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THE ONTOGENY OF BROWN ADIPOSE TISSUE IN *MICROTUS OCHROGASTER* IN VARIOUS THERMAL AND SOCIAL ENVIRONMENTS

By

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ABSTRACT

THE ONTOGENY OF BROWN ADIPOSE TISSUE IN *MICROTUS OCHROGASTER* IN VARIOUS THERMAL AND SOCIAL ENVIRONMENTS

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Short- and long-term brown adipose tissue (BAT) responses to postnatal cold exposure (PCE) and the effects of paternal care during PCE were examined in multiple BAT deposits in prairie voles (*Microtus ochrogaster*). PCE enhanced BAT recruitment in nestlings but not adults. Recruitment was higher when fathers were absent (versus present) during PCE. Responses in various BAT deposits were correlated regardless of treatments, suggesting that measures of single BAT deposits reflect responses in total BAT; however, deposits differed in relative lipid content. Prairie voles showed a short-term BAT recruitment response to PCE similar to that seen in murine rodents. However, prairie voles did not exhibit long-term BAT recruitment (into adulthood) in response to postnatal PCE. Nestling BAT recruitment was curtailed when paternal care was available during PCE, suggesting a possible mechanism for enhanced development observed in this species when paternal care is available.

To my parents, Gary White and Beverly Berini, with love.

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INTRODUCTION

Very little is known about the influences of low ambient temperature on postnatal brown adipose tissue (BAT) development in nonmurine altricial rodents. However, studies on murine rodents and other mammals have shed much light on BAT recruitment, and several predictions can be made for microtine rodents regarding 1) how BAT might respond over short (preweaning) and long (adult) time scales to cold exposure during the postnatal period, 2) how the presence or absence of paternal care during cold exposure might affect nestling BAT responses, and 3) how interrelated these responses might be as expressed in the various BAT deposits of an individual animal. Before elaborating on these predictions, I will present a general overview of the function, mechanisms, and process of BAT recruitment, focusing on altricial rodents. Recruitment time scales in nestlings and adults, methods of measuring BAT recruitment, the state of knowledge of microtine BAT responses, and the natural history of *Microtus ochrogaster* -- the focus of the studies presented here -- will also be briefly reviewed. First, BAT responses to postnatal cold exposure will be examined in preweanling prairie voles in the *short-term*

study and in adult prairie voles in the *long-term study*. Second, BAT responses to paternal care during cold exposure will be examined in preweanling prairie voles in the *paternal-care study*. Finally, responses of various BAT deposits will be compared in the study of *response relationships between BAT deposits*.

POSTNATAL NONSHIVERING THERMOGENESIS AND BAT

Studies of isolated nestlings have shown that a remarkable enhancement of thermoregulatory capacity occurs in small-bodied rodents during the postnatal period. The lack of a developed musculoskeletal system, high surface-area-to-volume ratio, and high rates of movement-related heat loss preclude shivering from playing a major role in thermoregulation in neonatal rodents (Jansky, 1973; Gordon, 1993). Experiments with surgical and pharmacological treatments to block shivering and nonshivering mechanisms have shown that nonshivering thermogenesis (NST) predominates during the postnatal period (e.g., Brueck and Wuennenberg, 1966). Brown adipose tissue (BAT) is the principal site of nonshivering thermogenesis (NST) in mammals.

BAT THERMOGENESIS MECHANISMS

The mechanisms of BAT thermogenesis have been well studied in laboratory rodents and reviewed recently by Himms-Hagen (1990) and Cannon (1995) (Figure 1). Norepinephrine (NE) stimulation of $beta_3$-adrenergic receptors on the surface of brown adipocytes elevates cyclic-AMP (cAMP) via G-proteins, activating protein kinases, which phosphorylate hormone-sensitive lipase (HSL). Intracellular triacylglycerol is hydrolyzed by activated HSL to free fatty acids (FFA), which enter the mitochondria and undergo beta-oxidation to produce acetyl-CoA and reduced cofactors NADH and $FADH_2$. Oxidation of these cofactors via the electron transport chain, in addition to those produced elsewhere, such as by acetyl-CoA oxidation in the tricarboxylic acid cycle, creates a proton electrochemical gradient between the mitochondrial matrix and intermembrane space. ATP synthetase content is unusually low in BAT mitochondria (Houstek et al., 1995), and this proton gradient is largely dissipated by a protein found only in the BAT inner mitochondrial membrane, called uncoupling protein (UCP).

UCP, also known as thermogenin, is unique to BAT. Its primary function is to allow protons accumulated in the intermembrane space to bypass ATP synthetase in returning to the mitochondrial matrix, thereby uncoupling

Figure 1. Schematic depiction of metabolic processes in a brown adipocyte.

Figure 1

NE
β
G
AC
plasma membrane
cAMP
protein kinases
lipase
lipase
P
4.
TAG
1. glucose
2. FFAs (blood)
3. plasma lipoproteins
– chylomicrons (dietary fat)
– VLDLs (liver)
lipogenesis
LPL
FFA
cytosol
outer membrane
FA Carn
Fatty acyl CoA
FFA
inner membrane
matrix
FA Carn
β-oxidation
acetyl CoA
Fatty acyl CoA
TCA
H+
ADP + Pi
ATP
ATP synthetase
ETC
UCP
intermembrane space
TAG

the electron transport chain from oxidative phosphorylation and degrading energy stored in the proton gradient to heat. Thus an increase in UCP concentration or activity raises the NST capacity of the tissue (Nedergaard and Cannon, 1994; Cannon, 1995). Oxidation of lipids to heat can occur very rapidly in BAT because ATP production, the usual rate-limiting step in aerobic catabolism, is attenuated (Figure 1, adapted from Horwitz, 1989 and Himms-Hagen, 1990).

BAT RECRUITMENT AND ITS REGULATION

The phenomenon of increased BAT growth and activity has been termed *recruitment* (Nedergaard et al., 1986). Recruitment results from physiological and biochemical enhancements of BAT, including increased sympathetic innervation, vascularization, cell proliferation and differentiation, mitochondriogenesis, and UCP activation and biosynthesis (Trayhurn and Milner, 1989).

Recruitment processes are primarily regulated by the central nervous system. Brown adipocytes and surrounding blood vessels are innervated by axons of sympathetic ganglia cells. These cells synapse with cholinergic cells in the spinal chord, linking BAT to the anterior hypothalamus, which stimulates BAT in response to lowered body temperature. BAT is also connected to the

ventromedial and lateral hypothalamus, and may function in the regulation of energy balance via diet-induced thermogenesis (DIT), also mediated by NE (Rothwell and Stock, 1986; Cannon, 1995).

Interestingly, NE promotes both acute and chronic changes in BAT. As described earlier, NE acts as a classical neurotransmitter, regulating acute metabolic activities via $beta_3$-receptors in mature brown adipocytes (Figure 1). NE also acts as a morphogenic factor earlier in adipocyte development. As a morphogen, NE first stimulates DNA replication and adipocyte mitosis through $beta_1$-receptors (proliferation), and later promotes UCP and lipoprotein lipase gene expression via $beta_3$-receptors (differentiation) (Nedergaard et al., 1995).

It is also becoming clear that endocrine factors interact with NE in BAT regulation, sometimes acting as synergists in recruitment. For instance, NE regulates an extrathyroid type II deiodinase in BAT in response to postnatal cold exposure, converting thyroxine to 3,3',5-triiodothyronine, which may enhance UCP production. Insulin also seems to facilitate UCP gene expression, and insulin levels increase in response to low postnatal ambient temperatures (Bertin et al., 1992). Furthermore, NE and insulin stimulate lipoprotein lipase mRNA transcription in rats (Mitchell et al., 1992).

RECRUITMENT STIMULI AND TIME SCALES

Laboratory mice and rats (e.g. Doi and Kuroshima, 1979; Obregon et al., 1989) and several domesticated ruminant species (Symonds et al., 1992; Symonds and Lomax, 1992) are known to respond to the cold exposure associated with birth by increasing BAT levels. It has been demonstrated in rats that postnatal recruitment of BAT will not occur if neonates are artificially shielded from low ambient temperatures at the time of birth (Obregon et al., 1989). BAT recruitment also occurs in many adult rodents (e.g., laboratory mice and rats) during cold acclimation, in response to short photoperiod (e.g., hamsters; Heldmaier and Hoffman, 1974) and under certain dietary conditions (e.g., diet-induced thermogenesis in rats).

The temporal pattern of postnatal BAT recruitment varies among species and depends in part on the degree of development at birth. As defined by Nedergaard et al. (1986), *immature* neonates (e.g. marsupials) experience a delay in recruitment up to several weeks after birth, whereas *altricial* neonates (e.g., rats, mice, voles) recruit BAT immediately after birth, and *precocial* neonates (e.g., guinea pigs, lambs) show recruitment (possibly stimulated by *in utero* NE) before birth.

In nonhibernating altricial and precocial species, BAT activity usually declines later during postnatal maturation, a phenomenon known as *involution* (Nedergaard et al., 1986); simultaneously, other thermoregulatory mechanisms (e.g., insulation and shivering) develop. However, prolonged exposure to low ambient temperatures beyond the neonatal period can delay BAT involution (Brueck and Wuennenberg, 1966; Lynch et al., 1976; Lacy et al., 1978). Furthermore, it has been shown in laboratory mice and rats that exposure to cold during adulthood results in recruitment of BAT, even when subjects had experienced no postnatal cold exposure (e.g., Lacy et al., 1978; Bertin et al., 1980; Senault et al., 1982).

Recruitment during cold acclimation in adults represents an unusual aspect of BAT as a mammalian tissue: its ability to proliferate and differentiate in adults after a period of quiescence (Cannon, 1995). By measuring DNA synthesis with tritiated thymidine, Cameron and Smith (1964) determined that BAT hyperplasia occurs by proliferation of reticuloendothelial cells (preadipocytes), rather than by mitosis of mature adipocytes, in the vascular spaces of BAT. Thus, postnatal recruitment does not appear to be a necessary precursor for adult cold acclimation, since preadipocytes can proliferate throughout the life span. However, in addition to the critical thermoregulatory role postnatal

recruitment plays in nestling thermoregulation, recruitment during the postnatal period may have long-term effects on BAT development.

Lynch et al. (1976) found a lasting recruitment effect of preweaning temperature on interscapular BAT weight-specific lipid-free weight in adult lab mice (*M. domesticus*) that were reared with mothers, siblings and nesting material; postweaning ambient temperature had no effect. Lacy et al. (1978) found that rearing lab mice in continuous cold from birth until 70 d resulted in lasting differences from controls, when the animals were adults, in weight-specific lipid-free dry weight of interscapular BAT and NST (assessed by measuring maximum O_2 consumption after NE injection). Likewise, Doi and Kuroshima (1979) determined that periodic daily cold exposure in lab rats in the first two weeks of life caused significant elevation of NST, lowering of the colonic temperatures required to induce shivering, and depression of shivering activity at up to 19 weeks of age, whereas the effects of identical cold treatment lasted only four weeks when applied to adult rats. However, observations made after obliterating interscapular BAT function led them to believe that interscapular BAT played a minor role in the prolonged responses they observed. In contrast, most studies of BAT suggest that NST elevation results from enhancement of BAT (e.g., Nedergaard et al., 1993). Thus,

it appears that more empirical evidence is necessary to elucidate the long-term consequences of postnatal BAT recruitment across various mammalian taxa.

In contrast to the remarkable "adaptability" BAT can exhibit during recruitment, BAT weight is negligibly heritable in quantitative genetic analyses (Lynch, 1992). Although changes in BAT within an individual's lifetime (i.e. changes over acclimatory and ontogenetic time scales) are not genetically transmitted to offspring, they can be important in a comparative or evolutionary context. Phenotypic plasticity can itself be heritable and evolve adaptively (Lynch, 1992); BAT recruitment responses may represent such a case. A clearer understanding of the time scales of physiological responses is necessary in order to determine how factors such as BAT recruitment affect behavior and ecology (Karasov and Diamond, 1988).

MEASUREMENT OF BAT RECRUITMENT

Many studies on BAT recruitment responses have focused on measurements taken on freshly dissected tissue. However, such measures confound several variables, including hydration state, energy (lipid) stores and thermogenic capacity of the tissue. Although it is not a direct index of brown adipocyte weight (as other cell types are present in BAT), lipid-free, dry BAT weight

expressed per unit body weight is a useful measure of the amount of functional BAT tissue, and permits determination of major changes in NST capacity (Trayhurn and Milner, 1989). Weight-specific, lipid-free dry weight of BAT is correlated throughout the year with NST capacity in adult white-footed mice, *Peromyscus leucopus* ($r=0.58$, $p<0.01$, Lynch, 1973). Hayward (*in* Chaffee and Roberts, 1971) found that, for several species of nonhibernating mammals, weight-specific, lipid-free BAT weight is directly proportional to NST capacity ($r=0.98$, $p<0.003$).

LIPID LEVELS AND COLD-INDUCED RECRUITMENT

Lipid stores in BAT are readily measured by subtracting the lipid-free dry weight from the dry weight prior to lipid extraction. Lipid content measurements provide a simple view of the net effect of lipolytic, lipogenic, and lipid import and export processes occurring in BAT. Because fatty acid synthesis displays an "anti-suckling" pattern in postnatal rats (i.e. lipogenesis is high just prior to birth, then decreases rapidly at birth, remaining low until weaning; Nedergaard et al., 1986), the lipid content of preweanling BAT largely reflects the balance between lipid utilization and restoration via milk.

In altricial rodents, prenatal triacylglycerol accumulation in BAT is low, and BAT lipid concentration rises rapidly after the first postnatal meal. As illustrated in Figure 1, BAT triacylglycerol stores are replenished via lipoprotein-lipase-mediated transfer of circulating chylomicrons and very-low-density lipoproteins (Carneheim et al., 1984). Lipolysis and lipid oxidation in BAT are enhanced by cold exposure (Bertin et al., 1980). Senault et al. (1982) have shown that postnatal rats housed with their mothers at 16°C from 1-21 d exhibit significantly lower BAT lipid concentrations than those housed with mothers at 28°C. This depletion associated with cold exposure has been interpreted to represent BAT thermogenic activity in cold-exposed nestlings (Nedergaard et al., 1986).

Lipid accumulation per unit of lipid-free BAT dry weight increases as involution proceeds with age in unstimulated BAT (which further complicates wet BAT weight interpretation, since a weight increase could be due to recruitment or involution; Nedergaard et al., 1986). In adults with stimulated BAT, lipid stores are often replenished as quickly as they are depleted via lipogenesis, dietary lipids, and translocation of FFAs from other adipose deposits. For instance, Cameron and Smith (1964) found that BAT lipid levels are restored within 24 h after 26°C-acclimated adult rats are exposed

to 6°C. Thus BAT lipid content in adults may remain relatively high in spite of cold exposure, providing a less reliable index of BAT thermogenic activity than postnatal BAT lipid content.

DEFINING COLD EXPOSURE

A common approach in previous studies of postnatal BAT responses to thermal stress (e.g., Lacy et al., 1978) has been to house neonates with parents, siblings, and nesting material during continuous cold exposure. However, short daily cold exposures are probably of greater ecological significance than continuous exposure, since free-living mammals typically experience daily fluctuations in ambient temperature. It has been shown that brief daily cold exposures can have effects similar to those of continuous cold on the growth of BAT during adult cold acclimation in lab mice, rats, ground squirrels, golden hamsters, and pigs (Heldmaier, 1975a).

A problem with exposing nestlings to cold in the presence of parents, siblings, or nesting material is that the exposure is not well defined. Thermal buffering and social thermoregulation can affect the level of cold exposure, and in fact there is some evidence that these factors can affect BAT mass by inhibiting recruitment (e.g., Heldmaier, 1975b). These influences, if left

unquantified, can confound results or cause difficulties in interpretation, and result in irreproducibility. Thus, it seems best to isolate young from siblings, parents, and nesting material during cold exposure, so that the exposures are well defined, unless social and nesting influences are the subjects of investigation.

PRIOR STUDIES OF BAT IN MICROTINE RODENTS

The vast majority of BAT research has been conducted on laboratory rats (*Rattus norvegicus*) and mice (*Mus domesticus*), both of which are Old-World altricial murine rodent species. Few studies exist on BAT in adult microtine rodents, and almost nothing is known about BAT development in nonmurine rodents. NST has been examined in adult winter-acclimatized red-backed voles, *Clethrionomys rutilus* (e.g., Feist and Rosenmann, 1976). In prairie voles, *Microtus ochrogaster*, NST is known to occur during cold acclimation (Wunder et al., 1977) and DIT has been examined in this species (Trier, 1995). The presence of BAT has been confirmed biochemically in *M. ochrogaster* (Trier, 1995) and *M. agrestis* (McDevitt and Speakman, 1994). McDevitt and Speakman (1994) found that the concentration of UCP per BAT deposit and the ratio of BAT to white adipose tissue increased over the course of 100 days exposure to 5^{o}C in short-tailed field voles, *Microtus agrestis*. Information on postnatal BAT

recruitment in microtines is very sparse. Nestling BAT development was examined in response to maternal-nestling interactions under different photoperiods in the meadow vole, *M. pennsylvanicus* (Reeves, 1994), but this study included only measurements of wet BAT weight, posing interpretation problems.

PRAIRIE VOLES: NATURAL HISTORY

Prairie voles (subfamily Arvicolinae) are New World microtines that range throughout the midwestern U.S. and breed facultatively from March through November (Keller and Krebs, 1970; Rose and Gaines, 1978). Gestation is about 21 d long, with litter sizes ranging between one and eight (mean = 3.9; lab conditions). Mean weight at birth is 2.9 g. Eyes open at 5-10 d of age, and weaning occurs around 21 d (Nadeau, 1985). Laboratory-reared prairie voles successfully breed by 40 days of age (personal observation). Average soil temperatures at the depth of prairie vole burrows in April and November in east-central Illinois are 15-16^{o}C (Solomon, 1991). Thus, nestling prairie voles are likely to experience ambient temperatures well below thermoneutrality during the postnatal period when the parents periodically leave the nest (e.g., Thomas and Birney, 1979) and when they begin to wander from the nest at around 14 d of age (Getz, personal communication).

EFFECTS OF LOW AMBIENT TEMPERATURE ON BROWN ADIPOSE TISSUE DEVELOPMENT

I. SHORT-TERM EFFECTS OF POSTNATAL COLD EXPOSURE

OBJECTIVES AND EXPERIMENTAL DESIGN

The primary goal of the short-term study was to test the hypothesis that postnatal brown adipose tissue recruitment is enhanced by cold exposure during the preweaning period in a free-ranging temperate-zone microtine rodent species (*Microtus ochrogaster*) which is likely to experience thermal stress during development.

To test this hypothesis, periodic cold exposures were employed. Lab-reared *M. ochrogaster* nestlings of each sex were exposed in isolation, from the day after birth through 20 days postpartum, to 10°C for 3 h/d. Data from both sexes were combined before analysis because no effect of sex was found by 21 days of age. When pups were 21 days of age, their weight-specific lipid-free BAT weights and lipid weights per lipid-free dry BAT weight were compared to the values for littermate controls maintained continuously at 30°C, the mean nest temperature as measured with an infrared radiometer. Based on previously published observations of nonmicrotine rodents, I hypothesized that the cold-exposed nestlings would possess

higher lipid-free dry BAT weights per gram body weight and lower lipid weights per lipid-free dry BAT weight than controls.

I also examined several presuppositions underlying these hypotheses. I presumed that lipid-free BAT dry weight and lipid weight per lipid-free BAT weight would vary as a function of body weight. I also presumed that the lipid content of a BAT deposit would vary as a function of its lipid-free dry weight. The correspondence of these presumptions with the data obtained was evaluated.

II. LONG-TERM EFFECTS OF POSTNATAL COLD EXPOSURE

OBJECTIVES AND EXPERIMENTAL DESIGN

The main goal of this study was to determine whether BAT recruitment induced by cold in the postnatal period is maintained into adulthood if cold-exposure is limited to the preweaning period.

Cold-exposed and control nestlings were treated up to 20 d of age exactly as in the study on short-term effects. After 20 d of age, however, all littermates were maintained continuously together as caged social groups in the animal colony until 20 weeks old. Data for both sexes

were combined for analysis based on the results of the short-term study, which indicated that sex had no effect on the variables of interest by 21 days of age. At 20 weeks, I compared weight-specific lipid-free BAT weights and lipid weights per gram lipid-free BAT weight in the prairie voles which had experienced either low or observed-nest temperatures during the first 20 days postpartum. I hypothesized that exposure to periodic cold during early development would result in higher weight-specific, lipid-free BAT weights in adult *M. ochrogaster*. Several presuppositions as described in the short-term study were likewise examined in the long-term study.

METHODS

SHORT- AND LONG-TERM STUDIES

ANIMAL SUBJECTS AND HUSBANDRY

The animals used were laboratory-born prairie voles (*M. ochrogaster*) from a colony at Michigan State University established in 1991 from animals originally captured near Urbana, Illinois. Throughout the study, breeding pairs were housed together at 21^{o}C on a 16:8 light:dark cycle in plastic cages (38x33x16 cm) provided with corn cob bedding (Andersons Industrial Products) and aspen laboratory-grade wood shavings (Northeastern

Products Corp.). Parents typically built nests from the shavings. Food (Teklad Rodent Diet 8640 and Rabbit Diet) and water were provided ad lib. In the long-term study, after weaning at 30 d, litters were housed as groups. Some weaned litters were housed with siblings from a prior or later litter to defray colony maintenance costs. Therefore, litter size was not examined as a variable in the long-term study.

PROCEDURE

All nestlings in a litter were toe-clipped (one toe on one foot) at 1 d of age for identification. Half of the individuals in each new litter were assigned at random to each of the two treatments. Cold-treated individuals were exposed alone in unused half-pint paint cans (8.5 cm diameter x 10 cm height) from one day of age (day after birth) through 20 days of age for 3 h/d during the lights-on period to 10^{o}C in a well-lit, climate-controlled incubator. This temperature is 5-6 degrees below the mean soil temperature across the breeding season at the depth of prairie vole burrows (Solomon, 1991). The other half of the individuals were exposed in an identical fashion to 31^{o}C as a control group. This temperature corresponds to nest temperature in our lab as measured with an infrared radiometer (Sensors Model ATR), and is within the thermoneutral zone of similar-sized adult rodents,

although it may be below that of neonates (Himms-Hagen, 1986).

Metal paint cans were used and individuals were isolated during treatments to prevent thermal buffering and social-thermoregulation effects, respectively, and to maintain constancy of experience with low ambient temperatures across subjects. To prevent different parental behavior toward chilled nestlings, all pups were kept at 31°C for 20 min at the end of each treatment period to equalize acute ambient influences on nestling temperature before they were returned to the parents. To reduce error variance due to differences among litters, individuals within each litter were assigned to sibling pairs. Each pair consisted of a cold-treated animal and a control animal that were as similar as possible in sex and weight, and analysis of variance (two-way ANOVA for paired comparisons) was carried out on pairs of siblings.

The number of nestlings per litter was recorded. Nestlings in the short-term study were weighed at regular intervals, whereas only weight at the time of dissection was examined in the long-term study. At 21 d, litters in the short-term study (n=26 sibling pairs) were euthanized with CO_2, weighed and sexed. BAT deposits were then removed under a dissecting microscope, placed in numbered glass vials and frozen for later drying, lipid extraction

and weighing. Samples were dried in a drying oven at 55°C until they reached a constant weight (about 24 hours). Lipids were extracted from the dry tissue samples per Lacy et al. (1978) using two changes of anhydrous ethyl ether (J. T. Baker, Inc.). It was determined in preliminary trials that two changes of ether produce a constant lipid-free dry weight which remains unaltered after additional ether changes.

In most studies of BAT, only responses in the interscapular tissue are examined. However, BAT is found in multiple isolated and semi-isolated deposits in most mammals; 13 have been clearly identified in the deer mouse, *Peromyscus maniculatus* (Rauch and Hayward, 1969). Various BAT deposits were positively identified in the prairie voles in our lab based on previous GDP binding assays (Trier, 1994), physical descriptions, and anatomical maps (Rauch and Hayward, 1969). Brown and white adipose tissue were easily distinguished by differences in color and location. In the short-term study, three deposits were collected from each individual (anatomical nomenclature follows Rauch and Hayward, 1969):

The first deposit removed was the "*interscapular*" BAT (n=26 pairs). This deposit is relatively large, bilobed, isolated from other BAT deposits, and located subdermally in the scapular depression.

The second removed was the squamo-occipito-cervical deposit, subsequently abbreviated as "*squamosal*" BAT (n=26 pairs). This is a smaller deposit, located more proximal to the cervical vertebrae than the interscapular deposit, and well-isolated from other deposits by the semispinalis muscles.

Last removed was a group of five individual deposits -- examined collectively -- abbreviated here simply as "*residual*" BAT. They included the subscapular, transverse cervical, axillary, jugular and carotid deposits. These residual deposits, named individually after their locations, accounted for most of the remaining BAT in the animal. Removal of residual BAT was painstaking and time-consuming (about 1.25 h per animal). Therefore only 22 individuals were examined for residual BAT (n=11 pairs).

In the long-term study, only the interscapular and squamosal (i.e., squamo-occipito-cervical) deposits were removed and analyzed. Litters in the long-term study (n=46 pairs of animals) were examined at 20 weeks of age for lasting responses in the two analyzed deposits of BAT.

DESIGN AND ANALYSIS

As noted earlier, to remove variation among litters as a potential confounding factor in both short- and

long-term studies, littermates were grouped in a randomized complete block design, blocking on pairs of siblings from the same litter. To minimize extraneous variation within blocks (pairs of littermates), siblings of the same sex and similar body weight at 20 d were paired where possible. Paired comparisons were performed using a two-way ANOVA to permit investigation of blocking effects in addition to treatment effects. Normality was examined using normal probability plots, and homogeneity of variances was evaluated using Cochran's C and Bartlett-Box F tests (SPSS PC+). Data were transformed using natural logarithms when such transformations were found to enhance normality and variance homogeneity. Outliers were identified by Dixon's or Grubb's tests (Sokal and Rohlf, 1995) for samples sizes below 26 or greater than 25, respectively, and removed prior to analysis.

The weight-specific, lipid-free dry BAT weight (LFDW/g), and the lipid weight per lipid-free BAT weight (LW/LFDW) were compared between treatments within pairs in both short- and long-term studies using the MANOVA procedure in SPSS PC+. Scattergrams were plotted to facilitate visual assessment of differences between cold-treated and control individuals within pairs.

Least squares linear regression analysis was used to test presuppositions by examination of relationships

between body weight and both lipid-free BAT dry weight and lipid weight per lipid-free BAT weight, and between the lipid content of a BAT deposit and the lipid-free dry weight of the deposit. Because rigorous use of linear regression implies assumptions which are often not met in functions among physiological variables (e.g., "independent" variables are often not fixed), correlation analysis was also employed to evaluate the degree of association among variables. Because the correlation results generally were commensurate with the results of the regression analyses, only the regression results are reported below.

RESULTS

I. SHORT-TERM STUDY

A PRIORI COMPARISONS:

The following results respond to the a priori hypotheses presented in the OBJECTIVES section.

The hypothesis was supported that cold exposure increases the weight-specific lipid-free BAT dry weight in nestlings. Cold-exposed nestlings at 21 d had significantly higher weight-specific levels of lipid-free, dry BAT (LFDW/g) than controls for all deposit types

examined (Table 1). Figures 2-4 show that, for each deposit type in over 90% of the pairs, the cold-treated littermate had a higher LFDW/g value than the control littermate (interscapular BAT: 24 of 26 pairs; residual BAT: 10 of 10 pairs; squamosal BAT: 24 of 26 pairs). In the residual BAT data, an outlier was identified and the pair which contained it was removed. Interscapular BAT and squamosal BAT data were log-transformed prior to analysis of variance. Pairing accounted for a significant part of the total variance for squamosal BAT data only (Table 1).

The hypothesis that cold exposure results in a significantly lower lipid content per deposit in nestlings was generally supported. The amount of lipid per lipid-free BAT (LW/LFDW) was lower in nestlings receiving periodic cold, although this difference was not significant for squamosal BAT (Table 1). As can be seen in Figures 5-7, only 17 (68%) of the 25 pairs had lower LW/LFDW values for the cold-treated pair members than control pair members for squamosal BAT, as opposed to 21 of 26 pairs (about 81%) for interscapular BAT and 8 of 11 (about 73%) for residual BAT. One outlier was identified and the pair removed from the squamosal BAT data before analysis. Pairing accounted for a significant variance component for residual BAT and squamosal BAT, but not for interscapular BAT (Table 1).

Table 1. Results from the short-term study. N is the number of pairs of animals (cold-treated and control) analyzed. Standard deviation (SD) is given in parentheses. The probability for treatment ("trtment") is the likelihood of no difference between cold-treated and control animals. That for pairs is the likelihood that none of the overall variance was attributable to differences among pairs. LFDW/g = weight-specific lipid-free BAT dry weight. LW/LFDW = lipid weight per lipid-free BAT dry weight.

		Mean (SD)		Probability	
Variable	N	Cold-treated	Control	Trtment	Pairs
LFDW/g (mg/g)					
Interscapular	26	0.88 (0.23)	0.62 (0.12)	<0.001	0.27
Squamosal	26	0.54 (0.17)	0.39 (0.13)	<0.001	<0.01
Residual	10	3.23 (0.50)	2.15 (0.39)	<0.001	0.27
LW/LFDW (g/g)					
Interscapular	26	3.62 (1.42)	4.72 (1.06)	0.001	0.07
Squamosal	26	2.42 (1.20)	2.74 (0.89)	0.194	0.04
Residual	11	3.83 (0.98)	4.63 (1.03)	0.016	0.02

Figure 2. Interscapular weight-specific lipid-free BAT dry weight (LFDW/g) for cold-treated and control animals of pairs in the short-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LFDW/g.

Figure 2

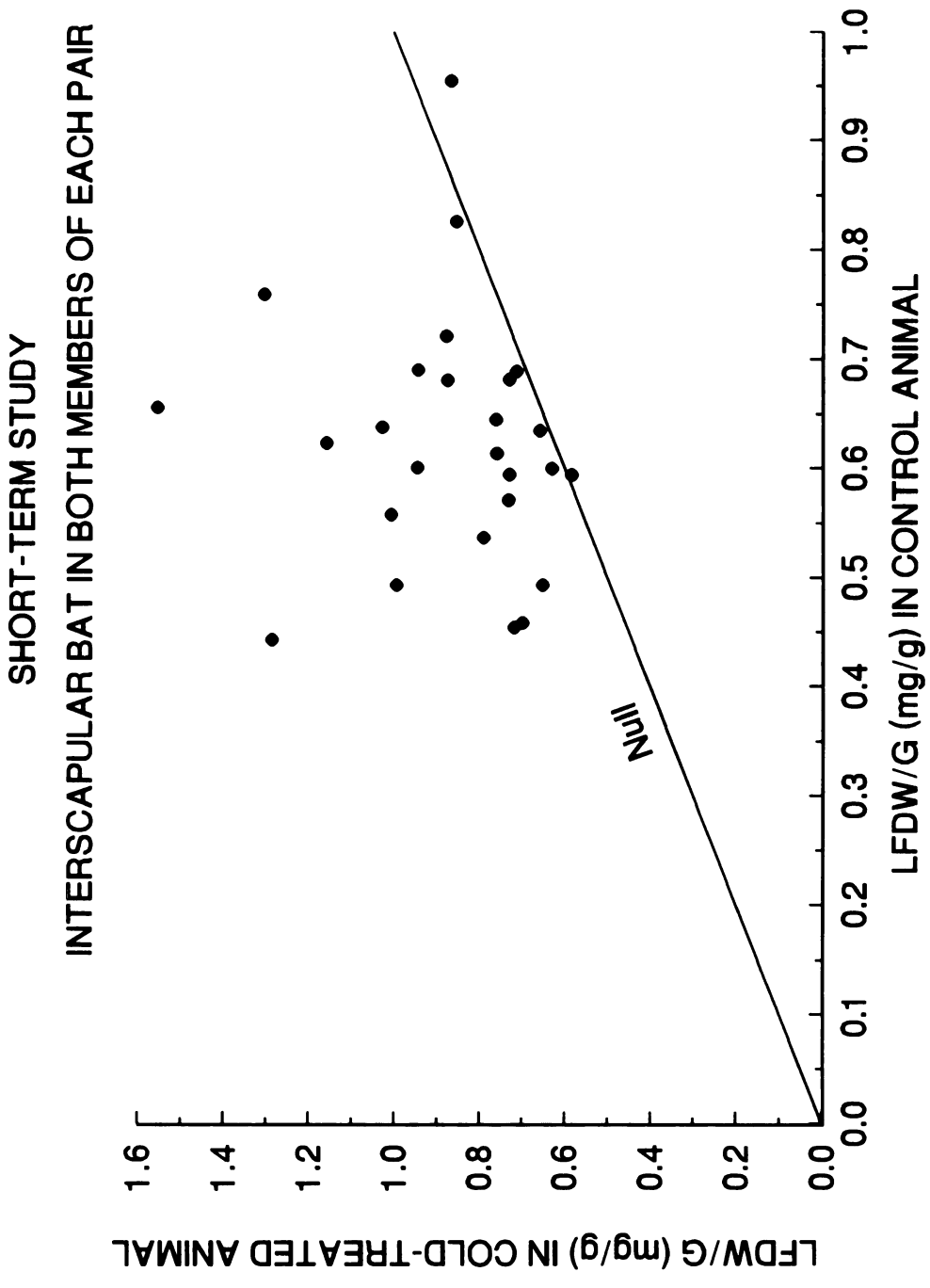

SHORT-TERM STUDY
INTERSCAPULAR BAT IN BOTH MEMBERS OF EACH PAIR
LFDW/G (mg/g) IN COLD-TREATED ANIMAL
LFDW/G (mg/g) IN CONTROL ANIMAL
Null
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0.1
0.3
0.5
0.7
0.9

Figure 3. Residual weight-specific lipid-free BAT dry weight (LFDW/g) for cold-treated and control animals of pairs in the short-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LFDW/g.

