The effects of day length and ambient temperature on the growth and structure of adipose tissue and other organs in the dwarf hamster *P. campbelli*

Thesis

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THE EFFECTS OF DAY LENGTH AND AMBIENT TEMPERATURE ON THE GROWTH AND STRUCTURE OF ADIPOSE TISSUE AND OTHER ORGANS IN THE DWARF HAMSTER *P. CAMPBELLI*

by

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Experimental Environments.

CSD	Cold short day.
WSD	Warm short day.
CLD	Cold long day.
WLD	Warm long day.

Adipose tissue.

BAT	Brown adipose tissue.
WAT	White adipose tissue.

Adipose tissue depots.

ATG	Mesenteric and ommental.
DWA	Dorsal wall of abdomen.
POP	Popliteal.
OV	Attached to ovaries.
EP	Attached to testes.
UNM	Under neck muscles.
TB	Thoracic brown.
TW	Thoracic white.
HB	Interscapular brown.
HW	Interscapular white.
BA	Behind fore-arm.
IFS	In front of shoulder.
GS	Groin side.
GV	Groin ventral.
BOT	Base of tail.

Pelt Areas.

FORE	Behind the ears.
RUMP	Above the tail.
V/THOR	Upper ventral thorax.

Physiological.

NST	Non-shivering thermogenesis.
BMR	Basal metabolic rate.

Chemicals, reagents, etc.

UCP	Uncoupling protein.
TBS	Tris. buffered saline.
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
EDTA	Ethylenediamine tetraacetic acid, sodium salt.
TEMED	Tetramethylethylenediamine.
APS	Ammonium persulphate.
SDS	Sodium dodecyl sulphate.
Tween 20	Polyoxyethylene-sorbitan monolaurate.
DAB	Diaminobenzidine tetrahydrochloride.
DSMP	Dried skimmed milk powder (e.g. Marvel).

ABSTRACT

The effects of photoperiod and ambient temperature on body mass and composition were studied in *Phodopus campbelli*.

Adult hamsters were studied for 102 d around the mid-winter solstice (1991-1994), under four different combinations of photoperiod and environmental temperature, 24 °C 16 h light/8 h dark, WLD ('simulated summer'), 24 °C (8 h light/16 h dark, WSD, cold 10 °C 16 h light/8 h dark, CLD, 10 °C 8 h light/16 h dark, CSD ('simulated winter'). Specimens were caged individually, with rat chow and water available *ad lib.*

Body mass and food intake were measured weekly. At the end of the experimental period, the mass and composition of 15 adipose depots, mass of the major organs, the colour, extractable lipid and hair length of the pelt were measured.

Females ate relatively more food than their male counterparts. Hamsters on CLD ate the most, followed by CSD hamsters, those on WSD and WLD ate significantly less. Males on short photoperiod lost most weight, due mainly to depletion of adipose tissue. In both long day environments, the males maintained their body mass. Maximum decrease in lipid content and increase in protein content occurred in the adipose tissue of hamsters on CSD, especially in brown adipose tissue. The pelt became light grey in 'winter', probably the best camouflage for an arid climate. Changes in extractable lipid may be related to insulation: the sparser the hairs, the more lipid recovered from them.

I conclude that photoperiod was the dominant influence on body size and lipid stores, but temperature interacted to affect many of the features studied. The hamsters' appetite adjusted to maintain body mass and composition. Photoperiod is the more reliable cue than weather for wild *Phodopus* for the physiological adaptations required for surviving in the cold Mongolian desert.

1.0 INTRODUCTION.

In order for small mammals to survive the extreme climatic changes that occur in their harsh natural environment, they need cues for physiological adaptation. These cues can take the form of the seasonal changes in daylength (photoperiod), that usually precede changes in environmental temperature.

1.1 Choice of species and its biology in the wild.

Phodopus campbelli, first described by Thomas in 1905, was chosen for this project, because it breeds readily under laboratory conditions and adapts easily to environmental extremes. *Phodopus sungorus,* first described by Pallas in 1770 has been more widely studied (Heldmaier and Ruf, 1992, Ruf, *et al.*, 1993, Youngstrom and Bartness, 1995) and has the advantage of providing opportunities for comparison between the two species. A literary search for the years 1994-98 yielded just 29 citations for *P. campbelli*, but 75 citations for *P. sungorus*.

