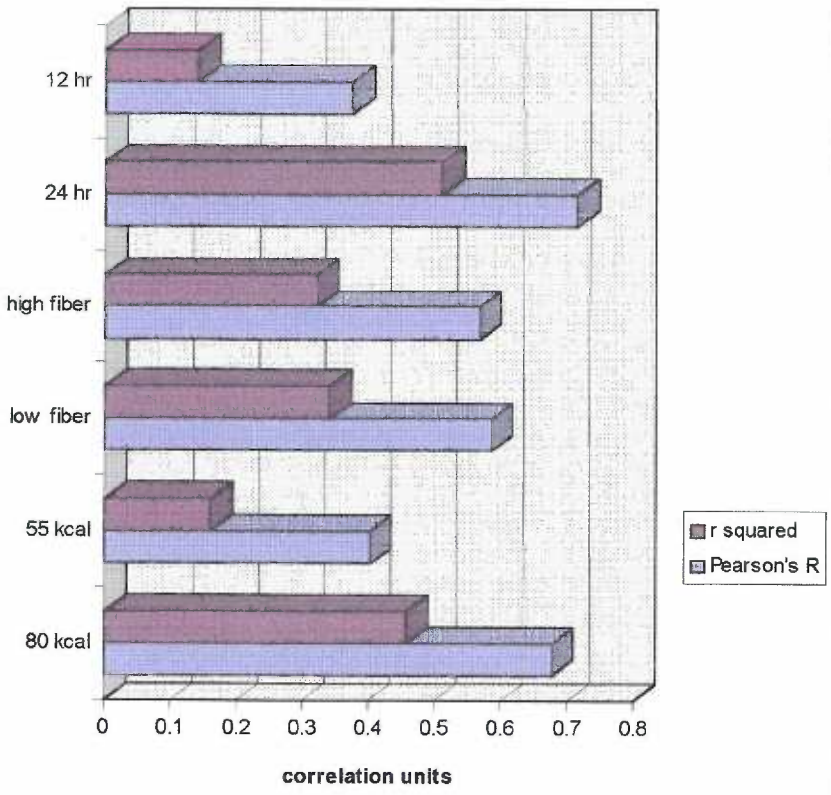


Figure 7: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: left lung weight to body weight Day 20.

Table 11:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Left Lung Weight in Rats: 3-Way ANOVA

Variable: Left Lung Weight **Three Way Analysis of Variance**

	MS	df	F Ratio	P Value
Main Effects	3859.62	3	2.953	0.047
Feeding Schedule	7155.63	1	5.476	0.026
Kilocalorie Level	3222.03	1	2.466	0.126
Fiber Content	1201.22	1	0.919	0.345
Two-Way Interaction	969.30	3	0.742	0.535
Sch x Kcal	2244.00	1	1.717	0.199
Sch x Fiber	253.01	1	0.194	0.663
Kcal x Fiber	410.88	1	0.314	0.579
Three-Way Interaction				
Sch x Kcal x Fiber	3724.90	1	2.850	0.101
Residual	1306.84	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 12:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Left Lung Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
80 kcal	428.42 mg ±9.36 (20)					
		BG	3222.03	1	2.155	NS
		WG	1494.96	38		
55 kcal	410.47 mg ±7.87 (20)					
Fiber Content						
Low	413.96 mg ±8.77 (20)					
		BG	1201.22	1	0.7759	NS
		WG	1548.14	38		
High	424.92 mg ±8.83 (20)					
Feeding Schedule						
24 Hour	432.82 mg ±7.81 (20)					
		BG	7155.63	1	5.143	NS
		WG	1391.45	38		
12 Hour	406.07 mg ±8.84 (20)					

Multiple Comparison Correction p .05/ 15= .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.7457	0.5224	0.8787	0.6666	0.6846	0.7131
r squared	0.5561	0.2729	0.7721	0.4444	0.4687	0.5085
n=	20	20	20	20	20	20

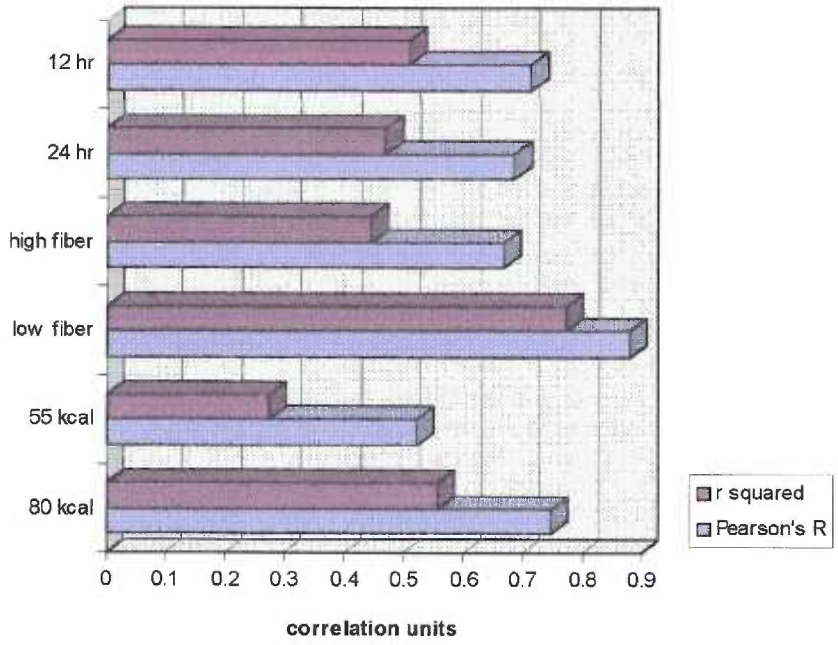


Figure 8: Pearson's R and r squared for kilocalorie level, fiber content, and feeding schedule: liver weight to body weight Day 20.

Table 13:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Liver Weight in Rats: 3-Way ANOVA

Variable: Liver Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	7259896.31	3	7.854	0.000
Feeding Schedule	6296660.55	1	6.812	0.014
Kilocalorie Level	10911578.22	1	11.804	0.002*
Fiber Content	4571450.16	1	4.945	0.033
Two-Way Interaction	1474709.33	3	1.595	0.210
FS x KL	33056.75	1	0.036	0.851
FS x FC	2542630.20	1	2.751	0.107
KL x FC	1848441.04	1	2.000	0.167
Three-Way Interaction				
FS x KL x FC	1070042.23	1	1.158	0.290
Residual	924393.19	32		

* Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 14:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Liver Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance			
	Mean±SE (N)	MS	df	F Ratio	P Value
Kcal Level					
80 kcal	10,896.26 mg ±278.50 (20)				
		BG 1091158.22 WG1209022.71	1 38	9.0251	.0047
55 kcal	9,851.68 mg ±208.19 (20)				
Fiber Content					
Low	10,712.03 mg ±322.43 (20)				
		BG 4571450.16 WG 1375868.19	1 38	3.3226	NS
High	10,035.91 mg ±183.37 (20)				
Feeding Schedule					
24 Hour	10,770.73 mg ±237.47 (20)				
		BG 6296660.55 WG 1330467.92	1 38	4.7327	NS
12 Hour	9,977.21 mg ±276.87 (20)				

Multiple Comparison Correction p .05/15= .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.1758	0.4683	0.5172	0.5559	0.4198	0.6097
r squared	0.0312	0.2193	0.2675	0.309	0.1762	0.3717
n=	19	20	20	19	19	20

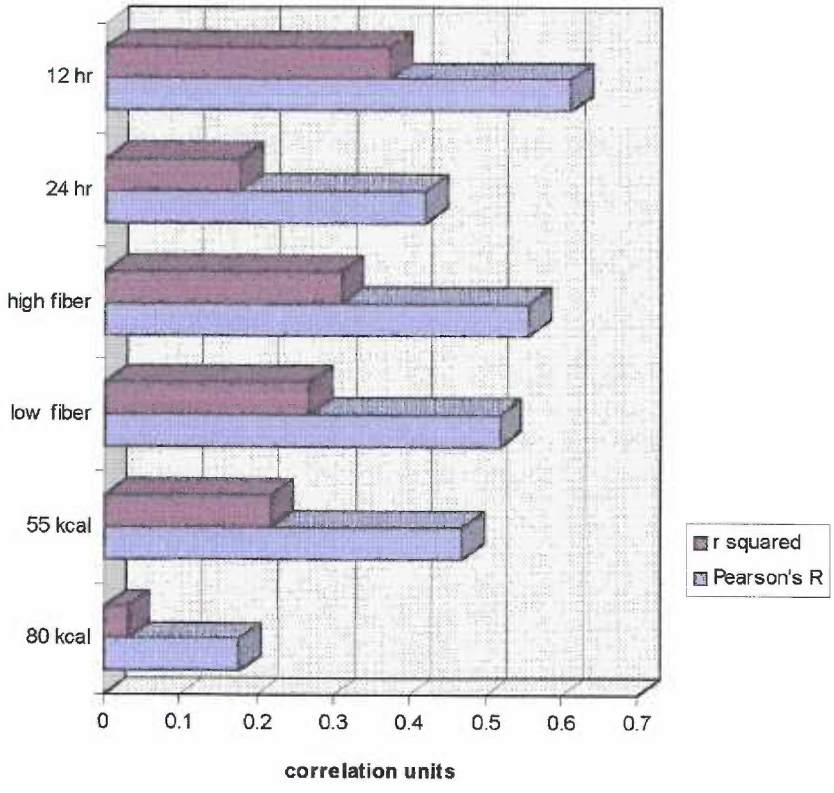


Figure 9: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: spleen weight to body weight Day 20.

Table 15:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Spleen Weight in Rats: 3-Way ANOVA

Variable: Spleen Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	27424.55	3	4.826	0.007
Feeding Schedule	6112.74	1	1.076	0.308
Kilocalorie Level	77251.15	1	13.594	0.001*
Fiber Content	19.76	1	0.003	0.953
Two-Way Interaction	4063.45	3	0.715	0.550
FS x KL	2033.84	1	0.358	0.554
FS x FC	5555.35	1	0.978	0.330
KL x FC	4278.57	1	0.753	0.392
Three-Way Interaction				
FS x KL x FC	3871.52	1	0.681	0.415
Residual	5682.54	31		

* Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 16:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Spleen Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
80 kcal	625.56 mg ±16.06 (19)					
		BG	76155.47	1	14.207	.0006*
		WG	5360.50	37		
55 kcal	537.15 mg ±17.02 (20)					
Fiber Content						
Low	580.99 mg ±19.09 (20)					
		BG	24.31	1	0.003	NS
		WG	7418.10	37		
High	579.41 mg ±19.94 (19)					
Feeding Schedule						
24 Hour	591.85 mg ±19.87 (19)					
		BG	5013.09	1	0.688	NS
		WG	7283.27	37		
12 Hour	569.17 mg ±18.81 (20)					

* Multiple Comparison Correction p .05/ 15= .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.7209	0.5201	0.6586	0.4705	0.5988	0.5413
r squared	0.5197	0.2705	0.4337	0.2214	0.3586	0.293
n=	20	20	20	20	20	20

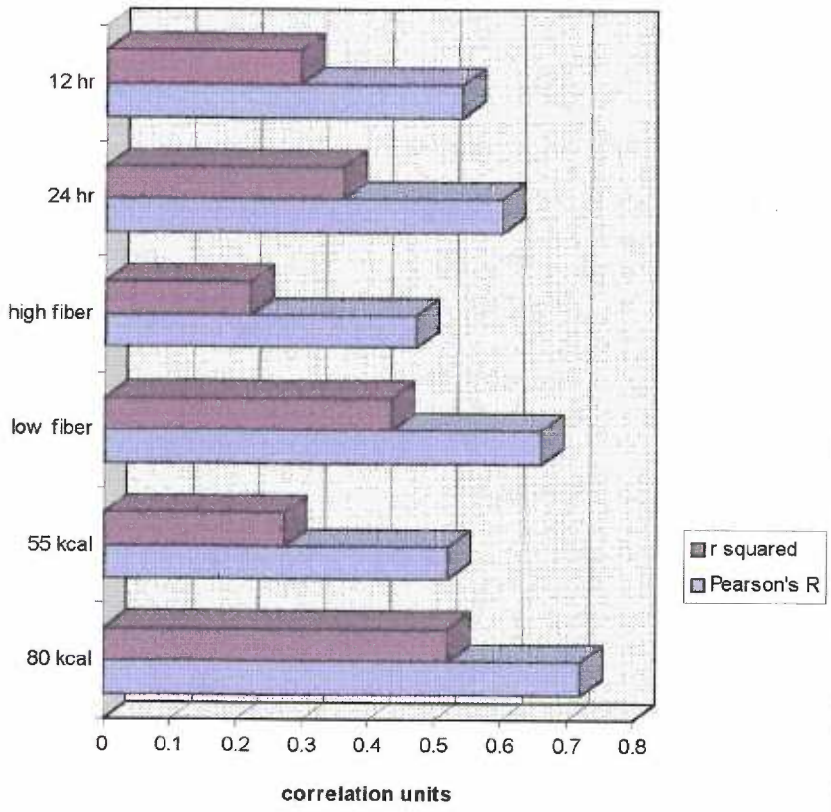


Figure11: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: stomach weight to body weight Day 20.

Table 18:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Stomach Weight in Rats: 3-way ANOVA

Variable: Stomach Weight	Three Way Analysis of Variance			
	MS	df	F Ratio	P Value
Main Effects	21015.20	3	1.040	0.388
Feeding Schedule	53619.01	1	2.654	0.113
Kilocalorie Level	9323.86	1	0.461	0.502
Fiber Content	102.72	1	0.005	0.944
Two-Way Interaction	10550.34	3	0.522	0.670
FS x KL	7488.43	1	0.371	0.547
FS x FC	20164.59	1	0.998	0.325
KL x FC	3998.00	1	0.198	0.659
Three-Way Interaction				
FS x KL x FC	4420.51	1	0.219	0.643
Residual	20204.00	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 19:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Stomach Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance			
	Mean±SE (N)	MS	df	F Ratio	P Value
Kcal Level					
80 kcal	1,330.45 mg ±39.02 (20)				
		BG 9323.86	1	0.481	NS
		WG 19376.87	38		
55 kcal	1,299.91 mg ±20.37 (20)				
Fiber Content					
Low	1,313.58 mg ±33.73 (20)				
		BG 102.72	1	0.005	NS
		WG 19619.53	38		
High	1,316.78 mg ±28.71 (20)				
Feeding Schedule					
24 Hour	1,351.79 mg ±35.36 (20)				
		BG 53619.01	1	2.944	NS
		WG 18211.21	38		
12 Hour	1,278.57 mg ±23.89 (20)				

Multiple Comparison Correction p .05/15 = .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.2765	0.0808	0.5494	-0.1535	0.2979	0.2147
r squared	0.0764	0.0665	0.3018	0.0236	0.0887	0.0461
n=	20	20	20	20	20	20

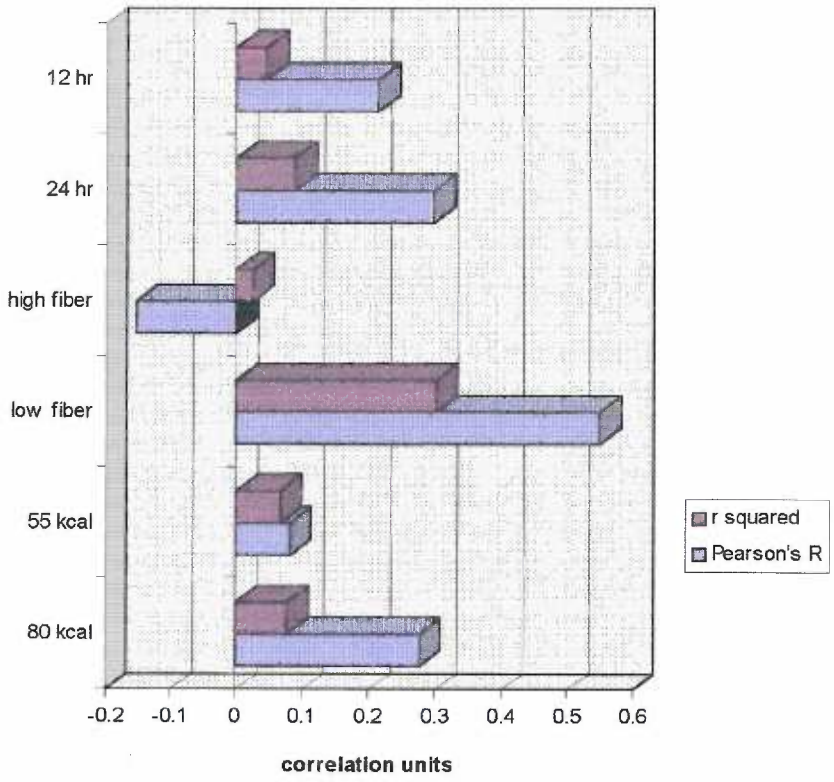


Figure 12: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: duodenum weight to body weight Day20.

Table 20:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Duodenum (standardized to 10 cm length) Weight in Rats: 3-Way ANOVA

Variable: Duodenum Weight Three Way Analysis of Variance

	MS	df	F Ratio	P Value
Main Effects	29277.02	3	0.788	0.510
Feeding Schedule	1907.54	1	0.051	0.822
Kilocalorie Level	20016.39	1	0.539	0.468
Fiber Content	65907.14	1	1.773	0.192
Two-Way Interaction	71099.63	3	1.913	0.147
FS x KL	24909.89	1	0.670	0.419
FS x FC	126940.28	1	3.415	0.074
KL x FC	61448.72	1	1.653	0.208
Three-Way Interaction				
FS x KL x FC	55089.64	1	1.482	0.232
Residual	37166.17	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 21:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Duodenum Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance			
	Mean±SE (N)	MS	df	F Ratio	P Value
Kcal Level					
80 kcal	837.88 mg ±34.74 (20)				
		BG 20016.39	1	0.499	NS
		WG 40145.28	38		
55 kcal	793.14 mg ±52.99 (20)				
Fiber Content					
Low	774.92 mg ±36.72 (20)				
		BG 65907.14	1	1.693	NS
		WG 38937.63	38		
High	856.10 mg ±50.45 (20)				
Feeding Schedule					
24 Hour	808.61 mg ±31.80 (20)				
		BG 1907.54	1	0.047	NS
		WG 40621.83	38		
12 Hour	822.42 mg ±55.24 (20)				

Multiple Comparison Correction p .05/15 = . 0033 Standardized to 10cm length.