Figure 3

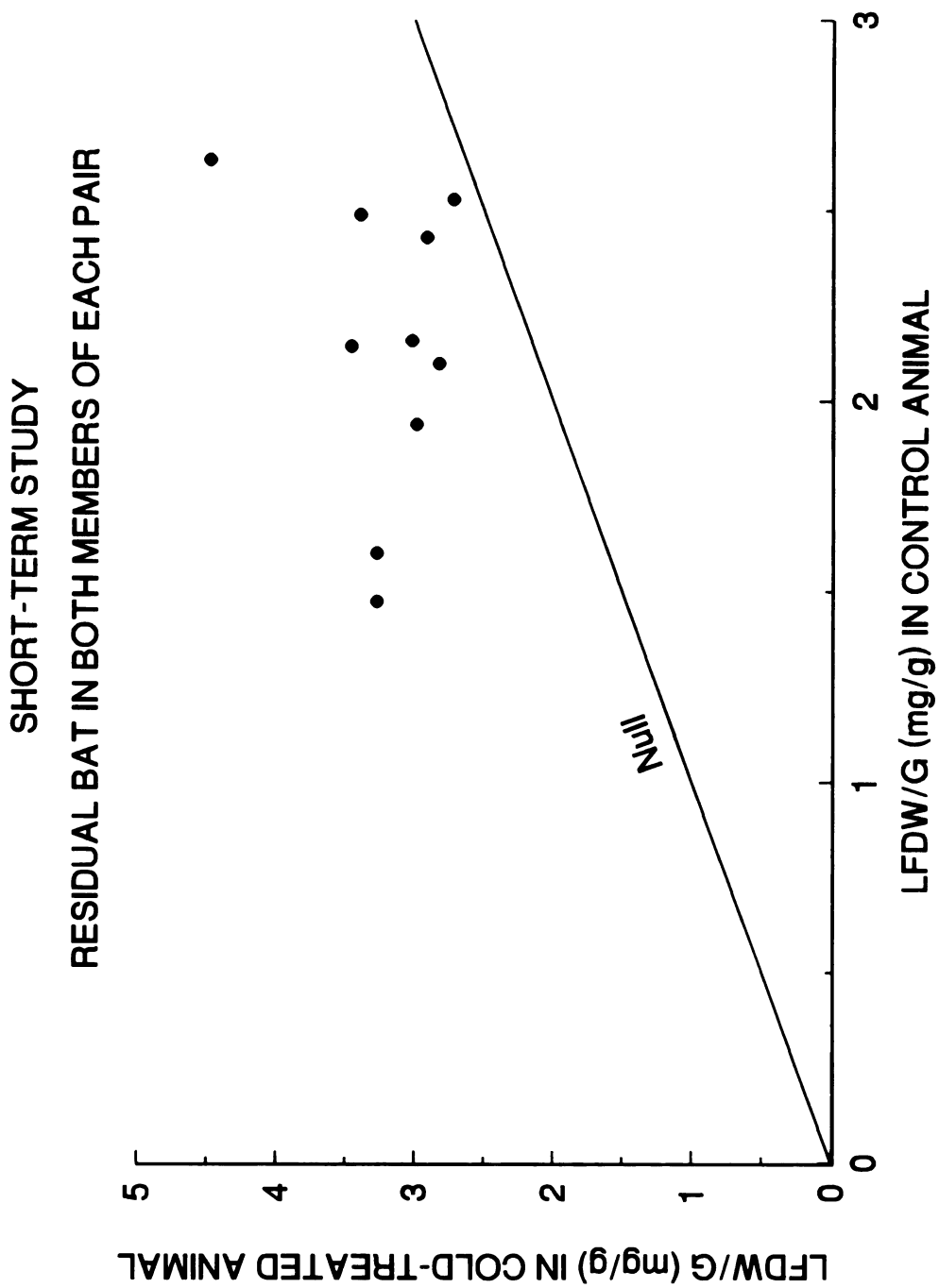

SHORT-TERM STUDY
RESIDUAL BAT IN BOTH MEMBERS OF EACH PAIR
LFDW/G (mg/g) IN COLD-TREATED ANIMAL
LFDW/G (mg/g) IN CONTROL ANIMAL
Null
0
1
2
3
4
5

Figure 4. Squamosal weight-specific lipid-free BAT dry weight (LFDW/g) for cold-treated and control animals of pairs in the short-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LFDW/g.

Figure 4

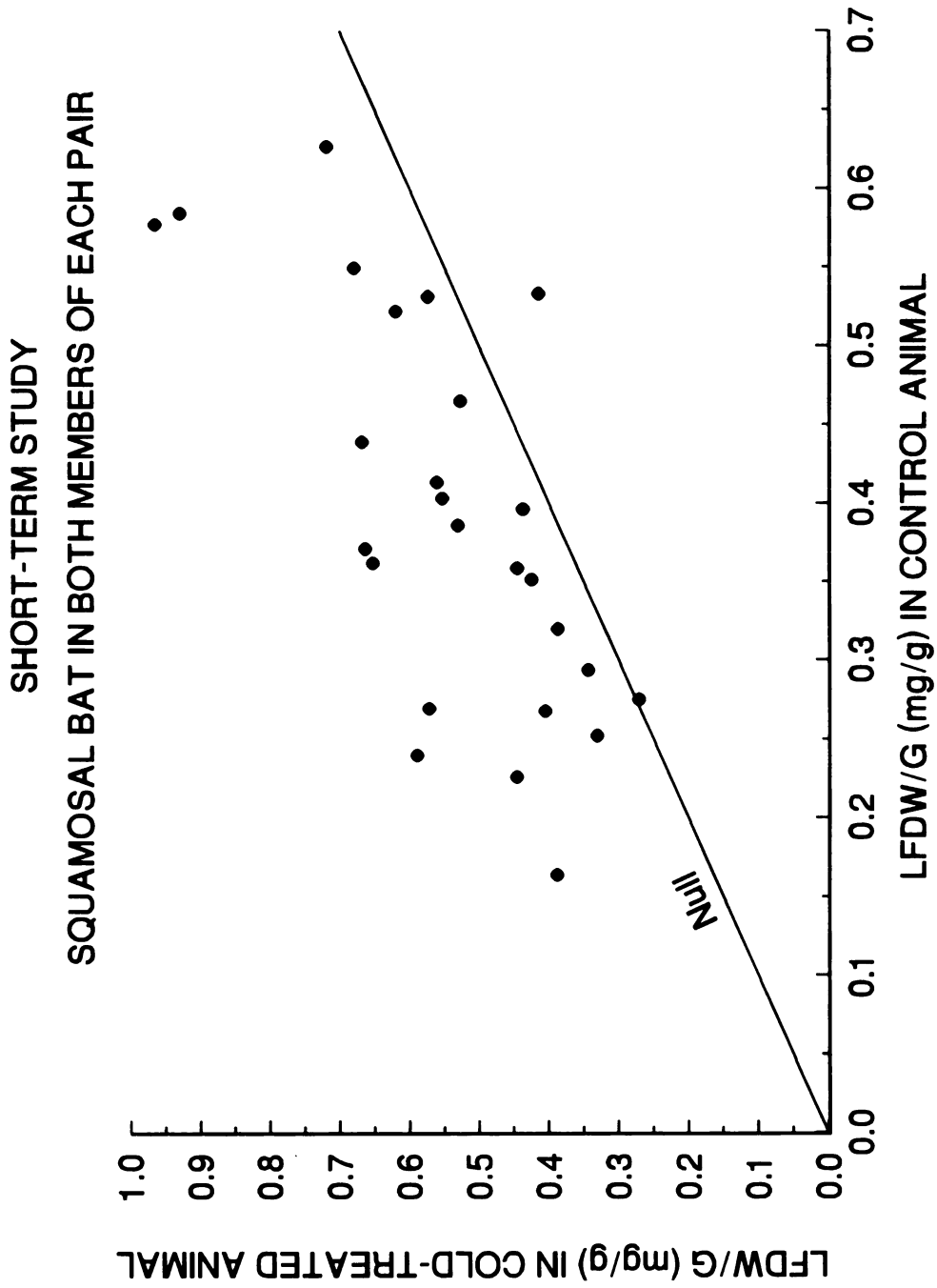

SHORT-TERM STUDY
SQUAMOSAL BAT IN BOTH MEMBERS OF EACH PAIR
LFDW/G (mg/g) IN COLD-TREATED ANIMAL
LFDW/G (mg/g) IN CONTROL ANIMAL
Null
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Figure 5. Interscapular BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated and control animals of pairs in the short-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LW/LFDW.

Figure 5

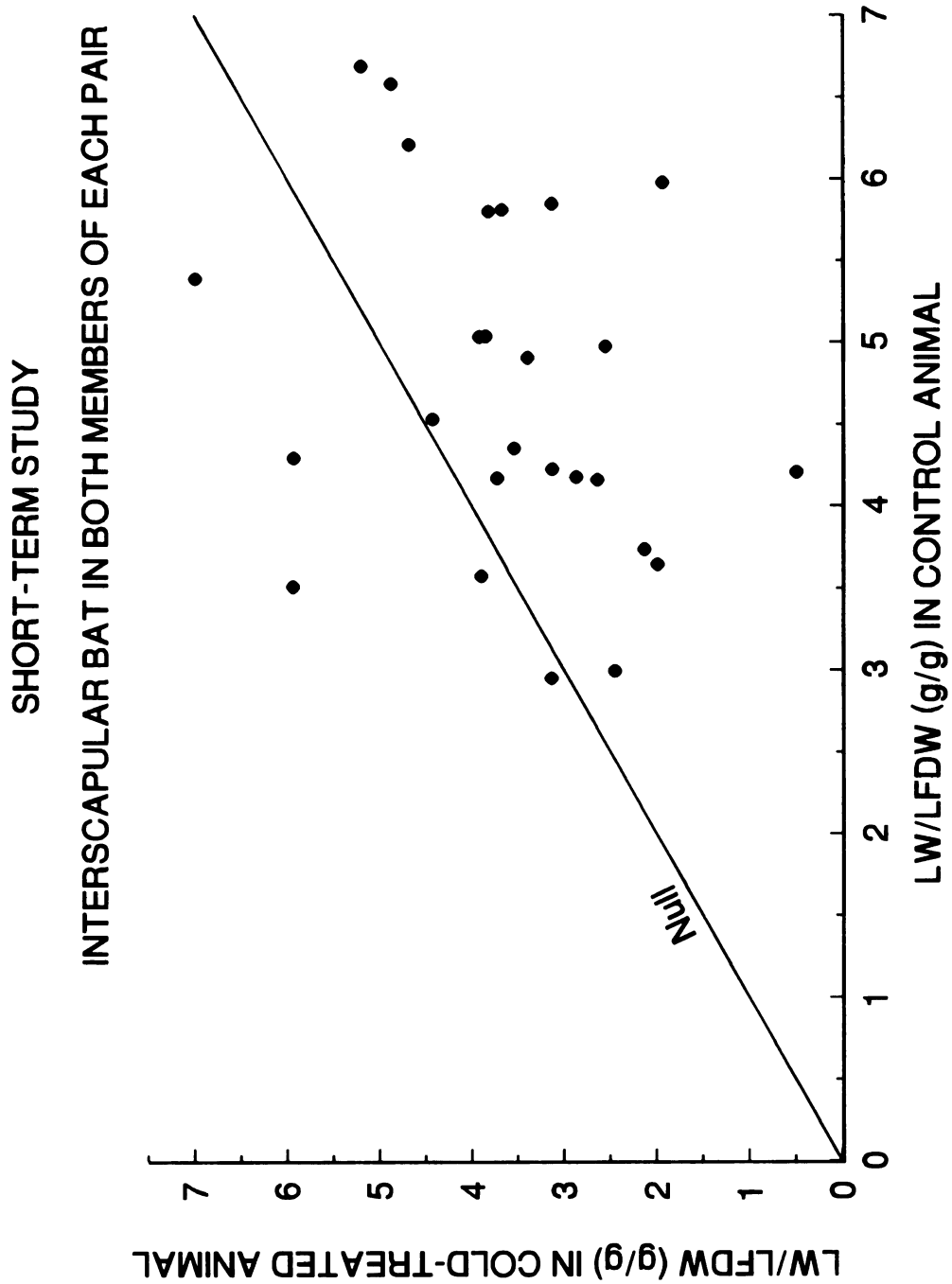

SHORT-TERM STUDY
INTERSCAPULAR BAT IN BOTH MEMBERS OF EACH PAIR
LW/LFDW (g/g) IN COLD-TREATED ANIMAL
LW/LFDW (g/g) IN CONTROL ANIMAL
Null
0
1
2
3
4
5
6
7

Figure 6. Residual BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated and control animals of pairs in the short-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LW/LFDW.

Figure 6

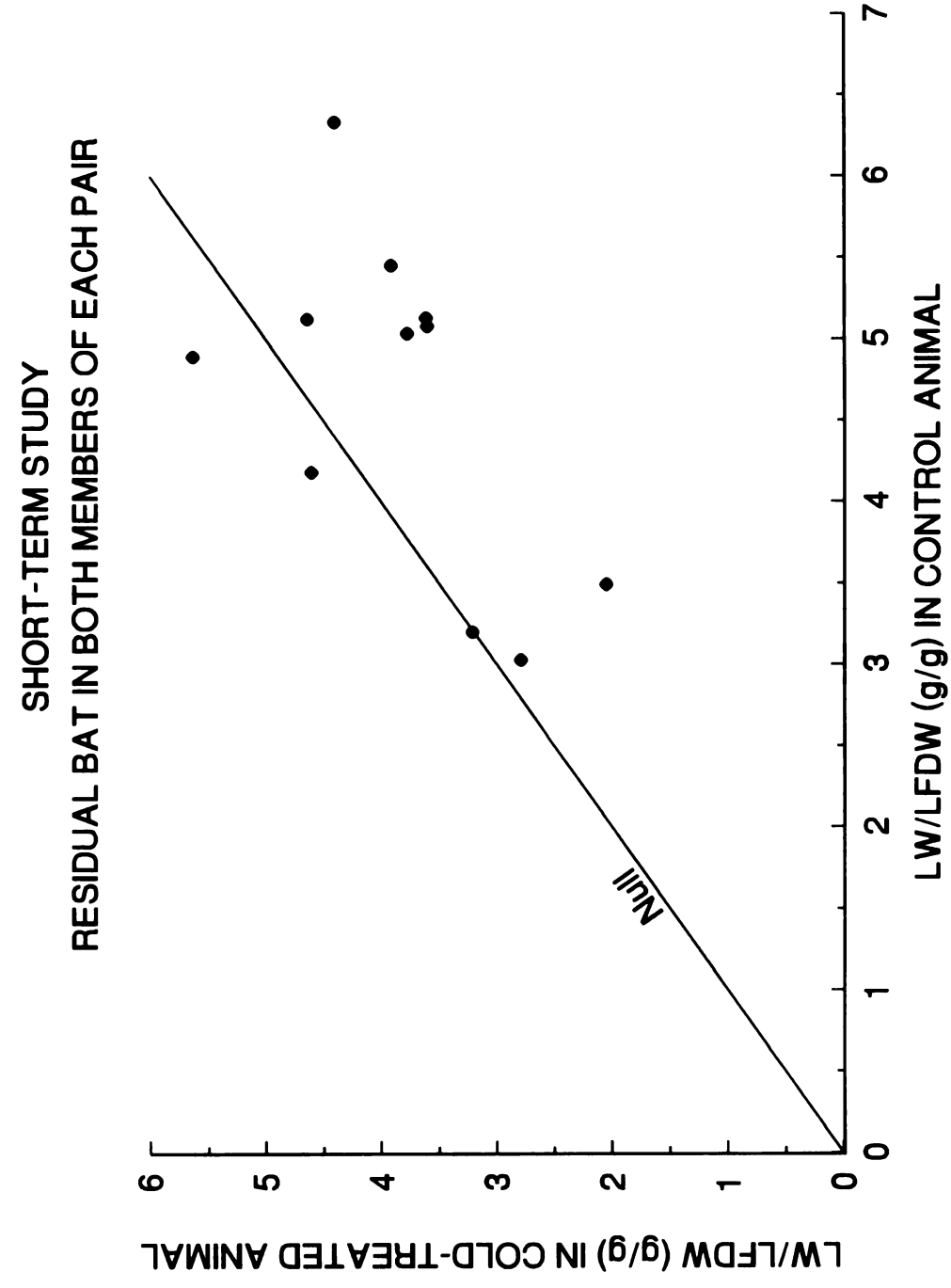
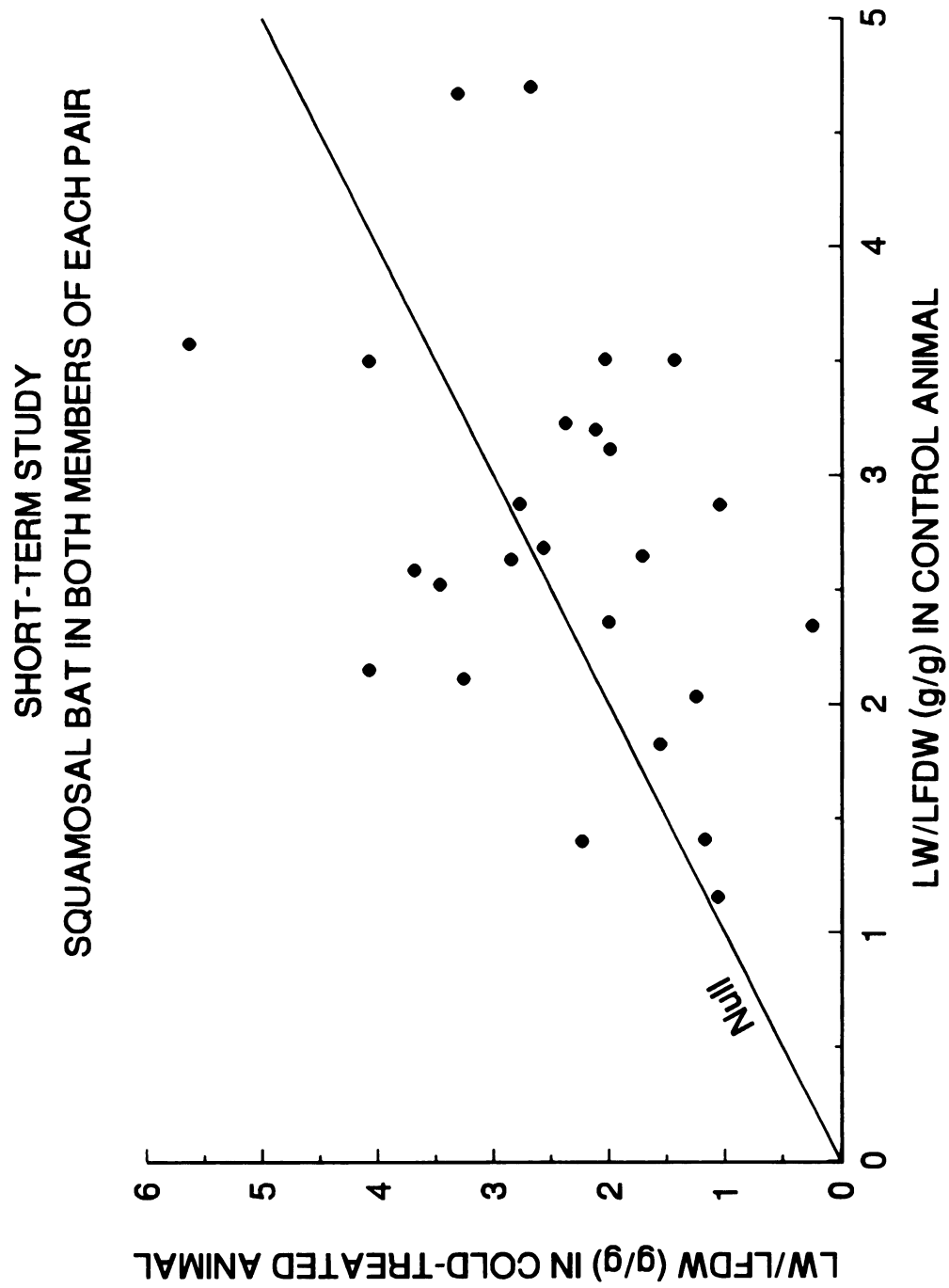

SHORT-TERM STUDY
RESIDUAL BAT IN BOTH MEMBERS OF EACH PAIR
LW/LFDW (g/g) IN COLD-TREATED ANIMAL
LW/LFDW (g/g) IN CONTROL ANIMAL
Null
0
1
2
3
4
5
6
7

Figure 7. Squamosal BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated and control animals of pairs in the short-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LW/LFDW.

Figure 7


SHORT-TERM STUDY
SQUAMOSAL BAT IN BOTH MEMBERS OF EACH PAIR
LW/LFDW (g/g) IN COLD-TREATED ANIMAL
LW/LFDW (g/g) IN CONTROL ANIMAL
0
1
2
3
4
5
6
N=11

The hypothesis that nestling lipid-free BAT dry weight is dependent on nestling body weight was generally not supported. Interscapular BAT LFDW in control nestlings showed a significant relation to BW (r^2=0.15, p<0.05), as did residual BAT LFDW in cold-treated nestlings (r^2=0.52, p=0.02). Otherwise, LFDW showed no significant relation to BW. As Figures 8-10 illustrate, there was a tendency for lower BW to result in lower LFDW in controls for all fat types. This tendency was eliminated, however, in interscapular and squamosal BAT in cold-exposed nestlings, where relatively high LFDW weights were observed in small animals as compared to the regression line of controls extrapolated into the low BW range. The lower BW range was occupied only by cold-treated nestlings, resulting in a wider BW range for cold-treated animals (cold-treated: 11.82 to 24.07 g, versus controls: 16.44 to 24.01 g).

The hypothesis that lipid weight per lipid-free BAT weight varies as a function of body weight was supported for cold-treated but not control nestlings. As shown in Figures 11-13, in all three deposits under cold-exposure conditions, a similar positive relationship existed between LW/LFDW and BW (interscapular BAT: r^2=0.47, p<0.001; residual BAT: r^2=0.67, p<0.01; squamosal BAT r^2=0.45, p=0.01). There was not a significant dependence of LW/LFDW on BW in control individuals, however. The data

Figure 8. Short-term study: Interscapular BAT lipid-free dry weight (LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 8

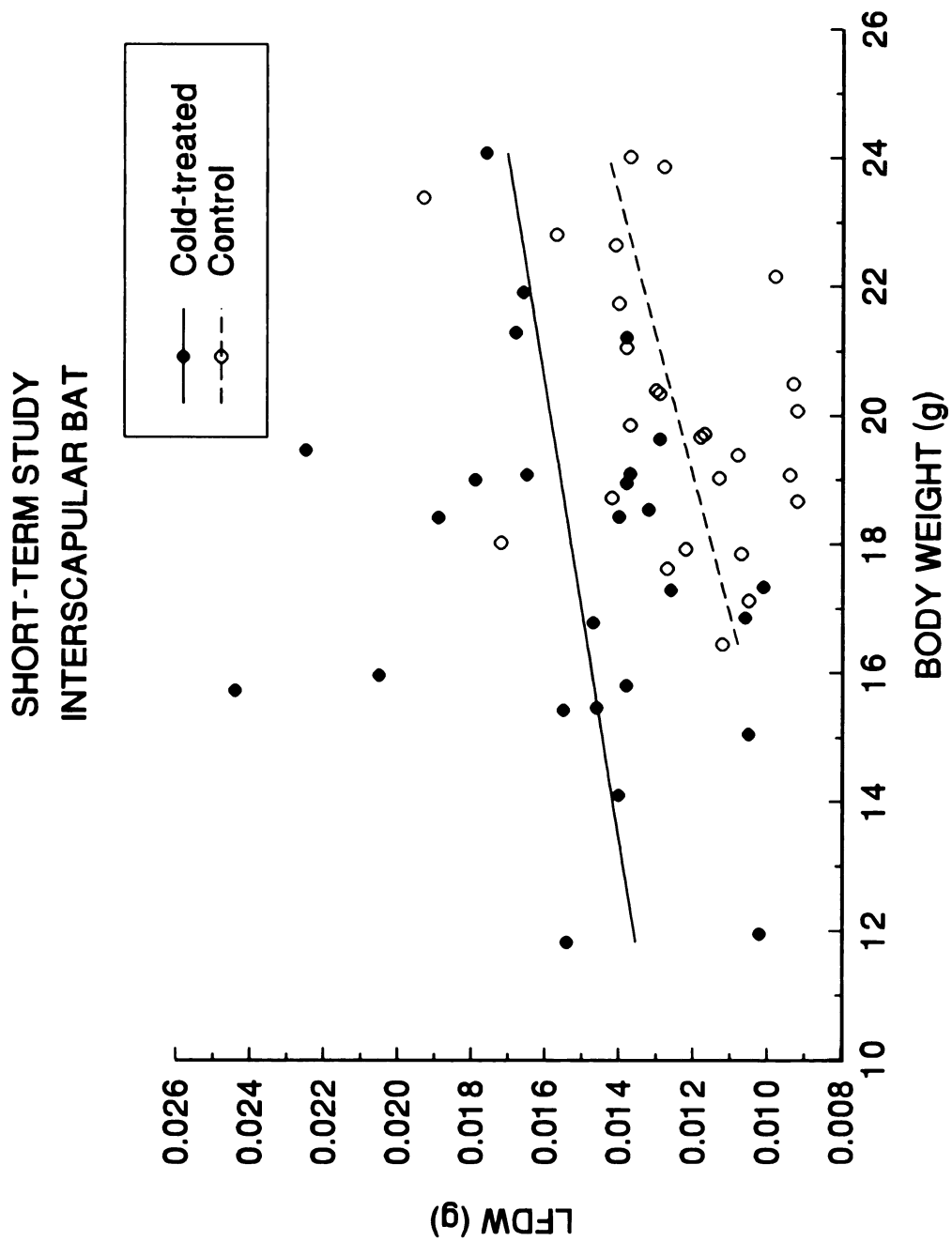

SHORT-TERM STUDY
INTERSCAPULAR BAT
Cold-treated
Control
LFDW (g)
0.026
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
0.008
10
12
14
16
18
20
22
24
26
BODY WEIGHT (g)

Figure 9. Short-term study: Residual BAT lipid-free dry weight (LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 9

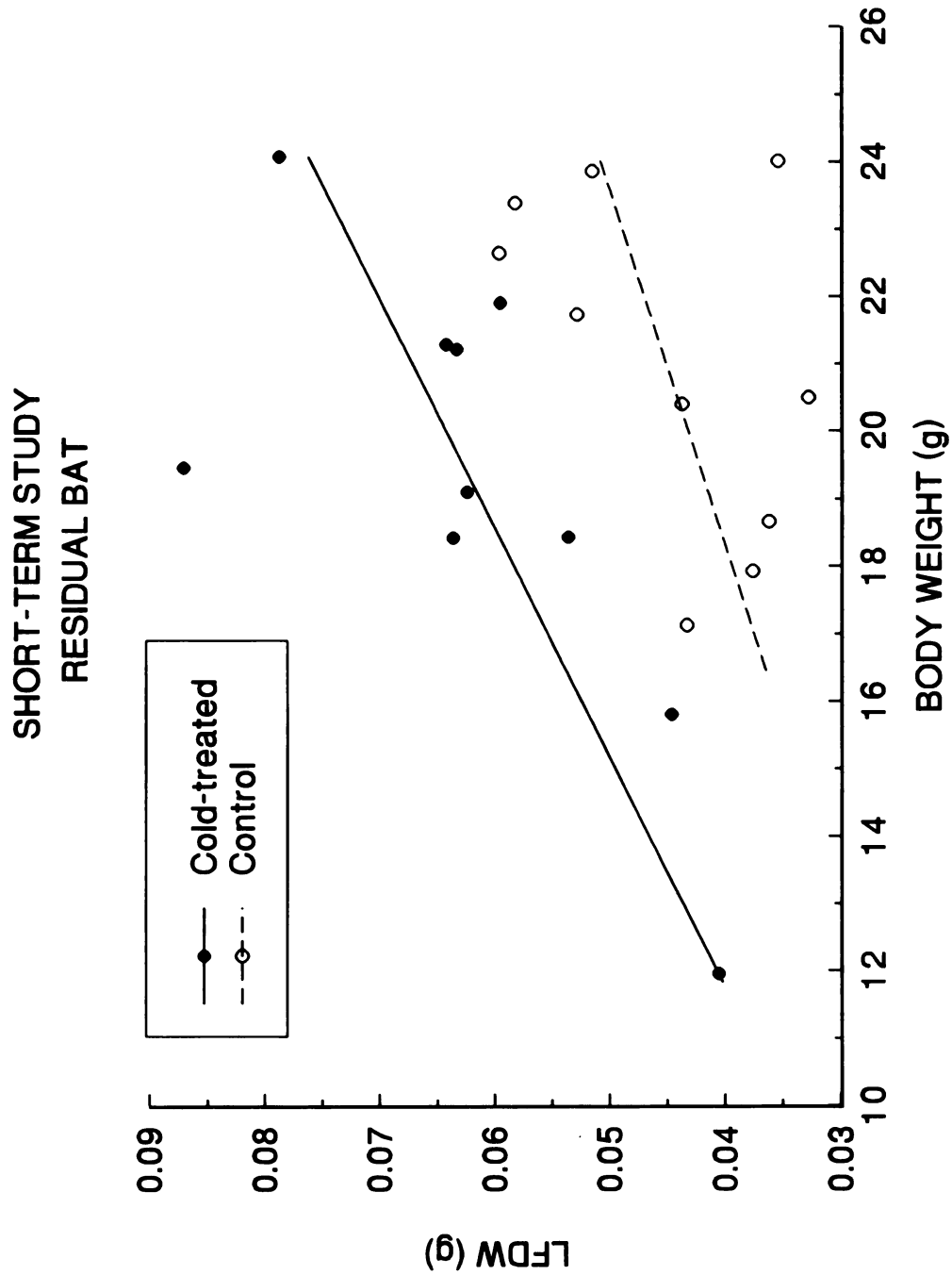

SHORT-TERM STUDY
RESIDUAL BAT
Cold-treated
Control
LFDW (g)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
10
12
14
16
18
20
22
24
26
BODY WEIGHT (g)

Figure 10. Short-term study: Squamosal BAT lipid-free dry weight (LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 10