P. sungorus is physically very similar to *P. campbelli*, hence they were once deemed sub-species. *P. campbelli*, also known as the Djungarian, dwarf, Russian, or furry-footed hamster. Information about this species has been mixed with that from what was once recognised as its sub-species *P. sungorus*. However *P. campbelli* is now deemed a separate species from *P. sungorus* by the majority of researchers in the west and the former USSR.

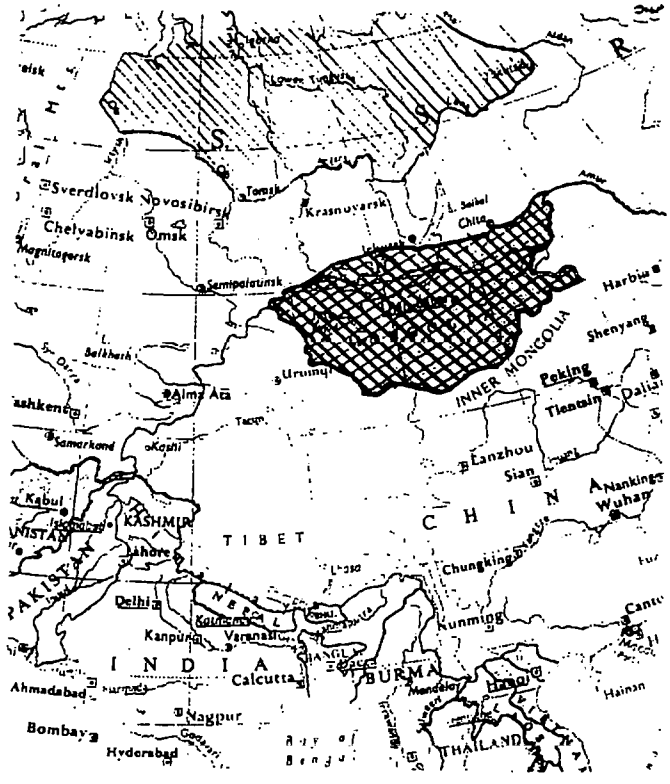
In spite of similarities in appearance, habits and range, *P. sungorus* and *P. campbelli* are true species. If they are allowed to interbreed in captivity, the hybrid offspring are infertile (Yudin *et al.*, 1979). Wynne-Edwards and Lisk (1987a) described several significant behavioural differences in the laboratory, between *P. campbelli* and *P. sungorus,* confirming their status as a distinct species.

The major well documented differences between the species are the effects of temperature and daylength on body mass and coat colour. The colour change of *P. sungorus* is extreme. *P. sungorus,* like *P. campbelli,* are dark grey in summer, but although after prolonged exposure to winter conditions, *P. sungorus* become nearly white (Figala *et al.*, 1973; Vorontsov *et al.*, 1967), there is no documented pelage colour change for *P. campbelli.* The classification was extremely confused, and in some

cases, it was only possible to decipher which species was used in an experimental paper if a pelage change was mentioned (Pilsborough, 1971; Heldmaier and Hoffmann, 1974; Hoffman and Illernova, 1986; Yellon and Goldman, 1984).

Weiner and Heldmaier (1987) observed that adult *P. campbelli* had different behavioural responses in the cold to *P. sungorus* under laboratory conditions. They suggested that the differences evolved because the former species is native to a less harsh environment, that extended far enough east to be under the influence of the temperate Pacific Ocean. Since, as Fig. 1.1.1 shows, the natural range of *P. campbelli* does not extend far beyond Mongolia, which has a severe climate, it seems more likely that these different responses to cold should be interpreted as fundamental differences between species, adapted to different habitats.

Fig. 1.1.1 Geographical range of *P. sungorus* and *P. campbelli.*



\\\\ *P. sungorus.* XXXX *P. campbelli.*

P. campbelli are now found wild in pockets of the arid desert steppes of Mongolia (Shaborte, latitude 42° 40' N) and southernmost parts of Siberia (Fig. 1.1.1), for example the Tuvinskaya and Trans-Baikal regions, with the Altai Sayan mountain range forming the extreme western and north-western border (personal communication, Wynne-Edwards). The natural range of *P. campbelli* has a severe

climate, with permafrost occurring in many places. The temperature ranges between 10 ^{o}C to 26.7 ^{o}C in summer and -15 ^{o}C to -30 ^{o}C in winter. Average summer rainfall was found to be 172±47 mm, measured during 1980-1988 at a weather station near Erzin, Tuva (latitude 50.16 ^{o}N), (Scribner and Wynne-Edwards, 1994). There is no recorded rainfall in the winter.