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.4875	0.0417	0.4573	0.0523	0.2501	0.2888
r squared	0.2376	0.0017	0.2091	0.0027	0.0626	0.0834
n=	20	20	20	20	20	20

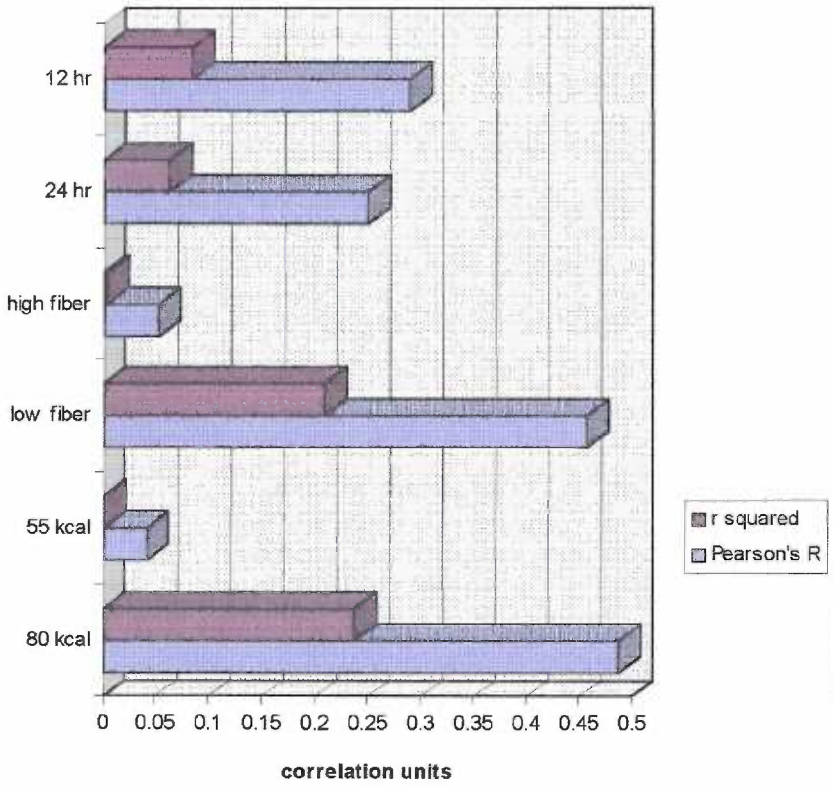


Figure 13: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: jejunum weight to body weight Day 20.

Table 22:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Weight for 10 cm of Jejunum in Rats: 3-Way ANOVA

Variable: Jejunum Weight	Three Way Analysis of Variance			
	MS	df	F Ratio	P Value
Main Effects	518.06	3	0.090	0.965
Feeding Schedule	144.40	1	0.025	0.875
Kilocalorie Level	776.16	1	0.135	0.716
Fiber Content	633.62	1	0.110	0.743
Two-Way Interaction	1299.98	3	0.225	0.878
FS x KL	1798.28	1	0.312	0.581
FS x FC	649.64	1	0.113	0.739
KL x FC	1452.03	1	0.252	0.619
Three-Way Interaction				
FS x KL x FC	117.65	1	0.020	0.887
Residual	5769.39	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 23:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Jejunum Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
80 kcal	358.62 mg ±14.91 (20)					
		BG	776.16	1	0.156	NS
		WG	4984.63	38		
55 kcal	349.81 mg ±16.62 (20)					
Fiber Content						
Low	350.23 mg ±14.03 (20)					
		BG	633.62	1	0.127	NS
		WG	4988.38	38		
High	358.19 mg ±17.72 (20)					
Feeding Schedule						
24 Hour	356.11 mg ±14.42 (20)					
		BG	144.40	1	0.029	NS
		WG	5001.26	38		
12 Hour	352.31 mg ±17.09 (20)					

Multiple Comparison Correction p .05/ = .0033 10cm length.

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.6129	0.1155	0.4879	0.0832	0.1691	0.3683
r squared	0.3756	0.0133	0.238	0.0069	0.0286	0.1356
n=	20	20	20	20	20	20

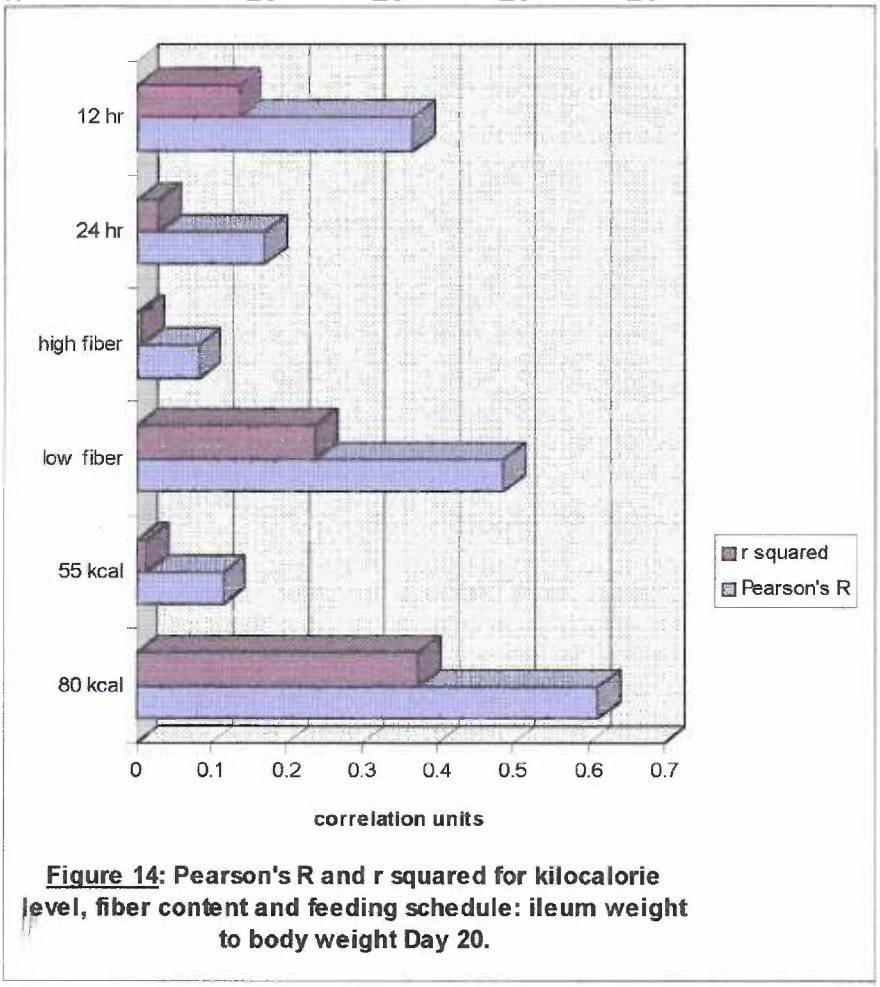


Figure 14: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: ileum weight to body weight Day 20.

Table 24:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Weight for 10 cm of Ileum in Rats: 3-Way ANOVA

Variable: Ileum Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	3148.54	3	0.577	0.635
Feeding Schedule	7972.15	1	1.460	0.236
Kilocalorie Level	1061.93	1	0.194	0.662
Fiber Content	411.52	1	0.075	0.785
Two-Way Interaction	4915.12	3	0.900	0.452
FS x KL	676.51	1	0.124	0.727
FS x FC	11394.00	1	2.087	0.158
KL x FC	2674.86	1	0.490	0.489
Three-Way Interaction				
FS x KL x FC	10416.76	1	1.908	0.177
Residual	5460.35	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 25:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Ileum Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
80 kcal	367.72 mg ±15.45 (20)					
		BG	1061.93	1	0.194	NS
		WG	5480.97	38		
55 kcal	357.42 mg ±17.59 (20)					
Fiber Content						
Low	365.78 mg ±19.11 (20)					
		BG	411.52	1	0.075	NS
		WG	5498.09	38		
High	359.36 mg ±13.59 (20)					
Feeding Schedule						
24 Hour	376.67 mg ±15.94 (20)					
		BG	7972.15	1	1.504	NS
		WG	5299.12	38		
12 Hour	348.45 mg ±16.61 (20)					

Multiple Comparison Correction p .05/15 = .0033 10cm length.

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.1695	0.2952	0.3888	0.1653	0.1505	0.4005
r squared	0.0287	0.0871	0.1512	0.0273	0.0227	0.1604
n=	20	20	20	20	20	20

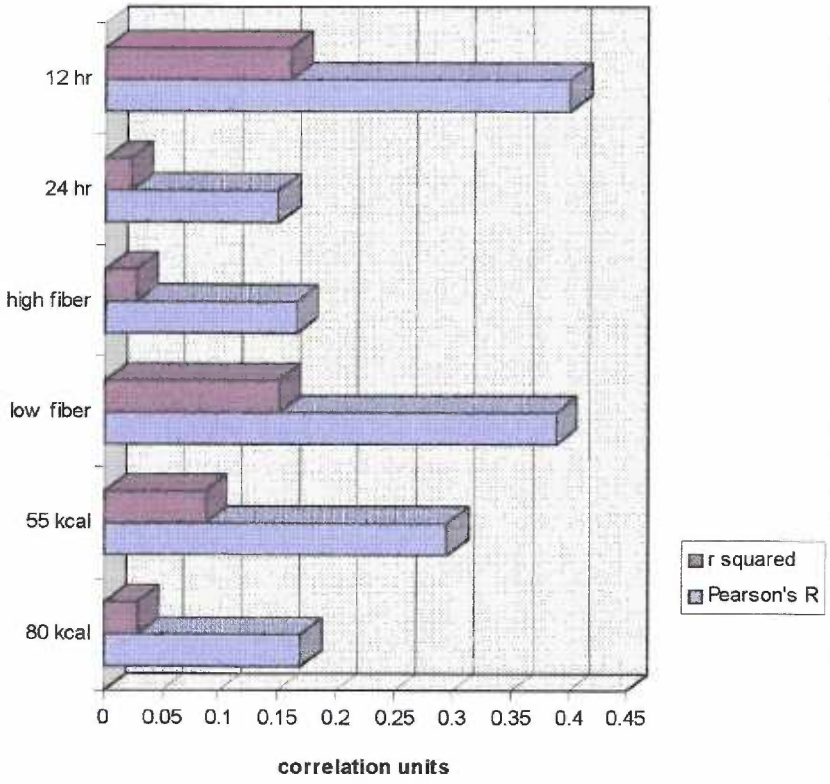


Figure 15: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: large intestine weight to body weight Day 20.

Table 26:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Weight for 10 cm of Large Intestine in Rats: 3-Way ANOVA

Variable: Colon Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	3596.03	3	0.871	0.466
Feeding Schedule	346.19	1	0.084	0.774
Kilocalorie Level	6344.36	1	1.537	0.224
Fiber Content	4097.54	1	0.993	0.327
Two-Way Interaction	9193.55	3	2.227	0.104
FS x KL	6674.44	1	1.617	0.213
FS x FC	20247.57	1	4.904	0.034
KL x FC	658.65	1	0.160	0.692
Three-Way Interaction				
FS x KL x FC	804.39	1	0.195	0.662
Residual	4128.40	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 27:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Large Intestine Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
	544.50 mg ±13.98 (20)					
		BG	6344.36	1	1.462	NS
		WG	4340.46	38		
55 kcal	519.31 mg ±15.08 (20)					
Fiber Content						
Low	521.78 mg ±14.09 (20)					
		BG	4097.54	1	0.931	NS
		WG	4399.59	38		
High	542.03 mg ±15.54 (20)					
Feeding Schedule						
24 Hour	534.85mg ±13.66 (20)					
		BG	346.19	1	0.077	NS
		WG	4498.31	38		
12 Hour	528.96 mg ±16.23 (20)					

Multiple Comparison Correction p .05/ 15= .0033 Standardized to10cm length.

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.6568	0.3674	0.8773	0.5701	0.6025	0.8181
r squared	0.4314	0.135	0.7696	0.325	0.363	0.6693
n=	20	20	20	20	20	20

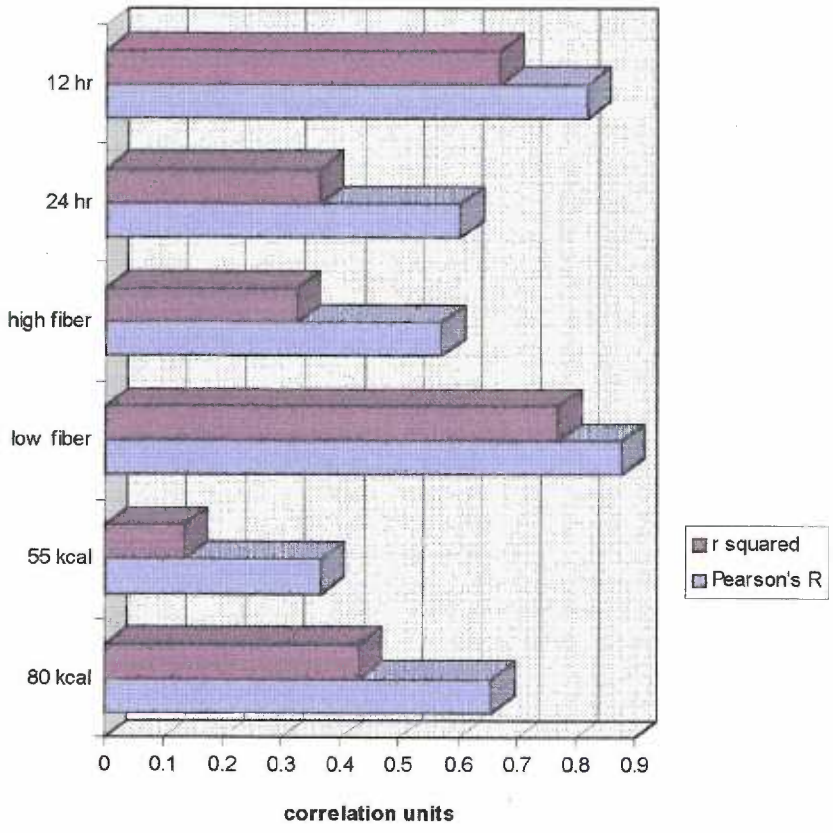


Figure 16: Pearson R and r squared for kilocalorie level, fiber content and feeding schedule: total kidney weight to body weight Day 20.

Table 28:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Kidneys Weight in Rats: 3-Way ANOVA

Variable: Kidneys Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	138166.62	3	7.799	0.000
Feeding Schedule	11319.86	1	0.639	0.430
Kilocalorie Level	303229.98	1	17.116	0.000*
Fiber Content	99950.01	1	5.642	0.024
Two-Way Interaction	32837.94	3	1.854	0.157
FS x KL	9582.12	1	0.541	0.467
FS x FC	38868.99	1	2.194	0.148
KL x FC	50062.70	1	2.826	0.103
Three-Way Interaction				
FS x KL x FC	5338.41	1	0.301	0.587
Residual	17716.62	32		

* Multiple Comparison Correction $p \leq 0.05/ 15 = .0033$

Table 29:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Kidneys Weight in Rats: 1-Way ANOVA

Independent Variable	Mean±SE (N)	One Way Analysis of Variance MS	df	F Ratio	P Value
Kcal Level					
	2,249.85 mg ±40.54 (20)				
		BG 303229.98	1	14.73	0.0005*
		WG 20580.37	38		
55 kcal	2,075.71 mg ±20.36 (20)				
Fiber Content					
Low	2,212.77 mg ±42.68 (20)				
		BG 99950.01	1	3.855	NS
		WG 25929.84	38		
High	2,112.79 mg ±27.78 (20)				
Feeding Schedule					
24 Hour	2,179.60 mg ±33.65 (20)				
		BG 11319.86	1	0.401	NS
		WG 28262.21	38		
12 Hour	2,145.96 mg ±41.15 (20)				

* Multiple Comparison Correction p .05/15 = .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	-0.0619	0.0123	0.0156	-0.196	-0.2437	-0.1641
r squared	0.0038	0.0001	0.0002	0.0384	0.0594	0.0269
n=	19	18	17	20	19	18

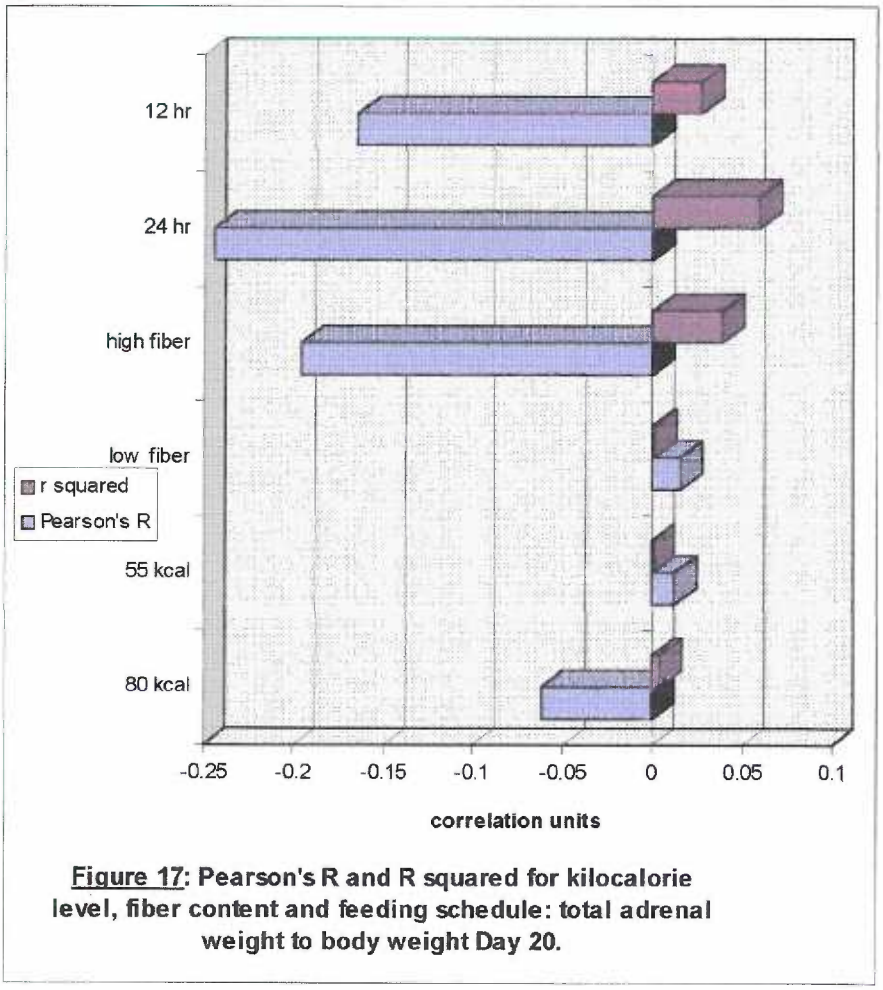


Figure 17: Pearson's R and R squared for kilocalorie level, fiber content and feeding schedule: total adrenal weight to body weight Day 20.