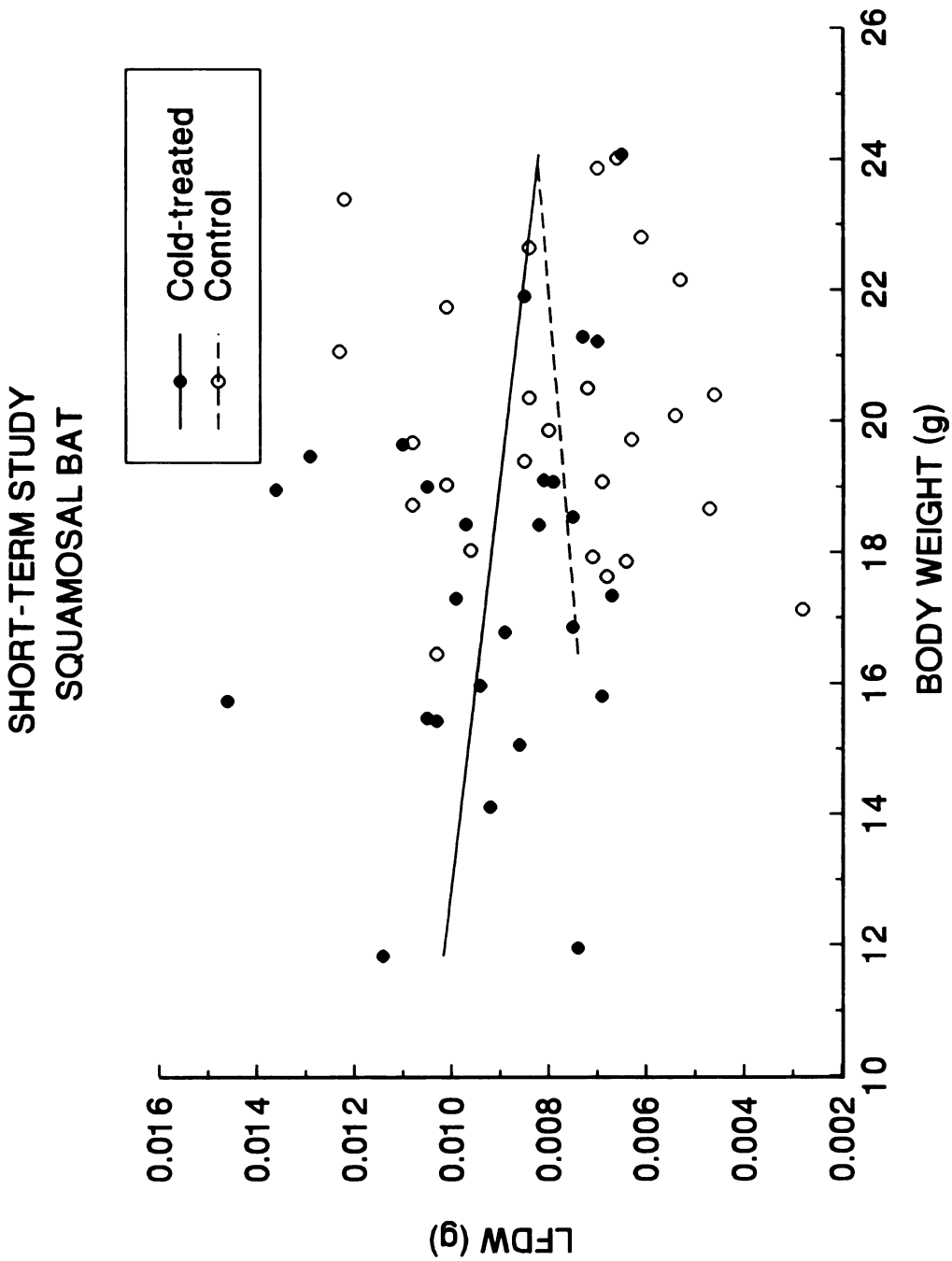

SHORT-TERM STUDY
SQUAMOSAL BAT
Cold-treated
Control
LFDW (g)
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
BODY WEIGHT (g)
10
12
14
16
18
20
22
24
26

Figure 11. Short-term study: Interscapular BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 11

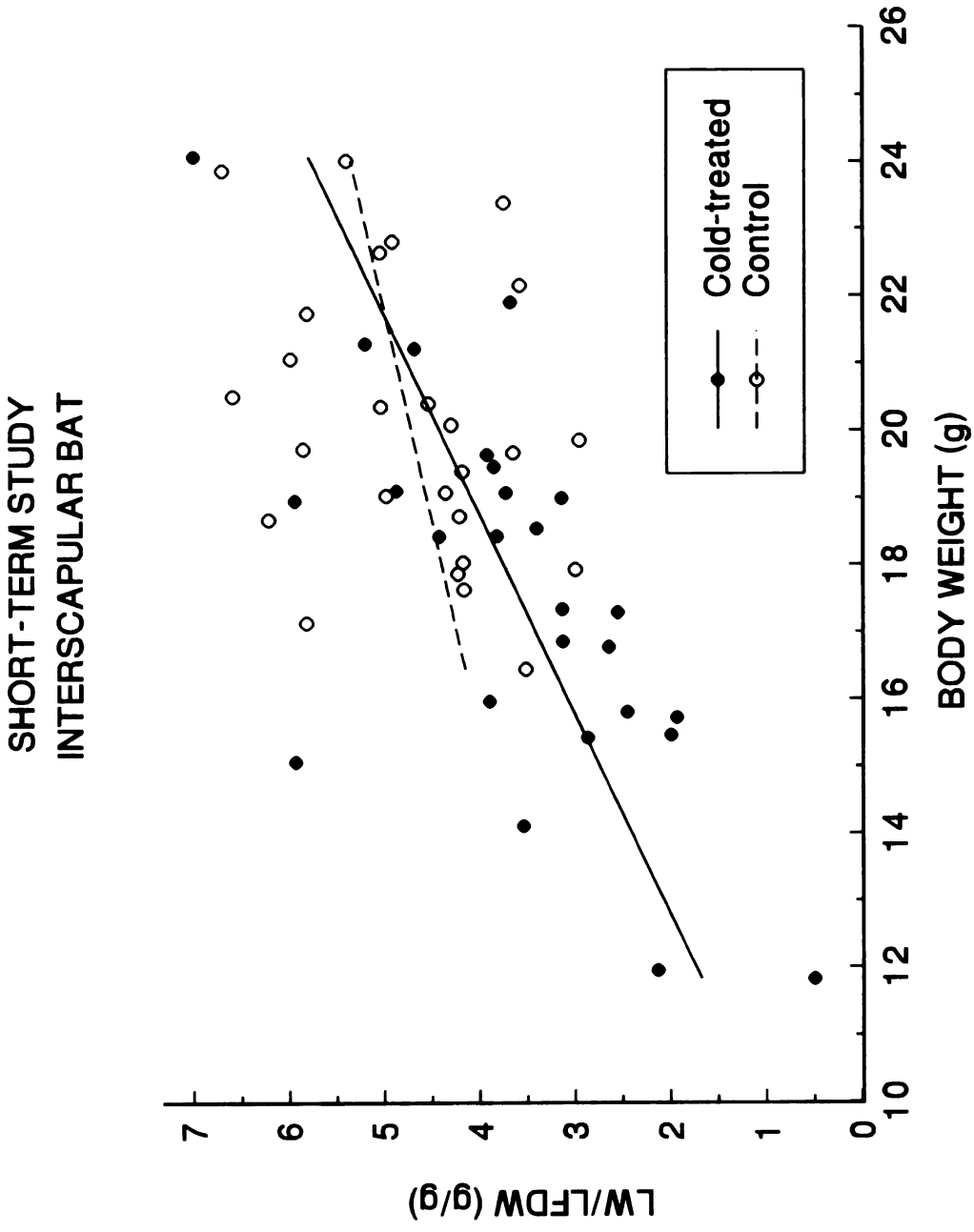

SHORT-TERM STUDY
INTERSCAPULAR BAT
Cold-treated
Control
LW/LFDW (g/g)
BODY WEIGHT (g)
0
1
2
3
4
5
6
7
10
12
14
16
18
20
22
24
26

Figure 12. Short-term study: Residual BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 12

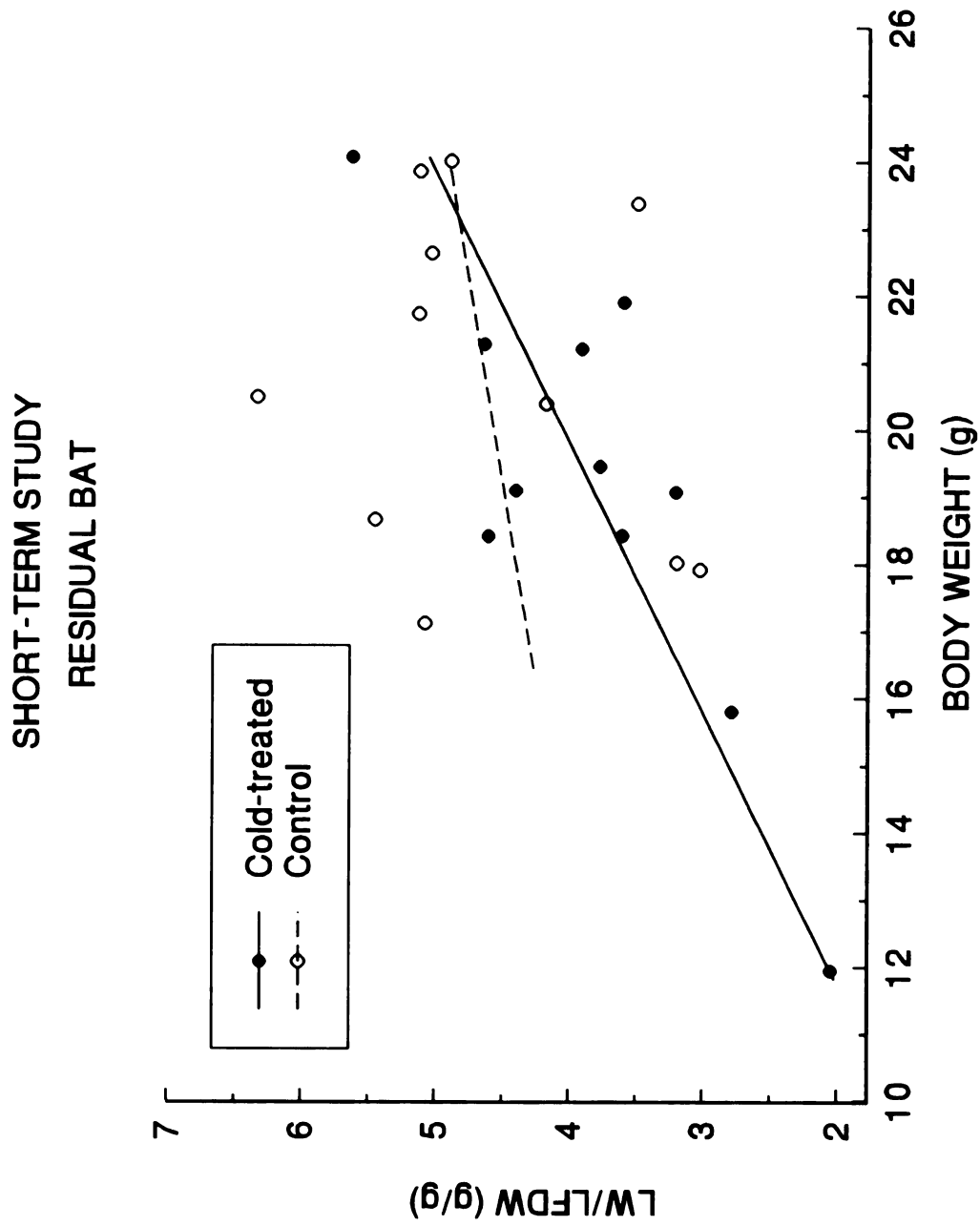
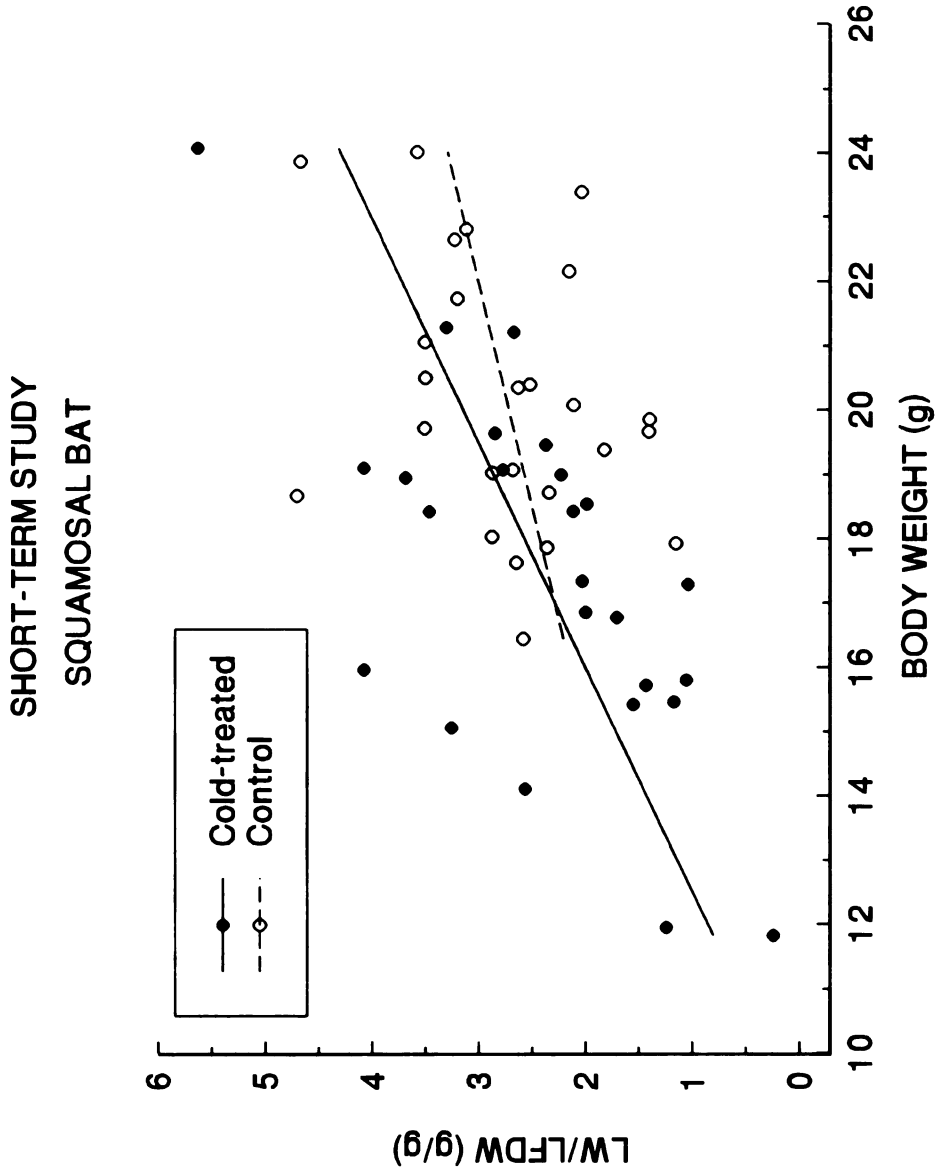

SHORT-TERM STUDY
RESIDUAL BAT
Cold-treated
Control
LW/LFDW (g/g)
BODY WEIGHT (g)
2
3
4
5
6
7
10
12
14
16
18
20
22
24
26

Figure 13. Short-term study: Squamosal BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 13


SHORT-TERM STUDY
SQUAMOSAL BAT
Cold-treated
Control
LW/LFDW (g/g)
BODY WEIGHT (g)
0
1
2
3
4
5
6
10
12
14
16
18
20
22
24
26

for control nestlings encompassed only the upper portion of the BW range of cold-treated nestlings. Figures 11-13 show much overlap between cold-treated and control nestling LW/LFDW for all three BAT deposits within the BW range of controls.

The hypothesis that BAT lipid weight is a function of lipid-free BAT dry weight was supported in most deposits, regardless of postnatal treatment. As can be seen in Figures 14-16, LW was dependent upon LFDW in all deposits except squamosal BAT from cold-treated nestlings (cold-treated interscapular BAT: $r^2=0.22$, $p=0.02$; cold-treated residual BAT: $r^2=0.77$, $p<0.01$; control interscapular BAT: $r^2=0.34$, $p<0.01$; control residual BAT: $r^2=0.44$, $p=0.03$; control squamosal BAT: $r^2=0.31$, $p<0.01$).

A POSTERIORI COMPARISONS:

Several variables were examined together in the absence of preformed hypotheses to determine whether propositions could be posed after studying their relationships. Paired comparisons were performed on body weights at ages 2, 10-11, and 19-20 d between cold-treated and control nestlings in each pair. The effects of sex on BW, LFDW/g, and LW/LFDW were examined by one-way ANOVA on unpaired data. The relationship between litter size and these variables was determined by linear regression

Figure 14. Short-term study: Interscapular BAT lipid weight (LW) for cold-treated (solid circles) and control animals (open circles) plotted against lipid-free dry weight (LFDW).

Figure 14

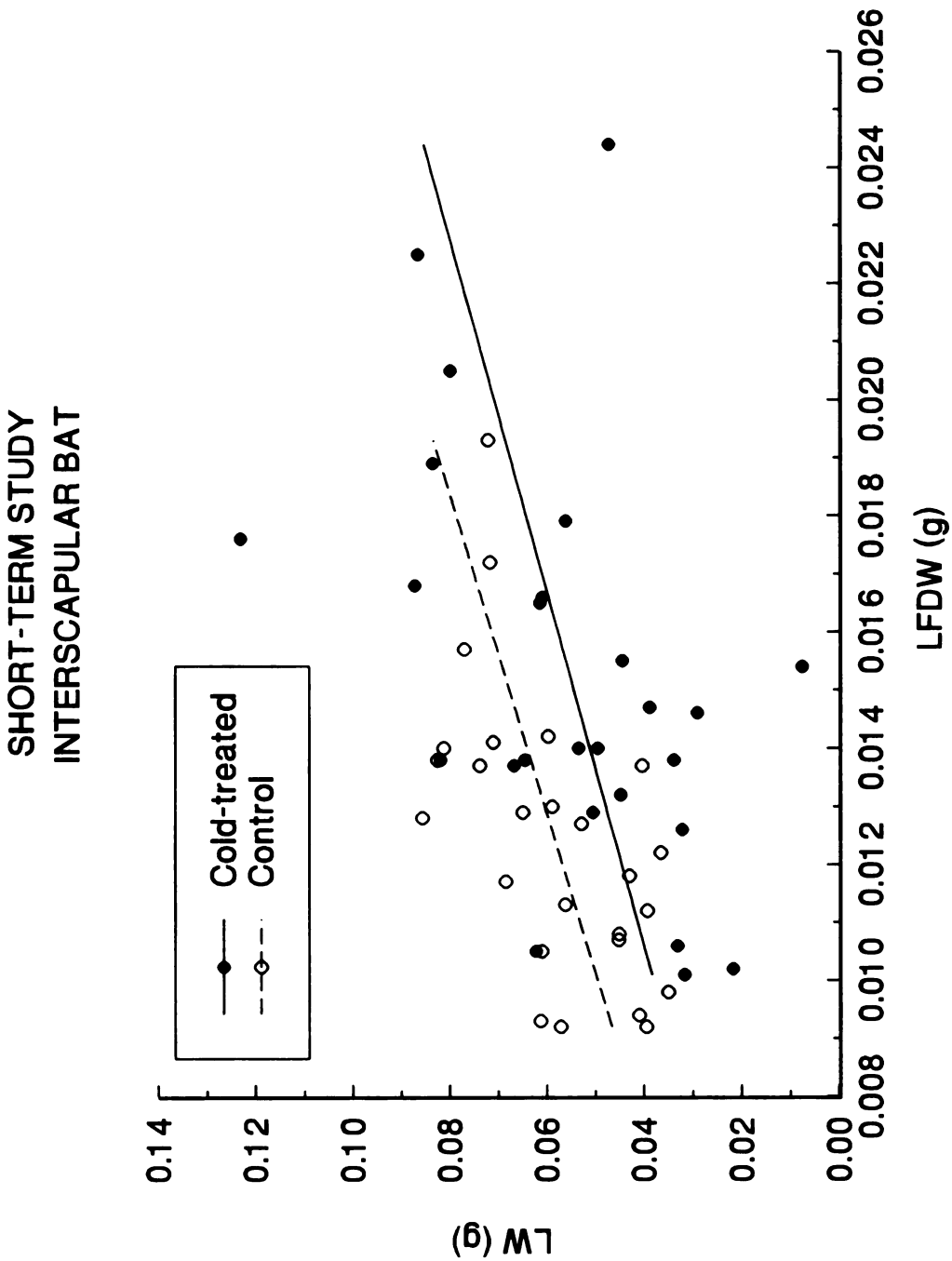

SHORT-TERM STUDY
INTERSCAPULAR BAT
Cold-treated
Control
LW (g)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.008
0.010
0.012
0.014
0.016
0.018
0.020
0.022
0.024
0.026
LFDW (g)

Figure 15. Short-term study: Residual BAT lipid weight (LW) for cold-treated (solid circles) and control animals (open circles) plotted against lipid-free dry weight (LFDW).

Figure 15

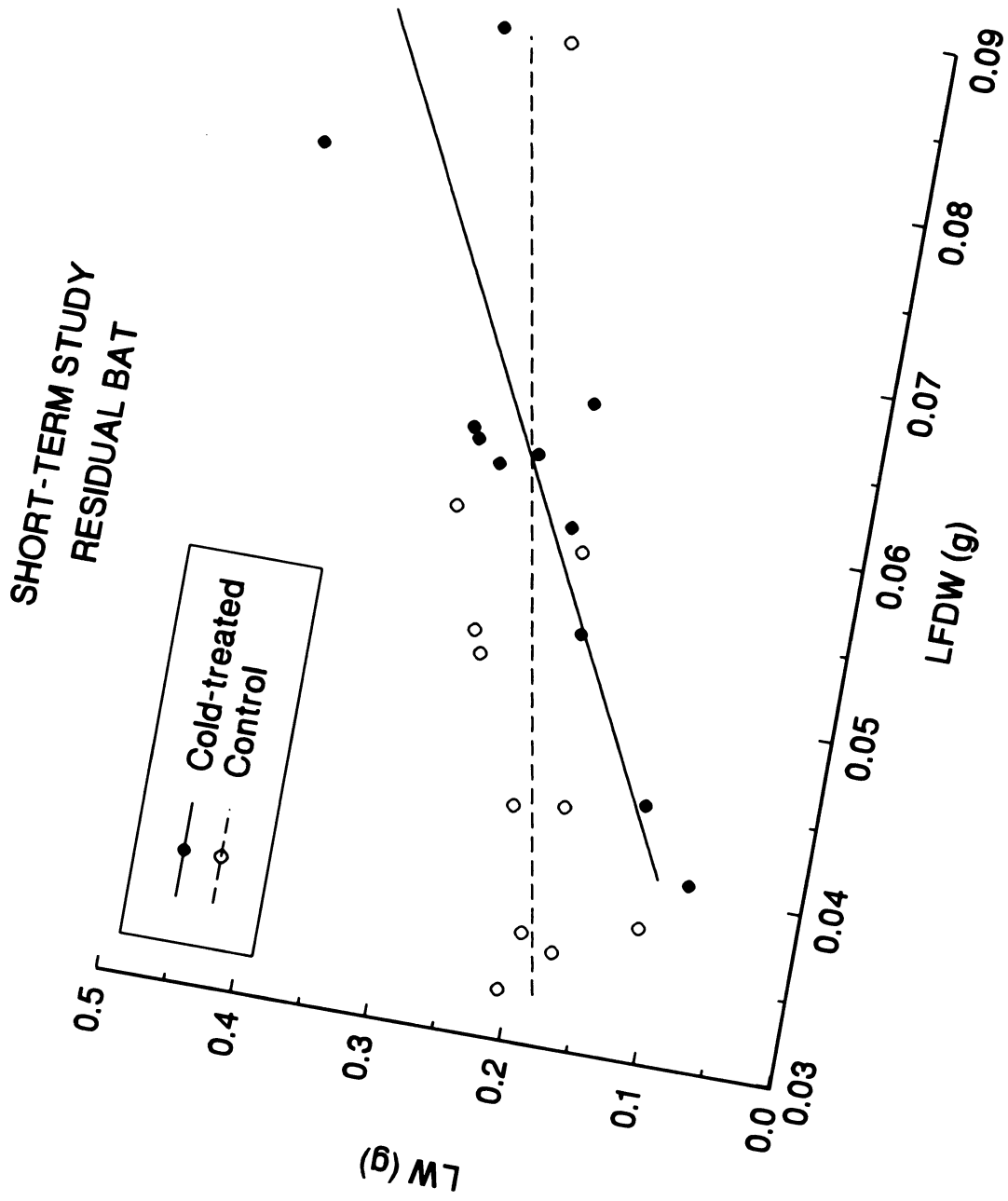
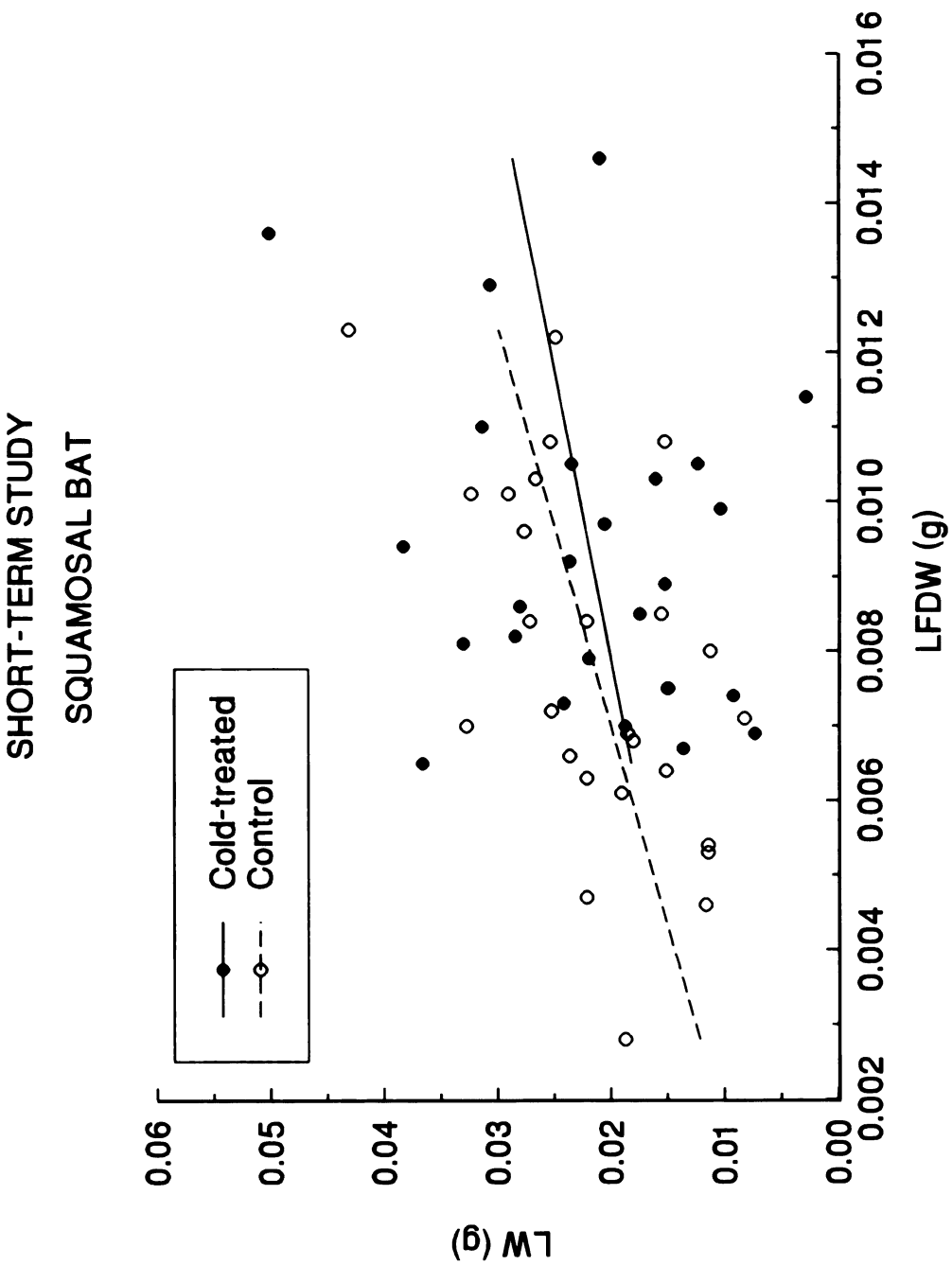

SHORT-TERM STUDY
RESIDUAL BAT
Cold-treated
Control
LW (g)
0.0
0.1
0.2
0.3
0.4
0.5
LFDW (g)
0.03
0.04
0.05
0.06
0.07
0.08
0.09

Figure 16. Short-term study: Squamosal BAT lipid weight (LW) for cold-treated (solid circles) and control animals (open circles) plotted against lipid-free dry weight (LFDW).

Figure 16


SHORT-TERM STUDY
SQUAMOSAL BAT
Cold-treated
Control
LW (g)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.002
0.004
0.006
0.008
0.010
0.012
0.014
0.016
LFDW (g)

analysis on unpaired data, with litter size treated as the independent variable.

Cold exposure resulted in lower nestling growth rates. Growth rate was faster for control than for cold-treated members of sibling pairs. Pairing accounted for most of the early variation in BW (ages 2 and 10-11 d), but the effects of cold-exposure increased over time, such that control nestlings weighed significantly more than cold-treated littermates by 19-20 d (Figure 17).

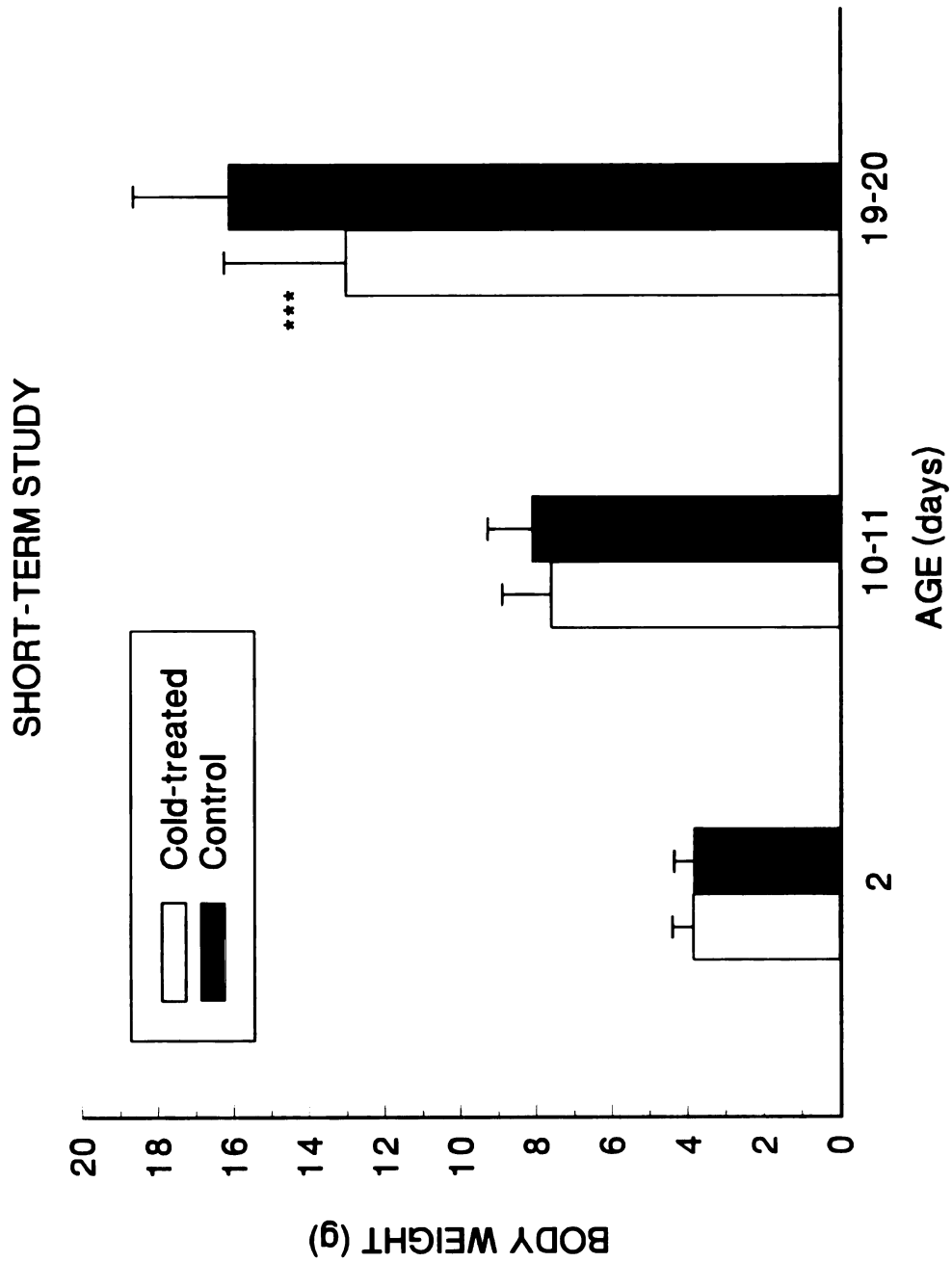
Nestling body weight at 21 days was not affected by sex. Due to heteroscedasticity, a Mann-Whitney U nonparametric test was used in lieu of a one-way ANOVA. Nestling BW was not affected by sex.

Nestling body weight at 21 days was not a function of litter size. BW was found not to vary significantly as a function of litter size.

Variance in nestling weight-specific lipid-free BAT dry weight was not explained by litter size. Variation in LFDW/g for all fat and treatment types was not dependent on litter size.