Wynne-Edwards (personal communication) was unable to find specimens in Khazakstan, northern China or Manchuria, where the species was once believed to occur. *P. sungorus* and *P. campbelli* are never sympatric in the wild (Flint, 1966).

P. sungorus naturally inhabit the Siberian steppes (Fig. 1.1.1), which is also an area with a severe climate and extreme temperatures, often above 35 ^{o}C in the summer and below -35 ^{o}C in the winter (Heldmaier *et al.*, 1985). Average summer rainfall was found to be 312±57 mm, measured during 1980-1988 at a weather station near Abakan, Hakasskaya region (latitude 53.43 ^{o}N), (Scribner and Wynne-Edwards, 1994).

1.2 General biology of *Phodopus* sp.

Phodopus sp. are small mammals of the order Rodentia, suborder Myomorpha, superfamily Muroidea, family Cricetidae and genus Cricetini (hamster). They have a body length of 53-102 mm, with a tail length of 6-18 mm (Piechocki, 1968). They are omnivorous and spend a lot of their time undercover - semi-fossorial (Flint, 1966 and Wynne-Edwards and Lisk, 1983). When caught in their natural habitat, their (usually full) cheek (buccal) pouches have been found to contain grass seeds, roots, shrubs, beetles, spiders, snails and other invertebrates. They only eat fresh plant material when their normal diet is in short supply (Piechocki, 1968). In the winter they inhabit burrows abandoned by other animals, especially pikas (*Ochotona sp.*), where they maintain a well-padded nest room and a food store (Piechocki, 1968). During the rest of the year, they shelter from the weather and predators under the stiff tundra grasses. In the wild, they are seasonal breeders, producing young from March through to September (Piechocki, 1968), but in the laboratory, on a photoperiod of 12 h light/12 h dark, they may breed all year round (Pilsborough, 1971). They do not hibernate, but may undergo daily torpor when the photoperiod is short.

P. campbelli is a small, compact, short-legged, and short-tailed hamster with 'furry feet' in the winter (Heldmaier and Steinlechner, 1981b). The buccal pouches that are characteristic of all hamsters are found in both *P. sungorus* and *P. campbelli*. *P. campbelli* has a mid-grey pelt with a black dorsal stripe and white underparts (including the tail and legs), that laterally forms three arches along each side between the fore and hind limbs and, where the grey and white fur merge, the fur has an orange tinge. There is sexual dimorphism in body mass (Fig. 1.2.1), with mature males being significantly larger than mature females (Sawrey *et al.*, 1984).

Fig. 1.2.1 Adult male and female *P. campbelli*, showing the large differences in body mass.

Herberg *et al.* (1980) and Wynne-Edwards (1987) found that in captivity, the breeding habits of *P. campbelli* and *P. sungorus* were also completely different, although Herberg *et al.* (1980) called *P. campbelli* a subspecies. *P. campbelli* has obligate monogamy as the preferred breeding system, where the continued presence of the male was necessary for successful pup rearing (Wynne-Edwards, 1987). It was observed by Wynne-Edwards and Lisk (1987b) that the preferred breeding system for *P. sungorus* in captivity was not with the father present; the females were successful

as solitary parents (Wynne-Edwards and Lisk, 1989). It was even possible to rear *P. sungorus* in colonies in captivity, where there was more than one breeding male in a harem (Jordan, 1971). Wynne-Edwards and Lisk (1989) investigated the role of the male in the rearing of pups in captivity and found that when the male was absent at warm (21 ^{o}C) ambient temperatures or present at low ambient temperatures (4 ^{o}C), pup survival was significantly lower in *P. campbelli* than with similarily treated *P. sungorus*. But when the male was present at a warm ambient temperature of 21 ^{o}C, pup survival was significantly higher in *P. campbelli* than *P. sungorus*. So in *P. campbelli* the continued presence of the male was important for the pups' survival.

1.3 Adaptations to seasonal changes in climate.

The thermobiology of *P. sungorus* has been studied extensively under laboratory conditions by Heldmaier and colleagues. Heldmaier *et al.* (1981a, 1982a) and Heldmaier and Steinlechner (1981b) kept *P. sungorus* under natural photoperiod for several seasons and divided them into two groups, (1) outside in Germany (Frankfurt, latitude 50^{o} 80' N) and (2) inside under laboratory conditions at a constant temperature of 23 ^{o}C and daylength matching the natural photoperiod. They measured changes in fur coloration, hair length, body mass and rectal temperature and found appropriate seasonal responses to photoperiod in both groups of hamsters, with the outside group showing 'improved precision of seasonality'. They concluded that photoperiod was the major cue for physiological adaptation to winter, with environmental temperature helping to strengthen acclimatization. The warm regime maintained in the laboratory simulated the outdoor condition sufficiently well to use them for experiments.