Table 30:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on Adrenals Weight in Rats: 3-Way ANOVA

Variable: Adrenals Weight	**Three Way Analysis of Variance**			
	MS	df	F Ratio	P Value
Main Effects	20.38	3	0.828	0.489
Feeding Schedule	37.79	1	1.536	0.225
Kilocalorie Level	7.43	1	0.302	0.587
Fiber Content	13.51	1	0.549	0.465
Two-Way Interaction	11.86	3	0.482	0.697
FS x KL	19.66	1	0.799	0.379
FS x FC	16.77	1	0.681	0.416
KL x FC	1.87	1	0.076	0.785
Three-Way Interaction				
FS x KL x FC	47.65	1	1.936	0.175
Residual	24.61	29		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 31:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on Adrenals Weight in Rats: 1-Way ANOVA

Independent Variable		One Way Analysis of Variance				
	Mean±SE (N)		MS	df	F Ratio	P Value
Kcal Level						
	47.83 mg ±1.14 (19)					
		BG	11.22	1	0.464	NS
		WG	24.19	35		
55 kcal	48.93 mg ±1.14 (18)					
Fiber Content						
Low	47.73 mg ±1.03 (17)					
		BG	12.81	1	0.530	NS
		WG	24.15	35		
High	48.91 mg ±1.21 (20)					
Feeding Schedule						
24 Hour	49.37 mg ±1.08 (19)					
		BG	39.53	1	1.961	NS
		WG	23.39	35		
12 Hour	47.31 mg ±1.17 (18)					

Multiple Comparison Correction p .05/15 = .0033

	80 kcal	55 kcal	low fiber	high fiber	24 hr	12 hr
Pearson's R	0.8741	0.4812	0.756	0.757	0.7919	0.762
r squared	0.764	0.2315	0.5715	0.573	0.6271	0.5806
n=	20	20	20	20	20	20

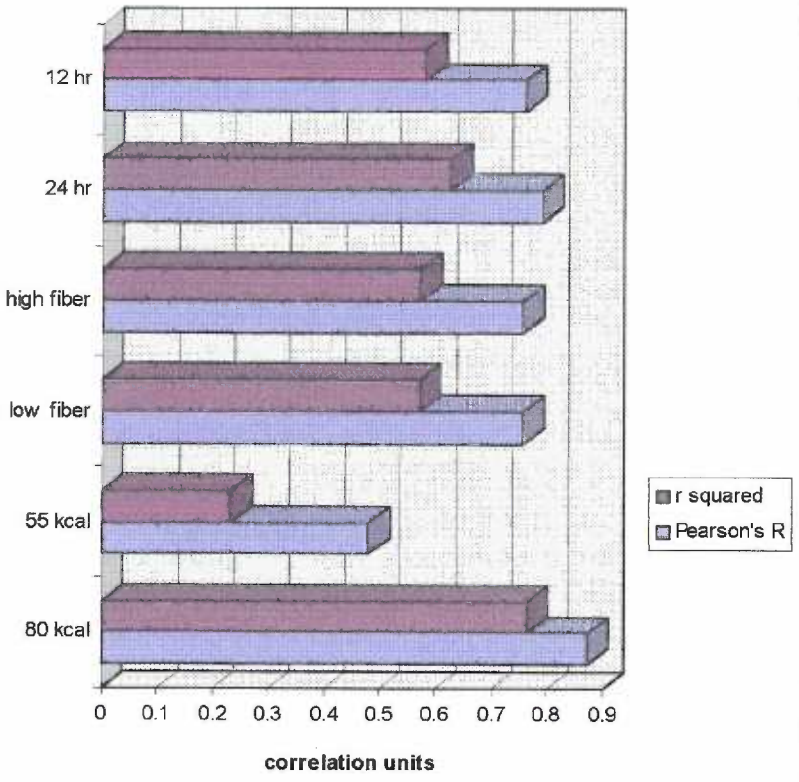


Figure 18: Pearson's R and r squared for kilocalorie level, fiber content and feeding schedule: muscle weight to body weight Day 20.

Table 32:

Effect of Different Feeding Schedules, Kilocalorie Levels, and Fiber Content on *Extensor Digitorum Longus* Muscle Weight in Rats: 3-Way ANOVA

Variable: Muscle Weight (***Extensor Digitorum Longus***)	**Three Way Analysis of Variance** MS	df	F Ratio	P Value
Main Effects	168.96	3	1.597	0.209
Feeding Schedule	104.01	1	0.983	0.329
Kilocalorie Level	401.32	1	3.794	0.060
Fiber Content	1.56	1	0.015	0.904
Two-Way Interaction	51.04	3	0.483	0.697
FS x KL	14.04	1	0.133	0.718
FS x FC	114.58	1	1.083	0.306
KL x FC	24.49	1	0.232	0.634
Three-Way Interaction				
FS x KL x FC	281.43	1	2.661	0.113
Residual	105.77	32		

Multiple Comparison Correction $p \leq 0.05/15 = .0033$

Table 33:

Effect of Different Kilocalorie Levels, Fiber Content, and Feeding Schedules on *Extensor Digitorum Longus* Muscle Weight in Rats: 1-Way ANOVA

Independent Variable	Mean±SE (N)	One Way Analysis of Variance				
			MS	df	F Ratio	P Value
Kcal Level						
	121.14 mg ±2.88 (20)					
		BG	401.32	1	3.886	NS
		WG	103.28	38		
55 kcal	114.80 mg ±1.42 (20)					
Fiber Content						
Low	118.17 mg ±2.75 (20)					
		BG	1.56	1	0.014	NS
		WG	113.80	38		
High	117.77 mg ±1.95 (20)					
Feeding Schedule						
24 Hour	119.58 mg ±1.85 (20)					
		BG	104.01	1	0.936	NS
		WG	111.11	38		
12 Hour	116.36 mg ±2.77 (20)					

Multiple Comparison Correction p .05/15 = . 0033

CHAPTER 5

Discussion

Overview

The primary purpose of this study was to determine if there was a linear relationship between final total body weights and selected organ and tissue weights in groups of rats after three controlled nutritional manipulations. From this determination a more informed judgment may be made regarding accurate reporting of organ weights. Therefore, the question driving this study is how to accurately represent or present organ and selected tissue weights. A secondary purpose of this study is to determine the effect of the three nutritional manipulations on final total body weight and selected organ and tissue weights.

The sample consisted of 40 healthy, Sprague-Dawley, postpubescent, male rats. Rats were randomly assigned to one of 8 feeding groups, for a total of 5 rats per cell in this 2x2x2 factorial, randomized block design study. All 40 animals completed the 21 day study. Twenty-one of the 40 animals had carotid artery catheters placed. The independent variables were kilocalorie level, fiber content and feeding schedule. The dependent variables were final total body weight and 14 organ and tissue weights.

After descriptive analysis, regression analysis, corresponding residual scatterplots and analysis of variance (ANOVA) were the primary inferential statistics used.

In this sample there was a strong linear relationship between liver weight and day 20 body weight. Residual plot patterns weakened the support for linearity between organ weight and body weight for organ weights with correlation coefficients of 0.5-0.8. Significant differences in weights ($p \leq .05$) were detected between kilocalorie levels for

the following dependent variables: Day 20 body weight, heart weight, liver weight, sleen weight and kidney weight. An interation ($p \leq .05$) between feeding schedule and kilocalorie level was present for the dependent variable of pancreatic weight.

Primary Research Purpose

Standardizing organ and tissue weights by body weights appears to be more defensible for some tissues, but somewhat less defensible for other tissues measured in this study. Both correlation coefficient values and residual plots were considered. Correlation coefficients for the entire sample (N=40) as well as for both levels of each independent variable (n=20) were done to examine the relationship between body weight or organ and tissue weight. For the total sample, the correlation coefficient values cluster into three groups. First, the correlation coefficients for the heart, pancreas, *extensor digitorum longus*, liver and kidneys weights ranged from .6998 to .8110. Second, the correlation coffecients for the stomach, left lung, right lung and spleen weights ranged from .5256 to .5812. And third, the correlation coefficients for the ileum, large intestine, jejunum, duodenum and adrenals weights ranged from -.0909 to .3369. (Table 34)

In addition to the correlation coefficient, the presence or absence of a pattern in the residual plots was considered. Even when correlations were moderately to quite high between final body weight and organ or tissue weight (.5256 to .8110), if there was a pattern in the plotted residual values, there may be a better fitting model than an unadjusted, simple, linear model. Interestingly, of the 9 organs and tissue weights having correlation coefficients of .52 or higher, only one had no pattern, or a randomness evident in the residual plot. The liver correlation coefficient was .7389 and the residual pattern

was random. Therefore, both correlation coefficient and the plotted residuals support a strong linear relationship between body and liver weight and standardization of liver weight by final total body weight.

In contrast, the ileum correlation coefficient was only .3369 but the residual pattern was random. The low correlation coefficient offers little support for single linearity between body weight and ileum weight, the underlying assumption for standardizing the ileum weight by the final body weight. In this study, using total body weight to adjust organ tissue weights for the large intestine, jejunum, duodenum and adrenals was not defensible because of the weak linear relationship between the organ or tissue weights and the body weight as well as the patterned residual plots. (Table 34)

The amount of stability across independent variables could only begin to be explored using total sample correlation coefficient 95% confidence intervals as possible boundries. More stability may be suggested for the left lung, stomach, jejunum and standardized large intestine. However, caution is advised when interpreting this finding because the 95% confidence intervals were calculated for the total sample (N=40), not for the smaller sample (n=20) groups.

Mean organ and tissue weights of this study were compared to organ and tissue weights from 6 current nutritionally based studies with similar final total body weights. (Table 35) Harris (1992) and Reif et al. (1993) reported liver weights to be 2 grams lighter than the mean liver weight in this study. Both liver weights from the published studies were from the control groups. Weights differences may be related to the length of the studies or gender differences by these researchers. The lighter weights may be related

to the longer study length of 7 weeks (Harris) and 8 weeks (Reif et al.), compared to 21 days for the current study. Additionally female rats were used rather than male rats.

Secondary research purpose

Significant differences using three-way ANOVAs were present for: kilocalorie level and feeding schedule in Day 20 body weight ($p \leq .05$), kilocalorie level in heart weight ($p \leq .05$), kilocalorie level in spleen weight ($p \leq .05$) and kilocalorie level in total kidney weight ($p \leq .05$).

An analysis of covariance for body weight on Day 20, while controlling for body weight the day after beginning the 12 hour feeding schedule (day 7), demonstrated significant differences between the kilocalorie groups ($p \leq .05$) but there was no longer a significant difference between the feeding schedule groups. Although it is not clear whether or not the statistical difference is from the schedule per se (24 hour versus 12 hour light period availability) or from the adjustment of the animals to the new schedule, both the lower feeding schedule F ratio with the covariate and the conservative multiple comparison correction lend support to the latter being the more likely explanation.

Kilocalorie level was the most significant independent variable influencing body weight. Animals receiving 80 kcals per day were heavier than animals receiving 55 kcals per day. After several hours of restricted food intake, glycogen stored in the liver and skeletal muscle are mobilized to provide glucose to meet energy needs. Gluconeogenesis is also stimulated, predominately from skeletal muscle, to serve as fuel for the brain, white blood cells and red blood cells (Cahill, 1970). Subsequently, body weight and muscle weight loss ensue.

Table34:

Findings by Dependent Variable in Rats: Correlation Coefficient, Residual Plot and Confidence Interval.

Organ/Tissue	n	Coefficient^	Confidence Interval	Residual Plot#
Heart	40	.8110	.895-.665	DP
Pancreas	25	.7842	.900-.565	DP
Muscle,EDL	40	.7495	.810-.575	DP
Liver	40	.7389	.855-.555	Ran
Kidneys	40	.6998	.830-.495	DP
Stomach	**40**	**.5812**	**.755-.325**	**DP**
Lung, left	**40**	**.5776**	**.755-.325**	**DP**
Lung, right	**40**	**.5393**	**.730-.275**	**DP**
Spleen	**39**	**.5256**	**.725-.255**	**DP**
Ileum (10 cm)	40	.3369	.585-.025	Ran
Large intestine	40	.2925	.555-(-).020	SP
Jejunum (10 cm)	40	.2561	.525-(-).060	SP
Duodenum	40	.1959	.480-(-).125	DP
Adrenals	37	-.0909	.335-(-).326	DP

^ Correlation coefficient with total body weight.

DP=definite pattern SP=some pattern Ran=random (assumptions met).

Table 35:

Comparison of Absolute Mean Organ Weight in 7 Studies.

STUDY	ABSOLUTE MEAN BODY WEIGHT	ABSOLUTE MEAN PANCREAS	LIVER	KIDNEYS	EDL
Schedler (1994)	255.9 G	1.11 G	10.37 G	2.16 G	118 G
Balaghi et al. (1992)	260 G	1.22 G			
Dhanakoti et al.(1992)	356 G			2.05 G	
Harris (1992)	256 G		8.1 G		
Levy et al. (1991)	278 G		10.6 G		
Nishio et al. (1992)	250 G				102 G
Reif et al. (1993)	227 G		8.1 G		

EDL = *Extensor digitorum longus* muscle

Though the mean muscle weight for the animals receiving 55 kcals per day was less than the animals receiving 80 kcals per day, (114.8 mg & 121.1 mg respectively) the difference was not statistically significant ($p>.05$). It may be that the *extensor digitorum longus* muscle is not the best muscle to sample for endogenous adjustment to starvation, or that the total muscle weight is not a sensitive enough measure for this study where 55 kcal equaled only a 39% kcal reduction and where restriction of kilocalories was for only 10 days.

Animals receiving 55 kcals per day had lighter liver weights than those animals receiving 80 kcals per day. A decrease in liver weight during early starvation may be posited from the mobilization of glycogen from the liver and the increase of gluconeogenesis in the liver. Furthermore, a study by Young et.al. (1988) demonstrated that animals fed 23% of their usual amount of calories have significantly lighter livers than those fed the required caloric intake. Though Young's et. al. (1988) study was 21 days long, this study supports Young's et. al. finding even after 10 days.

Animals receiving 80 kcals had heavier heart weights than animals receiving 55 kcals. The first phase of the starvation process lasts 5-10 days (Cahill, 1970). The animals in this study received a restricted caloric intake (i.e. 55 kcals per day) for 10 days. Thus by Day 10 the animals were at the end of the first phase of starvation or entering late starvation. At this time, gluconeogenesis is occurring predominately in the skeletal muscle and an increase in catabolism of endogenous fat is in process. An increase in free fatty acids during lipolysis serves as fuel for the heart, kidneys and muscle (Cahill, 1970). Thus it would seem that the heart would not weigh less after 10 days of restricted caloric intake.

However, if the animals were unduly stressed during this time, the stress response would be active. The sympathetic stimulation and a subsequent increase in circulation catacholamines leads to increased metabolism and may increase the progression of the starvation process (Cahill, 1970). Animals may have experienced stress during the 21 day study by such things as episodic hunger and the inability to find needed food. If sustained, the animals could have progressed into premorbid starvation and begun depleting protein stores for energy. However, if this were true, one would expected other visceral organs weighed to be significantly less in animals receiving 55 kcals per day compared to the animals receiving 80 kcals per day. However, this was not the case. It may be postulated that restricted caloric intake affect cardiac muscle differently than it affects smooth muscle. This study supports findings reported by Young et al. (1988). Young et al. (1988) demonstrated that heart weight was significantly less in animals fed a 23% calorie restricted diet over 21 days than those fed the required caloric intake over the same time period.

The starvation process is a likely contributing factor for the statistically significant differences in the spleen and kidneys. The animals receiving 80 kcals per day had a heavier spleen and kidneys than those animals receiving 55 kcals per day. Again, it may be that stress increased the progression of protein usage as a substrate for energy . The animals receiving 80 kcals/day had heavier spleen than the animals receiving 55 kcals/day. A possible explanation for this may be that the immune response may have been elicited by catheter placement six days before organ harvest. One of the functions of the spleen is to produce lymphocytes and monocytes. Thus, in an inflammatory or infectious state the

spleen may experience an increased work load or blood flow or both which may contribute to a heavier weight. Ten of the 20 animals in the 80 kcal group had catheters placed; 11 of the animals in the 55 kcal group had catheters placed. Though not significant, the mean spleen weights were higher in both 80 and 55 kcal levels for animals with catheters (652.6 mg and 562.8 mg respectively), compared with the 80 and 55 kcal level groups without catheters (601.2 mg and 505.7 mg respectively). The variablility between mean was greater in the catheterized group. This is an area deserving further study.

Another possible explanation for the mean heavier spleen weights in the 80 kcal group may be that a hemolytic event could have been elicited by the streptokinase given approximately 3-5 days prior to organ harvest. Another function of the spleen is to the storage of blood. Thus, a hemolytic reaction to the streptokinase may cause an enlarged spleen. Three of the 20 animals in the 80 kcal group received streptokinase and 3 of the 20 animals in the 55 kcal group received streptokinase. Though not significant, the mean spleen weights were higher in both the 80 kcal and 55 kcal levels for animals that received streptokinase (598.5 mg and 552.6 mg respectively), compared with the 80 and 55 kcal level groups that did not receive streptokinase (679.6 mg and 566.7 mg, respectively). Although, variability was higher in the group receiving streptokinase. This is also an area needing of further study.

Animals receiving 80 kcals/day had a statistically significant heavier total kidney weight compared to animals receiving 55 kcals/day. This may relate to increased glutamine production that occurs in the kidneys during the starvation process. An increase in this activity may lead to a decrease in total kidney weight.

In addition to the main effect differneces discussed above, when pancreatic weights were compared, a significant interaction between kilocalorie level and feeding schedule was present. In the 12 hour feeding schedule groups, both groups receiving 80 kcals/day had significantly heavier mean pancreas weights than the two groups receiving 55 kcals/day. A possible explanation for this may be that the pancreas had an increase work load during restricted feeding. There could be increased production of in enzymes and bicarbonate fluid. Because fluid adds weight, such increases could contribute to pancreatic weight increases. Lending further support to this possibility is the fact that all tissues were collected during the time animals on the restricted feeding schedule were being fed.

In summary, standardizing organ weights by body weight appears to be more defensible for liver weight but less defensible for other tissues measured in this study where kcals levels, fiber contents and feeding schedules were different. Furthermore, Day 20 body weight, heart, liver, spleen, and total kidney weight were significantly affected by kilocalorie level in this study. Pancreatic weights were also affected by kcal level, but only when the food was delivered during the restricted rest period.

Limitations of the Study

A limitation to this study is that the results represent only one point in the animals life. Brody and Ragsdale (1922) have demonstrated that growth in warm-blooded animals occurs in three cycles. At first the body weight increases sporadically then tapers off until maturity, and finally, either growth slowly increases or decreases during aging. Webster et al. (1947) reported a wide range of body weights with corresponding liver and kidney

weight changes. Webster et. al. illustrate that there is not a uniform linear relationship between liver or kidney weights and body weight over a broad range of body weights. However, the animals of this study are postpubescent males and thus were in a stable phase of growth. The results of this study are limited to one phase of growth. Further research is needed to secure additional data covering other age ranges (i.e. newborns to maturity) for these and additional organs.

Another limitation of this study is that correlation coefficients were not able to be performed for the individual feeding groups (n=5). A sample size of 5 is too small to give an accurate representation of correlation coefficients. Although n=20 is greater than n=5, 20 animals per group may still not be large enough to represent the data with confidence.

Furthermore, there were no exclusive groups in this study exceeding 5 animals per group. Future studies can be done that increase the sample size of each individual feeding group.

The same animals were partitioned several ways when performing statistical analyses. For example, when computing the effects of kilocalorie level on body, organ and tissue weights, this researcher also needed to consider the effects of fiber content and feeding schedule as well. Though done in consultation with a statistician, a larger sample size that would permit separate animals to be in each analysis group would strengthen the study.

Lastly, the effect of kilocalorie level on organ and tissue weights is perplexing and difficult to explain with confidence. This may be due to the fact that the animals received a calorie restricted diet for only 10 days or that the caloric restriction was only at the 61% level, or a combination of these factors. This may not be long enough or severe enough to

produce statistically significant organ and tissue weight changes. The literature suggests that a period longer than 10 days may be more appropriate to study restricted caloric intake. For example, Young et al (1988) studied their caloric restricted animals for 21 days and Nishio et al (1992) studied their caloric restricted animals until a 25% body weight loss was noted. Also, Cahill (1970) states that visceral proteins will not be used as a substrate for energy until premorbid starvation which occurs after 10 days.

Future Research

Future studies need to increase the sample size and conduct experimental manipulations on exclusive groups of animals in order to increase the power of the sample and increase the confidence in the accuracy of results. Furthermore, the length of future studies may need to be longer in order to detect organ weight changes in response to restricted caloric intake.

This study was asking only if there was a linear relationship between body weight and selected organ and tissue weights. Further statistical manipulations would need to be performed to determine what statistical model or models best fit theses data in order to accurately standardize these organ and tissue weights.

Future research may may also be directed toward how the calorie restricted heart responds to its environment. For example, is the calorie restricted heart more irritable or less strong than the well nourished heart? It may mean that another rat study could be designed to explore EKG patterns in nutritionally starved hearts.

Further research is also needed to determine the effects of a restricted diet on pancreatic weight and functions. Weight may not be a specific enough measure for this determination but pancreatic weight coupled with insulin levels may yeild more informative data. Thus, it may be appropriate to check blood glucoses in rats, receiving bolus feedings.