Figure 17. Short-term study: Body weight at three ages in cold-treated (white bars) and control animals (black bars). *** indicates significant difference in body weight at $p < 0.001$.

Figure 17


SHORT-TERM STUDY
Cold-treated
Control
***
BODY WEIGHT (g)
0
2
4
6
8
10
12
14
16
18
20
2
10-11
19-20
AGE (days)

Sex had no effect on nestling weight-specific lipid-free BAT dry weight. For all deposit types, LFDW/g was not significantly affected by sex.

Lipid weight per lipid-free BAT weight in nestlings did not vary as a function of litter size. Litter size did not explain a significant portion of variance in LW/LFDW.

Nestling lipid weight per lipid-free BAT weight was not affected by sex. LW/LFDW values were not significantly affected by sex.

II. LONG-TERM STUDY

A PRIORI COMPARISONS:

The following results respond to the a priori hypotheses presented in the OBJECTIVES section.

The hypothesis that postnatal cold exposure increases the weight-specific lipid-free BAT dry weight in adults was not supported. LFDW/g data were log-transformed prior to analysis. By 20 weeks of age, no detectable differences existed among the 46 pairs of siblings in postnatal-cold-treated and control groups in LFDW/g (Table 2). As can be seen in Figures 18 and 19, in the adults,

the numbers of pairs exhibiting a higher LFDW/g value in the postnatal-cold-treated individual (interscapular BAT: 25 of 46, about 54%; squamosal BAT: 24 of 46, about 52%) approximately equalled the numbers of pairs in which LFDW/g was higher in the control individual. Pairing accounted for a significant component of variation in squamosal BAT LFDW/g at 20 weeks (Table 2).

The hypothesis that adults exposed to cold during the postnatal period have lipid weight per lipid-free BAT dry weight values equal to individuals in the postnatal control group was supported. No significant differences were detected between treatment groups for LW/LFDW for either BAT deposit type (Table 2). Figures 20 and 21 demonstrate that the numbers of pairs exhibiting higher values for LW/LFDW for either treatment were approximately equal; for both interscapular and squamosal BAT, 22 (about 48%) of 46 pairs had higher adult LW/LFDW for cold-treated nestlings. LW/LFDW pairing effects were significant for both deposit types (Table 2).

The hypothesis that adult lipid-free BAT dry weight is dependent on adult body weight was supported, regardless of treatment. For both interscapular (cold-treated: $r^2=0.40$, $p<0.01$; control: $r^2=0.38$, $p<0.01$) and squamosal BAT (cold-treated: $r^2=0.37$, $p<0.01$; control: $r^2=0.18$, $p<0.01$), LFDW depended significantly on BW. Data

Table 2. Results from the long-term study. N is the number of pairs of animals (cold-treated and control) analyzed. Standard deviation (SD) is given in parentheses. The probability for treatment ("trtment") is the likelihood of no difference between cold-treated and control animals. That for pairs is the likelihood that none of the overall variance was attributable to differences among pairs.
LFDW/g = weight-specific lipid-free BAT dry weight.
LW/LFDW = lipid weight per lipid-free BAT dry weight.

		Mean (SD)		Probability	
Variable	N	Cold-treated	Control	Trtment	Pairs
LFDW/g (mg/g)					
Interscapular	46	0.36 (0.11)	0.35 (0.09)	0.707	0.19
Squamosal	46	0.17 (0.04)	0.17 (0.05)	0.506	<0.01
LW/LFDW (g/g)					
Interscapular	46	11.67 (4.14)	12.02 (3.49)	0.605	0.02
Squamosal	46	5.37 (2.12)	5.19 (1.90)	0.595	<0.01

Figure 18. Interscapular weight-specific lipid-free BAT dry weight (LFDW/g) for cold-treated and control animals of pairs in the long-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LFDW/g.

Figure 18

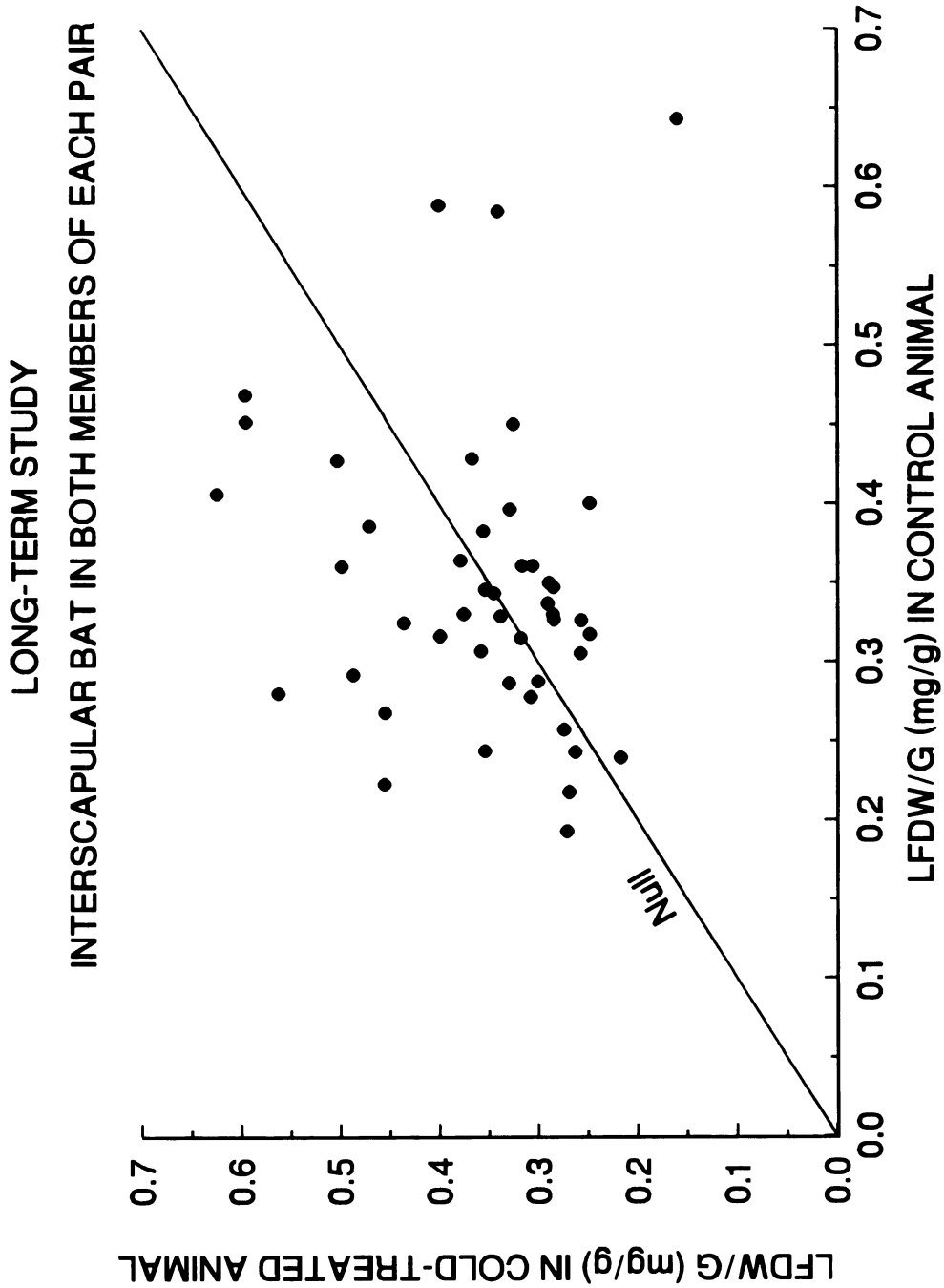

LONG-TERM STUDY
INTERSCAPULAR BAT IN BOTH MEMBERS OF EACH PAIR
LFDW/G (mg/g) IN COLD-TREATED ANIMAL
LFDW/G (mg/g) IN CONTROL ANIMAL
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
N=111

Figure 19. Squamosal weight-specific lipid-free BAT dry weight (LFDW/g) for cold-treated and control animals of pairs in the long-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LFDW/g.

Figure 19

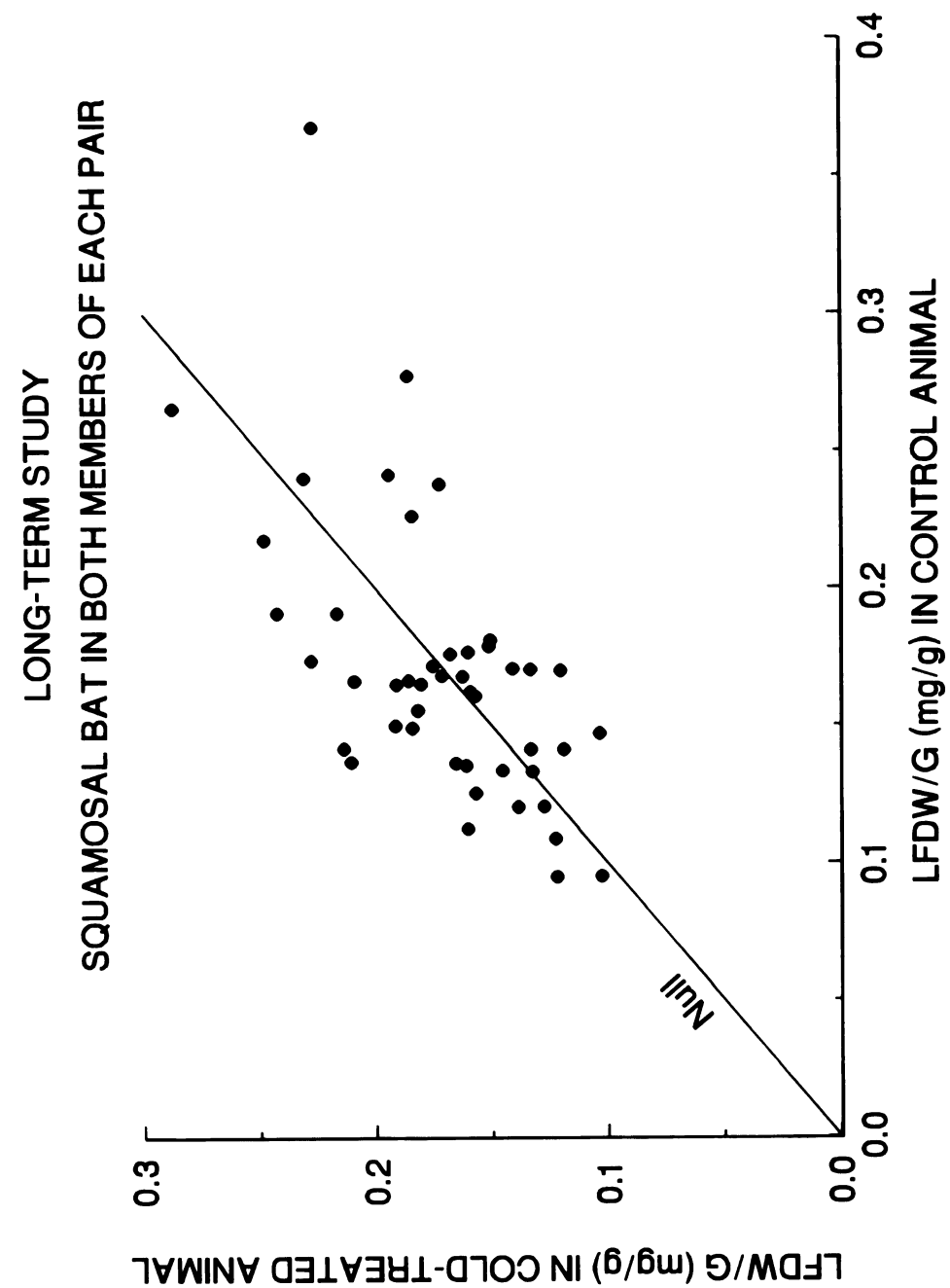

LONG-TERM STUDY
SQUAMOSAL BAT IN BOTH MEMBERS OF EACH PAIR
LFDW/G (mg/g) IN COLD-TREATED ANIMAL
LFDW/G (mg/g) IN CONTROL ANIMAL
0.0
0.1
0.2
0.3
0.4
Null

Figure 20. Interscapular BAT lipid weight per lipid-free BAT dry weight (LW/LFDW) for cold-treated and control animals of pairs in the long-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LW/LFDW.

Figure 20

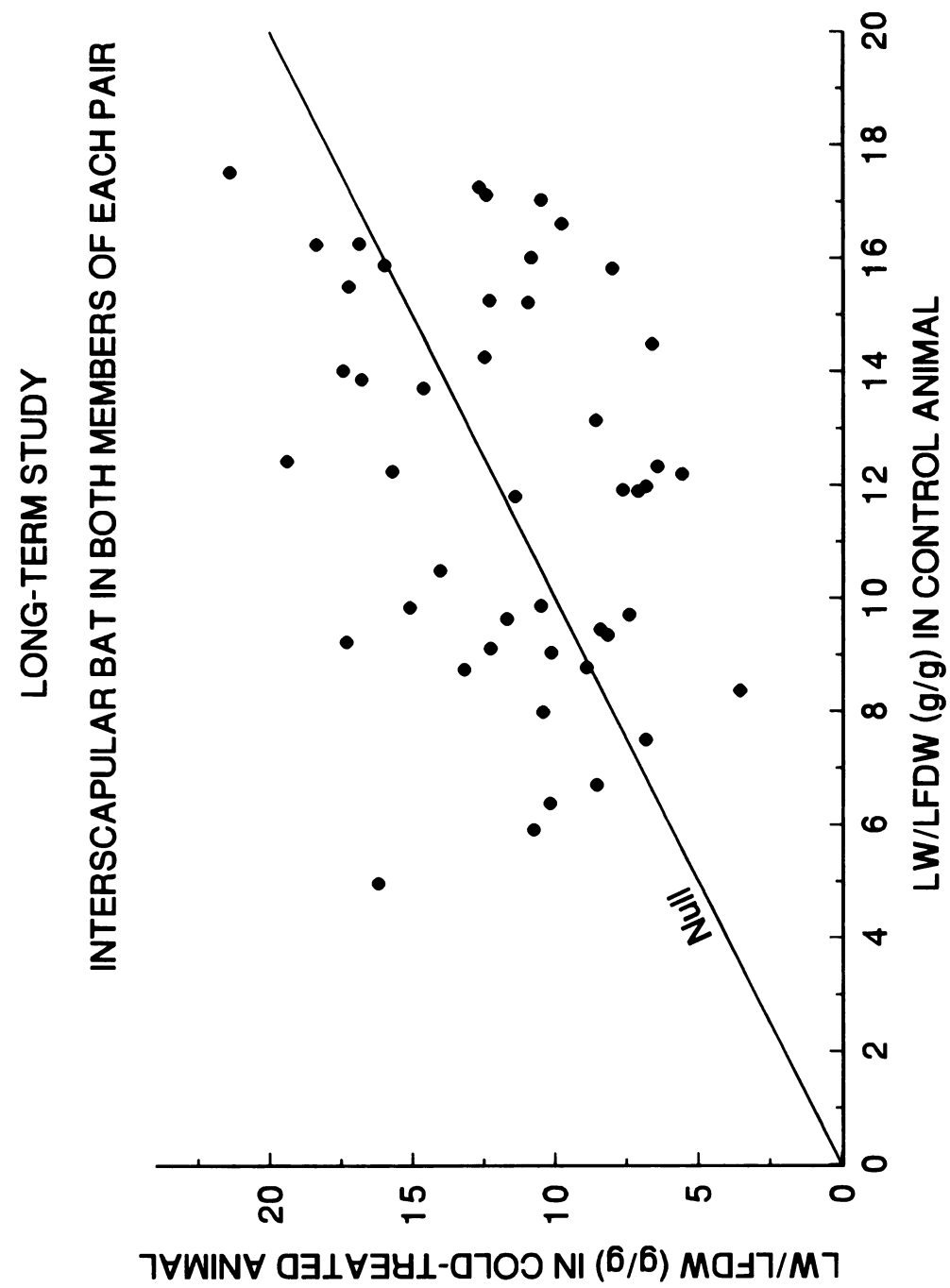
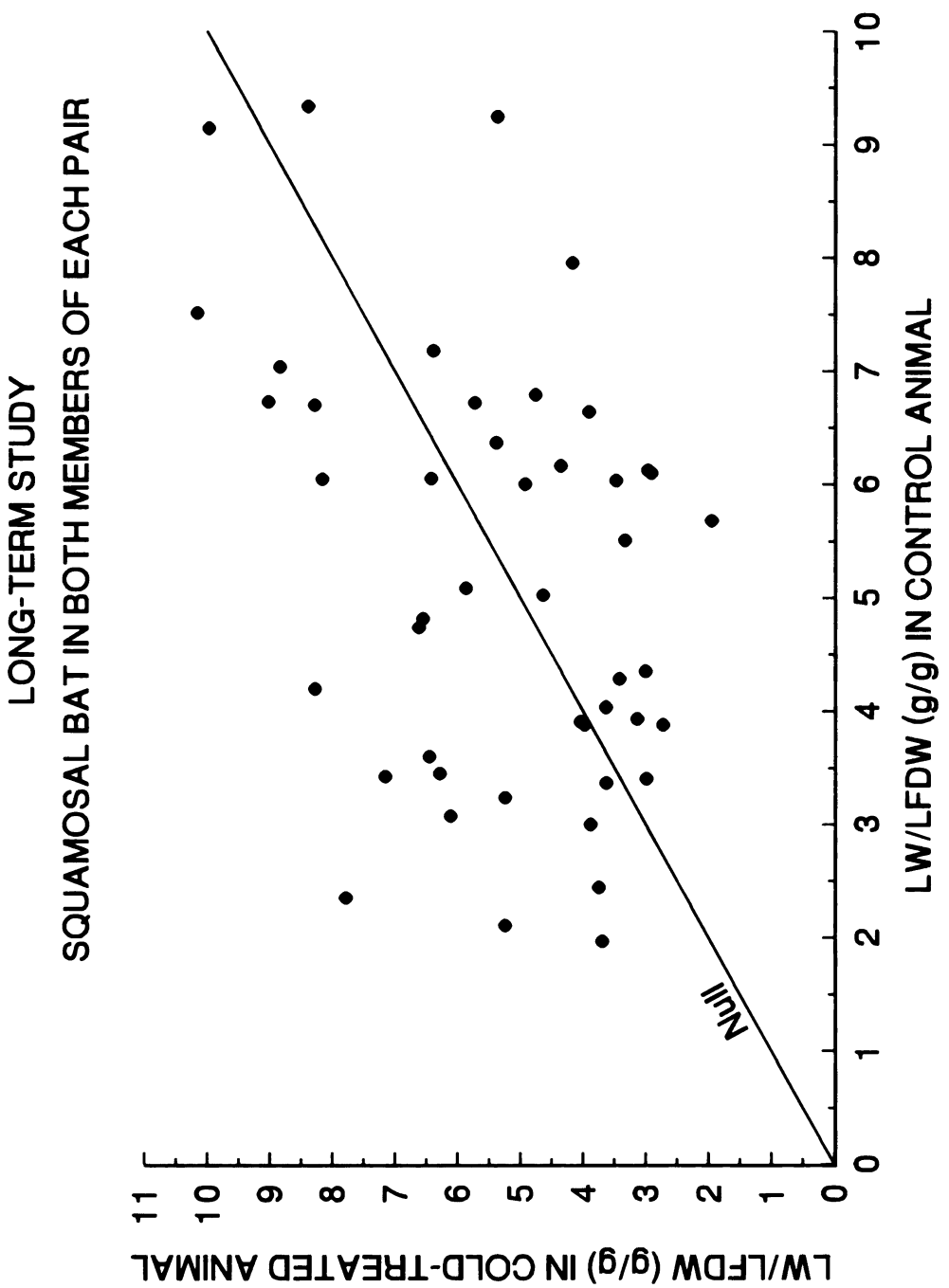

LONG-TERM STUDY
INTERSCAPULAR BAT IN BOTH MEMBERS OF EACH PAIR
LW/LFDW (g/g) IN COLD-TREATED ANIMAL
LW/LFDW (g/g) IN CONTROL ANIMAL
Null
0
5
10
15
20
0
2
4
6
8
10
12
14
16
18
20

Figure 21. Squamosal BAT lipid weight per lipid-free BAT dry weight (LW/LFDW) for cold-treated and control animals of pairs in the long-term study. The line marked "null" indicates expected location of points for each pair if there were no differences among cold-treated and control nestlings for LW/LFDW.

Figure 21


LONG-TERM STUDY
SQUAMOSAL BAT IN BOTH MEMBERS OF EACH PAIR
LW/LFDW (g/g) IN COLD-TREATED ANIMAL
LW/LFDW (g/g) IN CONTROL ANIMAL
Null
0
1
2
3
4
5
6
7
8
9
10
11

for interscapular BAT LFDW are shown in Figure 22 (squamosal BAT showed a similar pattern).

The hypothesis that lipid weight per lipid-free BAT weight varies as a function of body weight in adults was supported for both treatments. For both deposit types there was a significant functional dependence of LW/LFDW on BW. Figure 23 shows this relationship for interscapular BAT LW/LFDW (cold-treated: r^2=0.26, p<0.01; control: r^2=0.14, p=0.01), which was related to body weight much like squamosal BAT LW/LFDW (cold-treated: r^2=0.40, p<0.01; controls: r^2=0.17, p<0.01).

The hypothesis that BAT lipid weight depends on lipid-free BAT dry weight was supported, irrespective of treatment. As can be seen in Figures 24 and 25, a significant amount of variation in LW was explained by LFDW in both interscapular (cold-treated: r^2=0.62, p<0.01; control: r^2=0.67, p<0.01) and squamosal BAT (cold-treated: r^2=0.66, p<0.01; control: r^2=0.45, P<0.01).

A POSTERIORI COMPARISONS:

Several variables were examined together in the absence of preformed hypotheses to determine whether propositions could be posed after studying their relationships. Paired comparisons were performed on body weights (BW) at 20

Figure 22. Long-term study: Interscapular BAT lipid-free dry weight (LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 22

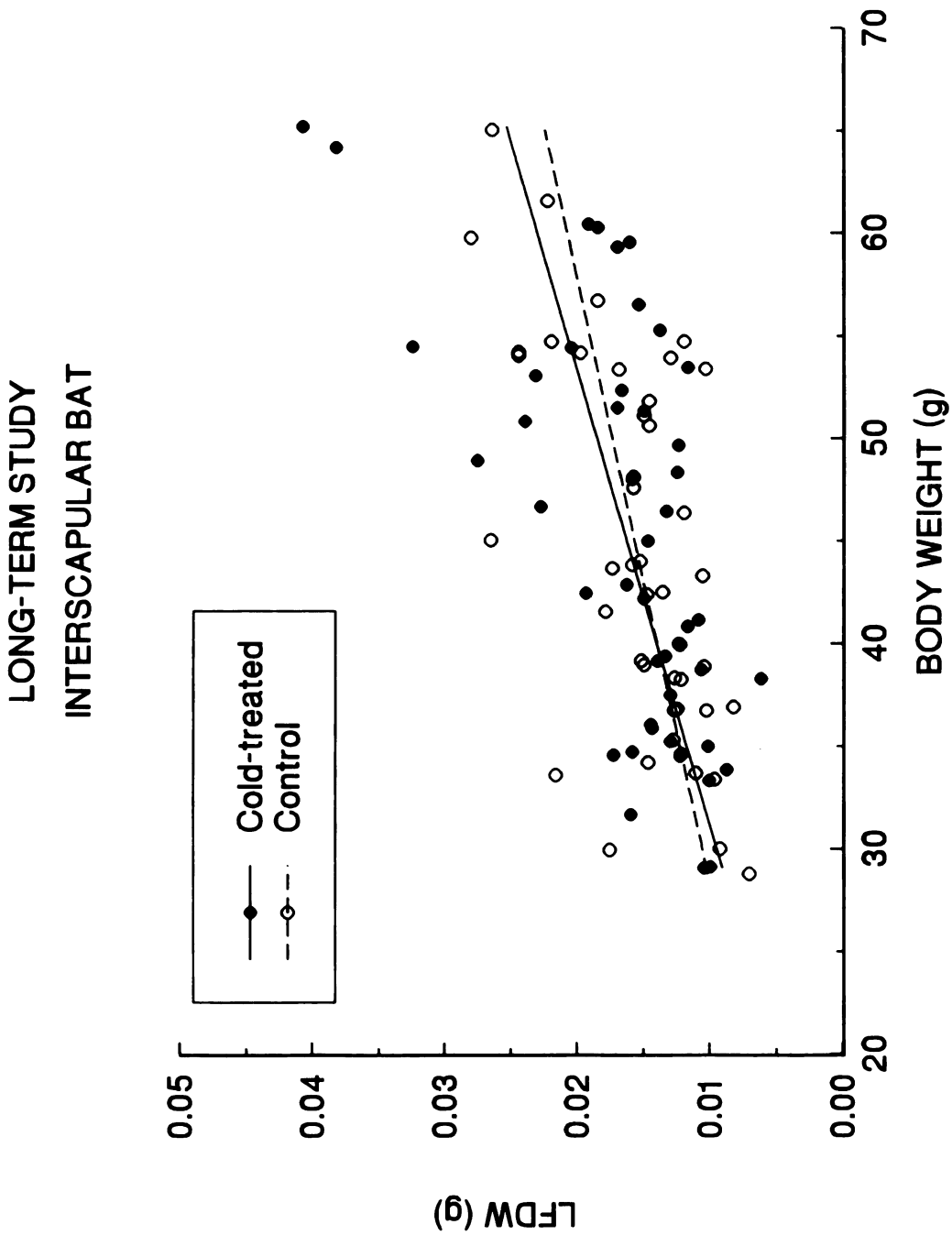

LONG-TERM STUDY
INTERSCAPULAR BAT
Cold-treated
Control
LFDW (g)
0.05
0.04
0.03
0.02
0.01
0.00
20
30
40
50
60
70
BODY WEIGHT (g)

Figure 23. Long-term study: Interscapular BAT lipid weight per lipid-free dry weight (LW/LFDW) for cold-treated (solid circles) and control animals (open circles) plotted against body weight.

Figure 23

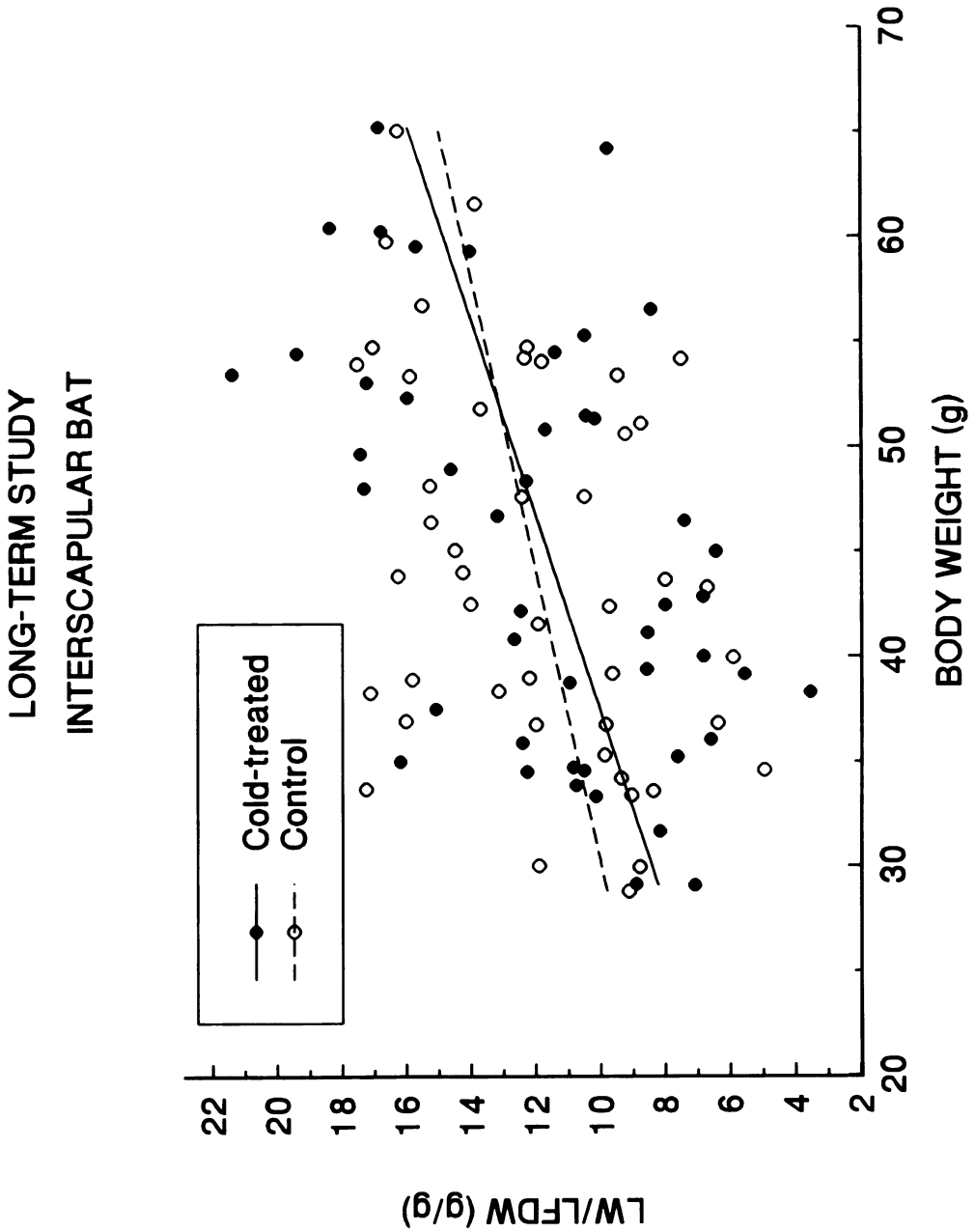

LONG-TERM STUDY
INTERSCAPULAR BAT
Cold-treated
Control
LW/LFDW (g/g)
BODY WEIGHT (g)
22
20
18
16
14
12
10
8
6
4
2
20
30
40
50
60
70

Figure 24. Long-term study: Interscapular BAT lipid weight (LW) for cold-treated (solid circles) and control animals (open circles) plotted against lipid-free dry weight (LFDW).