Weiner and Heldmaier (1987) measured oxygen consumption and basal metabolic rate (BMR) in *P. campbelli* and *P. sungorus* using a controlled temperature chamber. They found that the cold limit (the lowest ambient temperature that can be tolerated, below which raised metabolism by non-shivering thermogenesis (NST) can no longer maintain body (core) temperature) for winter acclimatized *P. campbelli* was 6-10 ^{o}C higher than for *P. sungorus*. The behavioural responses to the cold were strikingly different in the two species. *P. campbelli* responded to the cold by vigorously

attempting to escape from the experimental temperature chamber, whereas *P. sungorus* adopted a heat-saving posture, relying on insulation (Weiner and Heldmaier, 1987).

1.4 Changes in body mass and food intake.

Seasonal changes in body mass are characteristic of *Phodopus* sp. Hamsters of both species are significantly heavier in the summer than the winter, with *P. campbelli* always the smaller in body mass than *P. sungorus*. However most of the data available about seasonal changes in body mass are from *P. sungorus*, not *P. campbelli* and as shown in Table 1.1, these reported values differ.

Table 1.1 Reported body mass of *P. sungorus* and *P. campbelli* when kept under different environmental regimes.

Species + sex	Photoperiod	Environ.	Body Mass*	Reference
P. sungorus males	12 h l /12 h d	warm	54 g*	Pilsborough, 1971
P. campbelli males	12 h l /12 h d	warm	40 g*	Herberg *et al.*, 1980
P. sungorus females	12 h l /12 h d	warm	30 g*	Pilsborough, 1971
P. campbelli females	12 h l /12 h d	warm	27 g*	Herberg *et al.*, 1980
P. sungorus males	8 h l /16 h d	cold	30 g*	Hoffmann, 1973, 1978
P. sungorus sex not stated	8 h l /16 h d	cold	25 g*	Heldmaier and Steinlechner, 1981b
P. sungorus sex not stated	16 h l /8 h d	warm	42 g*	Heldmaier and Steinlechner, 1981b

* It was only Hoffmann (1973, 1978) that stated mean body mass, all other values for body mass were stated as approximate.

Over many generations in closed colonies differences in mean litter size, body mass, physiological capacities (Richardson *et al.*, 1994) or behaviour patterns (Smith, 1985) appear. Such processes are accepted as a normal occurrence in isolated colonies of animals that slowly become in-bred (Festing, 1987).

Table 1.2 Reported changes in body mass in different environmental conditions.

Species	P/period	Environ.	Body Mass change	Reference
P. sungorus	short	cold	-36%	Rafael *et al.*, 1985a
P. sungorus	long	cold	-13% (by week 3)	Rafael *et al.*, 1985a
P. sungorus	short	cold	-20% *	Ruf *et al.*, 1993
P. sungorus	short	warm	-20% *	Ruf *et al.*, 1993
P. campbelli	long	warm	+4% (by week 16)	Mercer *et al.*, 1994
P. campbelli	short	warm	-29% (by week 16)	Mercer *et al.*, 1994

*After 28 days on short photoperiod, body mass decreased more rapidly in both male and female *P. sungorus* being kept in the cold, than those kept in the warm, where body mass reduction differed substantially between individuals.

The data in Table 1.2. show that in both species of hamster it was photoperiod that triggered changes in body mass, with temperature strengthening the response.

Ruf *et al.* (1991) investigated the physiological function of changes in body mass. The smaller winter acclimatized hamsters were energetically better off, with up to 37% total energy savings (Ruf *et al.*, 1991). Even though they spent more time searching for scarce supplies of food, because they were moving a lighter body mass about, energy expenditure and therefore energy requirements, were reduced (Heldmaier and Steinlechner, 1981b, Steinlechner *et al.*, 1983). Even when food was available *ad lib.* under laboratory conditions at a constant 22±1 ^{o}C, less food was consumed by *P. sungorus* on short photoperiod, than by *P. sungorus* on long photoperiod (Wade and Bartness, 1984, Bartness and Wade, 1985). The mean quantity of pelleted diet consumed by *P. sungorus* (sex not mentioned) at a constant 23±1.5 ^{o}C was 4 g /hamster /day on long photoperiod (*i.e.* simulated summer) and 2.8 g /hamster /day on short photoperiod (*i.e.* warm short day) (Steinlechner *et al.*, 1983). The significant loss in body mass by *P. sungorus* on short photoperiod at 22±1 ^{o}C preceded any reduction in food intake. Therefore photoperiod-induced changes in food intake may be a consequence of changes in body mass, rather than vice versa (Wade and Bartness, 1984). Mercer *et al.* (1994) studied *P. campbelli* for 9 weeks on long and short