Further research is also needed to determine the effects of a restricted diet on the rat spleen. Perhaps a T-cell count in combination with the splenic weight may better determine the immune status of the rat.

Implications for Practice

Nurse researchers build on other sciences as they work to generate nursing's unique knowledge base. Thus, nurses are consumers of research from multiple related fields. Nurses must educate themselves as to the ways data can be presented accurately. This is a necessary step in determing scientific soundness of research. This study provides a perspective for helping nurses critique studies done with rats that report organ or tissue weights by helping to determine an aspect of their scientific soundness.

Conclusion

Standardizing organ weights by body weights may be more defensible for the liver than for the heart, right lung, left lung, pancreas, spleen, stomach, duodenum, jejunum, ileum, large intestine, kidneys, adrenals and *extensor digitorum longus* muscle. Even when

correlations are relatively high, if there is a pattern in the residual plots there may be a better fitting model than an unadjusted, simple linear one.

Kilocalorie level was statistically significant for the organ weights of the heart, spleen, liver, kidneys and total body weight on Day 20, with the group receiving more kcals having higher mean weights than the group consuming fewer kcals. In only one instance, pancreatic weight, was feeding schedule a significant factor, and even then it was in a 2 way interaction with kcals.

Both the study duration and the limited number of animals impose caution in generalizing these results to populations. However, the trends evident provide insight and strong support for research beyond this small scale study.

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Appendix A
Journal Articles Meeting Inclusion Criteria

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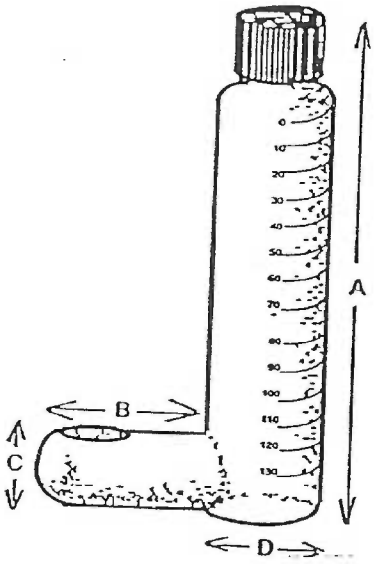
Appendix B
Feeder



Bioserv. Frenchtown, NJ

Appendix C

Daily Handling and Feeding Protocol
DAILY CARE PROTOCOL
June, 1993

FEEDING

Preparation of Food Before 0645, the time lights come on

- On tray assemble the following equipment
  - Water container
  - Jevity food
  - Osmolite HN food
  - Can opener when using large cans
  - 60 ml syringe for Jevity
  - 60 ml syringe for Jevity
  - 12 ml syringe for H_2O
  - 2 clean, empty containers in which to mix each food with calculated H_2O
  - Clean feeding tubes from Bioserve Company
  - Tray covered with clean paper towels
  - Clipboard with current cage and animal #'s locations, previous day's Daily Formula Flowsheet, and present day's Daily Formula Flowsheet
  - Room disinfectant spray container to spray down cart wheels before returning cart to room
  - Means to cover open cans that will be placed in refrigerator (Room 280)
- Move cart with tray, tubes, and above equipment into hall just outside room
- Begin with Jevity food
  - Shake can well before opening
  - Place liquid food into clean container
  - Mix calculated amount of H_2O to make soln 1ml=1kcal (240ml can + 10.6ml H_2O)
  - Use 60ml syringe to mix water and formula
  - Draw up 45 or 85 ml (depending on diet option) in the 60ml syringe
  - **Place thumb over feeding tube opening**
  - With thumb firmly in place empty syringe contents into feeding tube
  - Screw feeding tube top firmly in place
  - Slowly release thumb from feeding tube opening
  - Gently place tube into designated slot for same numbered animal
  - On today's Daily Formula Flowsheet enter information including amount for designated animal
  - Follow the above sequence to fill all feeding tubes for animals receiving Jevity
  - If any food remains, place it back into Jevity container, cover, write name, date, and time opened and place in refrigerator in room 280
- Prepare Osmolite HN food
  - Follow the above listed sequence for Jevity food
- Spray wheels of cart just before returning cart with food to room

Food exchange

- Place sheet with current animal # and location on rack on the top of the rack
- When lights come on, start replacing food containers beginning on left side of rack with animals closest to the end of the study (2nd and 3rd rows from the top)
- Talk quietly to the animal as you pull the cage out from the rack
- Remove present feeding tube and hanger from the hanging cage
- *If 2 people present*, hold this feeding tube level and note the amount left in the tube;
  - -Remove the tube from the hanger and hand to the 2nd person
  - -Report the **animal number and** amount remaining so the 2nd person can record the amount on the preceding day's Daily Formula Flowsheet
  - -Carefully clip the hanger onto the new feeding tube, keeping the filled feeding tube level

- *If 1 person is present*, separate hanger from old feeding tube;
  - -place old feeding tube in space immediately above where the current tube is;
  - -carefully remove new feeding tube from preparation container, keeping it level;
  - -carefully attach the new tube to the hanger and place it in the right hand corner of the animal's cage
  - -After all tubes have been exchanged, then record the amounts remaining in each of the removed tubes on the preceding day's Daily Formula Sheet
- Continue down each rack row exchanging the old for the new feeding tubes using the above procedure

WEIGHING

Equipment

From locked door in cart:
- A&D balance
- Cord
- Plastic basket
- Handling cloth
- Notebook with daily weight recording sheet
- Pen

Sheet with current animal # and location on rack (on clipboard)

Set Up

- Turn on lights and airflow on work bench
- Wash down work table with spray bottle disinfectant on top of hood
- Position balance at right hand side of work table so readout faces left hand side
- Plug cord into outlet behind work table and then plug cord into balance (right side)
- Adjust balance so bubble in center of red circle (back left hand side of balance)

•Place Sheet with current animal # and location on rack on clipboard and position it so you can clearly see it during weighing procedure
• Open notebook to daily weight recording sheet and place on work table
• Turn balance on, place weighing basket on scale and press reset so readout value is 0 (If need to use top to weighing basket, be sure to 0 with top on basket

Procedure

• Using handling cloth, pick up an animal, leaving his cage open enough so he can easily be placed back in cage
•Note amount of liquid food gone from feeding tube as each ml=~1gm
Check daily forumla flowsheet for amount of intake provided to animal
Subtract amount gone from beginning amount and record intake prior to being weighed on weight flow sheet)
• Talk quietly to animal as you bring him to work table
• Verify readout value with basket is 0
• Gently place him in weighing basket
If need be, place top on basket to get more stable weight
• Read the value when the animal is centered in the basket (as opposed to hanging on the side of the basket)
• Record the body weight under the study day it is for this animal
• Pick him up using the handling cloth, stroking and talking quietly to him
• Return him to his cage
• When sliding the cage flush to the rack, do so evenly to avoid spilling food and startling animal
• Continue down each row, handling each animal according to the above steps
• Be sure to zero balance before each weighing
• If unable to zero balance with air flow on, turn air flow off
• When the last animal has been weighed,
-unplug balance;
-clean weighing basket;
-return weighing basket, plug, and balance to locked drawer in cart
-place handling cloth in covered container in locked cabinet
• Remove animal # chart and notebook from work table
• Wash down work table with disinfectant
• Turn off work table lights and airflow

CONTINUING DATA COLLECTION

• Move animal # chart back to rack
• On a separate sheet of paper (e.g. paper towel) make squares to correspond to each animal on the rack and note date
• Pull out the tray under each cage and note stool consistency, color, and amount
• On the paper with squares, note stool information (consistency, color & amount)

CLEANING UP

Rack
- Bring trash can over close to rack
- Put on gloves
- Pull tray under the cages out and roll paper up to keep stool & liquid contained
- Place in trash can
- Place new paper on tray and smoothly move tray back to original position

Feeding Tubes
- Take from room the following items
  - -used feeding tubes
  - -tube rack
  - -wash basin (from locked cupboard)
  - -used syringes
  - -containers used to mix food and water
  - -detergent (in locker)
- When possible, use room 280
- Use very hot water
- Soak feeding tubes in hot soapy water
- Be sure to get the bottoms of the feeding tubes clean
- Rinse well to remove all detergent from the feeding tubes
- Position cleaned tubes so they can drain
- Wash and rinse feeding tube tops
- Place so they can drain dry

SET UP
- Cover tray with paper towels and place the following items on the tray:
  - -containers used to mix food (upside down to drain dry)
  - -syringes (separated barrel from plunger for each to drain try)
  - -water container (upside down to dry)
  - -12ml syringe (separated to drain barrel and plunger)
  - -if anticipate needed a new can (s) of food, place the new can on the tray
  - -if new can(s), can opener
- Place tray on cart and cover this tray with a green cloth (ready to use)

APPENDIX D

Harvest Protocol

ALL ORGANS WILL BE CLEANED OF CONNECTIVE TISSUE AND EXTERNAL DEBRIS VISIBLE TO THE EYE BEFORE WEIGHING.

WET WEIGHTS WILL BE OBTAINED.

PANCREAS Whitish-gray and heavily lobulated shortly after death. One part embedded in the mesoduodenum and the beginning of mesojejunum (body and right lobe), a branched flattened part (left lobe) runs along dorsal aspect of stomach, embedded in dorsal part of greater omentum, and along the lienal artery toward the intestinal surface of the spleen. *Hepatic duct surrounded by pancreas along almost entire length. (p. 103) If unable to harvest entire organ then clip where it is the most difficult space to extricate and measure the length. *Dedicate boat and dissecting space to this organ.

STOMACH Lies in left cranial part of abdominal cavity. Esophagus enters the middle of the lesser curvature. *Papillary process of the liver comes in immediate contact with the visceral face of the stomach. *Medial surface of spleen is ventrally in contact with greater curvature.

Clip at end of esophagus and at end of pyloric sphincter/beginning of duodenum. Split along greater curvature to empty and flush.(p. 48)

DUODENUM Clip at stomach exit and measure 10 cm and clip. Split to empty and flush.

SPLEEN Lies left dorsal abdominal cavity. Dorsally attached with kidney, cecum and jejunum. About 1 cm long, elongated and flat. (p.116)

Clip at entry and exit of splenic artery and vein and trabecular vein.

LIVER 4 lobes. Visceral surface contacts the stomach, descending duodenum, transverse colon, jejunum and spleen. Dorsal cuadate in contact with right kidney. Clip at entry and exit of vena cava caudalis, hepatic artery and portal vein. Clip falciform ligament between left medial and medial lobe and coronary ligament which inserts around the exit of the caudal vena cava. Dissect away esophagus, small caudate lobe fits around it. (p. 50) *Dedicate boat and dissection space for liver.

KIDNEY Rt. higher than lt. Fat surrounds hilus and sides but not dorsal and ventral aspects. Clip at entry and exit of renal artery and vein. Clean of fat. (p. 62)

ADRENAL Lies in retroperitoneal fat close to cranial pole of each kidney. Brownish color and firm. Clip at entrance of central vein and clean of thin, irregularly indented connective tissue. (p. 86)

HEART Clip at entrance site of vena cava caudalis, vena cava cranialis and vena cava cranialis sin. Clip at exit of ascending aorta. (p. 92) Split and remove visible blood clots from 4 chambers of heart and weight.

LUNGS Lt.-1 lobe and rt.-4 lobes. Clip at entrance site of bronchus. (p. 60)

EXT. DIG. LONGUS Origin-lateral epicondyle of femur. Insertion-proximally at tarsus it divides into 4 tendons. (p. 37)

Clip at tendon of m. ext. dig. longus (p. 41) and tendon near lateral epicondyle of femur. (p.21) *Use right leg only. Protocol: Remove pelt--Remove facsia--Remove superficial muscles of upper leg--Find tendon behind calcaneuos, dissect out *gastrocnemius* and *soleus* and clip out--Dissect out *lateralis*, lateral to calcaneous--Dissect out *tibia cranialis*, front of tibia with big belly--Dissect out *extensor digitorum longus*, in between *lateralis* and *tibia cranialis*, 1/4 of *ext. dig. longus* runs under *tibia cranialis*--Clip *ext. dig. longus* at lateral epicondyle--Clip where tendon meets muscle at origin so only weigh muscle.

(Hebel & Stomberg, 1976 pp. 21, 37, 41, 50, 60, 62, 86, 92 & 116)

Appendix E
Data Collection Form

Animal number:_____ Last day Wt.:_____ Date:_____
Feeding schedule:_____
Time:_____ Fiber:_____
Days on protocol:_____ Kilocalories:_____
Last food:_____

Comments

Pancreas:_____mg/G

Stomach-Full/Empty:_____mg/G

Duodenum-Full/Empty:_____mg/G
Length:_____mg/G

Spleen:_____mg/G

Liver:_____mg/G

Jejunum Empty:_____mg/G
Length:_10_cm

Ileum Empty:_____mg/G
Length:_10_cm

Large intestine Empty:_____mg/G
Length:_____cm

Kidney-Rt.:_____mg/G
Lt.:_____mg/G

Adrenal-Rt.:_____mg/G
Lt.:_____mg/G

Heart:_____mg/g

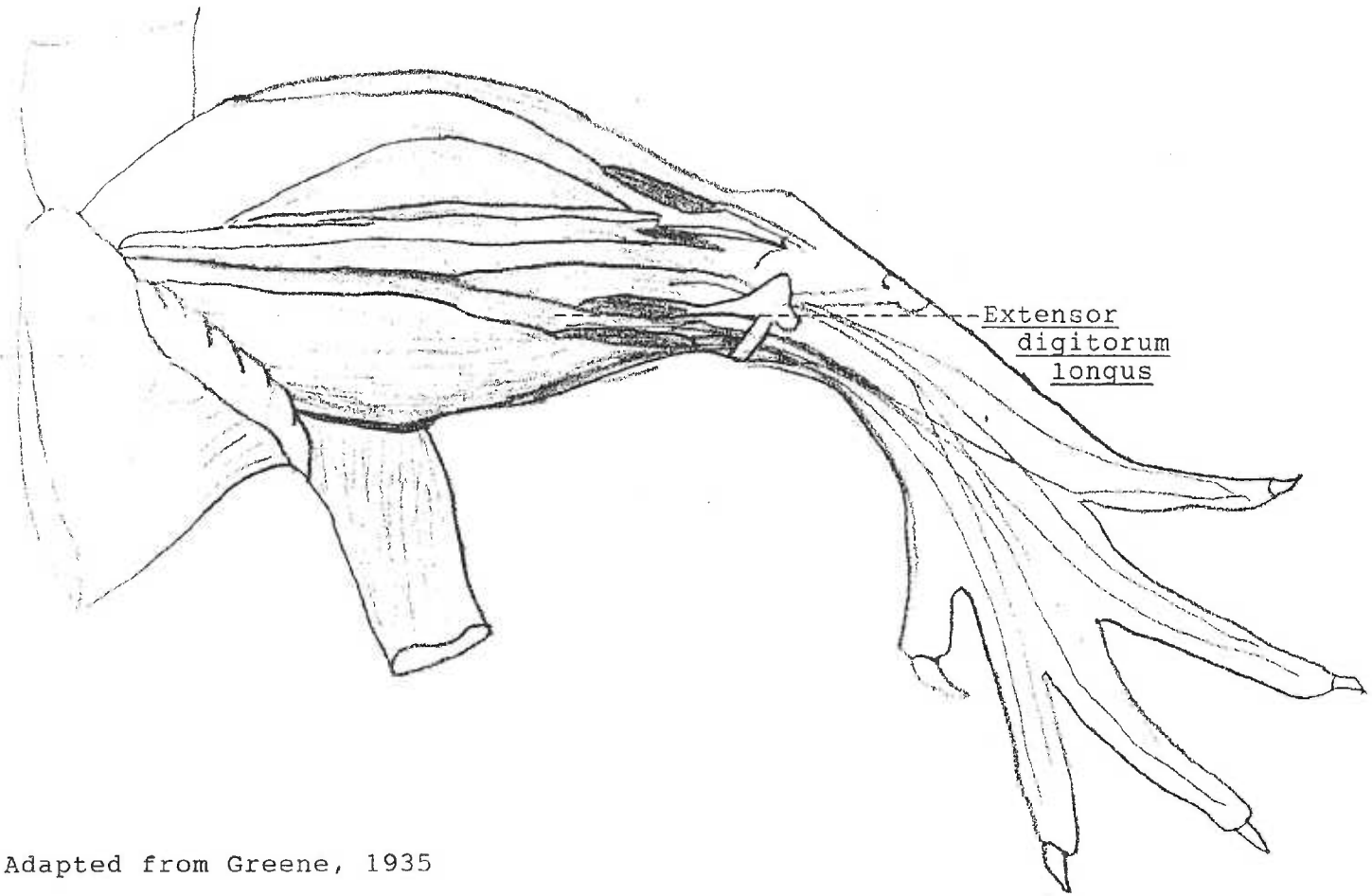
Lung-Rt.:_____mg/G (4 lobes)
Lt.:_____mg/G (1 lobe)

Ext. Dig. Longus:_____mg/G

Appendix F
Location of Organs

Adapted from Greene, 1935

Appendix G
Location of Muscle



Adapted from Greene, 1935

Appendix H
Approval of Animal Care and Use Committee

OREGON
HEALTH SCIENCES UNIVERSITY
3181 S.W. Sam Jackson Park Road, Portland, OR 97201-3098
Mail Code L106, (503) 494-7784, FAX (503) 494-7787
Office of Research Services

July 20, 1992

Dr. Ethel Jackson
PHS NIH NCNR
Room 5B25, Building 31
9000 Rockville Pike
Bethesda, MD 20892

SUBJECT: FOLLOW-UP ON ANIMAL CARE CERTIFICATION/REVIEW

The application described below was sent to your agency with animal care certification and review date pending. Review and approval have taken place by this institution's Animal Care and Use Committee.

Project Title: Selected Physiologic Responses to Dietetic Manipulations

Principal Investigator: Una Westfall, RN, PhD

Agency Number: 1R15 NR03337-01

Date of Approval: June 19, 1992

This institution has an Animal Welfare Assurance on file with the Office for Protection from Research Risks. The assurance number is A3304-1. Please feel free to call this office if you have any questions regarding this certification.

Katey Lust
Grants Proposal Specialist

KL:ors

cc: Una Westfall, RN, PhD (SN-AHI)

Date Received by Protocol Office: 6-4-92 147

Instructions: This must be completed and returned to the Department of Animal Care for review and approval by the OHSU Animal Care Committee before animals can be purchased, boarded or used for teaching or research at OHSU. Answers must be typed.