Figure 24

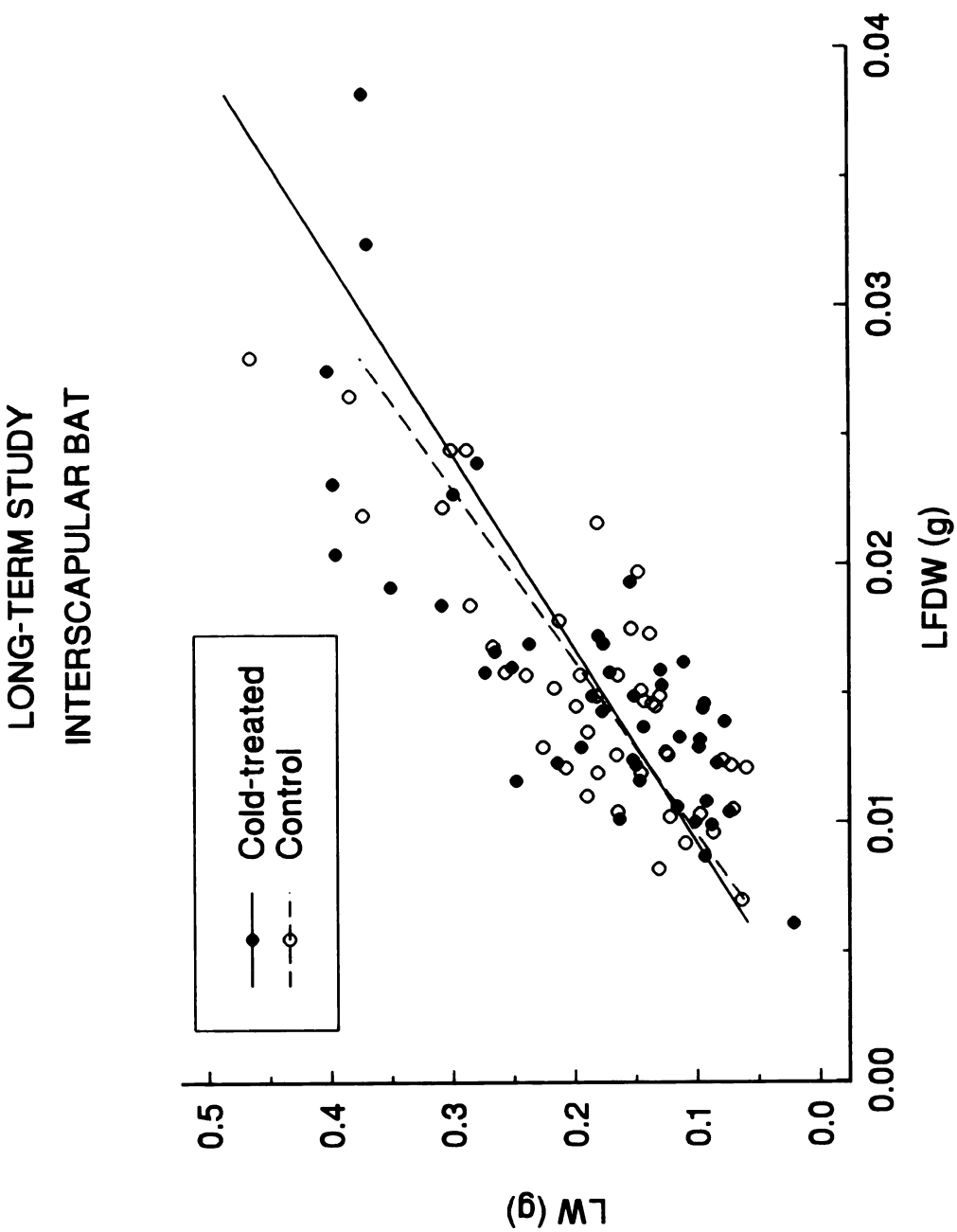
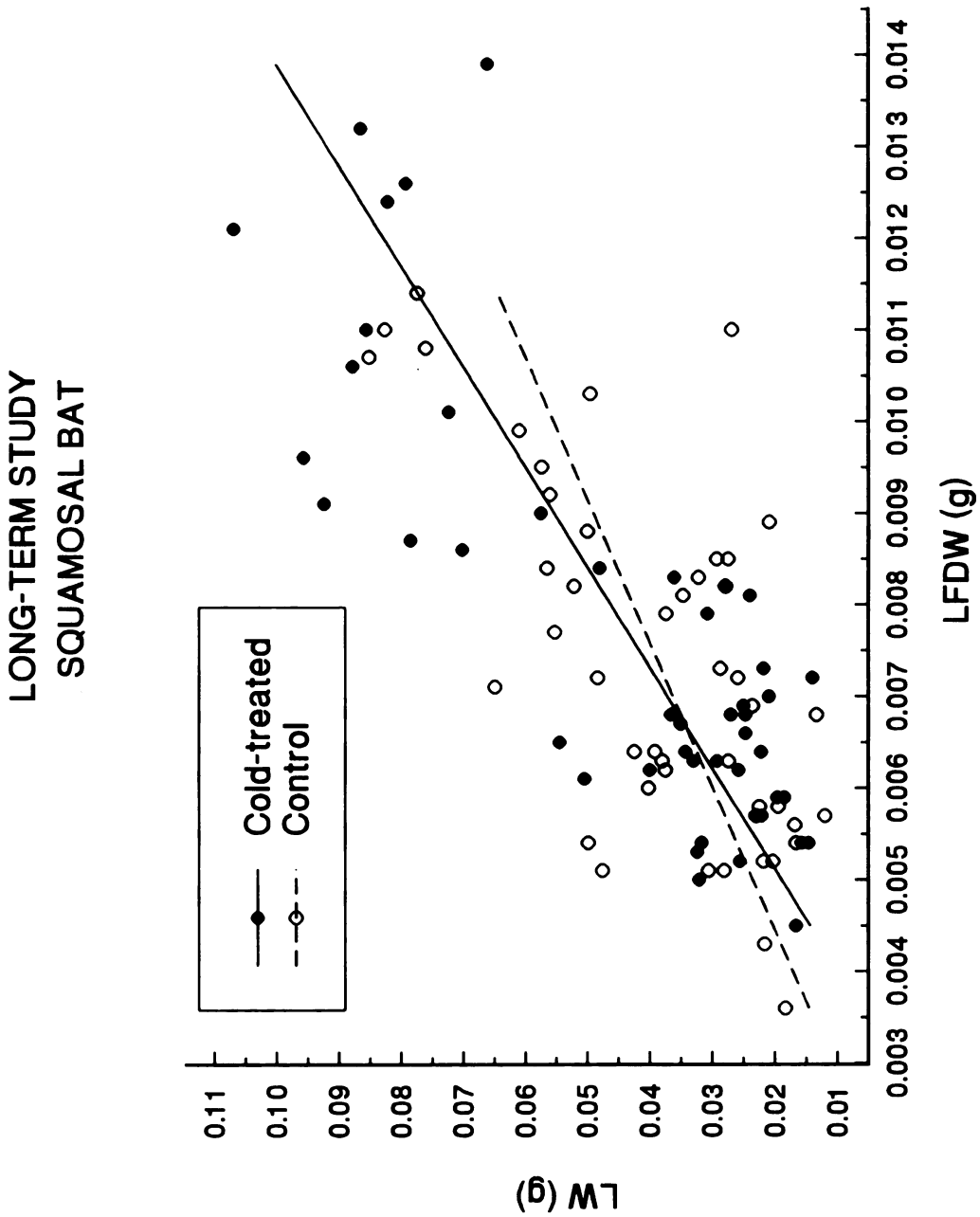

LONG-TERM STUDY
INTERSCAPULAR BAT
Cold-treated
Control
LW (g)
LFDW (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.00
0.01
0.02
0.03
0.04

Figure 25. Long-term study: Squamosal BAT lipid weight (LW) for cold-treated (solid circles) and control animals (open circles) plotted against lipid-free dry weight (LFDW).

Figure 25


LONG-TERM STUDY
SQUAMOSAL BAT
Cold-treated
Control
LW (g)
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
LFDW (g)
0.003
0.004
0.005
0.006
0.007
0.008
0.009
0.010
0.011
0.012
0.013
0.014

weeks. The effects of sex on BW, LFDW/g, and LW/LFDW were examined by one-way ANOVA on unpaired data. As earlier noted, potential relationships between litter size and these variables were not examined because litters sometimes lived with other, related litters between 30 d and 20 weeks of age.

Adult body weight was not affected by postnatal cold exposure. No significant effect of treatment on BW was found in paired comparisons between cold-exposed and control adults. Mean BW for 20-week-old cold-treated individuals (mean = 45.23 g +/- 9.85 S.D.) was not different from BW of controls at 20 weeks (mean = 44.33 g +/- 9.14 S.D.).

Body weight by 20 weeks of age was affected by sex. Males (mean = 48.68 +/- 7.24 g) weigh significantly more than females (mean 40.58 +/- 9.35 g) when examined by a Wilcoxon Rank Sum W test ($p<0.0001$).

Sex had an effect on adult weight-specific lipid free BAT dry weight for interscapular but not squamosal BAT. LFDW/g was significantly higher in females than in males for interscapular BAT ($p=0.012$). Although squamosal BAT showed the same trend (females: mean = 0.1803 mg/g +/- 0.0528; males: mean = 0.1633 mg/g +/- 0.384), it was not significant when examined by Wilcoxon Rank Sum W test.

Lipid weight per lipid-free BAT weight was not affected by sex. Due to variance heterogeneity, interscapular lipid weight per lipid-free BAT dry weight was examined nonparametrically and found not to be significantly affected by sex by Wilcoxon Rank Sum W test. Likewise, sex had no effect on squamosal lipid content per lipid-free deposit weight, as determined by one-way ANOVA.

DISCUSSION

I. SHORT-TERM STUDY

As appears to be true of other altricial rodent species examined previously, 21-day-old nestling prairie voles exposed to cold during the postnatal period have higher weight-specific lipid-free BAT weights than those maintained at nest temperature. Because no BAT measurements were taken at an earlier stage of development under these environmental conditions, it is unclear whether the difference at 21 d reflects enhanced recruitment or interruption of involution in *M. ochrogaster*, although studies on altricial murine rodent nestlings suggest the difference reflects mostly the latter.

In postnatal rats at regular laboratory temperatures, wet BAT weights expressed per neonate surface area (i.e.,

per gram body weight$^{0.67}$) peak around 5 d, followed by rapid decline (Nedergaard et al., 1986). This initial decline in wet BAT weight, however, primarily reflects a depletion of BAT lipid stores in response to cold exposure at birth; more direct measurements of thermogenic activity (e.g., cytochrome c oxidase activity) suggest that postnatal BAT involution occurs more gradually, coinciding with the onset of shivering around 9 d (Mouroux et al., 1990; Gordon, 1993).

Postnatal prairie voles in this study visibly shivered following cold exposure at approximately 8 days of age (occasionally seen as well in controls at about the same age). Thus if involution occurs concomitantly with shivering onset in prairie voles, as seen in murine rodents, then the difference in weight-specific lipid-free BAT dry weights between cold-treated and control littermates in the present study probably reflects involution in control nestlings which has been at least partially delayed in cold-exposed littermates.

Regarding nestling lipid weight per lipid-free BAT dry weight, the results of the present study again provide only a static view of differences between cold-exposed and control nestlings, but are consistent with previous findings of lower BAT lipid content associated with higher thermogenic activity in response to cold rearing (e.g.,

Senault et al., 1982, Mouroux et al, 1990). It is unclear why squamosal BAT deposits did not exhibit this pattern; the retention of lipid in squamosal BAT deposits relative to lipid-free dry weight in cold-treated nestlings in this study (also seen in litter average responses to postnatal cold exposure during paternal absence, as discussed later in the study of paternal care effects on BAT development), may reflect underlying functional specialization between different BAT deposits (discussed later in the study of response relationships among deposits).

In general, lipid-free BAT dry weight appears not to depend on nestling body weight. This was an unexpected result which might raise the question of whether weight-specific measurements of lipid-free BAT dry weight are an appropriate variable for assessing recruitment responses in nestlings. However, there is a trend for control nestlings of lower body weight to have lower lipid-free BAT dry weight, and the fact that the regression is significant for only interscapular BAT may be partly a function of the narrow range of body weight values observed for nestlings in the control group and the small sample size for residual BAT; both factors would reduce the power to detect a regression which deviates from a slope of zero (Figure 9).

Other factors are likely to operate in cold-treated individuals to mask an underlying relationship between lipid-free BAT dry weight and body weight. Cold-treated nestlings span a wider body weight range (Figure 9). Compared to the regression for controls, small cold-treated nestlings often appear to have relatively high lipid-free interscapular and squamosal BAT dry weights (Figures 8 and 10, respectively), reducing the slope of the regression to a nonsignificant value. In other words, for the low ambient temperature applied in the present study, the positive relationship between body weight and the absolute dry weight of lipid-free BAT may be counterbalanced by an inverse relationship between sensitivity to cold (and thus the magnitude of BAT recruitment) and body weight. Both inter- and intraspecifically, smaller mammals tend to show larger BAT-mediated responses to cold (Horwitz, 1989) due to increased rates of heat loss across a relatively large surface area and greater limits on insulation (Cannon, 1995). Smaller nestling voles therefore may develop relatively large interscapular and squamosal BAT deposits in response to cold. However, a single measure of lipid-free BAT dry weight does not provide information on the relative extent of BAT recruitment for a given nestling. Furthermore, the residual BAT lipid-free BAT dry weight is explained to a significant extent by the nestling's body

weight, although this may reflect a difference between deposits in response to cold.

The dependence of lipid weight per lipid-free BAT weight on body weight in cold-treated nestlings, with larger animals displaying higher BAT lipid content, could be interpreted as further evidence for relatively enhanced thermogenic activity in smaller individuals. Alternatively, heavier individuals might have similar thermogenic demands but relatively large lipid stores. Body size differences may have less of an effect on lipid stores in the absence of thermal stress, resulting in a nonsignificant relationship between lipid weight per lipid-free BAT dry weight and body weight for nestlings treated at nest temperatures. Additionally, the narrow range of body weight values for control animals may have limited the power of the regression slope test. This latter explanation seems more likely in light of the significant relationship found between lipid content per lipid-free deposit and body weight in adults in the absence of cold-treatment effects, discussed below.

In most instances, lipid content is a function of BAT deposit size. Although cold-exposed nestling BAT generally becomes more depleted of lipid than BAT in controls, the weight of lipid per lipid-free tissue dry weight varies in a predictable fashion irrespective of

treatment; larger deposits have more lipid than smaller deposits.

Interestingly, periodic cold exposure resulted in lower body weight between 19 and 20 days of age, suggesting that either nestling vole growth becomes limited by energy supplies during cold exposure, or that periodic hypothermia slows growth by thermally slowing key biochemical processes. As will be discussed, these hypotheses are not mutually exclusive.

Perrigo and Bronson (1985) found that adult house mice (*Mus domesticus*, which, like nestling prairie voles, lack the energy-conserving mechanisms of torpor and food hoarding) direct assimilated energy preferentially towards meeting *primary* demands of thermoregulation, maintenance, and food-gathering, before investing surplus in growth, fat storage, and other *secondary* demands. The growth (or maintenance) of BAT in cold-exposed nestling voles in the present study was observed simultaneously with lower body weights, suggesting that limited energy supplies might be allocated to maintenance of thermoregulation preferentially instead of growth in cold-stressed nestlings. If energy supply in the form of milk was inadequate in the present study to meet both primary and secondary demands, nutritional supplementation would be expected to curtail the weight loss by permitting

allocation of extra assimilated energy to growth. As will be discussed, however, nestling energy supply appeared to be unlimited by about 16 days of age.

A second hypothesis to explain the concurrent reduction of body weight and increase in lipid-free BAT dry weight is that biochemical processes which regulate postnatal body growth are more inhibited by low body temperatures than are processes which regulate postnatal BAT development. Isolated neonatal altricial rodents, which cannot defend their body temperature in the face of severe thermal stress, are subject to what Barcroft termed the "tyranny of the Arrhenius equation" (*in* Hill and Wyse, 1989); their metabolic rates fluctuate with ambient temperature. In spite of an adequate resource supply to meet the energetic requirements of body growth, a reduction of molecular kinetic energy due to lower body temperature could slow rate-limiting enzyme-catalyzed reactions necessary for growth, resulting in stunting. At the same time, stimulation of NE release in response to low body temperature could result in enhanced BAT recruitment. Presumably, a failure of nutritional supplementation to equalize growth rates among cold-treated and control nestlings would indicate that those systems which regulate body growth in nestling prairie voles are unable to acclimate to hypothermia (e.g., by quantitative or kinetic modulation of key enzymes).

The two hypotheses are not mutually exclusive. The latter hypothesis might secondarily entail cold-induced energy limitation: in spite of adequate energy supply, energy assimilation processes may be slowed by low body temperature. The resulting energy limitation could result in preferential energy allocation to thermoregulation as in the first hypothesis.

Furthermore, the two proposed growth-stunting mechanisms might exert their effects at different stages of postnatal development. At the end of the three hour cold treatment, nestlings generally were inactive and felt cool to the touch until about 10 days of age, whereas by about 16 days of age, they typically were active and felt warm to the touch after cold treatment (personal observation). Thus, nestling prairie voles in this study appeared to be unable to defend body temperature before 10 days of age during periodic exposures to low ambient temperature. This inability might result in the slowing of biochemical processes in the early postnatal period, stunting growth. Furthermore, as nestlings develop the ability to defend body temperature during cold exposure, energy costs should increase. This increase in primary energy demands (i.e. thermoregulation) later in the postnatal period might result in the allocation of limited energy away from secondary demands, including growth.

Since no significant differences in body weight were observed until 19 to 20 days of age, it appears unlikely that biochemical processes affecting growth are slowed substantially in response to cold exposure during early postnatal development in *M. ochrogaster*. The remaining hypothesis (that energy limitation results in allocation of assimilated energy away from growth) requires further examination, however, because nestling voles were observed to consume solid food -- an unlimited energy supply -- by about 13 days of age.

Thus, it remains unclear why cold-treated nestlings show a reduction in body weight by 19 to 20 days of age. Food quality and the ontogeny of digestive physiology might be important; nestlings may not be able to assimilate enough energy from solid foods by 20 days of age to sustain body growth under thermally stressful conditions. It is also possible that cold exposure negatively affects energy intake in nestling voles by influencing feeding performance, appetite, or satiety. Quantification of energy consumed and assimilated by nestlings would provide insight into these factors.

A posteriori examination revealed no significant effects of sex and litter size on any of the variables examined in this study. Because measurements were taken on prereproductive nestlings in the short-term study, the

lack of sex effects is not surprising. It is possible that the litter size range observed for *M. ochrogaster* in the present study (2 to 8 nestlings per litter) was too narrow to permit detection of litter size effects, as might be seen in rodent species which bear larger litters (e.g., *M. domesticus*).

II. LONG-TERM STUDY

Contrary to the results of previous studies on lab rats and mice (Lynch et al., 1976; Lacy et at., 1978; Doi and Kuroshima, 1979), no lasting effect of early cold exposure is observed in *M. ochrogaster* lipid-free BAT dry weight in response to postnatal cold exposure. This unexpected result may reflect idiosyncrasies in method between the present study and previous studies, or real variation between murine and microtine rodent recruitment and involution responses. Both possibilities shall be considered.

The cold-treatment temperature used in the present study (10^{o}C) was higher than that applied in previous research on lasting cold effects on NST thermoregulatory patterns (5^{o}C: Lynch et al., 1976; Lacy et al., 1978; Doi and Kuroshima 1979), and exposures were periodic, as opposed to the continuous exposure used by Lynch et al. (1976) and Lacy et al. (1978). However, nestling mice

were housed with mothers, littermates, and nesting material in previous studies (Lynch et al., 1976; Lacy et al., 1978), which likely decreased the cold stress experienced by the pups. (It is unclear whether rats used in the investigation by Doi and Kuroshima in 1979 were treated in isolation or with littermates; the authors merely state that litters were separated into two groups for daily treatment at 5 or 25°C.) Furthermore, it was shown earlier in the short-term study that 10°C is sufficient to cause significant recruitment in nestling prairie voles. Differences in the length of postnatal treatment (25 days: Lynch et al., 1976; 70 days: Lacy et al., 1978; 14 days: Doi and Kuroshima, 1979; versus 20 days in the present study) and the age at examination (50-56 days: Lynch et al., 1976; 115 days: Lacy et al., 1978; 133 days: Doi and Kuroshima, 1979; versus 137-143 days in the present study) may have affected the present outcome, although these differences would appear to be too insignificant to fully account for the lack of detectable response in adult prairie voles. For instance, the length of treatment and age at dissection of prairie voles were within a week of those used by Doi and Kuroshima (1979).

It is possible, albeit unlikely, that the ambient temperature at which prairie voles were housed until 20 weeks of age (21°C) may have masked some of the effects of early cold exposure in one of two ways. Incidental mild

hypothermia induced by colony room temperature, which is usually below the thermoneutral zone of small mammals, can confound BAT studies by stimulating recruitment in supposedly non-cold-treated animals (Himms-Hagen, 1986). Wunder et al. (1977) found that the thermoneutral zone for adult prairie voles varies between 27 and 34°C. Although littermates and nesting material with which weaned voles were housed after postnatal treatment probably raised the effective ambient temperature, controls in the long-term study may have been partially cold acclimated, which could have masked lasting differences caused by early environmental temperature, if these differences were weak. Of course, such weak lasting effects of early cold would probably be biologically unimportant. On the other hand, if postnatal cold exposure enhances the *capacity to respond* to cold later in life by preparing BAT for a more rapid or complete recruitment in response to subsequent cold, the temperature to which adults were acclimated may have been too close to thermoneutrality to detect such a predisposition.

It seems unlikely that such methodological idiosyncrasies affected the results of the present study, however, since lasting recruitment responses were observed in previous studies when rats or lab mice were housed at similar ambient temperatures in the weeks prior to dissection. Therefore, long-term BAT responses to

postnatal cold stress in the present study do not appear to differ from past investigations due to idiosyncrasies of method.

It is not possible to determine from the results presented whether murine rodents examined in the previous studies exhibited greater differences at the time of weaning in recruitment between postnatal cold-treated versus control animals than *M. ochrogaster* did in the present study. If preweaning responses to cold are greater in murine than in microtine rodents, this difference may partly explain the persistences of early-cold-exposure effects observed in murine but not microtine rodents. On the other hand, it is possible that BAT recruitment in *M. ochrogaster* differs from that in lab rats and mice in that it does not exhibit permanent changes in response to early cold.

Lynch et al. (1976) and Lacy et al. (1978) were interested in delineating genetic and environmentally-imposed thermoregulatory components of an individual's phenotype so that quantitative genetic analyses could be applied to the genetic components to study evolutionary adaptation. In this context, irreversible developmental responses observed in lipid-free BAT dry weight were viewed mainly as nuisances to investigators wishing to perform comparative evolutionary studies on wild-caught

species. However, as shown in the present study, the flexibility of BAT recruitment and involution may vary among species. It would seem energetically costly, and thus maladaptive, for BAT to be maintained permanently in an active state in response to early cold stress when it can be recruited later in life when necessary. Thus the permanent response observed in lab rats and mice but not in prairie voles may indicate that arvicoline rodents have evolved energy-saving (and thus adaptive) phenotypic plasticity in a thermoregulatory trait which displays developmental irreversibility in murine rodents.

As was expected, postnatal cold exposure had no lasting effect on lipid weight per lipid-free BAT dry weight in either interscapular or squamosal deposits in adult prairie voles. Although this prediction was based on the rapid replenishment of lipid stores observed previously in BAT of cold-acclimated adult rats (Cameron and Smith, 1964), lipid levels in adult prairie voles reared with periodic exposure to cold may equal those of adults reared at warmer temperatures due to the lack of permanent BAT recruitment effects in this species as described above.

Adult lipid-free BAT dry weight depends on adult body weight. This relationship is clearly displayed in adults in the long-term study, in contrast to nestlings in the

short-term study, probably because the sample size was larger and because the effects of early cold-exposure did not significantly influence the relationship (Figure 22). Thus, in the absence of treatment effects, heavier adult voles have heavier lipid-free BAT deposits.

Likewise, the amount of lipid per lipid-free BAT deposit varies as a function of adult body weight in this species. As was presupposed, larger adult prairie voles have more lipid per BAT deposit than smaller animals when acclimated as adults to ambient temperatures near thermoneutrality, regardless of postnatal thermal conditions. A predisposition towards lipid storage might have resulted concurrently in higher body weight and higher lipid weight per lipid-free BAT dry weight. Body composition analyses could be conducted to test this hypothesis, which would be supported by evidence of relatively high lipid levels in heavier individuals.

As seen in the short-term study, the lipid weight of a BAT deposit increases with lipid-free BAT dry weight in adult prairie voles, irrespective of rearing temperature. This observation might reflect a relationship between the lipid content of BAT cells and their volume at a given level of thermogenic activity in a well-provisioned adult animal, such that an increase in cell number or size leads to a rise in lipid-free BAT dry weight and total lipid

content. Body weight, however, should affect this relationship, and presumably accounts for some of the variation seen in Figures 24 and 25.

In contrast to findings from the short-term study, a posteriori observation revealed that adult body weight was not affected by postnatal cold exposure, but was affected by sex. The latter finding corroborates the observations of sex effects on body weight in mice by Lacy et al. (1978); male *M. domesticus* were found to weigh more than females.

Unlike the present study, however, Lacy et al. found no relationship between body weight and lipid-free BAT dry weight, which led them to believe that sex differences they observed in thermoregulatory traits reflected fundamental differences between male and female thermoregulatory mechanisms. Lacy et al. found that females scored higher than males on NST indices including weight-specific lipid-free BAT dry weight of the interscapular deposit. Although lipid-free BAT dry weight was found to increase with body weight in the present study, adult male prairie voles, which were heavier than adult females, had less weight-specific lipid-free BAT in the interscapular deposit than did females (squamosal BAT showed a similar, though nonsignificant, trend). This discovery supports and extends to adult microtine rodents

the interpretation of Lacy et al. (1978) that sex affects thermoregulation via mechanisms other than the effect sex has on body weight.

The amount of lipid per lipid-free BAT weight, on the other hand, was found not to be affected by sex for either interscapular or squamosal deposits. Thus in spite of the fact that adult female prairie voles were smaller and had relatively high levels of thermogenically active BAT (measured as weight-specific lipid-free BAT dry weight), the lipid concentration in BAT of adult females was not depleted to a greater extent than males under the conditions of the present study. It would be interesting to determine whether adult females are able to maintain BAT lipid stores to the same extent as males under thermally or energetically stressful conditions as well.

EFFECTS OF PATERNAL CARE ON BAT DEVELOPMENT

GENERAL OVERVIEW OF THE PROBLEM: POSTNATAL BAT RECRUITMENT IN RESPONSE TO PATERNAL CARE

The goal of this study was to determine whether postnatal brown adipose tissue (BAT) recruitment is reduced in altricial nestlings when paternal care is available during periodic cold exposure in a free-ranging

microtine rodent, the prairie vole (*Microtus ochrogaster*). This temperate-zone species is likely to experience thermal stress during development. Recent studies have shown that paternal care in this species facilitates offspring development, but the mechanisms by which paternal care aids postnatal development are unclear. It has been demonstrated in the first section of this thesis that BAT recruitment in nestling prairie voles is enhanced by periodic exposure to low ambient temperatures during the first 20 days postpartum. For studies of paternal care, BAT recruitment may thus be useful as an index of both thermoregulatory ontogeny (specifically, NST) and thermal stress experienced by nestlings in the presence or absence of their father during periodic postnatal cold exposure.

MONOGAMY AND PATERNAL CARE IN MICROTINE RODENTS

An especially intriguing aspect of the biology of *M. ochrogaster* is its monogamous mating system, in which both parents share responsibilities of rearing young. Juveniles remain in the natal nest after weaning and also assist in rearing the next litter (Gruder-Adams and Getz, 1985; Wang and Novak, 1992; Solomon, 1994). Alloparental care by juveniles results in higher body weights and earlier eye opening in the next litter (Solomon, 1991; Solomon, 1994).

Wang and Novak (1992) recently demonstrated that paternal care facilitates development of lab-reared neonatal prairie voles maintained on ad lib food, at 20°C ambient temperature. They found that nestlings raised with both parents ate solid food and exited the nest sooner than those raised in the absence of fathers, even when juveniles were present. Although not a statistically significant difference, eye opening tended to occur earlier in nestlings with fathers present. Several entire litters died in the absence of the father and were excluded from analysis. The remaining litters showed no differences in either nestling weight at 20 days, or pup mortality.

Paternal care seems likely to have more profound effects on nestling survivorship under natural conditions, which might result in selection for paternal investment, a concept known as the Male Care Hypothesis (Kleiman, 1977). It was demonstrated by Gubernick et al. (1993) that, in cold (8.5-10.5°C) conditions or when parents were required to forage for food (by means of a dispenser linked to a running wheel), nestling survival in the California mouse (*Peromyscus californicus*) was substantially reduced when the father was absent. *P. californicus*, like *M. ochrogaster*, is noteworthy for exhibiting exceptional levels of paternal care relative to other species of its genus.

Developmental acceleration by paternal care may be mediated by thermoregulatory enhancement. Thus a goal of this study was to evaluate the influence of the father's presence on the development of BAT in *M. ochrogaster*. Because brown adipose tissue simultaneously affects thermoregulation and responds to thermal stimuli in quantifiable ways, it provides a means to investigate whether thermoregulatory aspects of paternal care may impinge on the development of thermoregulatory systems in postnatal nestlings. If energy supplies limit growth and maturation among altricial rodents such that tradeoffs in energy allocation exist, the effect of paternal care on BAT recruitment might have profound effects on other developmental parameters.

OBJECTIVES AND EXPERIMENTAL DESIGN

To test the hypothesis that the absence of the father during exposure to periodic postnatal cold has a stimulatory effect on BAT recruitment in nestlings, I exposed lab-reared *M. ochrogaster* litters from the day after birth through 20 days of age to periodic low ambient temperatures, with or without the father present. Litter averages for several variables were examined. I compared lipid-free, weight-specific BAT weights and lipid weights per lipid free dry BAT weight in the two treatments. Based on earlier observations, I hypothesized that the

average lipid-free dry BAT weight per gram body weight for litters with fathers absent would be higher than the average litter value with fathers present (controls). I further hypothesized, based on the earlier studies on lipid content and postnatal cold exposure, that father-absent litters would have lower average lipid weights per lipid-free dry BAT weight than would controls.

I also examined several presuppositions underlying these hypotheses. I presumed that lipid-free BAT dry weight and lipid weight per lipid-free BAT weight would correlate positively with body weight. I also presumed that the lipid content of a BAT deposit would correlate positively with its lipid-free dry weight.

METHODS

ANIMAL SUBJECTS AND HUSBANDRY

The animals used were laboratory-born prairie voles (*M. ochrogaster*) from a colony at Michigan State University established in 1991 from animals originally captured near Urbana, Illinois. Throughout the study, all breeding pairs were housed together at 21^{o}C on a 16:8 light:dark cycle in plastic cages (38x33x16 cm) provided with a standardized volume (about 960 mL) of corn cob bedding (Andersons Industrial Products) and a standardized

weight (44-46 g) of aspen laboratory wood shavings (Northeastern Products Corp.). Parents typically built nests from the shavings. Food (Teklad Rodent Diet 8640 and Rabbit Diet) and water were provided ad lib.

PROCEDURE

Two litters from each breeding unit (mother and father) were assigned to father-present (FP) or father-absent (FA) treatment groups. These two litters were paired in a randomized complete block design to control for variation among breeding units. To control for possible parity effects, the order of assignment (i.e. FP first or FA first) was determined randomly, such that about half of the pairs (13 out of 28) were assigned FA treatment first, and the other half FP first. Most litters in the study for both FA and FP treatments (23 of 25 pairs) were from multiparous parents. Fathers from FA-assigned litters were removed from the cage to a holding chamber provided with ad lib food and water during cold treatment, whereas fathers from FP-assigned litters were left in the cage during cold treatment. To control for possible disturbance effects, fathers from FP-assigned litters were handled briefly and returned to the cage prior to and following cold treatment. For both treatment groups, the entire cage (including the mother) was exposed from 1 day of age through 15 d for 5 h/d during the

lights-on period to 10°C. During all non-treatment hours, both parents remained in the cage with the litter. Attempts were made at all times to minimize disturbance. Two incubators were used in this study for cold treatments. However, all litters from a given breeding unit were treated in a single incubator, so that incubator differences would be controlled by the blocked design.

At 16 d of age, litters were euthanized with CO_2, weighed and sexed, and BAT deposits were removed under a dissecting microscope, placed in numbered glass vials and frozen for later drying, lipid extraction and weighing. All nestlings in a litter were dissected up to a total of four; litters with more than four young were briefly sorted by sex just prior to the first dissection, and four animals were chosen at random while balancing the numbers of each sex whenever possible. A total of 210 animals was dissected. Mean litter size was 4.94 (+/- 1.53 SD). The interscapular and squamo-occipito-cervical deposits of BAT were located as described by Rauch and Hayward (1969) and dissected from each animal (the squamo-occipito-cervical deposit is hereafter called simply *squamosal*). BAT samples were dried in a drying oven at 55°C until they reached a constant weight (about 24 hours). Lipids were extracted from the tissue samples per Lacy et al. (1978) using two changes of anhydrous ethyl ether (J. T. Baker, Inc.). It was determined in preliminary trials that two

changes of ether produce a constant lipid-free dry weight which remains unaltered after additional ether changes.

DESIGN AND ANALYSIS

Litters from the same parents and representing each treatment were paired in a randomized complete block design (n=27 pairs). Several litters could not be paired and thus were left out of the analysis. To avoid non-orthogonality effects caused by unequal litter sizes (disproportional, unequal subclasses), paired comparisons were performed on litter means. Thus, for all variables, *the average response of the litter* was examined in this study, as opposed to individual responses or variability of individual responses within litters. Paired comparisons were performed using a two-way ANOVA to permit investigation of blocking effects in addition to treatment effects. Normality was examined using normal probability plots, and homogeneity of variances was evaluated using Cochran's C and Bartlett-Box F tests (SPSS PC+). Data were transformed using natural logarithms when such transformations were found to enhance normality and variance homogeneity. Outliers were identified by Grubb's test per Sokal and Rohlf (1995) and removed prior to analysis. Data which remained heteroscedastic or non-normal after these attempts were analyzed using a Wilcoxon

matched-pairs signed-ranks test, a nonparametric test for paired-comparisons (SPSS PC+).

The weight-specific, lipid-free dry BAT weight (LFDW/g), and the lipid weight per lipid-free BAT weight (LW/LFDW) were compared between treatments within pairs using the MANOVA procedure in SPSS PC+ (unless a nonparametric test had to be used). Scattergrams were plotted to facilitate visual assessment of differences between father-absent and father-present litters within pairs of litters.

Least squares linear regression analysis was used to test presuppositions by examination of functional relationships between body weight and both lipid-free BAT dry weight and lipid weight per lipid-free BAT weight, and between the lipid content of a BAT deposit and lipid-free dry weight. Because rigorous use of linear regression implies assumptions which are often not met in functions among physiological variables (e.g., "independent" variables are often not fixed), correlation analysis was also employed to evaluate the degree of association among variables. Because the correlation results generally were commensurate with the results of the regression analyses, only the regression results are reported.

RESULTS

A PRIORI COMPARISONS

The following results respond to the a priori hypotheses presented in the OBJECTIVES section.