photoperiod at a constant 22 °C and also found that the significant loss in body mass on short photoperiod (week 4 onwards) preceded a reduction in food intake (week 6 onwards).

The experiments of Steinlechner *et al.* (1983) and Bartness and Wade (1985) where restricted feeding was imposed on groups of *P. sungorus*, housed under natural photoperiod, have demonstrated that the lower body mass seems to be regulated actively. In both groups reduced body mass resulted and upon refeeding *ad lib.*, both groups regained body mass to a level appropriate to the season, matching that of their unrestricted counterparts, in which body mass was elevated in summer and depressed in winter.

Masuda and Oishi (1988) also compared the effects of temperature and photoperiod on body mass in *P. sungorus*. They found that taking the body mass at the beginning of the experiment as 100%, the hamsters kept on long day (16 h light /8 h dark) in a warm (25 °C) and cold (7 °C) environment both increased to over 120% of initial mass within 25 weeks. The short day (8 h light /16 h dark) warm and cold groups both dropped to 80% within 8-10 weeks. The cold short day group then increased body mass back to 100% within 20 weeks and continued to 120% by week 45. By week 31, the warm short day group had returned to 100% of the original body mass which then remained constant.

With the original food intake being taken as 100%, the investigation found that the cold, long and short day groups doubled their intake of pelleted food within the first week and intake continued to climb to 300% of the initial quantity by week 30. The warm short day group hovered around 100% for the whole of the experiment and the warm long day group reached an intake of 150% of original requirement by week 15 and remained constant. They calculated that relative food intake in the cold, long and short day regimes after the first week (approximately 1.5 g /g body mass), was twice as much as that under warm, long and short day regimes (approximately 0.7 g /g body mass) Since oxygen consumption measured at 7 °C was twice as high as that at 25 °C (Heldmaier and Steinlechner, 1981), Masuda and Oishi (1988) concluded that the change in food intake by environmental temperature was probably due to changes in the metabolic rate.

So the winter acclimatized *Phodopus* become smaller, but eat more, presumably to fuel heat production.

Herberg *et al.* (1980) studied many things including plasma insulin (IRI) levels in their colony of *P. campbelli* that were kept at 25±1 ^{o}C on a 12 h light /12 h dark cycle. They found that the majority of their hamsters excreted ketone bodies or glucose in their urine (measured by test tapes). They measured IRI levels using 100 µl of plasma by solid phase immunoassay in both fasted (16 h) and nonfasted hamsters.

IRI levels were the closest between nonfasted and fasted (16 h) hamsters, that had negative results to both glucose and ketone bodies in their urine. These hamsters also had a significantly lower body mass than the other groups, whether this feature was related to their metabolic condition was not stated. The highest levels were found in fasted and nonfasted hamsters that tested positive to glucose. Their colony of hamsters have an atypical metabolism, whether this is true of all *P. campbelli* is not known.

1.5 Torpor and hypothermia.

Many small mammals and a few adult birds undergo varying periods of adaptive hypothermia, in response to environmental conditions. For animals to remain euthermic under cold-stress, they must have a good supply of food, good energy stores and very good insulation. However many small animals would not be able to eat enough food to provide the energy to remain euthermic, hence they undergo adaptive hypothermia. Hibernation is extreme and prolonged hypothermia, an adaptation for animals whose food supply is unavailable for long periods of time. Only animals that lay down large energy stores can survive hibernation and re-warm themselves. Hibernators usually arouse occasionally and replenish their energy stores. The less extreme form is torpor, where animals become hypothermic for short periods of time either regularly or as necessary, during periods of extreme food shortage.

P. sungorus do not hibernate, because as with many other mammals under 100 g in body mass, they are unable to have large enough stores of fat to overwinter. Instead they undergo several brief episodes of torpor, which normally occur every third day on a regime of short photoperiod (Heldmaier and Ruf, 1992).