1. Principal Investigator: Una Elizabeth Westfall
2. Department: Adult Health & Illness 3. Mail Code: SON-AHI
4. Institution: SOD ☐ SOM ☐ SON ☒ VIABR ☐ CROET ☐ SHCC ☐ VA ☐ Other:
5. Degree: Ph.D. in Nsg Science 6. Academic Rank: Associate Professor
7. Telephone: Work: 494-3739 Home: 452-0987

8. Person to be contacted in case of project emergency if you cannot be reached: Margaret VonDreele
Telephone: Work: 494-3743 Home: 293-7819

9. Project Title: Selected Physiologic Responses to Dietetic Manipulations

10. Funding Source(s): National Center for Nursing Research (NIH)
Date of Submission to Funding Agency: 6/19/92 Deadline for Verification of Approval: June if possible

11. Type of Project: RESEARCH ☒ INSTRUCTION ☐

12. Project Start Date: March, 1993 Project End Date: February, 1996

13. Animal Information:
13a. Animal Species: Rat
13b. Strain/Breed: Male, Sprague-Dawley (Simonsen or Batman-Kingman); 170-190 initial weight
13c. Number: 40 (2 mon period/year) 120 total (per Month ☐ / per Year ☒ / entire project ☒)
This number is for conclusion of data collection

CHECK ONE: NEW PROTOCOL ☒ ONE YEAR RENEWAL ☐ THREE YEAR RENEWAL ☐ OTHER ☐

Animal Care Department/Committee Use Only

Animal Usage Category B
Date(s) of Previous Approval(s) *if applicable*

Committee Decision: ✓
Extension of Previous Approval
Assign for Expedited Review
Committee Review Date:
Approved
Approved with Modification *see attachment*
Approval Withheld *see attachment*

George A Pantely
Chairperson, Animal Care Committee

19 June 92
Date

Keyed: P___ IA___ A___ AM___ AW___ Sent to Office of Research Services ✓

If the PI is informed that the Committee decision is "Approved with Modification", the PI must sign the following statement and return this form to the Animal Care Department before verification of approval is sent to any outside agency.

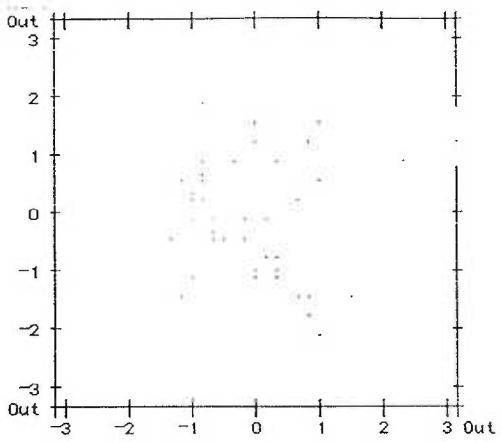
PI's Statement: "I agree to make the modifications to my protocol required by the Animal Care Committee."

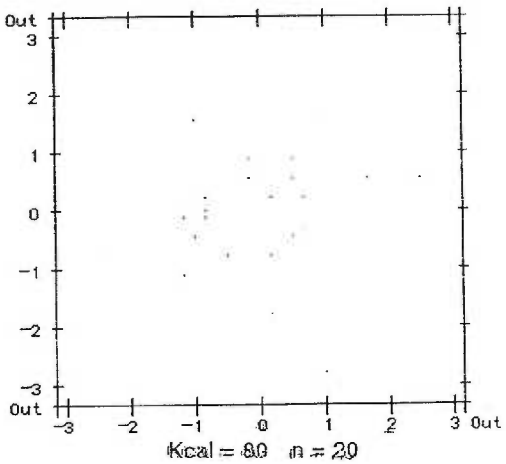
PI's Signature Date

APPENDIX I
Standardized
Scatterplot
Across = Resid
Down = Pred

Organ:
Heart

Total Sample
N = 40





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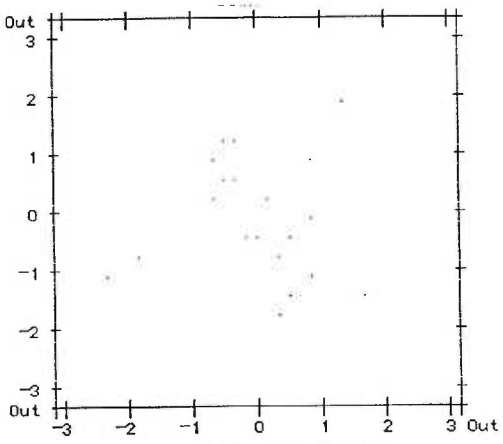

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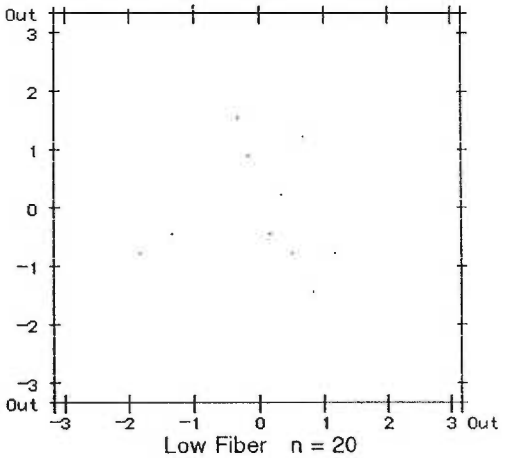
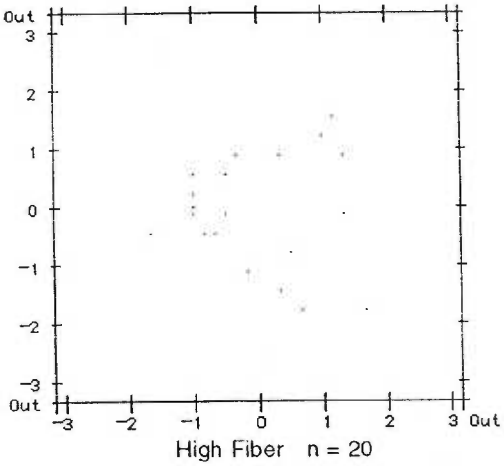


Low Fiber n = 20



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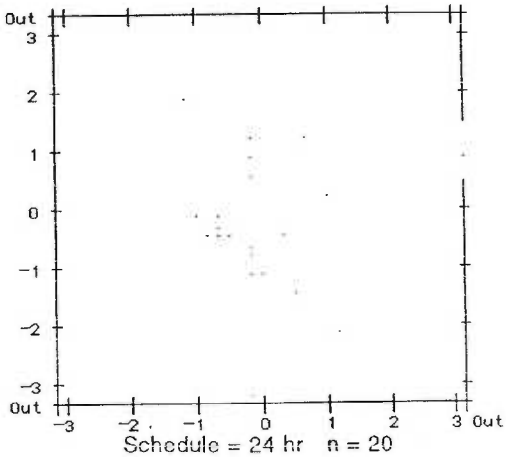

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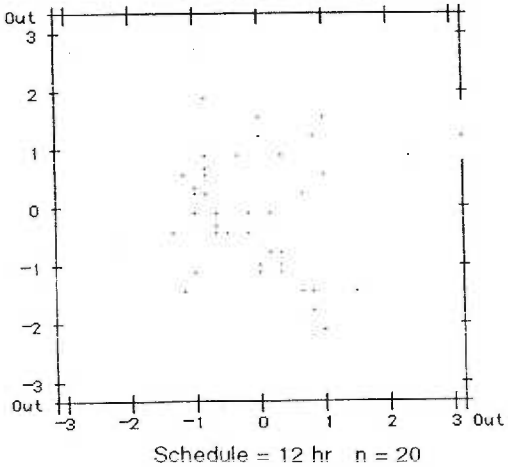
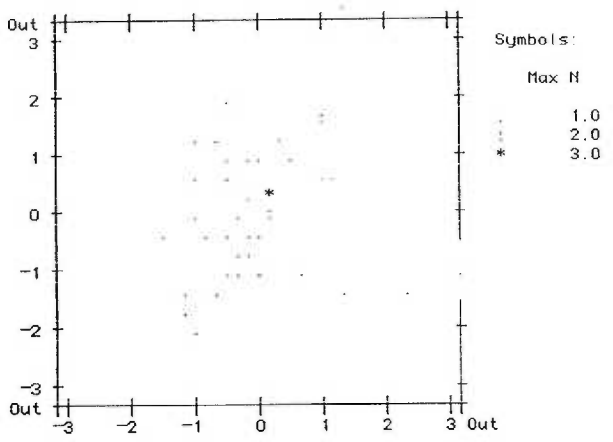


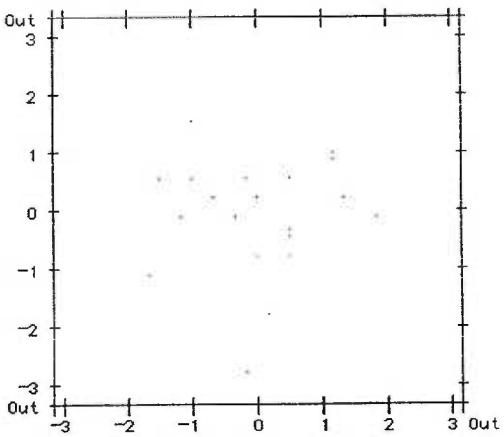
Schedule = 12 hr n = 20

Standardized
Scatterplot
Across = Resid
Down = Pred

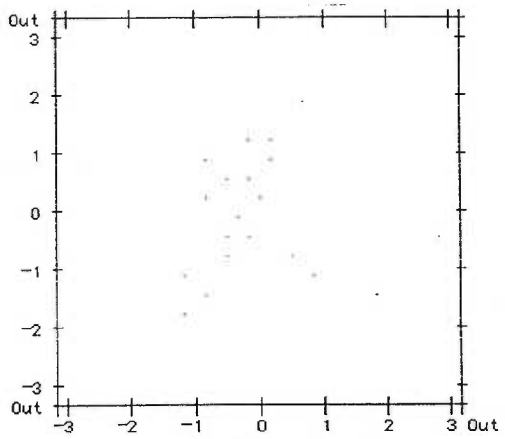


Organ:
Right Lung

Total Sample
N = 40



Kcal = 80 n = 20



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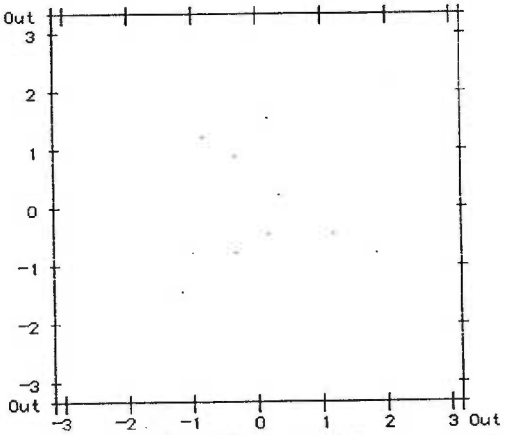
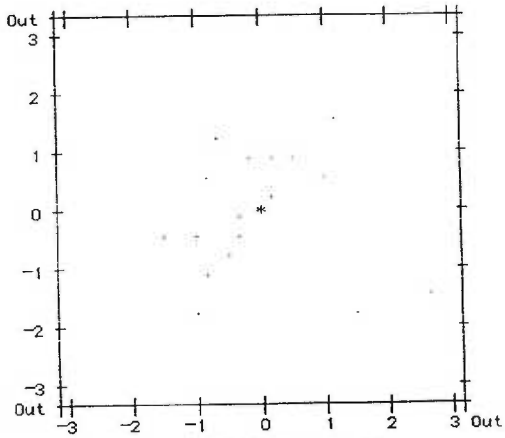
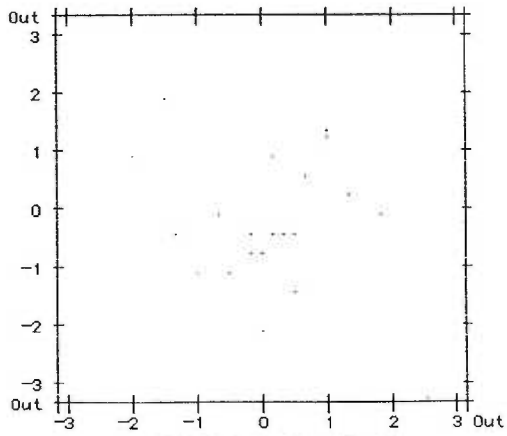


Low Fiber n = 20



High Fiber n = 20



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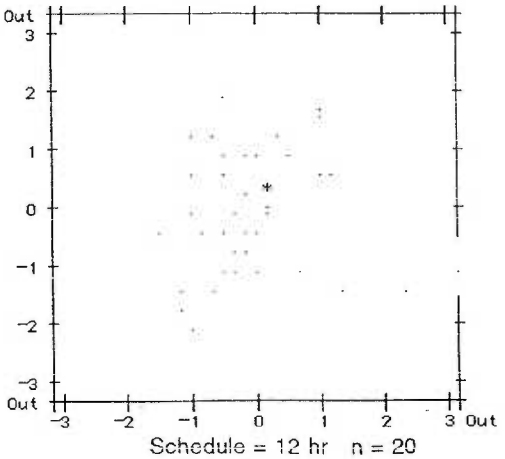
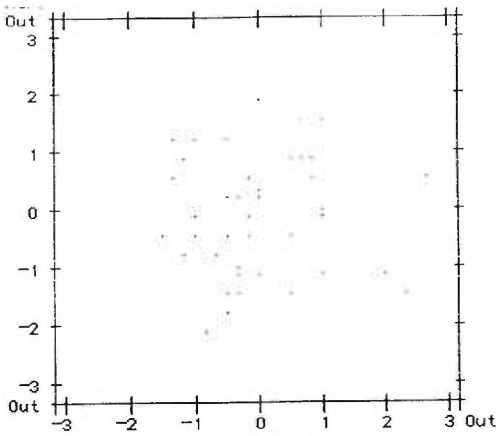


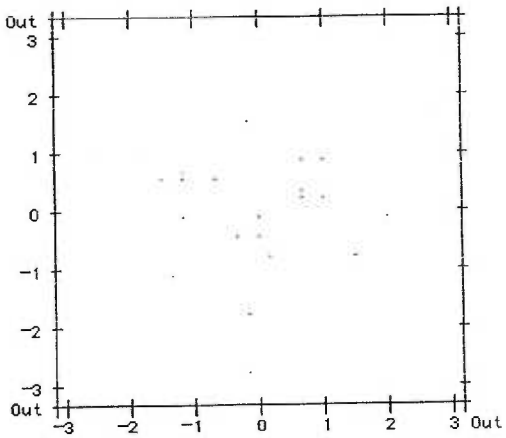
Schedule = 12 hr n = 20

Organ:
Left Lung

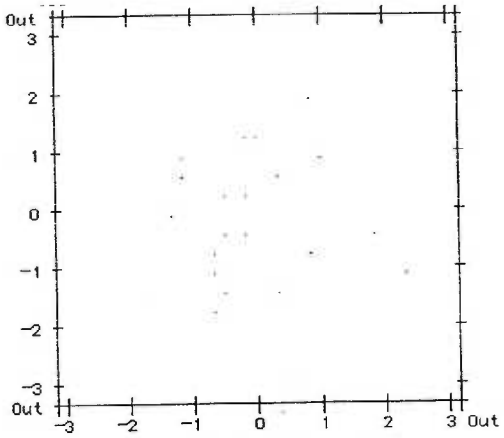
Standardized
Scatterplot
Across = Resid
Down = Pred

Total Sample
N = 40





Kcal = 80 n = 20



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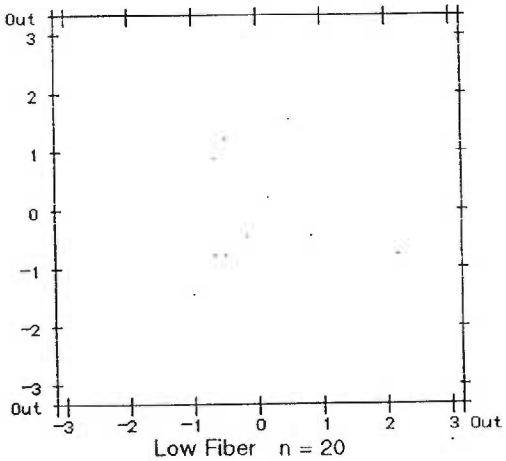
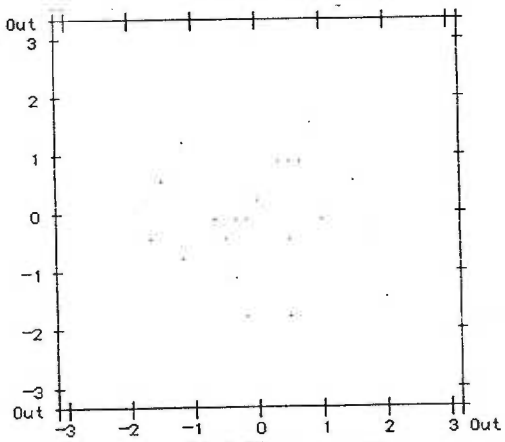
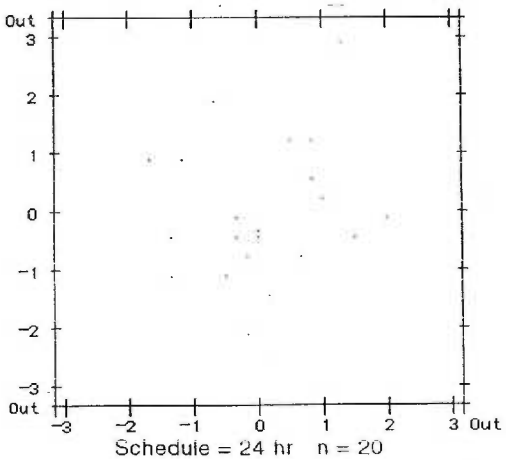


Low Fiber n = 20



High Fiber n = 20



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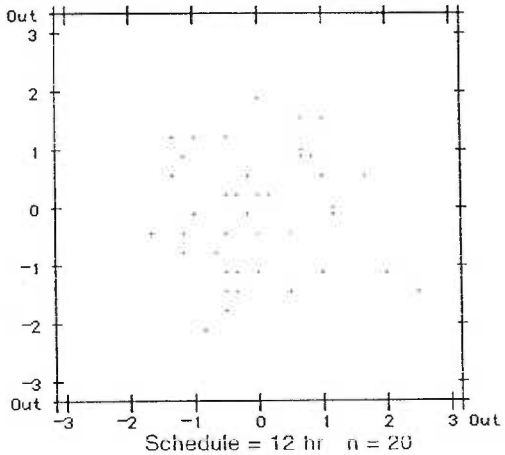
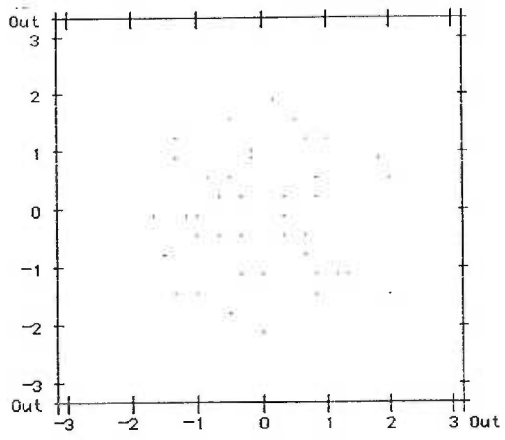


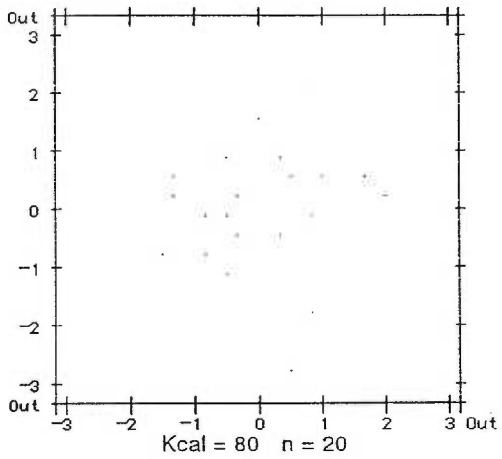
Schedule = 12 hr n = 20

Organ:
Liver

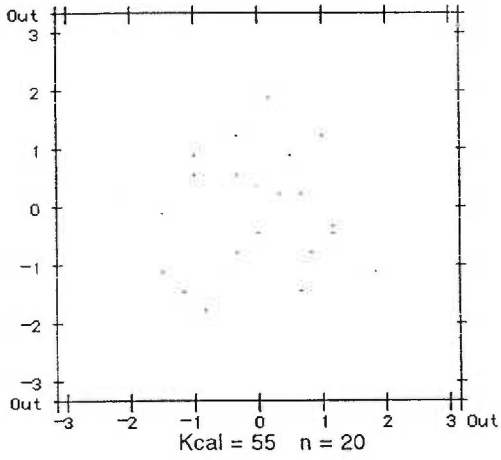
Standardized
Scatterplot
Across = Resid
Down = Pred