The hypothesis that paternal absence during periodic postnatal cold-exposure increases the weight-specific lipid-free BAT dry weight in litters was supported. Paternal absence had a significant stimulatory effect on LFDW/g litter averages for both interscapular and squamosal BAT deposits, as seen in Table 3. Figures 26 and 27 indicate that about 77 percent (20 of 26, one outlier pair removed) of the litter pairs had higher LFDW/g values for father-absent than father-present treatments for interscapular BAT, and about 67 percent (18 of 27) had higher father-absent that father-present LFDW/g for squamosal BAT. Significant variance existed among pairs of litters for interscapular and squamosal BAT LFDW/g (Table 3).

The hypothesis that paternal absence results in a depletion of nestling BAT lipid per lipid-free BAT dry weight, measured as litter averages, was only partly supported. Results are presented in Table 3 and Figures 28 and 29. Interscapular BAT LW/LFDW was significantly

Table 3. Results from the paternal-care study. N is the number of pairs of litters (father-absent and father-present) analyzed. Standard deviation (SD) is given in parentheses. The probability for treatment ("trtment") is the likelihood of no difference between father-absent (FA) and father-present (FP) litters. That for pairs is the likelihood that none of the overall variance was attributable to differences among pairs. NPAR indicates that the Wilcoxon test (a nonparametric test) was used; this test yields no probability for pairs. LFDW/g = weight-specific lipid-free BAT dry weight. LW/LFDW = lipid weight per lipid-free BAT dry weight.

		Mean (SD)		Probability	
Variable	N	FA	FP	Trtment	Pairs
LFDW/g (mg/g)					
Interscapular	26	0.67 (0.12)	0.57 (0.14)	0.001	<0.03
Squamosal	27	0.43 (0.06)	0.38 (0.08)	0.009	0.03
LW/LFDW (g/g)					
Interscapular	26	4.33 (1.01)	4.98 (1.09)	0.016	<0.10
Squamosal	28	3.01 (0.62)	3.26 (1.09)	<0.388	NPAR

Figure 26. Interscapular weight-specific lipid-free BAT dry weight (LFDW/g) for father-absent (FA) and father-present (FP) litters of pairs in the paternal-care study. The line marked "null" indicates expected location of points for each pair if there were no differences among FA and FP litters for LFDW/g.

Figure 26

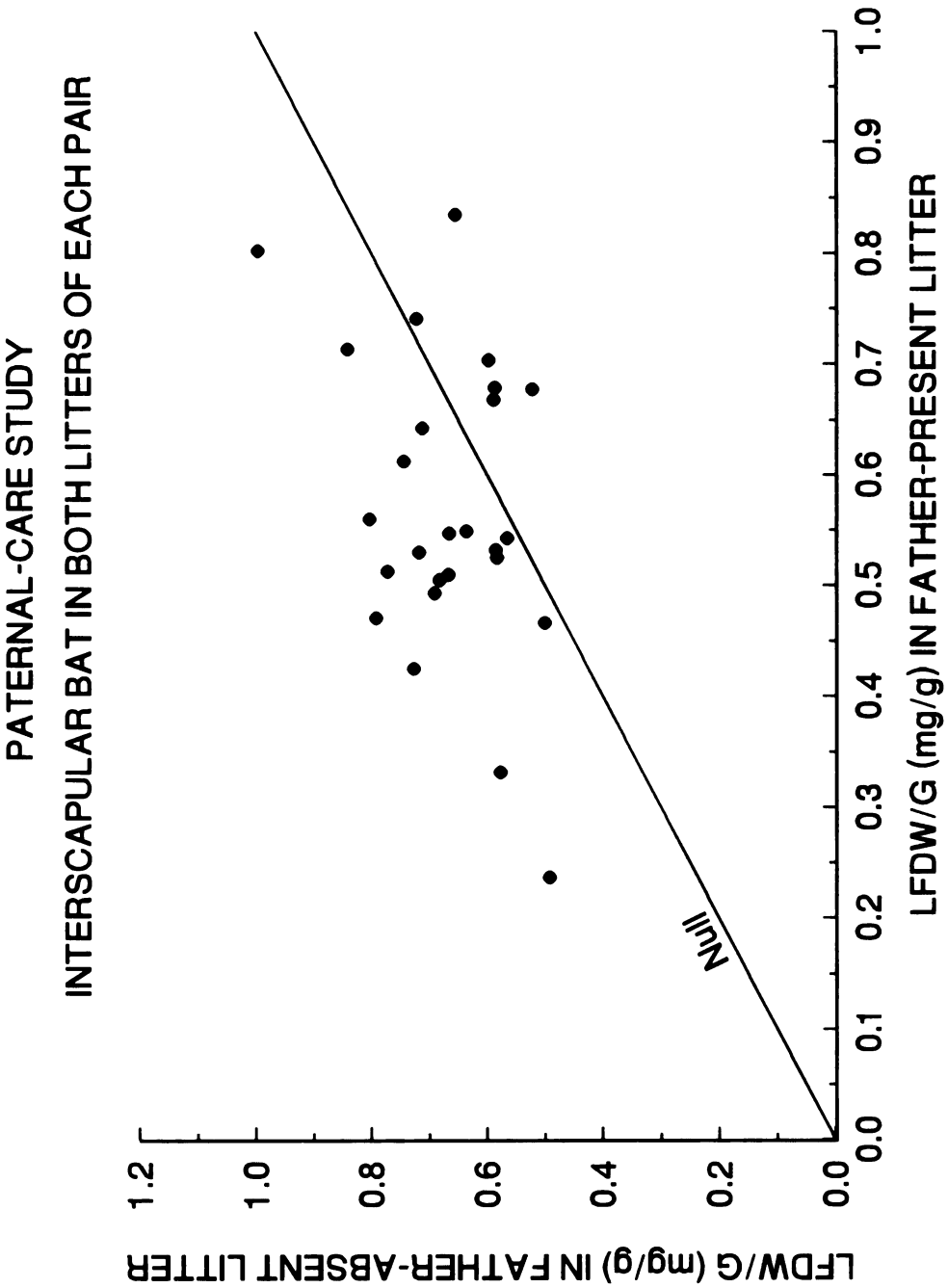

PATERNAL-CARE STUDY
INTERSCAPULAR BAT IN BOTH LITTERS OF EACH PAIR
LFDW/G (mg/g) IN FATHER-ABSENT LITTER
LFDW/G (mg/g) IN FATHER-PRESENT LITTER
Null
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.1
0.3
0.5
0.7
0.9

Figure 27. Squamosal weight-specific lipid-free BAT dry weight (LFDW/g) for father-absent (FA) and father-present (FP) litters of pairs in the paternal-care study. The line marked "null" indicates expected location of points for each pair if there were no differences among FA and FP litters for LFDW/g.

Figure 27

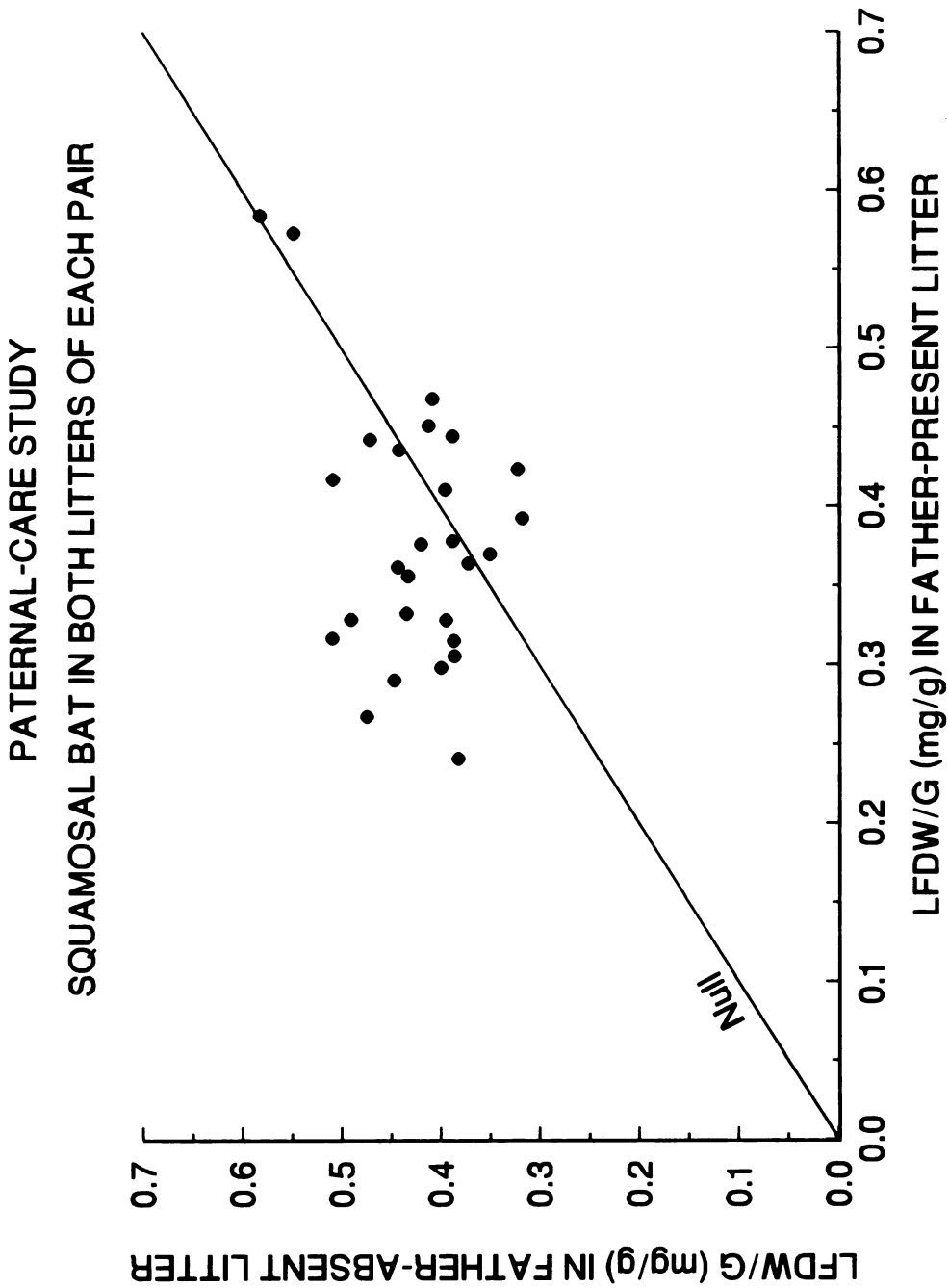

PATERNAL-CARE STUDY
SQUAMOSAL BAT IN BOTH LITTERS OF EACH PAIR
LFDW/G (mg/g) IN FATHER-ABSENT LITTER
LFDW/G (mg/g) IN FATHER-PRESENT LITTER
Null
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Figure 28. Interscapular lipid weight per lipid-free BAT dry weight (LW/LFDW) for father-absent (FA) and father-present (FP) litters of pairs in the paternal-care study. The line marked "null" indicates expected location of points for each pair if there were no differences among FA and FP litters for LW/LFDW.

Figure 28

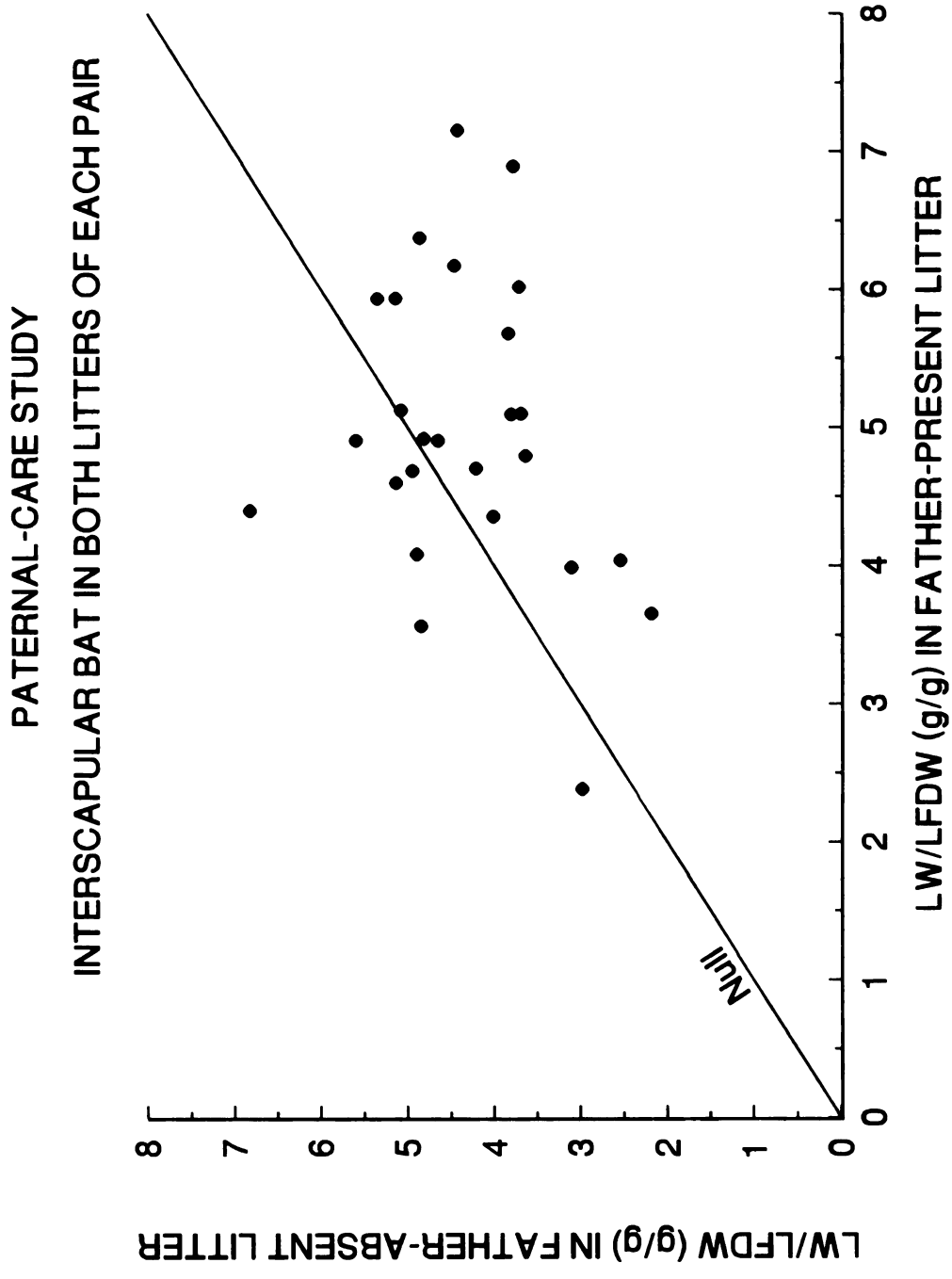
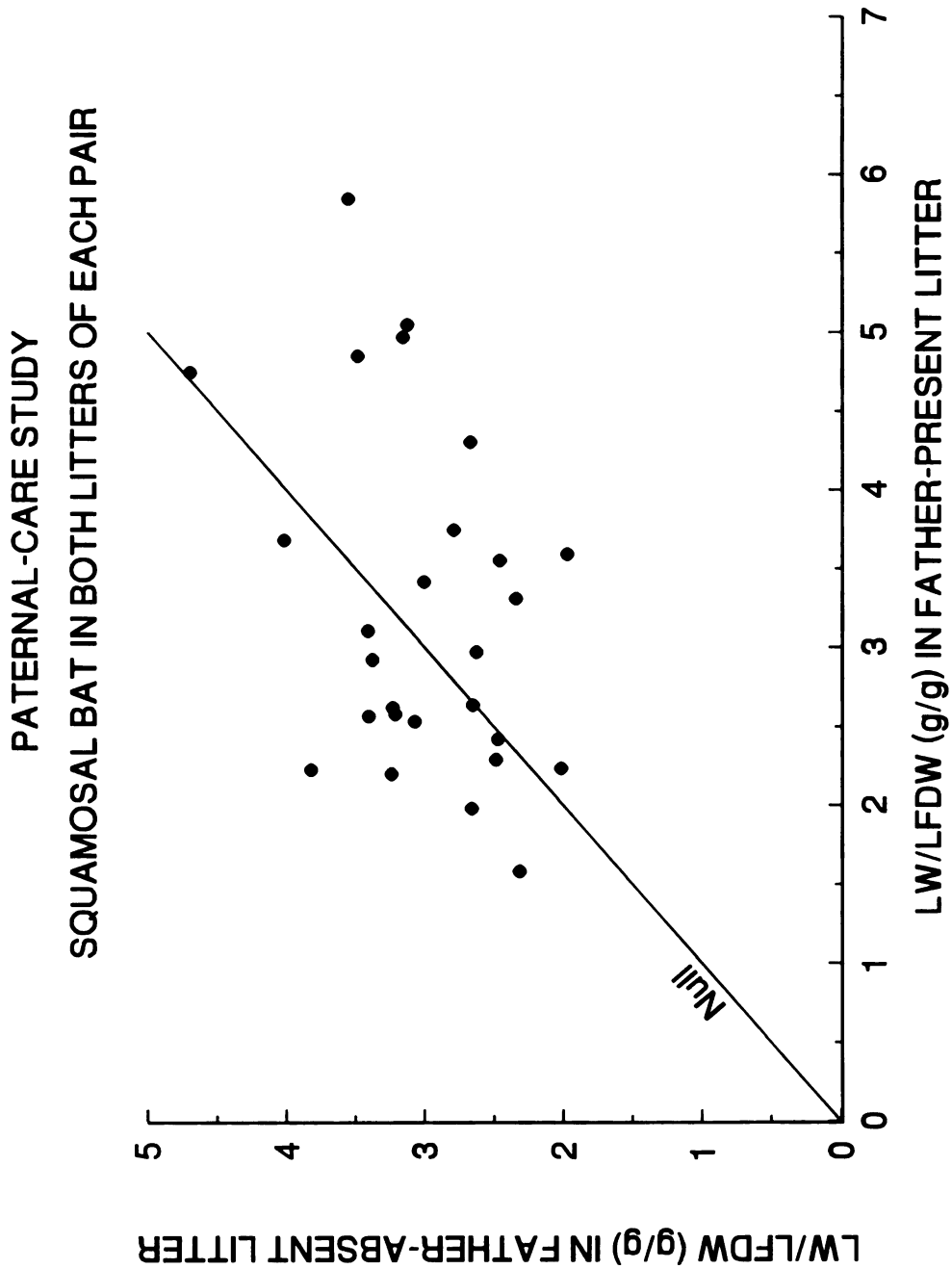

PATERNAL-CARE STUDY
INTERSCAPULAR BAT IN BOTH LITTERS OF EACH PAIR
LW/LFDW (g/g) IN FATHER-ABSENT LITTER
LW/LFDW (g/g) IN FATHER-PRESENT LITTER
Null
0
1
2
3
4
5
6
7
8

Figure 29. Squamosal lipid weight per lipid-free BAT dry weight (LW/LFDW) for father-absent (FA) and father-present (FP) litters of pairs in the paternal-care study. The line marked "null" indicates expected location of points for each pair if there were no differences among FA and FP litters for LW/LFDW.

Figure 29


PATERNAL-CARE STUDY
SQUAMOSAL BAT IN BOTH LITTERS OF EACH PAIR
LW/LFDW (g/g) IN FATHER-ABSENT LITTER
LW/LFDW (g/g) IN FATHER-PRESENT LITTER
Null
0
1
2
3
4
5
6
7

higher in father-present (FP) litters than father-absent (FA) pair members (Table 3); about 65 percent of pairs (17 of 26, one outlier pair removed) exhibited a higher average LW/LFDW in the FP litter. No significant pairing effects were detected. Due to variance heterogeneity, lipid levels per lipid-free dry weight in squamosal BAT were analyzed using Wilcoxon matched-pairs signed-ranks test. Squamosal BAT LW/LFDW did not differ between treatments (Table 3), and only 12 of 27 pairs (approximately 44 percent) had higher values for FP litters.

The hypothesis that nestling lipid-free BAT dry weight is dependent on nestling body weight was supported. A significant functional relationship between LFDW and BW was evident for both interscapular (FA: r^2=0.56, $p<0.01$; FP: r^2=0.47, $p<0.01$) and squamosal BAT (FA: r^2=0.46, $p<0.01$; FP: r^2=68, $p<0.01$), regardless of treatment.

The hypothesis that lipid weight per lipid-free BAT dry weight varies as a function of body weight was not supported for any deposit or treatment. In FA and FP litters for both interscapular and squamosal deposits, LW/LFDW was not affected by BW.

Support was found for the hypothesis that variation in BAT lipid weight is dependent upon variation in

lipid-free BAT dry weight. For both deposits and treatments, LW was a function of LFDW. Interscapular BAT for FA litters (r^2=0.52, $p<0.01$) and FP controls (r^2=0.74, $p<0.01$) and squamosal BAT FA litters (r^2=0.50, $p<0.01$) and FP controls (r^2=0.39, $p<0.01$) all showed this pattern.

A POSTERIORI COMPARISONS

Several variables were examined together in the absence of preformed hypotheses to determine whether propositions could be posed after studying their relationships. Paired comparisons among father-present and father-absent litters within pairs were performed on litter-averaged body weight (BW) at 16 d. The effects of sex on BW, LFDW/g, and LW/LFDW were examined by one-way ANOVA on unpaired data. The relationship between litter size and these variables was determined by linear regression analysis on unpaired data, with litter size treated as the independent variable. The following results on "nestlings" refer to litter averages, as opposed to individuals within litters.

Paternal presence or absence had no effect on body weight at 16 days of age in nestlings. No significant difference was detected between FA and FP litters with regard to nestling BW. Variance attributable to pairs was also nonsignificant.

Sex had no effect on nestling body weight at age 16 days. Male (14.57 g +/- 3.13) and female (14.40 g +/- 2.97) BW means were not significantly different.

Nestling body weight did not vary as a function of litter size. No significant variation in BW at 16 days of age was accounted for by litter size.

Variance in nestling weight-specific lipid-free BAT dry weight was not explained by litter size. LFDW/g values were not functions of litter size for either interscapular or squamosal BAT.

Sex did not affect weight-specific lipid-free BAT dry weight in nestlings. Due to heteroscedasticity, a Mann-Whitney U test was used in lieu of a one-way ANOVA on interscapular BAT LFDW/g values. No significant effect of sex on LFDW/g was observed for interscapular or squamosal BAT.

Lipid weight per lipid-free BAT weight in nestlings did not vary as a function of litter size. LW/LFDW variance was not explained by litter size to a significant extent for interscapular or squamosal BAT.

Sex had no effect on lipid weight per lipid-free BAT weight in nestlings. For both interscapular and squamosal

BAT, no significant influence of sex on LW/LFDW was observed.

DISCUSSION

Hill (1992) has explained how the energetic costs to altricial nestlings can be divided into those that can be met only by the young using their own resources (e.g., maintenance, growth, and development), those that can be met only by the parents (e.g., foraging), and those that can be met either by the young or by the parents (e.g., thermoregulation). In the latter case, utilization of parental resources could theoretically conserve the young's own energy resources for maintenance and maturation. As Hill has shown, this argument assumes that the parents would not be required to sacrifice limited energy or foraging time to maintain the nestlings' body temperature; otherwise incubated young may be deprived of milk energy. The argument further assumes that the nestlings are energy-limited, that high body temperatures facilitate growth and development, and that high growth and maturation rates are advantageous. Although the young of many altricial placental small mammals are able to thermoregulate as a litter in the absence of parental warming, facultative suspension of thermogenesis via active mechanisms (e.g., preweanling torpor) could permit

energy conservation in the presence of the parents (Hill, 1992).

The inhibited BAT development (weight-specific lipid-free BAT dry weight) seen in father-present litters in this study suggests that fathers provide a less-demanding thermal environment resulting in curtailment of thermogenesis in nestling *M. ochrogaster*. Consequent energy savings may permit the accelerated postnatal development witnessed by Wang and Novak in litters receiving paternal care (1991). Testing this scenario from the perspective of paternal investment and preweanling thermoregulation, however, would require consideration of the assumptions raised above, and the degree to which laboratory studies can be extrapolated to natural conditions (e.g., parents are presumably less food- and foraging-time limited in the lab than in the field).

Interscapular BAT deposits of litters deprived of paternal care during cold-exposure had lower lipid weights per lipid-free dry tissue weight than did those of litters receiving paternal care. This observation suggests that litters deprived of paternal care maintain higher rates of NST than litters in which paternal care is available. It seems unlikely that slowed lipid transport to BAT would be responsible for depletion of lipid stores in litters

lacking paternal care, since low ambient temperatures stimulate lipoprotein lipase activity, enhancing lipid transport to BAT (Carneheim et al., 1984). It is unclear why squamosal BAT deposits do not exhibit the same pattern of lipid depletion, however. The retention of lipid relative to lipid-free dry weight in squamosal BAT deposits of nestlings lacking access to paternal care during cold stress (also observed in individual nestlings in response to isolated postnatal cold exposure in the short-term study), may reflect underlying functional specialization of different BAT deposits.

As was observed in the long-term study (and much less clearly in the short-term study) lipid-free BAT dry weight varies as a function of the litter-averaged body weight whether or not paternal care is available during cold exposure. Presumably nestlings in the present study, compared to those in the short-term study, experienced a more benign drop in ambient temperature during cold treatments due to the presence of mothers, littermates, and nesting material. Even in the absence of paternal care, this thermal buffering seems to have prevented litters in the paternal care study from recruiting lipid-free BAT to the extent seen in nestlings in the short-term study; mean interscapular weight-specific lipid-free BAT recruitment, for example, was greater for cold-treated individuals in the short-term study than for father-absent

litters in the paternal-care study (Tables 1 and 3). The more benign ambient temperature experienced in the paternal-care study may have permitted the underlying relationship between lipid-free BAT dry weight and body weight to show as a significant regression slope. This argument presumes that it is only under more extreme postnatal thermal stress, as in the short-term study, that smaller nestlings exhibit relatively high levels of BAT recruitment; this supposition remains untested.

The lipid weight per lipid-free BAT dry weight did not vary as a function of litter mean body weight in either deposit or treatment. As was suggested in the short-term study, perhaps body size differences have less of an effect on lipid stores when thermal stress is relatively benign, as it appeared to be in the paternal-care study, resulting in a nonsignificant relationship between lipid weight per lipid-free BAT dry weight and body weight for nestlings treated at nest temperatures. It is unclear, however, why body weight would assume a more important role in determining the lipid content per lipid-free BAT deposit in adult prairie voles in the long-term study.

As revealed by a posteriori examination, nestling voles in this study apparently did not become severely energy-limited due to paternal absence. Litter-averaged

father-absent body weights of 16-day-old nestlings did not differ from father-present litter-mean body weights (unlike body weights observed in the short-term study after 20 days of periodic cold exposure of isolated nestlings). In addition to thermal buffering provided by mothers, littermates, and nesting material described earlier, maternal presence during daily cold exposure may have provided more opportunities for suckling, resulting in a higher energy supply to the nestlings in the paternal care study than in the short-term study. These two factors combined may have resulted in assimilated energy resources in surplus of those needed to meet the primary demands of thermoregulation, and the excess could have been allocated to growth and storage (Perrigo and Bronson, 1985), resulting in equal mean body weights between father-absent and father-present litters. Alternatively, the cold stress experienced by the entire litter in the present study may not have been severe enough to slow key biochemical processes that would have resulted in stunted growth in father-absent litters.

As was observed in the short-term study, sex had no effect on average values for nestlings in a litter for any variables examined in the paternal-care study. Furthermore, litter size did not explain a significant component of the variance for any of these variables: an interesting observation, considering that nestlings were

cold-treated with littermates. Evidently, one littermate, in the presence of a nest of shavings and the mother at 10°C, had the same effect on the ontogeny of BAT in a sibling as would eight littermates.

RESPONSE RELATIONSHIPS BETWEEN BAT DEPOSITS

GENERAL OVERVIEW OF THE PROBLEM

Interscapular BAT is particularly simple to access for removal or manipulation and, because of this, is the only deposit of BAT examined in most research on this tissue. However, 13 regularly occurring BAT deposits have been described in mice and voles (Rauch and Hayward, 1969). The location of these deposits in the cervical and thoracic areas results in regional heterothermy, suggesting preferential warming of vital organs, including the brain (Tarkkonen and Julku, 1968). It seems likely that organs differ in their requirements for thermal homeostasis, and plausible that BAT deposits could show a local "fine tuning" in their thermogenic responses. It has been demonstrated that white adipose tissue dynamics vary regionally in response to short photoperiod in Siberian hamsters and to fasting in humans (Bartness et al. 1989). Mounting evidence suggests that, although easily measurable, changes in interscapular BAT may not

reflect responses of other BAT deposits to environmental stimuli (Gemmell et al., 1978). Anderson and Rauch (1984) found that in adult free-ranging red-backed voles (*Clethrionomys gapperi*) and meadow voles (*Microtus pennsylvanicus*), the ratio of interscapular BAT to total brown fat wet mass varied seasonally, while composition (lipid, water, and protein content) changed only slightly.

It would appear that studies focusing upon multiple BAT deposits could add significantly to our knowledge of BAT ontogeny. Thus a primary goal of this study was to investigate potential variation in responses between different deposits. BAT deposits were positively identified in the prairie voles in our lab based on previous GDP binding assays (Trier, 1994), physical descriptions, and anatomical maps (Rauch and Hayward, 1969). Differentiation between brown and white adipose tissue was facilitated by the distinct coloration and location of each type of tissue. Several BAT deposits were collected:

The first removed was the "*interscapular*" BAT. This deposit is relatively large, bilobed, isolated from other BAT deposits, and located subdermally in the scapular depression.

The second removed was the squamo-occipito-cervical deposit, abbreviated as "*squamosal*" BAT. This is a smaller deposit, located more proximal to the cervical vertebrae than the interscapular deposit, and well-isolated from other deposits by the semispinalis muscles.

Last removed was a group of five individual deposits -- examined collectively -- abbreviated here simply as "*residual*" BAT. Residual BAT included the subscapular, transverse cervical, axillary, jugular and carotid deposits. These deposits, named individually after their locations, accounted for most of the remaining BAT in the animal.

All voles were examined for interscapular and squamosal BAT responses. Because residual BAT was painstaking and time-consuming (1.25 h per animal) to remove, it was measured in only 22 voles, in the short-term study.

OBJECTIVES

The primary goal of this part of the research was to determine whether responses of BAT in the short-term, long-term, and paternal-care studies in nestling *M. ochrogaster* occurred similarly in various deposits. I hypothesized that a positive correlation would exist among

interscapular, squamosal, and residual deposits in weight-specific lipid-free BAT dry weight (LFDW/g) and lipid weight per lipid-free BAT dry weight (LW/LFDW).

A presupposition of this study was that treatment effects in each of the prior studies would have no significant effects on the relationships between deposit types. Treatments included isolated postnatal cold exposure (at 1-20 d of age) assessed after short (21 d) or long (20 week) time periods, and exposure of whole litters, with their mothers, to cold with and without their fathers during the postnatal (1-16 d) period.

METHODS

This study includes a compilation of methods and results from the previous short-term, long-term, and paternal-care studies, with a specific focus on interrelationships among BAT deposit responses. The methods of these studies were described earlier.

To test the presupposition that treatment effects do not affect the relationships among BAT deposit responses in any of the three studies (short-term, long-term, and paternal-care), one-way ANOVAs (SPSS PC+) were computed on ratios of variables for deposit types (e.g., interscapular LW/LFDW divided by squamosal LW/LFDW) to determine whether

treatment effects in the study affected the relationships between deposit types for LFDW/g or LW/LFDW variables.

Using data combined from both treatments, correlation analysis (i.e. scattergrams, product-moment correlation coefficients, and *t*-tests of significance of correlation) was used to examine the degree of association among BAT deposit types for weight-specific lipid-free BAT dry weight (LFDW/g) and lipid weight per lipid-free BAT dry weight (LW/LFDW).

RESULTS

A PRIOR COMPARISONS

The following results respond to the a priori hypotheses presented in the OBJECTIVES section.

The presupposition that treatment effects do not affect the relationships among BAT deposit responses in any of the three studies was supported. The relationships between deposit types were not significantly affected by treatment in the short-term, long-term, and paternal-care studies, even when treatment effects on BAT within individual deposits were significant. Therefore, data were pooled across treatments in each study for the remaining analyses.

The hypothesis that a positive correlation exists among interscapular, squamosal, and residual deposits for weight-specific lipid-free BAT dry weight was supported for data pooled across treatments for each study. Table 4 displays the correlation coefficients and probability statistics for each of these comparisons. Scattergrams are plotted for each of the associations among deposit types in Figures 30-32 for short-term LFDW/g data, Figure 33 for long-term LFDW/g data, and Figure 34 for paternal-care study data.

The hypothesis that a positive correlation exists among interscapular, squamosal, and residual deposits for lipid weight per lipid-free BAT dry weight was supported for data pooled across treatments for each study. Correlation coefficients and probability statistics for each of these comparisons are presented in Table 5. Scattergrams are plotted for each of the associations among deposit types in Figures 35-37 for short-term LW/LFDW data, Figure 38 for long-term LW/LFDW data, and Figure 39 for paternal-care study LW/LFDW data.

A POSTERIORI COMPARISON

Per lipid-free BAT dry weight, residual BAT deposits may contain more lipid than interscapular BAT, and interscapular BAT more than squamosal BAT. While

Table 4. Results from the response-relationships study for weight-specific lipid free BAT dry weight (LFDW/g). N is the number of animals analyzed (cold-treated and control groups combined). r is the product-moment correlation coefficient. p is the probability that LFDW/g values for the two deposit types examined are uncorrelated.

Study	Deposit types	r	p	N
Short-term	Interscapular x Residual	0.854	<0.001	20
	Interscapular x Squamosal	0.742	<0.001	52
	Residual x Squamosal	0.610	0.002	20
Long-term	Interscapular x Squamosal	0.549	<0.001	97
Paternal-care	Interscapular x Squamosal	0.719	<0.001	52

Figure 30. Short-term study: Correlation between weight-specific lipid-free BAT dry weight (LFDW/g) values for squamosal and interscapular BAT in cold-treated (closed circles) and control animals (open circles).

Figure 30

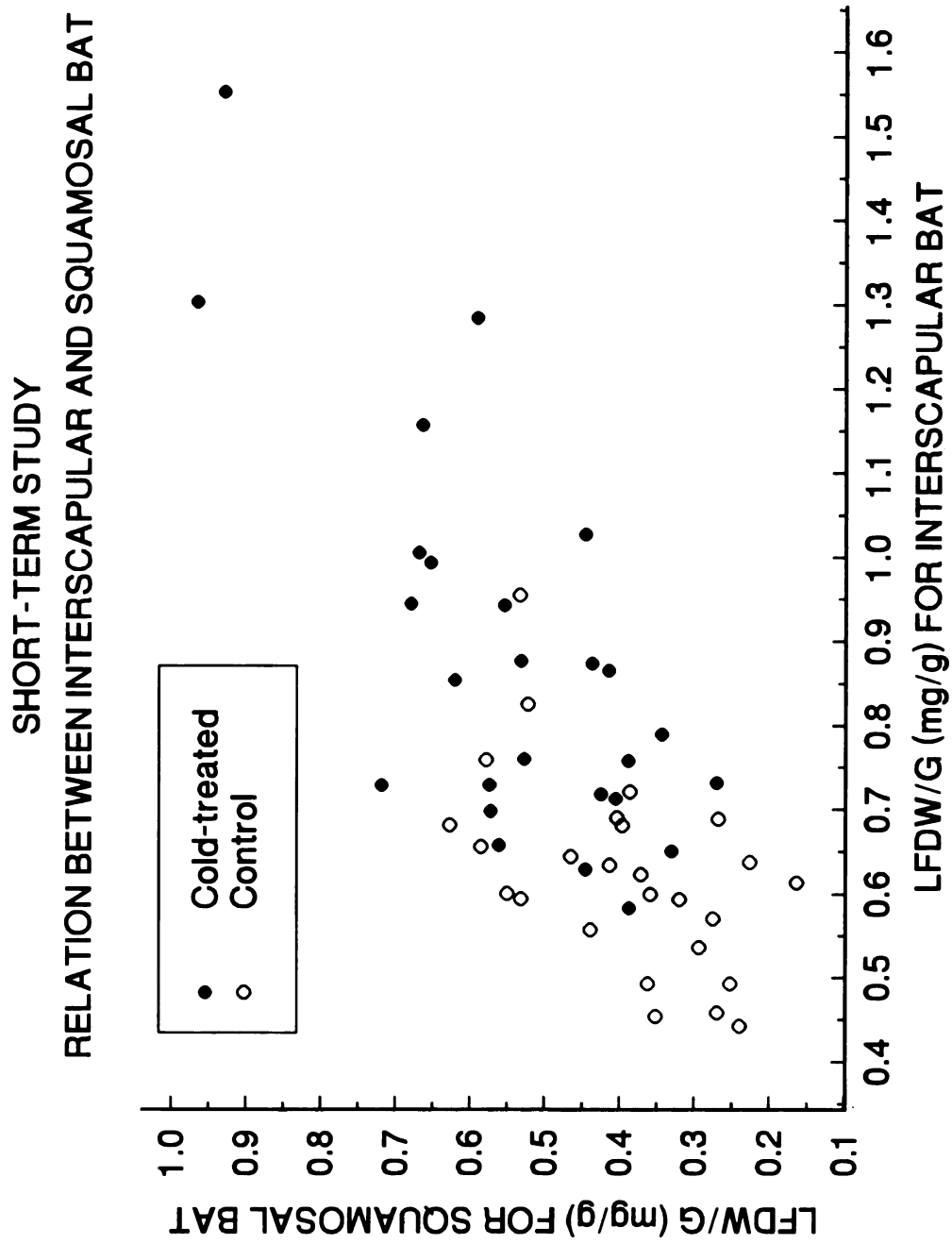

SHORT-TERM STUDY
RELATION BETWEEN INTERSCAPULAR AND SQUAMOSAL BAT
Cold-treated
Control
LFDW/G (mg/g) FOR SQUAMOSAL BAT
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
LFDW/G (mg/g) FOR INTERSCAPULAR BAT

Figure 31. Short-term study: Correlation between weight-specific lipid-free BAT dry weight (LFDW/g) values for squamosal and residual BAT in cold-treated (closed circles) and control animals (open circles).

Figure 31

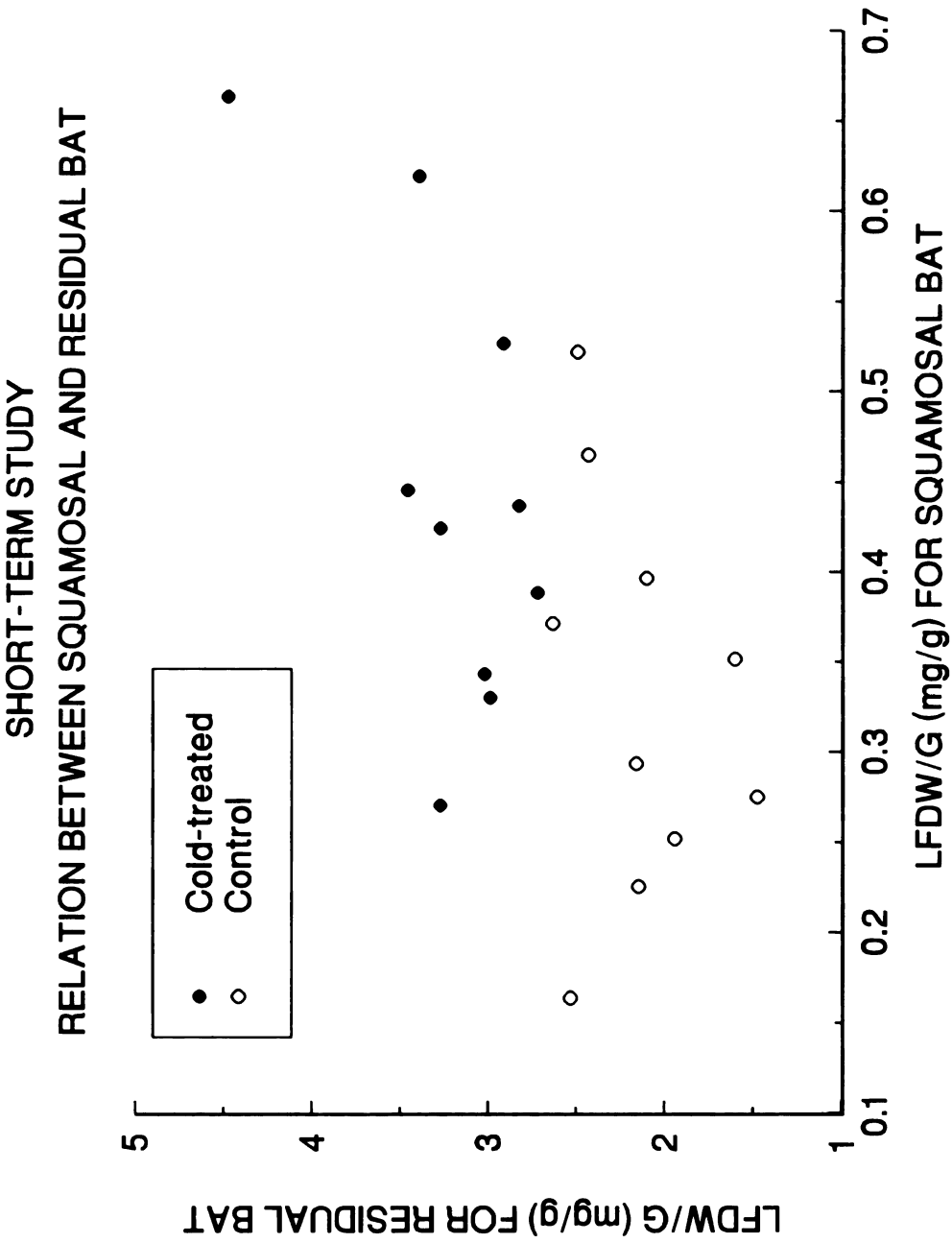

SHORT-TERM STUDY
RELATION BETWEEN SQUAMOSAL AND RESIDUAL BAT
Cold-treated
Control
LFDW/G (mg/g) FOR RESIDUAL BAT
LFDW/G (mg/g) FOR SQUAMOSAL BAT
1
2
3
4
5
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Figure 32. Short-term study: Correlation between weight-specific lipid-free BAT dry weight (LFDW/g) values for interscapular and residual BAT in cold-treated (closed circles) and control animals (open circles).

Figure 32

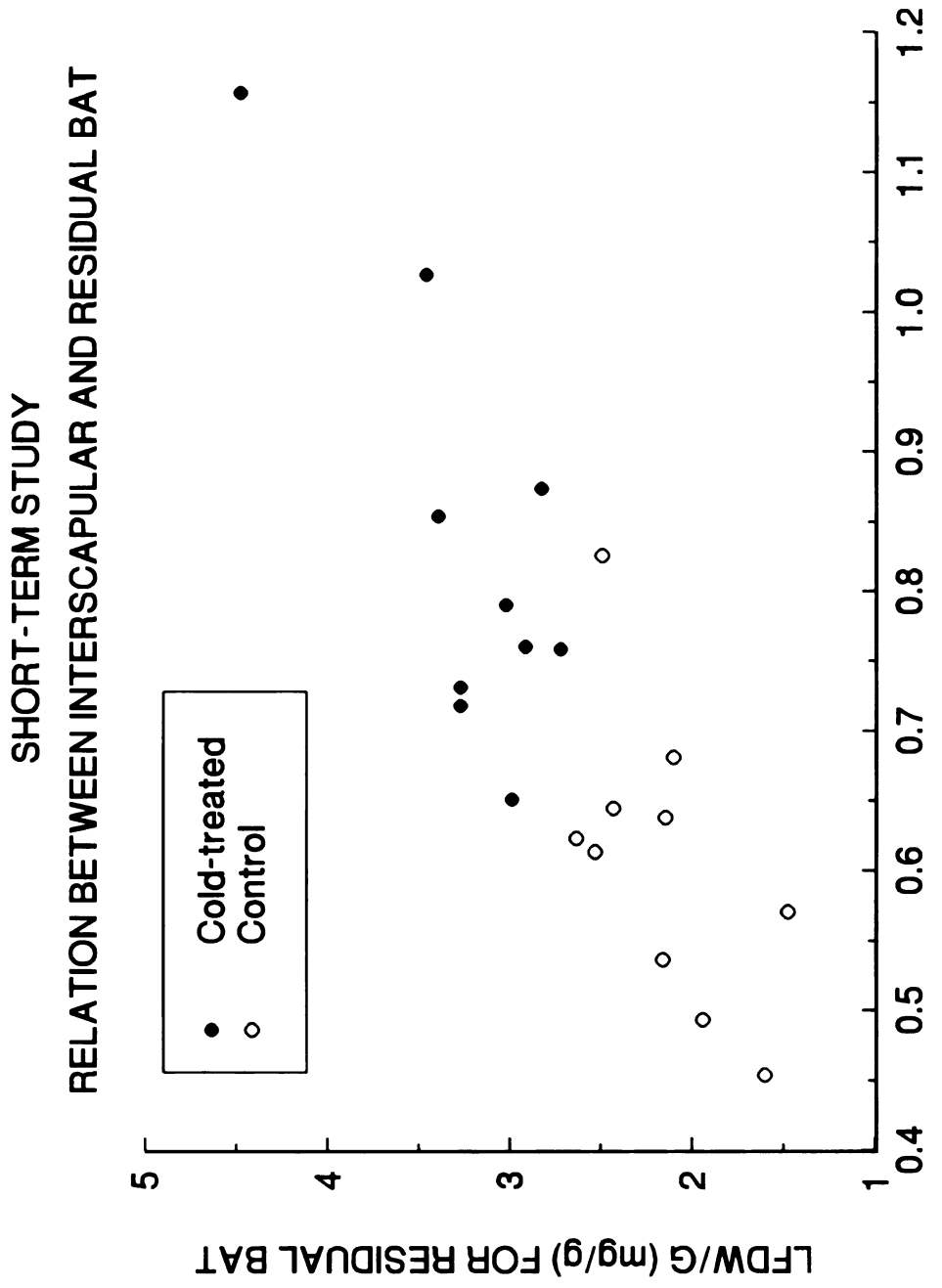

SHORT-TERM STUDY
RELATION BETWEEN INTERSCAPULAR AND RESIDUAL BAT
Cold-treated
Control
LFDW/G (mg/g) FOR RESIDUAL BAT
LFDW/G (mg/g) FOR INTERSCAPULAR BAT
1
2
3
4
5
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2

Figure 33. Long-term study: Correlation between weight-specific lipid-free BAT dry weight (LFDW/g) values for interscapular and squamosal BAT in cold-treated (closed circles) and control animals (open circles).

Figure 33

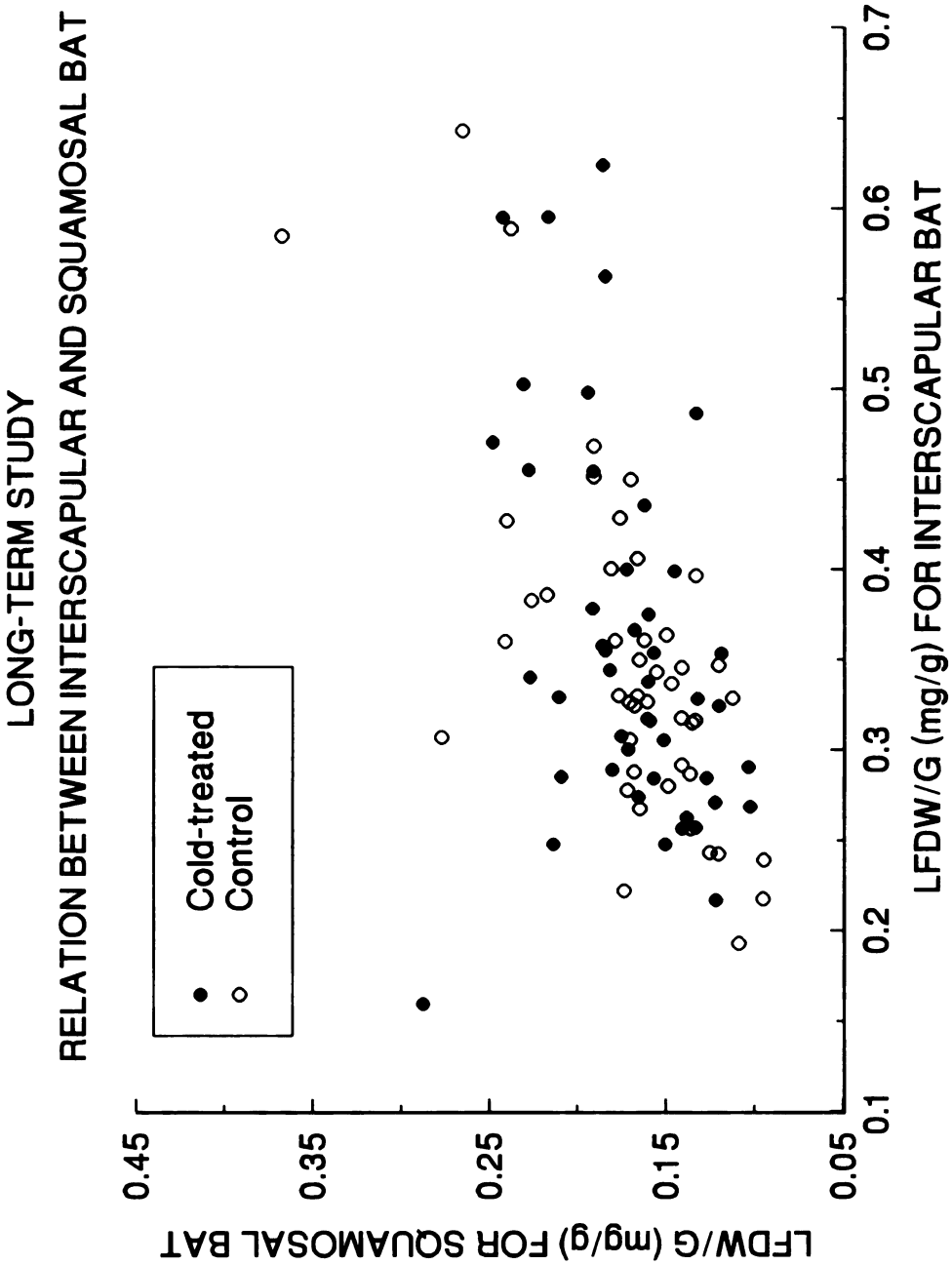

LONG-TERM STUDY
RELATION BETWEEN INTERSCAPULAR AND SQUAMOSAL BAT
Cold-treated
Control
LFDW/G (mg/g) FOR SQUAMOSAL BAT
0.45
0.35
0.25
0.15
0.05
0.1
0.2
0.3
0.4
0.5
0.6
0.7
LFDW/G (mg/g) FOR INTERSCAPULAR BAT

Figure 34. Paternal-care study: Correlation between weight-specific lipid-free BAT dry weight (LFDW/g) values for interscapular and squamosal BAT in father-absent (closed circles) and father-present litters (open circles).

Figure 34

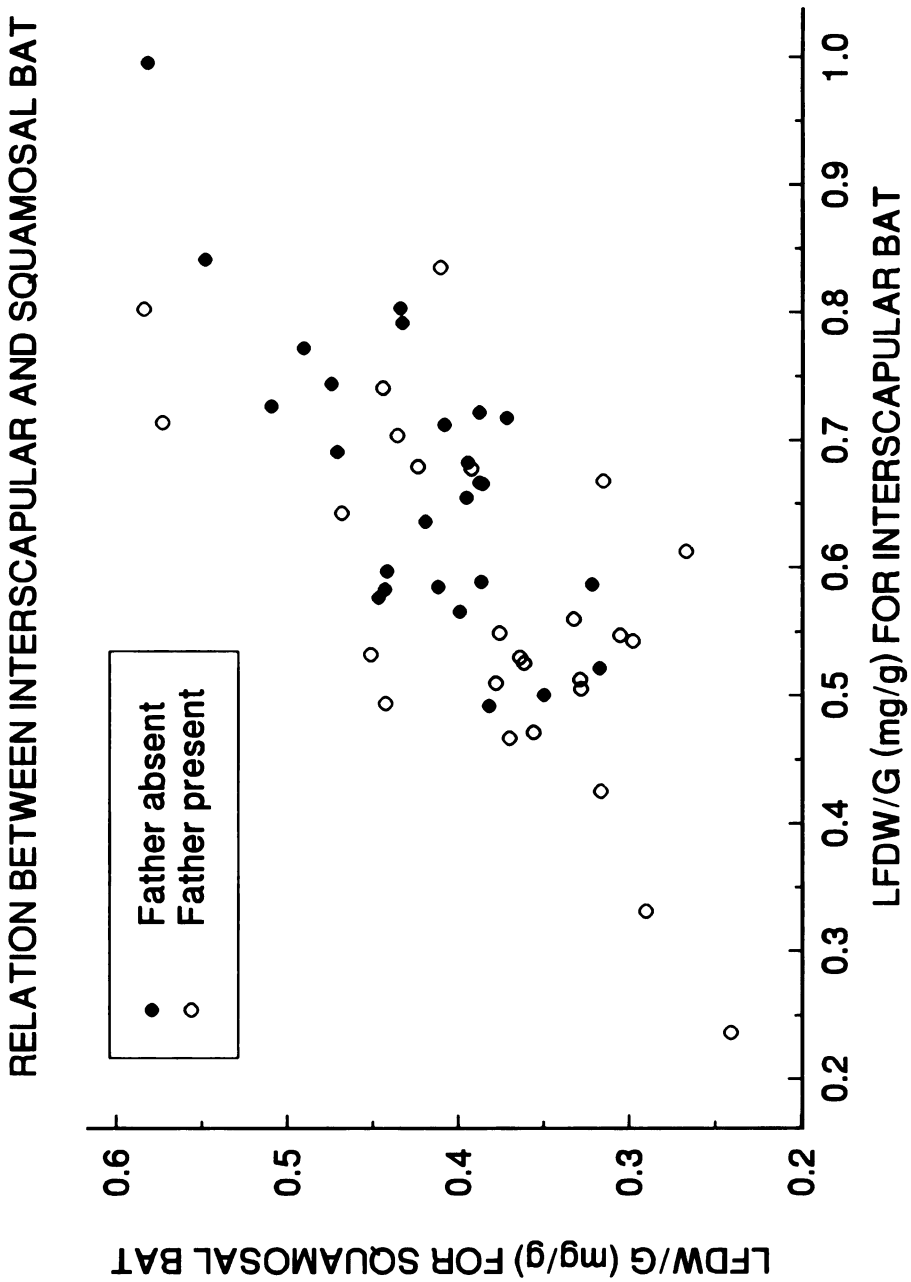

PATERNAL-CARE STUDY
RELATION BETWEEN INTERSCAPULAR AND SQUAMOSAL BAT
Father absent
Father present
LFDW/G (mg/g) FOR SQUAMOSAL BAT
LFDW/G (mg/g) FOR INTERSCAPULAR BAT
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Table 5. Results from the response-relationships study for lipid weight per lipid-free BAT dry weight (LW/LFDW). N is the number of animals analyzed (cold-treated and control groups combined). r is the product-moment correlation coefficient. p is the probability that LW/LFDW values for the two deposit types examined are uncorrelated.

Study	Deposit types	r	p	N
Short-term	Interscapular x Residual	0.084	<0.001	20
	Interscapular x Squamosal	0.847	<0.001	52
	Residual x Squamosal	0.715	<0.001	20
Long-term	Interscapular x Squamosal	0.801	<0.001	97
Paternal-care	Interscapular x Squamosal	0.677	<0.001	52

Figure 35. Short-term study: Correlation between lipid weight per lipid-free BAT dry weight (LW/LFDW) values for interscapular and residual BAT in cold-treated (closed circles) and control animals (open circles).

Figure 35

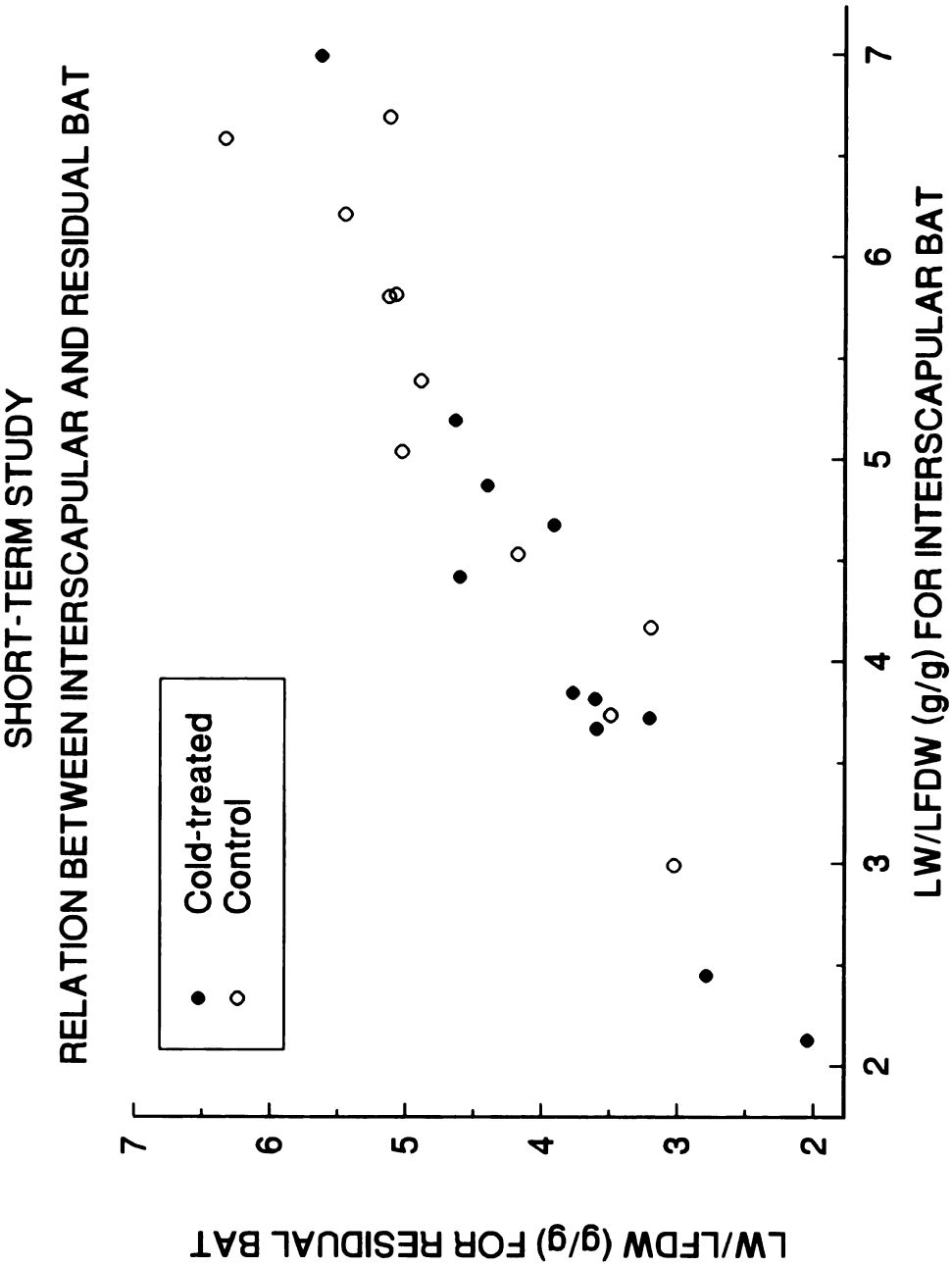

SHORT-TERM STUDY
RELATION BETWEEN INTERSCAPULAR AND RESIDUAL BAT
Cold-treated
Control
LW/LFDW (g/g) FOR RESIDUAL BAT
LW/LFDW (g/g) FOR INTERSCAPULAR BAT
2
3
4
5
6
7

Figure 36. Short-term study: Correlation between lipid weight per lipid-free BAT dry weight (LW/LFDW) values for interscapular and squamosal BAT in cold-treated (closed circles) and control animals (open circles).

Figure 36

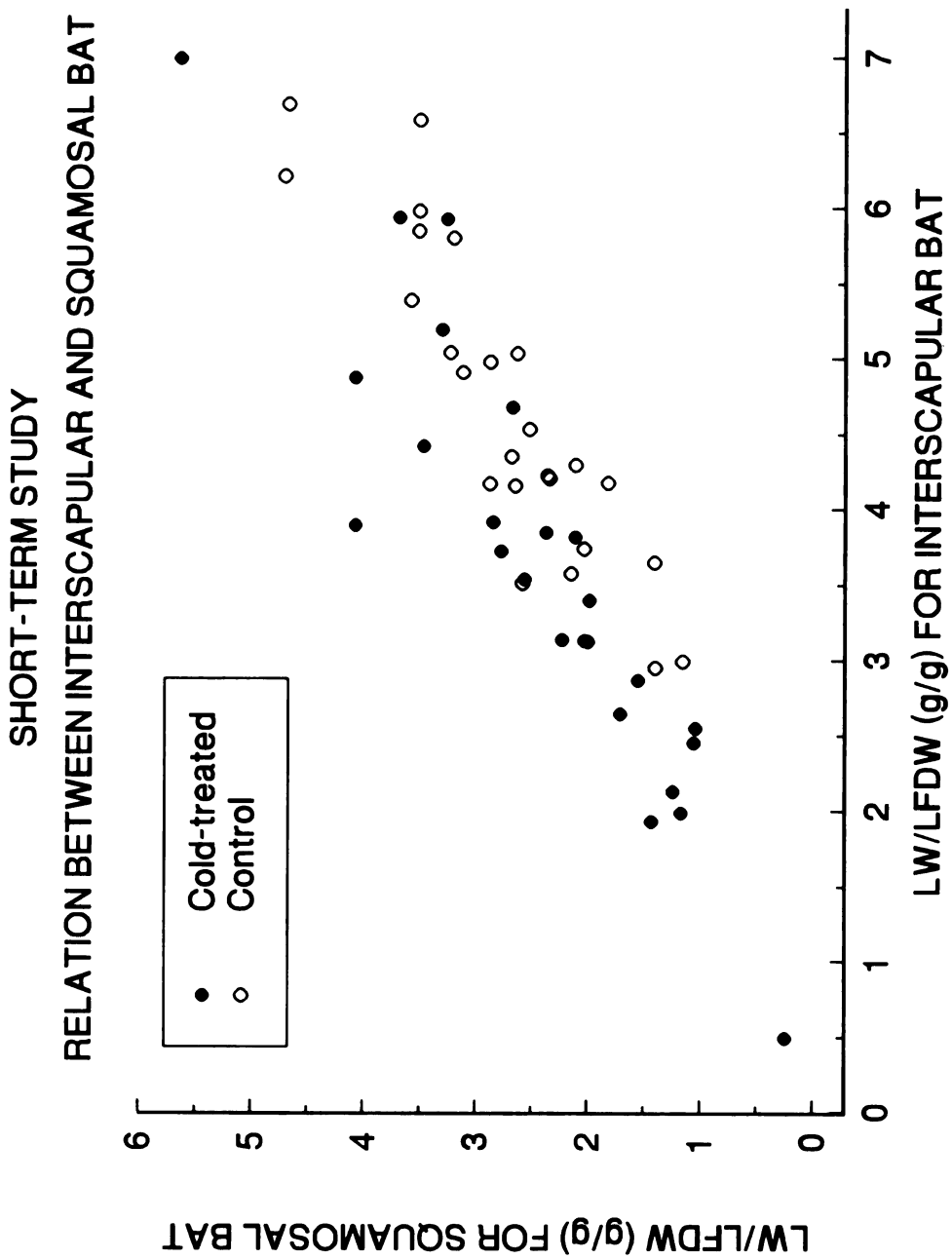

SHORT-TERM STUDY
RELATION BETWEEN INTERSCAPULAR AND SQUAMOSAL BAT
Cold-treated
Control
LW/LFDW (g/g) FOR SQUAMOSAL BAT
LW/LFDW (g/g) FOR INTERSCAPULAR BAT
0
1
2
3
4
5
6
7

Figure 37. Short-term study: Correlation between lipid weight per lipid-free BAT dry weight (LW/LFDW) values for residual and squamosal BAT in cold-treated (closed circles) and control animals (open circles).