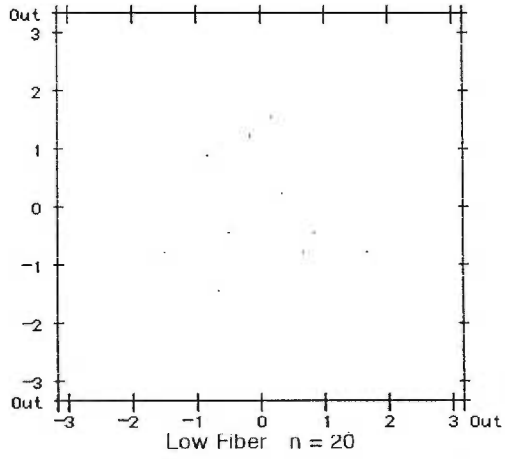
Total Sample
N = 40



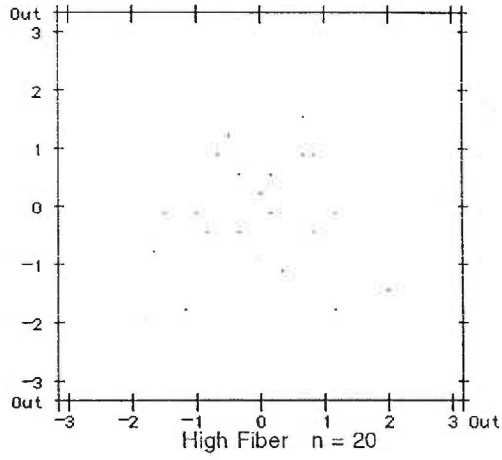
Kcal = 80 n = 20



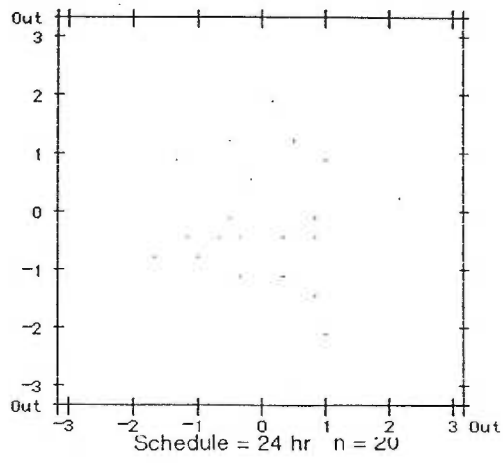
Kcal = 55 n = 20



Low Fiber n = 20



High Fiber n = 20



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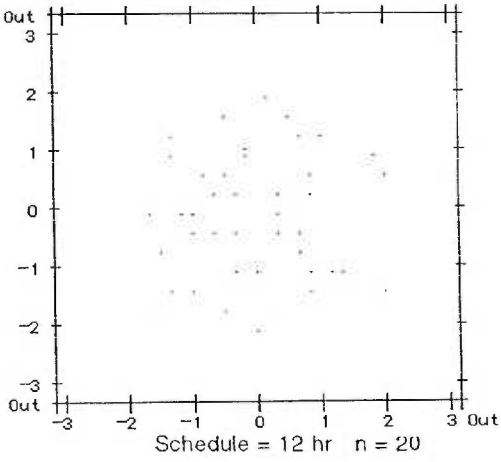
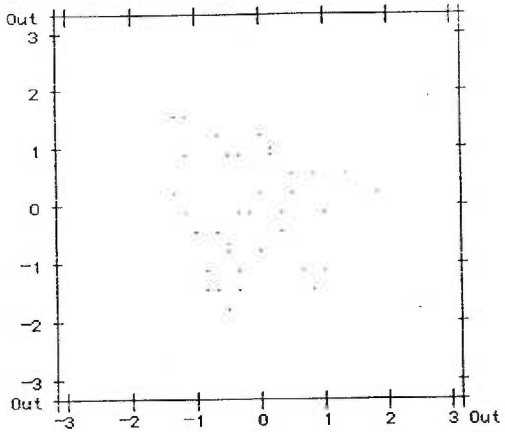


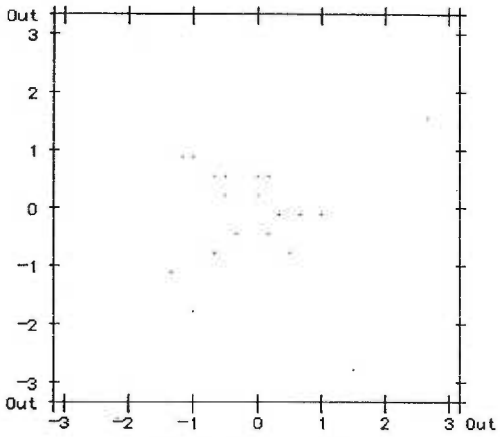
Schedule = 12 hr n = 20

Organ:
Spleen

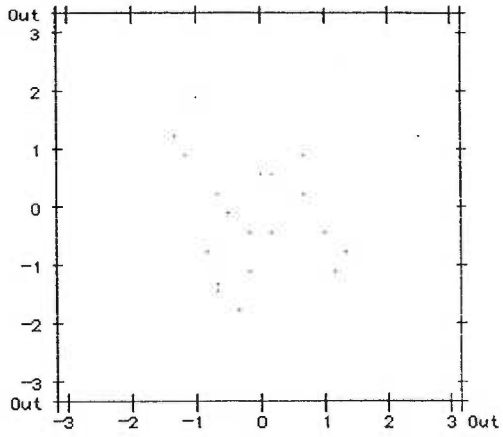
Standardized
Scatterplot
Across = Resid
Down = Pred



Total Sample
N = 39



Kcal = 80 n = 19



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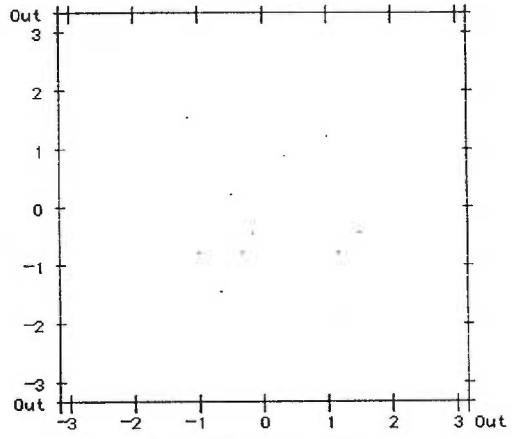
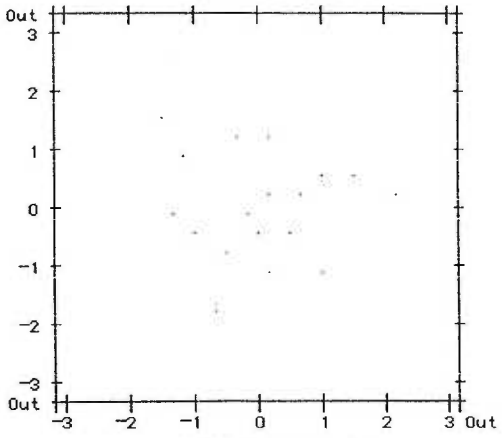
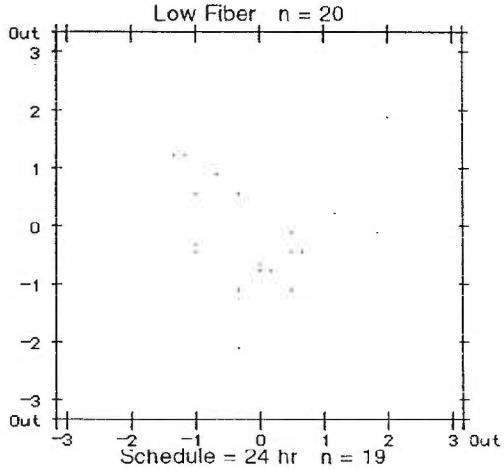


Low Fiber n = 20



High Fiber n = 19



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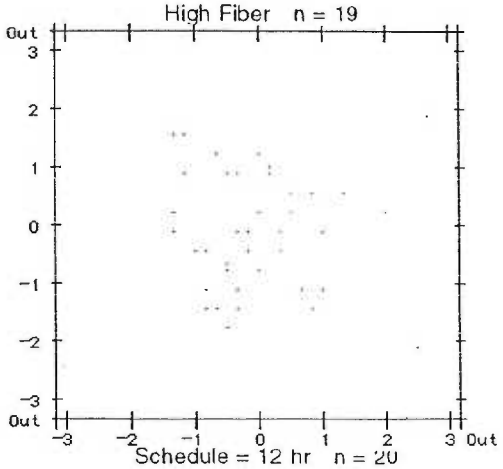
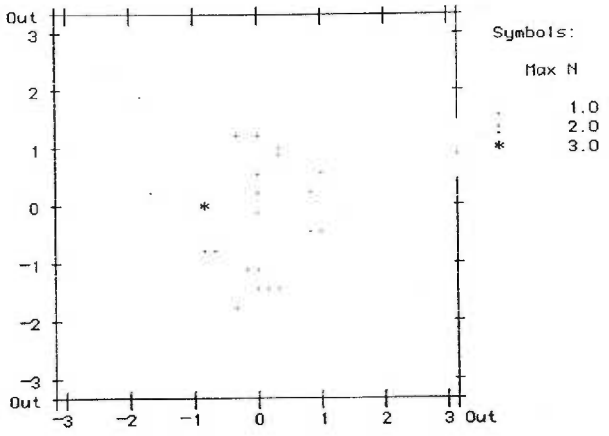


Schedule = 12 hr n = 20

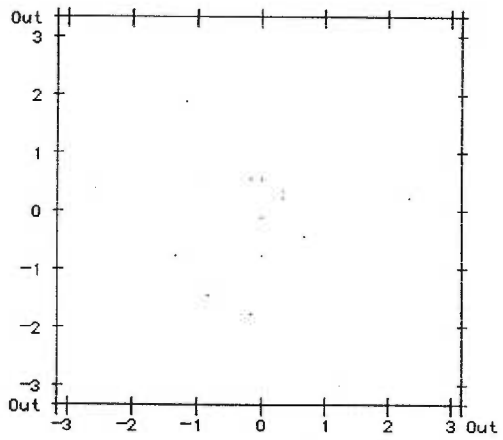
Standardized
Scatterplot
Across = Resid
Down = Pred

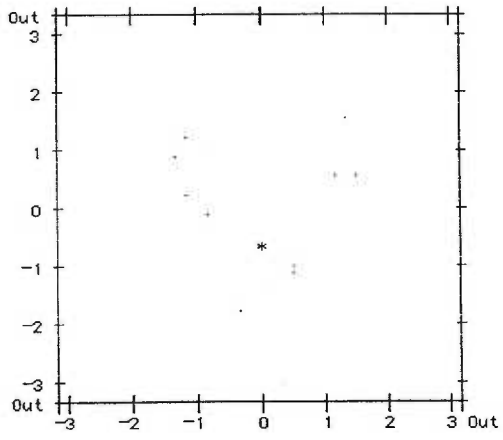


153

Organ:
Pancreas

Total Sample
N = 25





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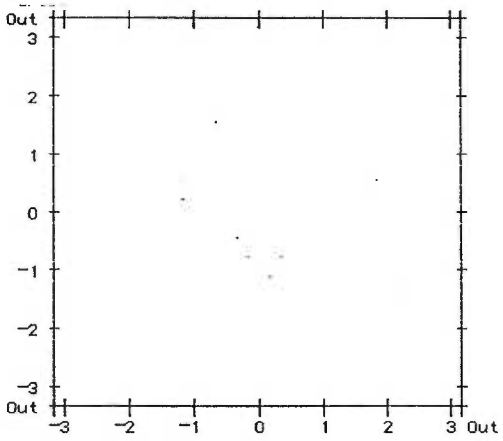

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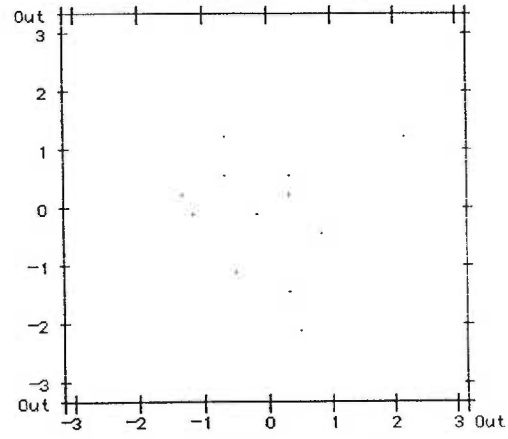
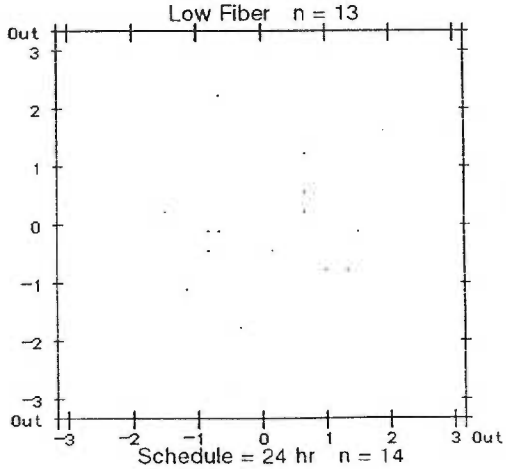
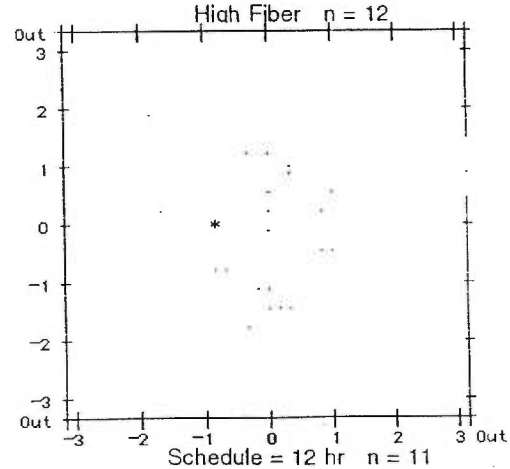


Low Fiber n = 13



Schedule = 24 hr n = 14

High Fiber n = 12

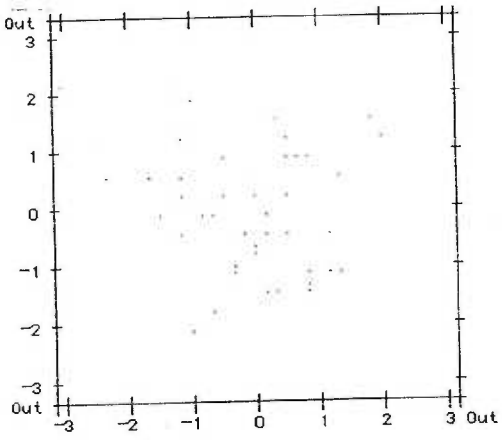


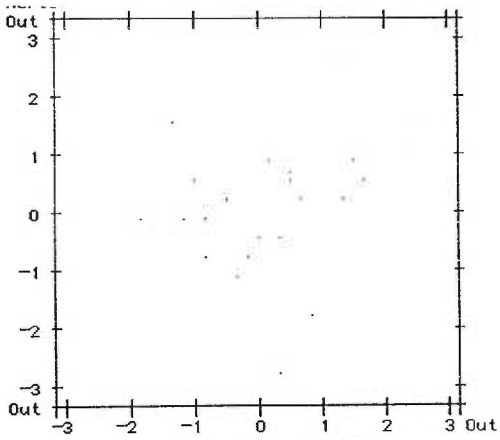
Schedule = 12 hr n = 11

Organ:
Stomach

Standardized
Scatterplot
Across = Resid
Down = Pred

Total Sample
N = 40





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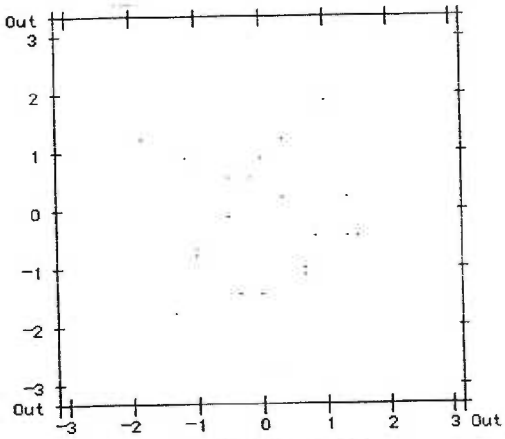

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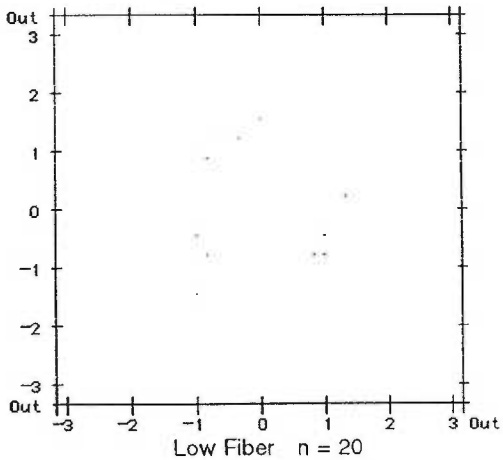
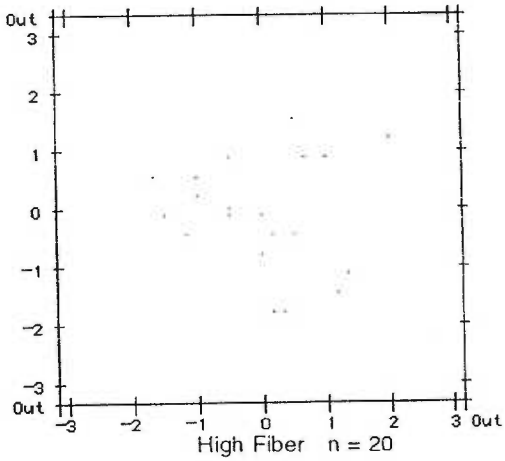
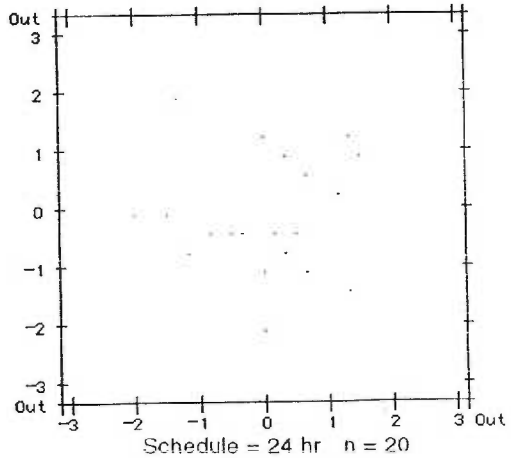