Figure 37

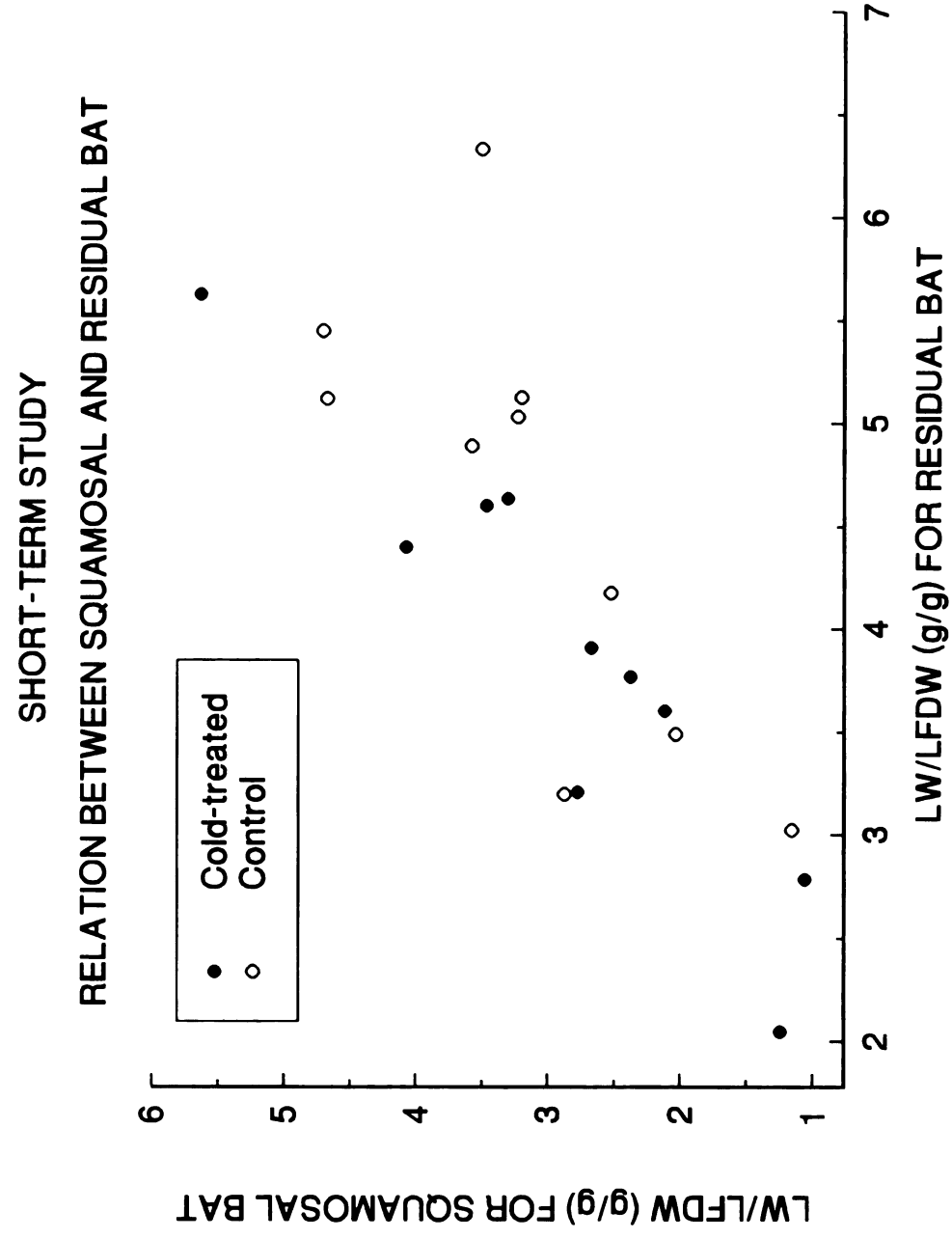

SHORT-TERM STUDY
RELATION BETWEEN SQUAMOSAL AND RESIDUAL BAT
Cold-treated
Control
LW/LFDW (g/g) FOR SQUAMOSAL BAT
LW/LFDW (g/g) FOR RESIDUAL BAT
1
2
3
4
5
6
2
3
4
5
6
7

Figure 38. Long-term study: Correlation between lipid weight per lipid-free BAT dry weight (LW/LFDW) values for interscapular and squamosal BAT in cold-treated (closed circles) and control animals (open circles).

Figure 38

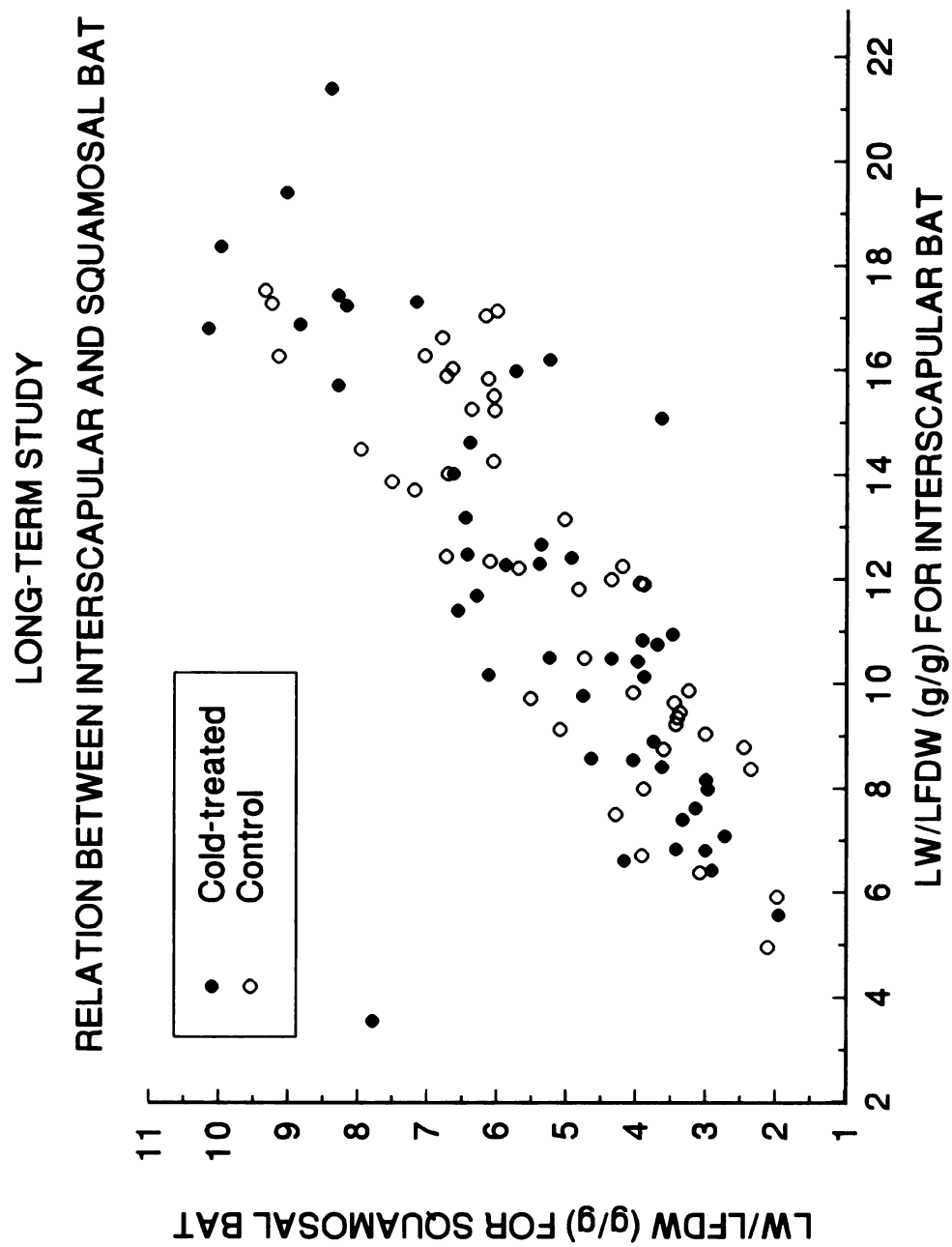

LONG-TERM STUDY
RELATION BETWEEN INTERSCAPULAR AND SQUAMOSAL BAT
Cold-treated
Control
LW/LFDW (g/g) FOR SQUAMOSAL BAT
LW/LFDW (g/g) FOR INTERSCAPULAR BAT
11
10
9
8
7
6
5
4
3
2
1
2
4
6
8
10
12
14
16
18
20
22

Figure 39. Paternal-care study: Correlation between lipid weight per lipid-free BAT dry weight (LW/LFDW) values for interscapular and squamosal BAT in father-absent (closed circles) and father-present litters (open circles).

Figure 39

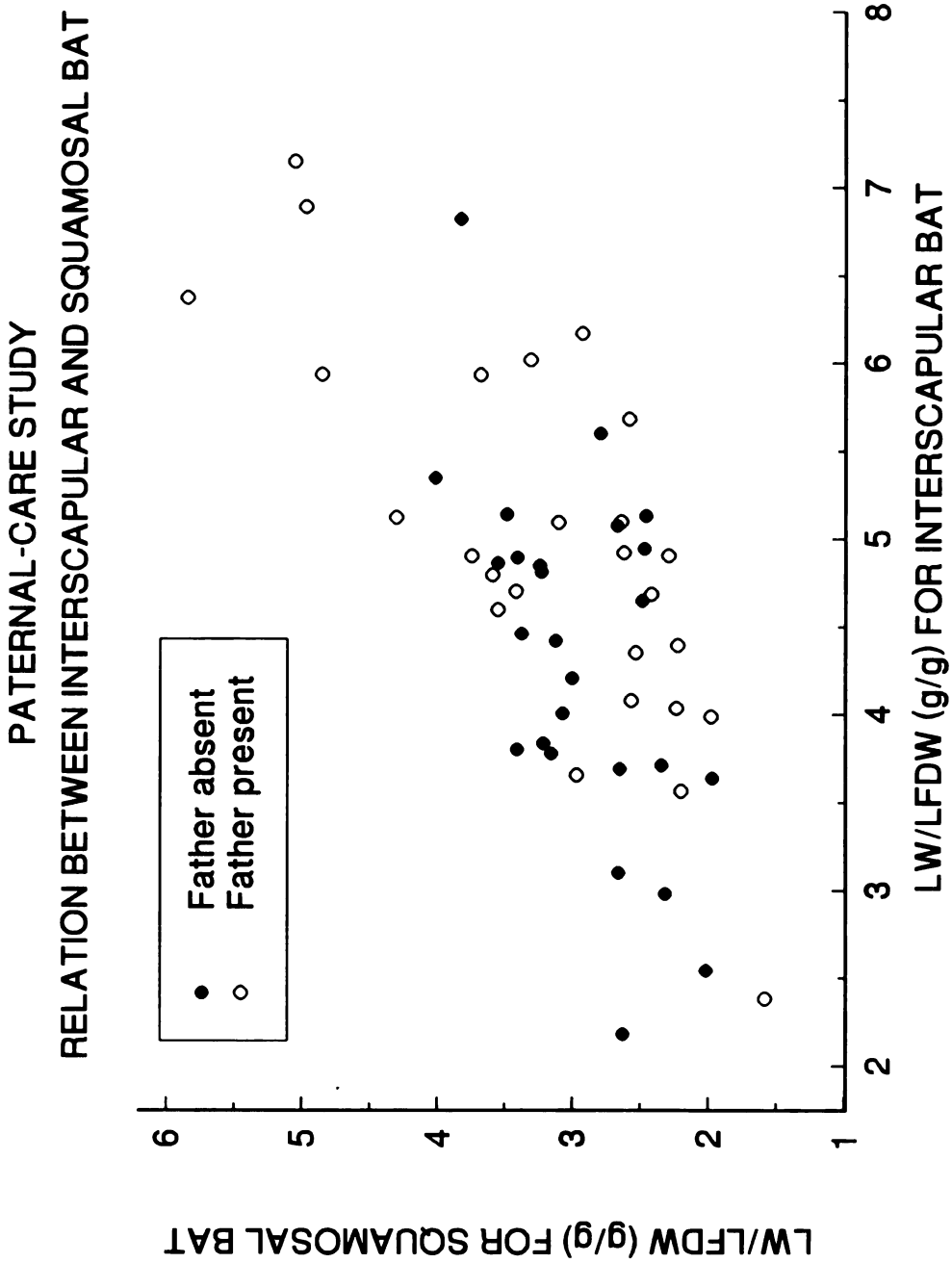

PATERNAL-CARE STUDY
RELATION BETWEEN INTERSCAPULAR AND SQUAMOSAL BAT
Father absent
Father present
LW/LFDW (g/g) FOR SQUAMOSAL BAT
LW/LFDW (g/g) FOR INTERSCAPULAR BAT
1
2
3
4
5
6
7
8
2
3
4
5
6

Figure 40. Short-term study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles) and squamosal BAT (open circles) for control animals.

Figure 40

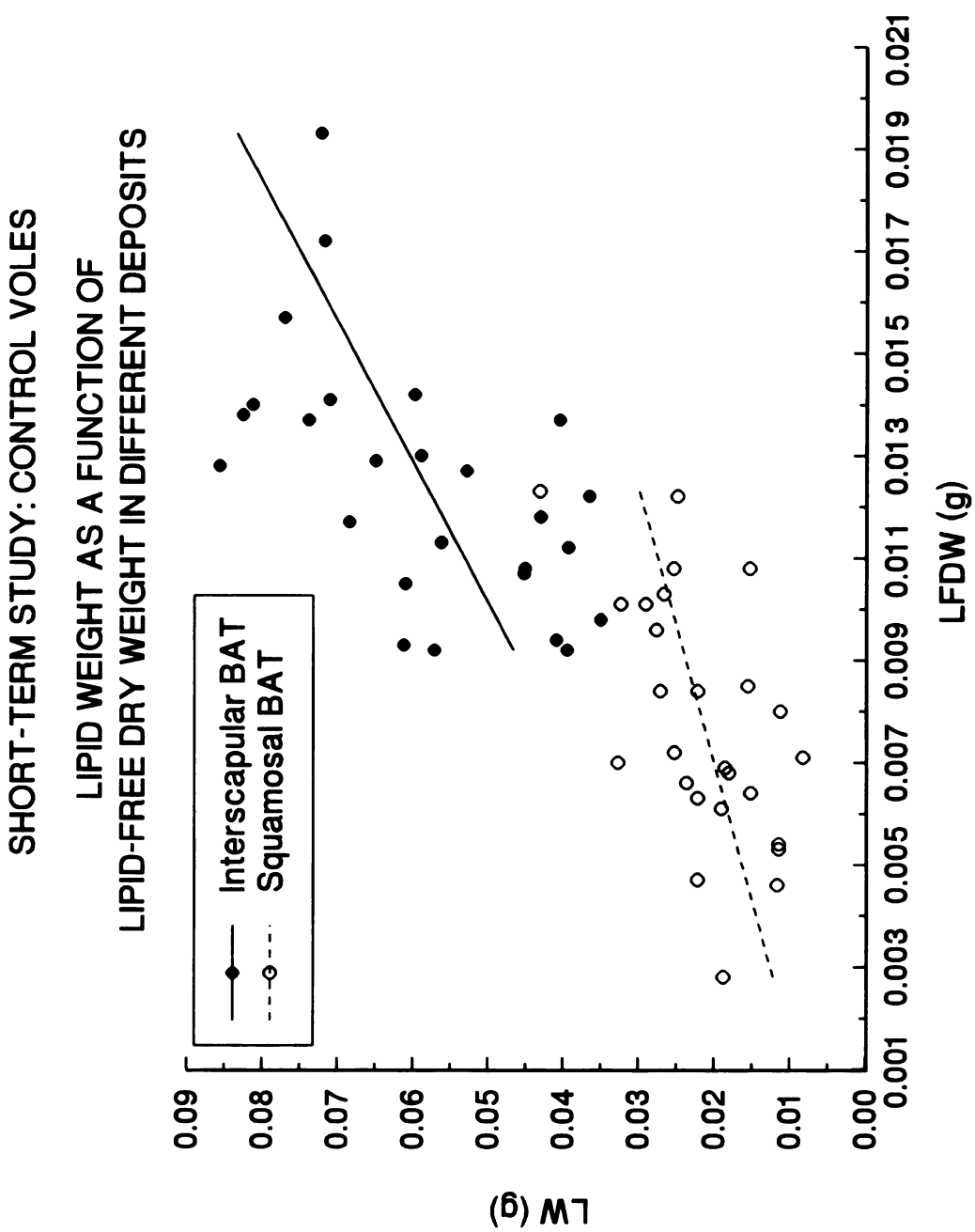

SHORT-TERM STUDY: CONTROL VOLES
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
LW (g)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.001
0.003
0.005
0.007
0.009
0.011
0.013
0.015
0.017
0.019
0.021
LFDW (g)

Figure 41. Short-term study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles) and squamosal BAT (open circles) for cold-treated animals.

Figure 41

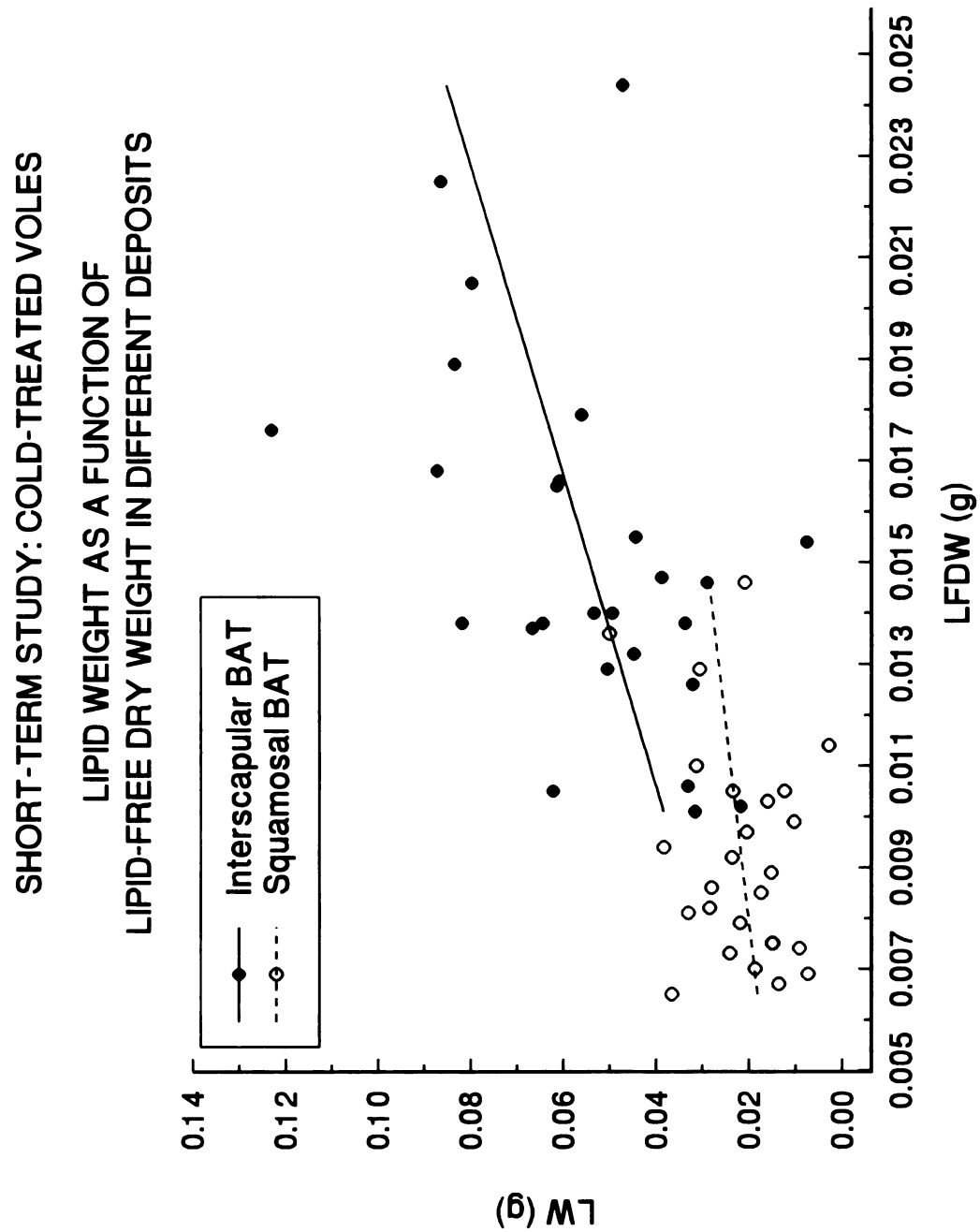

SHORT-TERM STUDY: COLD-TREATED VOLES
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
LW (g)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.005 0.007 0.009 0.011 0.013 0.015 0.017 0.019 0.021 0.023 0.025
LFDW (g)

Figure 42. Long-term study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles) and squamosal BAT (open circles) for control animals.

Figure 42

LONG-TERM STUDY: CONTROL VOLES
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
LW (g)
LFDW (g)
0.5
0.4
0.3
0.2
0.1
0.0
0.000
0.005
0.010
0.015
0.020
0.025
0.030

Figure 43. Long-term study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles) and squamosal BAT (open circles) for cold-treated animals.

Figure 43

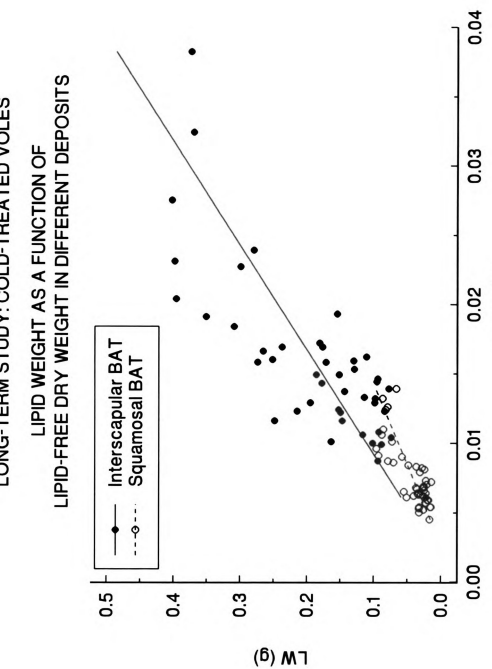

LONG-TERM STUDY: COLD-TREATED VOLES
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
LW (g)
LFDW (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.00
0.01
0.02
0.03
0.04

Figure 44. Paternal-care study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles) and squamosal BAT (open circles) for father-absent (FA) litters.

Figure 44

PATERNAL-CARE STUDY: FATHER-ABSENT LITTERS
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
LW (g)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.002 0.004 0.006 0.008 0.010 0.012 0.014 0.016 0.018 0.020
LFDW (g)
Interscapular BAT
Squamosal BAT

Figure 45. Paternal-care study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles) and squamosal BAT (open circles) for father-present (FP) litters.

Figure 45

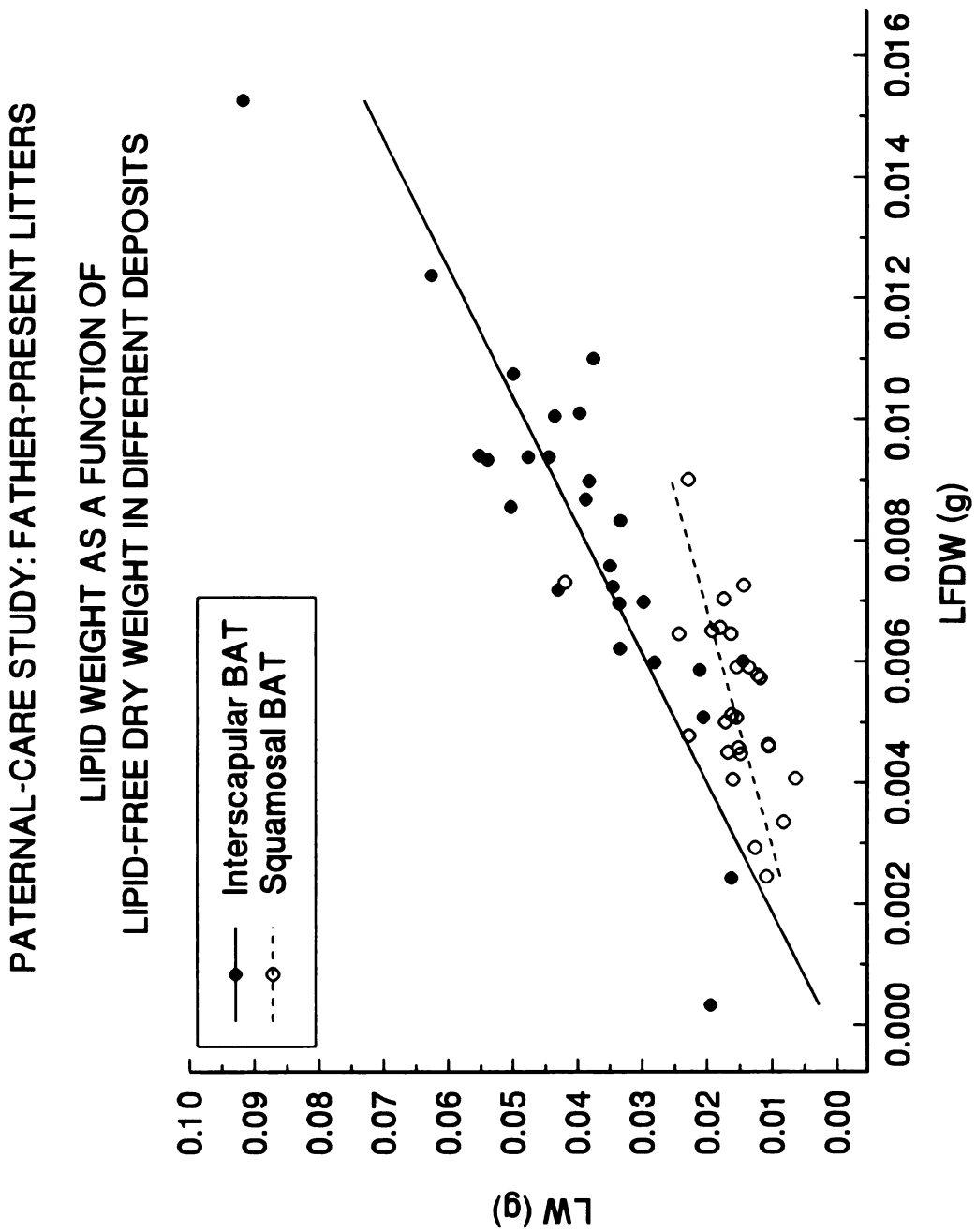

PATERNAL-CARE STUDY: FATHER-PRESENT LITTERS
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
LW (g)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0.014
0.016
LFDW (g)

Figure 46. Short-term study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles), squamosal (open circles) and residual (triangles) BAT for control animals.

Figure 46

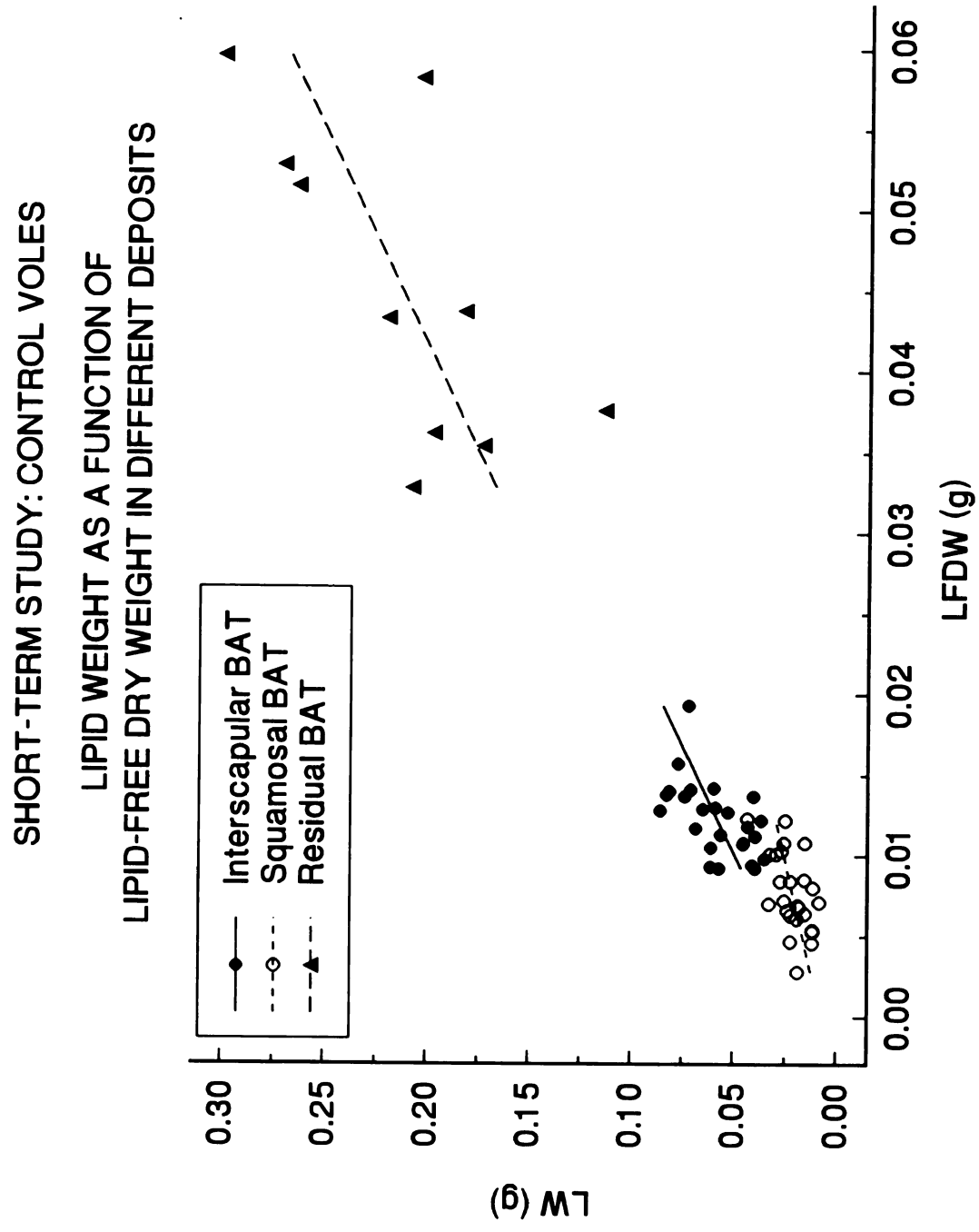
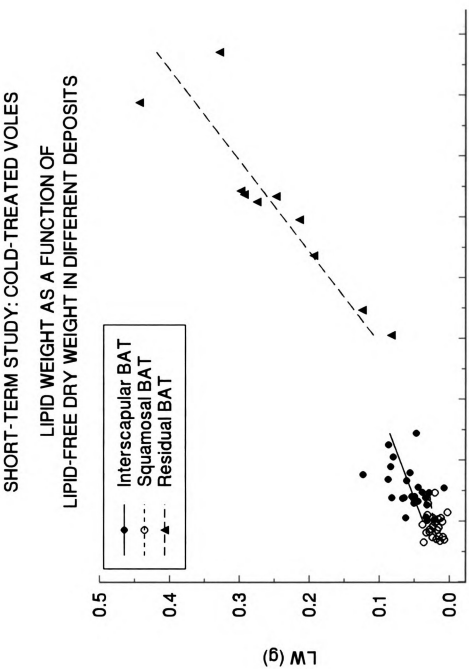

SHORT-TERM STUDY: CONTROL VOLES
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
Residual BAT
LW (g)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.00
0.01
0.02
0.03
0.04
0.05
0.06
LFDW (g)

Figure 47. Short-term study: Lipid weight (LW) plotted against lipid-free dry weight (LFDW) for interscapular (closed circles), squamosal (open circles) and residual (triangles) BAT for cold-treated animals.

Figure 47


SHORT-TERM STUDY: COLD-TREATED VOLES
LIPID WEIGHT AS A FUNCTION OF
LIPID-FREE DRY WEIGHT IN DIFFERENT DEPOSITS
Interscapular BAT
Squamosal BAT
Residual BAT
LW (g)
LFDW (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09

examining scattergrams for the a priori comparisons just described, it was noticed that lipid weight relative to lipid-free BAT dry weight appeared to differ consistently between deposit types across the short-term, long-term, and paternal-care studies. To explore this possibility, regressions of LW against LFDW for different deposit types were plotted together. As seen in Figures 40-45, in every treatment in each of the three studies, interscapular tissue seemed to have higher LW per LFDW than squamosal tissue. Residual BAT may be even higher in lipid content than interscapular BAT per LFDW (Figures 46-47).

DISCUSSION

In an investigation of changes in BAT in response to various ambient temperatures in adult albino mice, Heldmaier (1974) observed differences in response between deposits dissected from four regions. Because he measured weights on freshly dissected tissue, however, it is difficult to interpret whether these differences represent regional BAT thermogenic specialization, or differences in lipid content or degree of hydration. The results of the present study indicate that *responses* in the various deposits are associated; therefore, changes in weight-specific lipid-free BAT dry weight and lipid weight per lipid-free BAT dry weight measured in the interscapular deposit probably are indicative of responses in other BAT

deposits in the cervical and thoracic regions. Associations among BAT deposit responses are seen in both nestlings and adults, and do not appear to depend on the degree of thermal stress in *M. ochrogaster*.

However, a few differences among deposits were observed as well. Interscapular tissue appears to contain an intermediate concentration of lipid per lipid-free BAT dry weight in prairie voles, regardless of age and thermal conditions. Furthermore, during cold stress in nestlings, squamosal BAT exhibits greater retention of lipid content per lipid-free BAT dry weight than interscapular BAT. These differences may reflect unique processes and functional specializations in different BAT deposits which await further study.

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