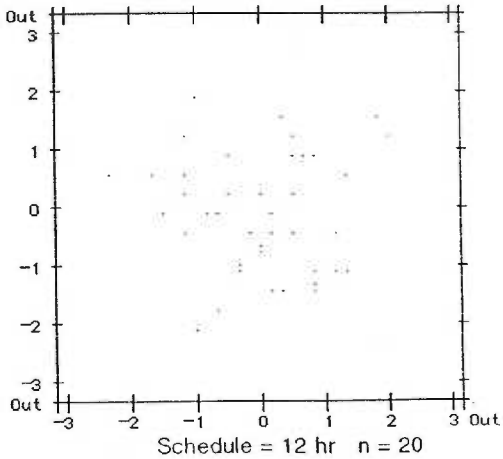
Low Fiber n = 20



High Fiber n = 20



Schedule = 24 hr n = 20

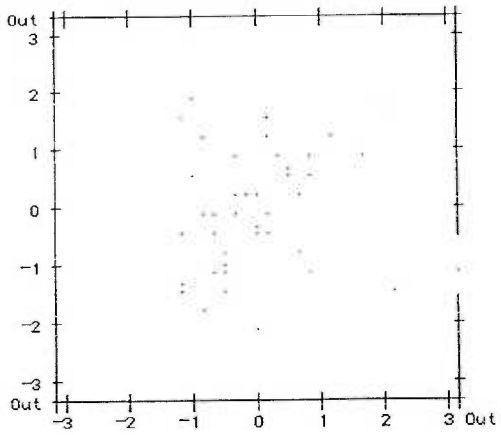


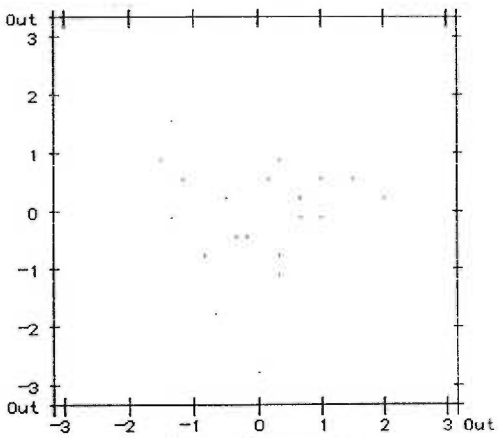
Schedule = 12 hr n = 20

Organ:
Duodenum

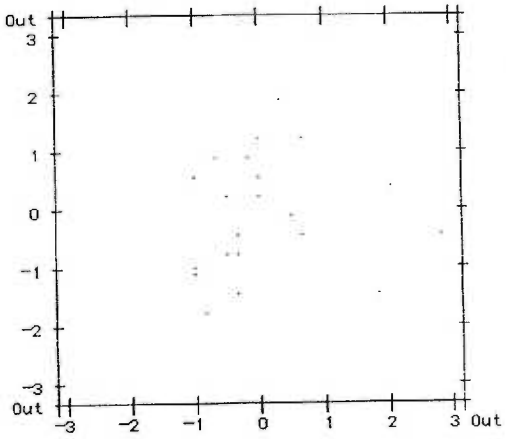
Standardized
Scatterplot
Across = Resid
Down = Pred

Total Sample
N = 40

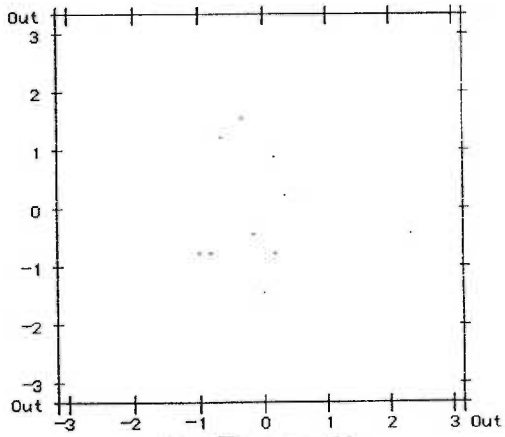




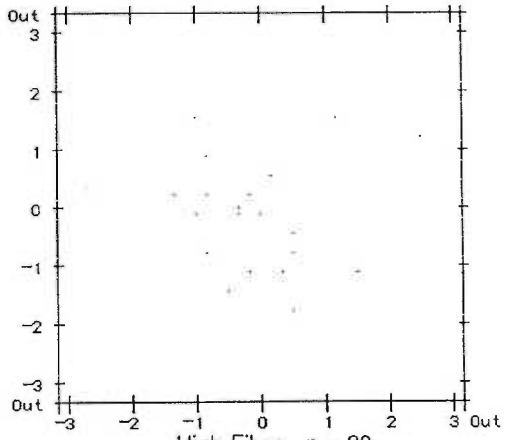
Kcal = 80 n = 20



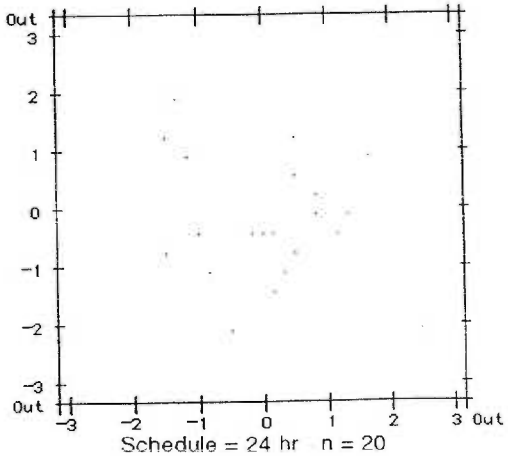
Kcal = 55 n = 20



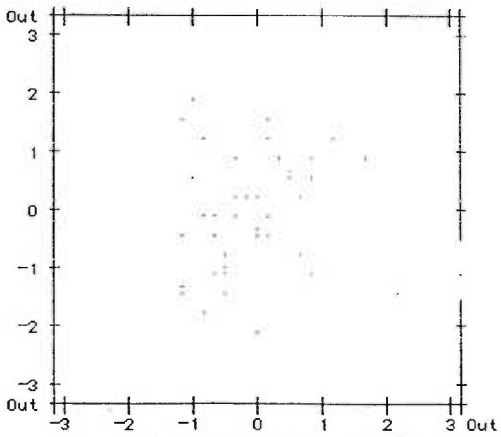
Low Fiber n = 20



High Fiber n = 20



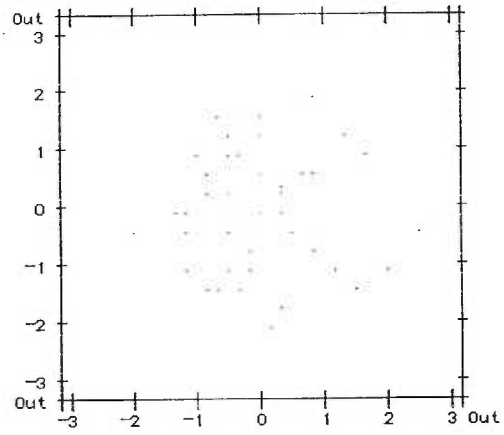
Schedule = 24 hr n = 20



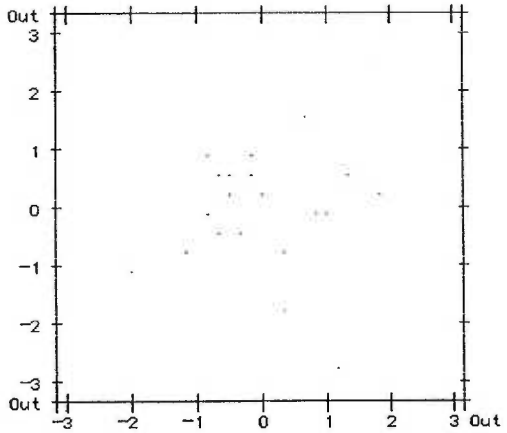
Schedule = 12 hr n = 20

Organ:
Jejunum

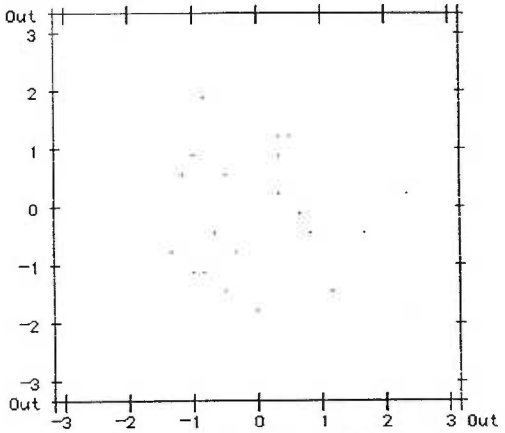
Standardized
Scatterplot
Across = Resid
Down = Pred



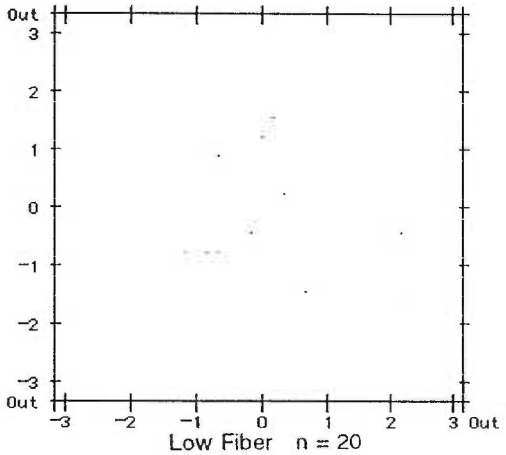
Total Sample
N = 40



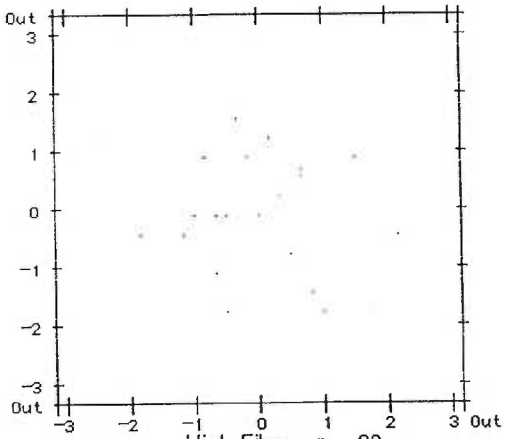
Kcal = 80 n = 20



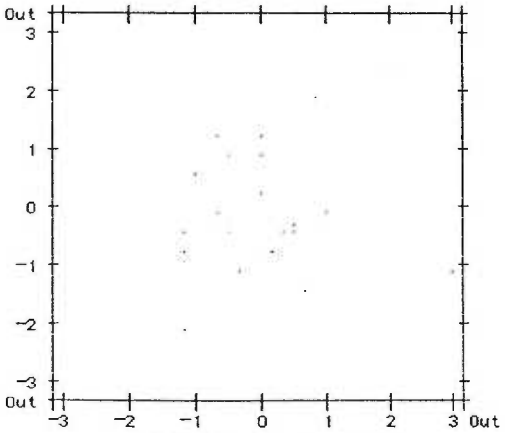
Kcal = 55 n = 20



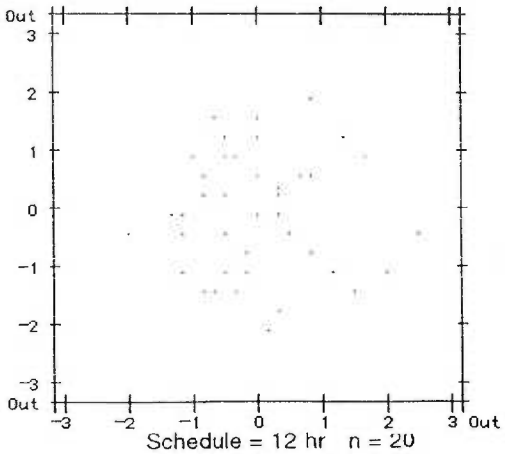
Low Fiber n = 20



High Fiber n = 20



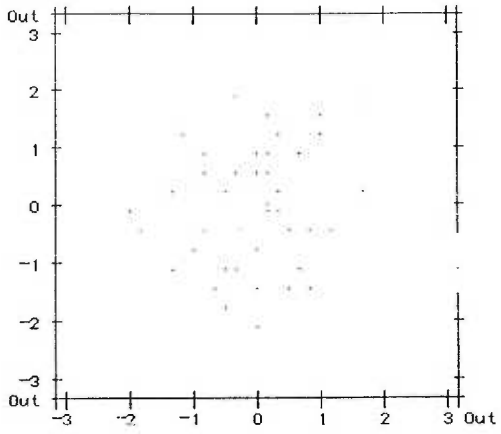
Schedule = 24 hr n = 20



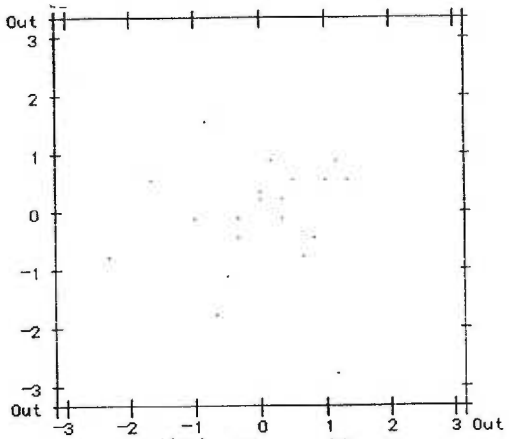
Schedule = 12 hr n = 20

Organ:
Ileum

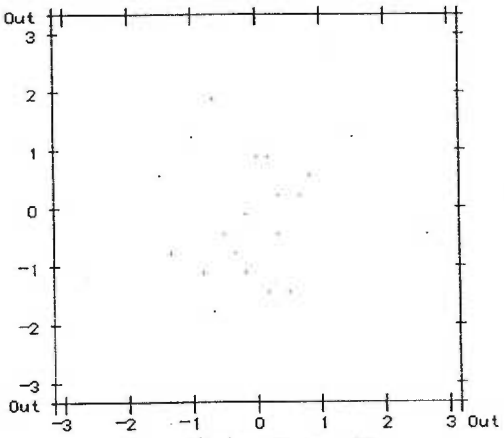
Standardized
Scatterplot
Across = Resid
Down = Pred



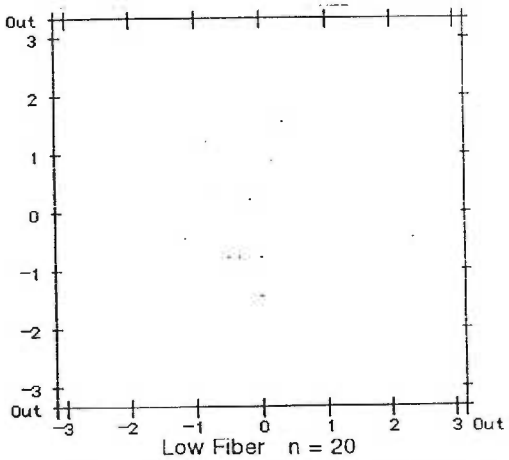
Total Sample
N = 40



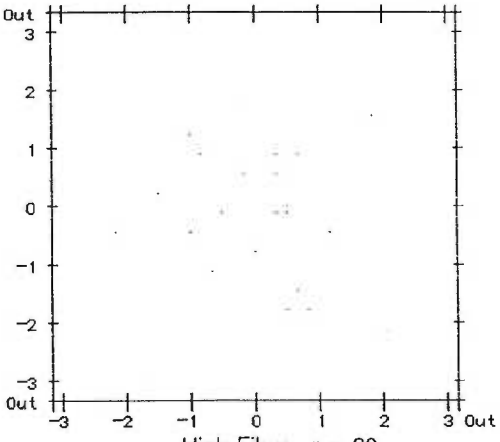
Kcal = 80 n = 20



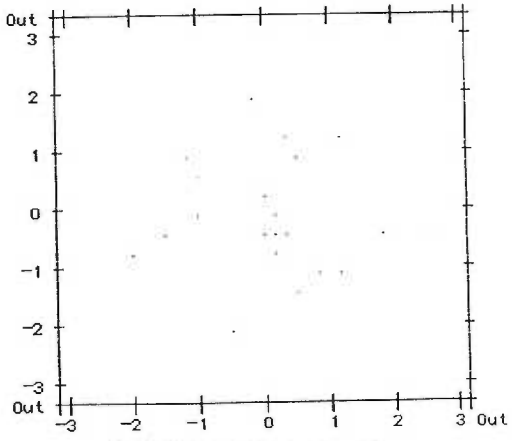
Kcal = 55 n = 20



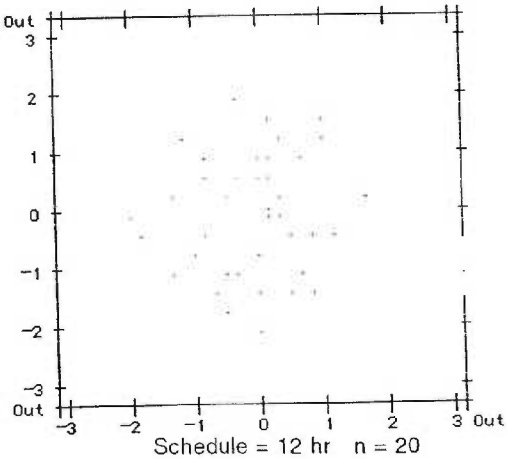
Low Fiber n = 20



High Fiber n = 20



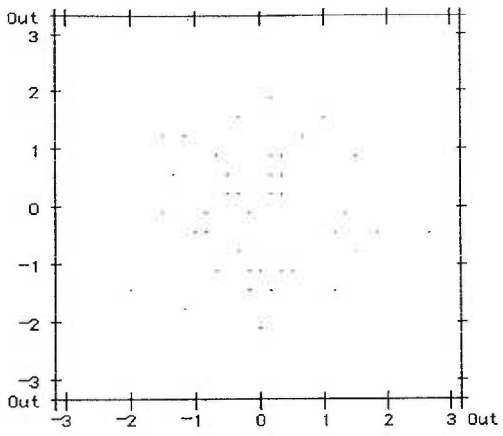
Schedule = 24 hr n = 20



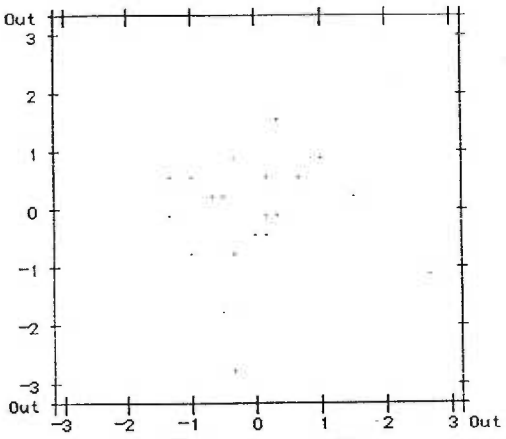
Schedule = 12 hr n = 20

Organ:
Large Intestine

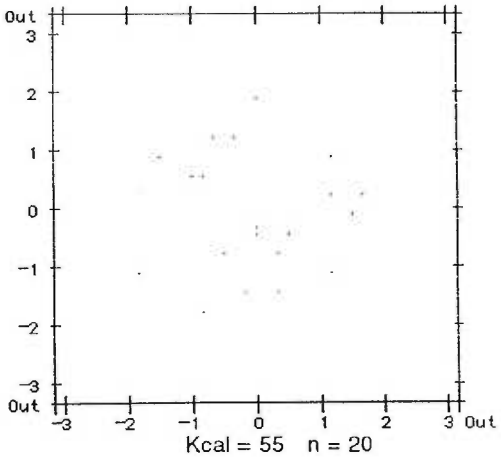
Standardized
Scatterplot
Across = Resid
Down = Pred



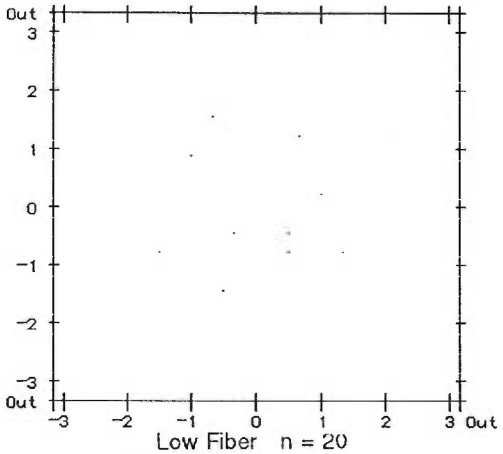
Total Sample
N = 40



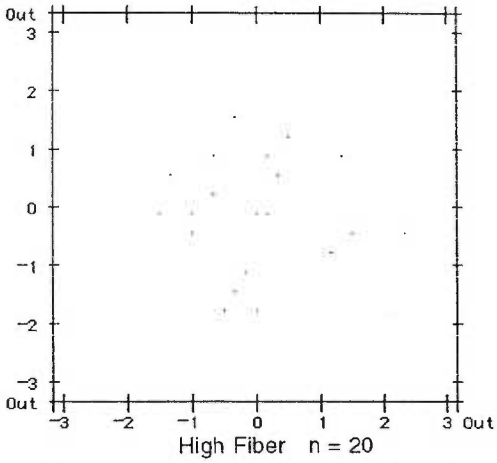
Kcal = 80 n = 20



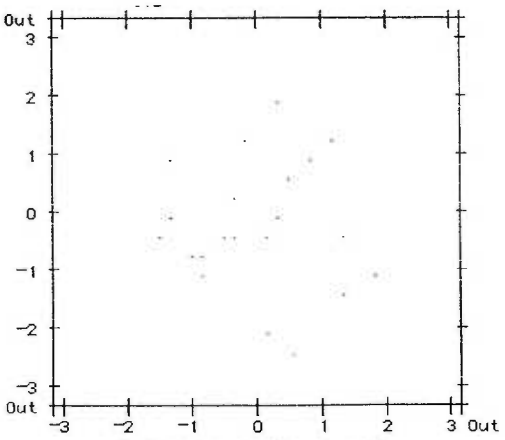
Kcal = 55 n = 20



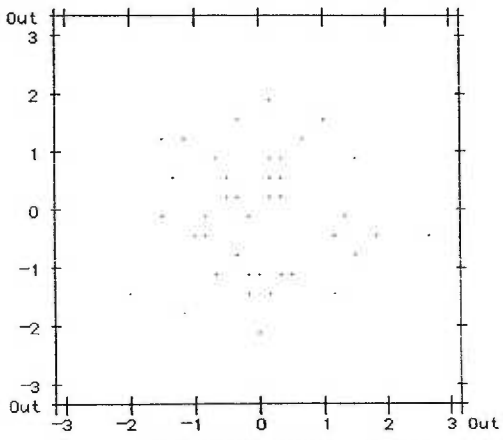
Low Fiber n = 20



High Fiber n = 20



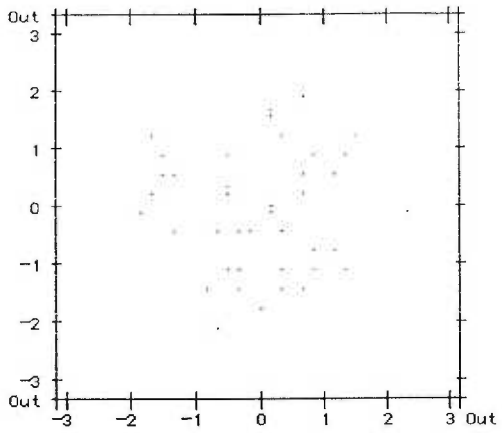
Schedule = 24 hr n = 20



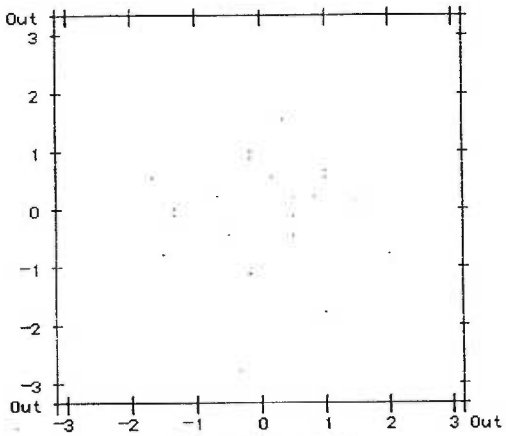
Schedule = 12 hr n = 20

Organ:
Kidneys

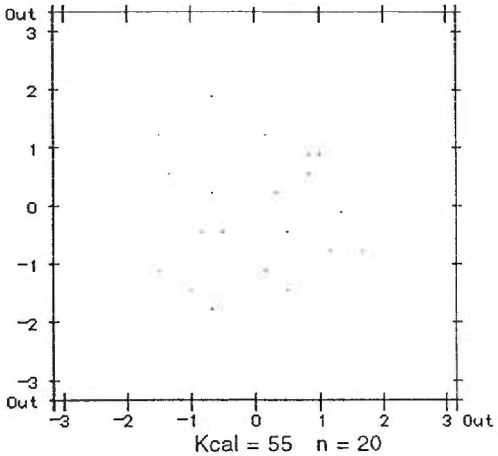
Standardized
Scatterplot
Across = Resid
Down = Pred



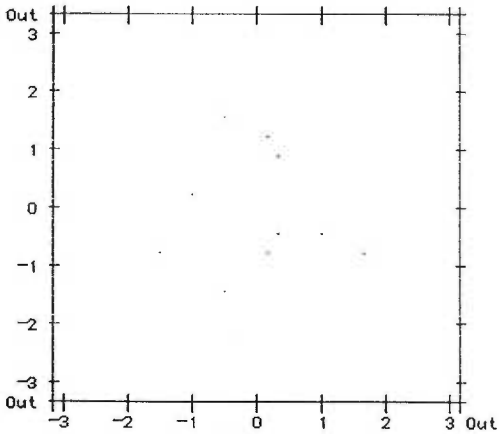
Total Sample
N = 40



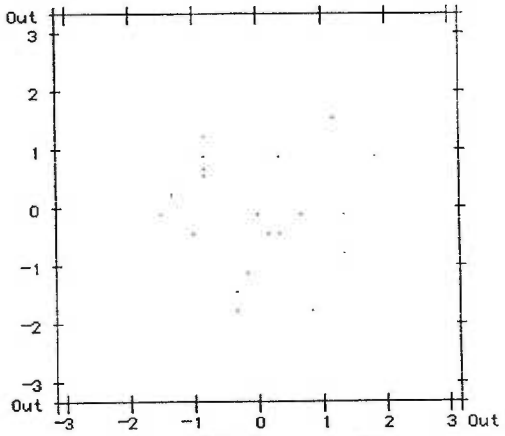
Kcal = 80 n = 20



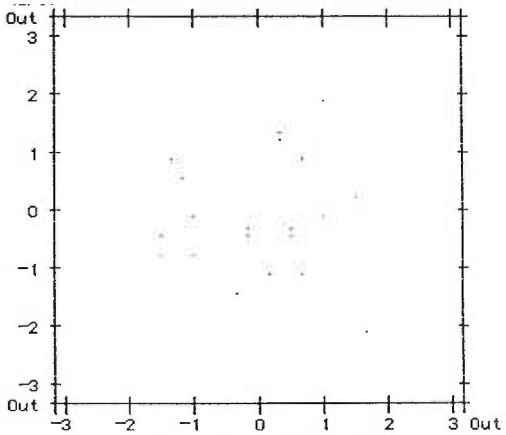
Kcal = 55 n = 20



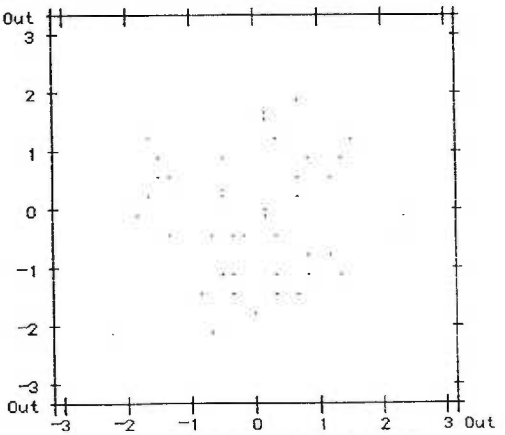
Low Fiber n = 20



High Fiber n = 20



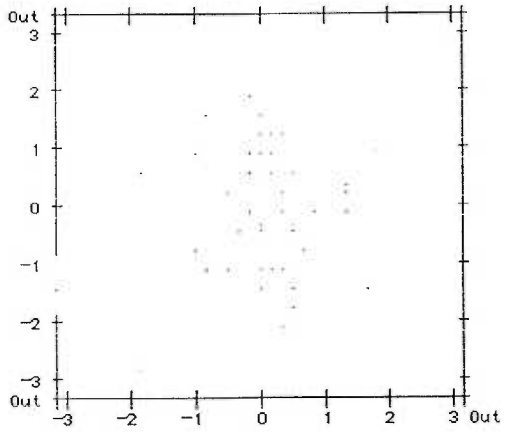
Schedule = 24 hr n = 20



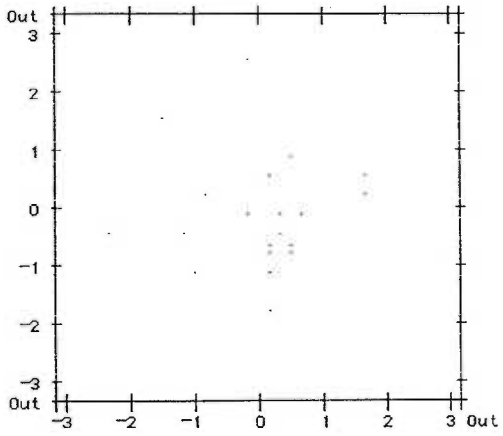
Schedule = 12 hr n = 20

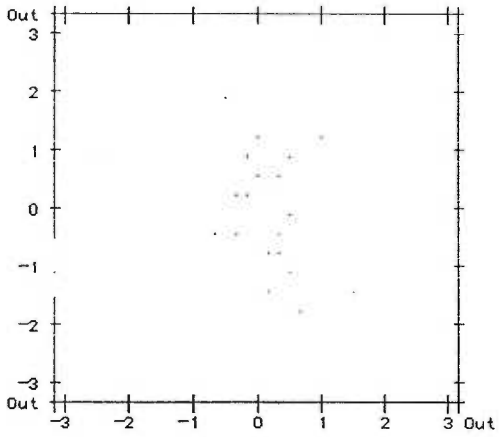
Organ:
Adrenals

Standardized
Scatterplot
Across = Resid
Down = Pred

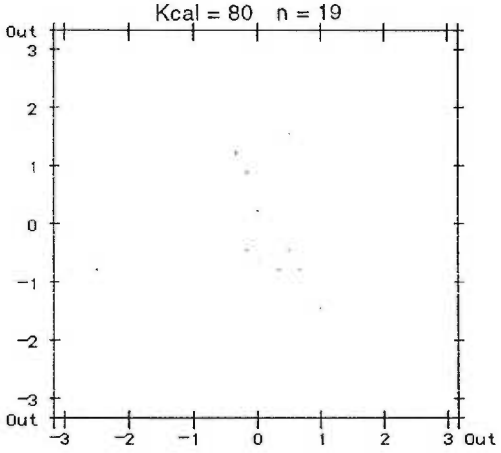


Total Sample
N = 37

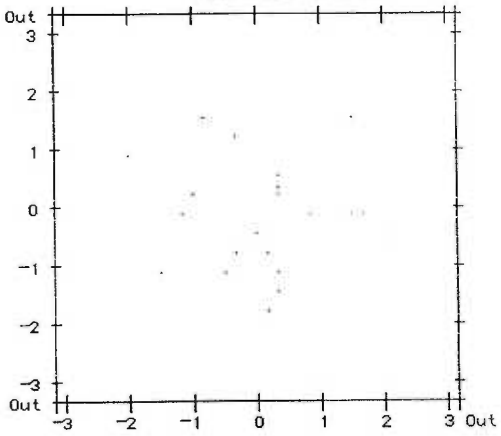




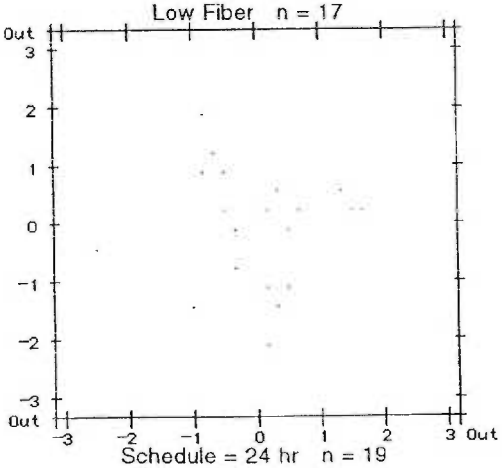
Kcal = 80 n = 19



Kcal = 55 n = 18

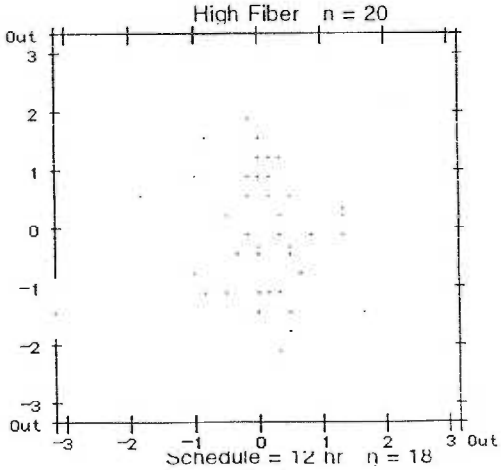


Low Fiber n = 17



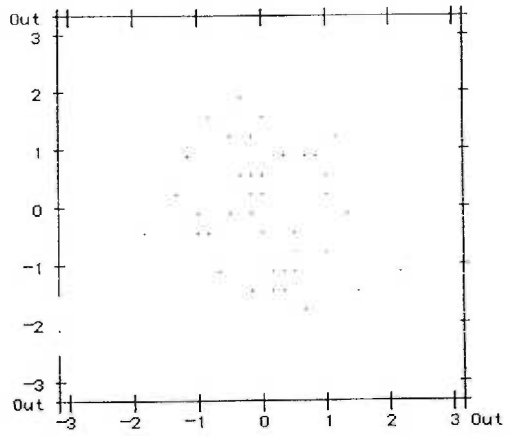
Schedule = 24 hr n = 19

High Fiber n = 20

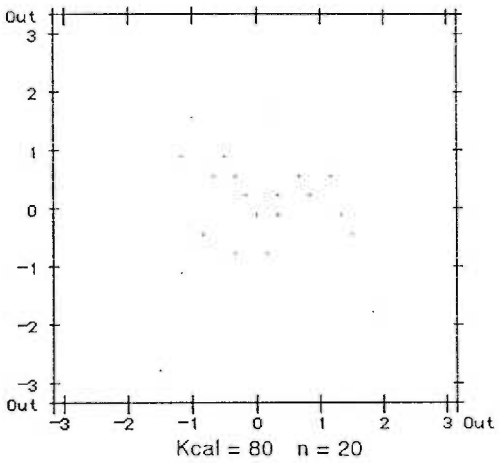


Schedule = 12 hr n = 18

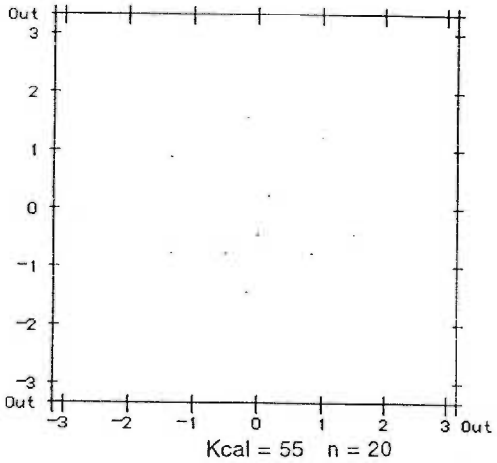
Standardized
Scatterplot
Across = Resid
Down = Pred



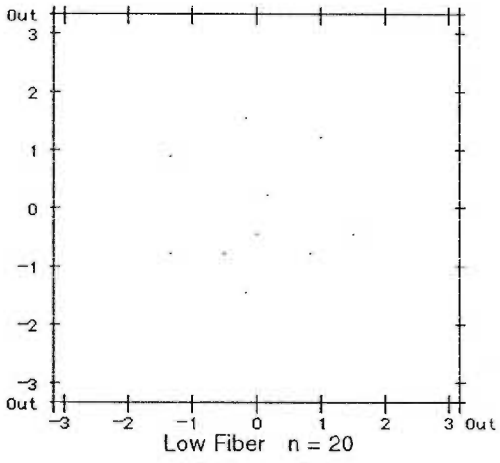
Organ:
Muscle
Extensor
Digitorum
Longus
Total Sample
N = 40



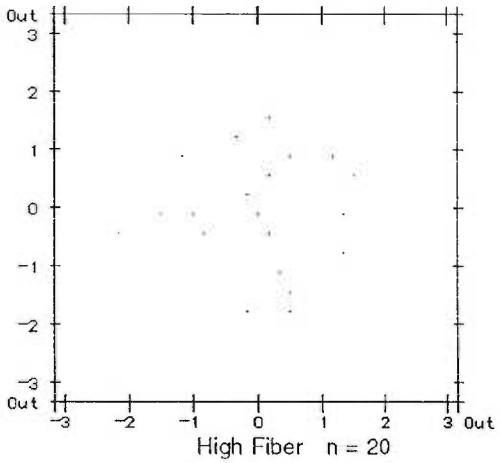
Kcal = 80 n = 20



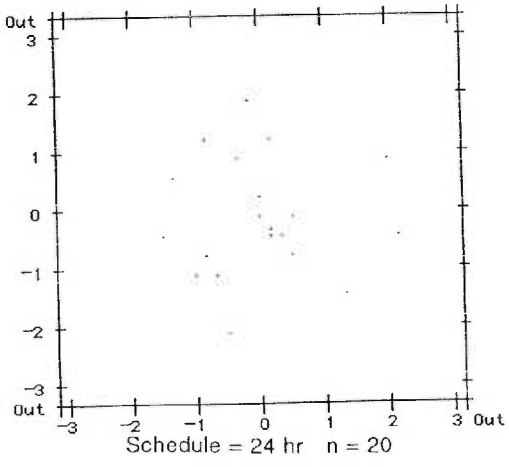
Kcal = 55 n = 20



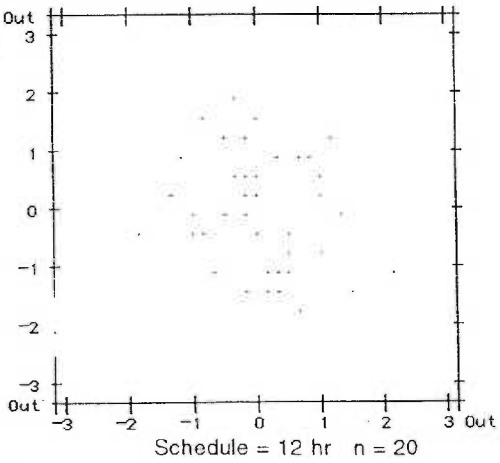
Low Fiber n = 20



High Fiber n = 20



Schedule = 24 hr n = 20



Schedule = 12 hr n = 20