Bartness *et al.* (1989) listed criteria for assessing torpor in *P. sungorus*;

(a) cool to the touch,

(b) non-responsive,

(c) decreased respiration rates, relative to both awake and sleeping hamsters.

(d) the hamsters were considered to have recently come out of torpor, if they were cool to the touch, but moving and unable to perform the righting reflex, observed in hamsters that were fully awake or recently aroused from sleep.

By implanting temperature transmitters intraperitoneally into a few hamsters, Heldmaier and Ruf (1992) found that torpor in *P. sungorus* occurs only on short photoperiod and is mainly restricted to daylight hours. It normally begins just before sunrise /lights on (Ruf *et al.*, 1993). The torpor season was found to run in captivity from October through March in the cold acclimatized hamsters and from November through March in the hamsters on short photoperiod at 23 ^{o}C. Torpor in *P. sungorus* appears during the thirteenth week of exposure to short photoperiod at temperatures of 10, 15 and 20 ^{o}C and disappears during the twenty-fourth week (Ouarour *et al.*, 1991).

Ruf *et al.* (1993) studied incidences of torpor in *P. sungorus* and found that as the number of torpor episodes experienced by an individual hamster increased, so their duration increased (mean torpor duration ranged from 4.5±0.64 h to 10.9±0.52 h in hamsters housed at 5 ^{o}C) and body temperature decreased (mean minimal body temperature of hamsters in torpor ranged from 22.7±0.80 ^{o}C to 13.7±0.41 ^{o}C, when housed at 5 ^{o}C). Ruf *et al.* (1993) found 10-35% of *P. sungorus* kept at 23 ^{o}C on a short photoperiod and 15-95% of those kept at 5 ^{o}C on a short photoperiod exhibited torpor on any given day and that torpor was mainly restricted to the daylight hours.

1.5.1 Function of torpor and hypothermia.

Periods of torpor in *P. sungorus* become longer in duration and of more frequent incidence with increasing cold exposure and food shortage (Ruf *et al.*, 1991), creating further energy savings of 5-26% (Heldmaier, 1989). On natural photoperiod, maximum torpor frequency of *P. sungorus* was found to occur in January, with 30% of all hamsters (either inside at 23 ^{o}C or outside at ambient temperature) being torpid at a time. These conclusions were based on the rectal temperatures of hamsters, measured

weekly throughout the year (Heldmaier and Steinlechner, 1981a). It was calculated that torpor would reduce the long-term energy requirements by only 5%, due to the high energetic costs of arousal. Heldmaier and Steinlechner (1981a) concluded that the main function of torpor was not to reduce total energy requirements over the whole winter, but as a means of survival during short periods of extreme cold or inaccessability of food (Heldmaier and Steinlechner, 1981a).

Kirsch *et al.* (1991) studied torpor frequency in both male and female *P. sungorus* housed at 20±1 °C on long photoperiod, then transferred to a short photoperiod of 10 h light /14 h dark at the same temperature. They found the mean torpor frequency (ratio of number of torpor bouts to number of days of experiments) in males to be 19±14.5% and in females to be 8.15±11.6%. There was large inter-individual variation, with males found to have between 1-11 torpor bouts over a 16 week period and females having between 0-9 torpor bouts over the same period. They calculated that the energy savings of 2.4% in males and 1% in females was negligible due to the low incidence of torpor and the high energy costs of arousal. However, when males were exposed to continuous darkness, torpor frequency increased by 71%, giving gross energy savings of 14.2% for the period, which they suggested was a survival mechanism for extreme conditions.

Experimentally restricting the diet of *P. sungorus* to 60% of normal food intake increased torpor incidence 1.8-2.6 fold at all ambient temperatures (Ruf *et al.*, 1993).

A similar relationship between nutrition and hypothermia has been reported in other murid rodents, whether they undergo torpor or hibernation. Bartness *et al.* (1991) found that Turkish hamsters, *Mesocricetus brandtii,* that had high body mass at the outset of exposure to short photoperiod, underwent fewer incidences of hibernation, as did those on high-fat diets. Similar effects on torpor were found in the deer mouse, *Peromyscus maniculatus* (Geiser, 1991). These habits are probably also a survival mechanism for extreme conditions, i.e. food shortage.

Winter acclimatized *P. sungorus* reduce heat loss by slight hypothermia at moderately cold temperatures. Heldmaier *et al.* (1985) calculated that by reducing the body temperature by 2 ° to 34 °C, the hamsters may save up to 15% of energy requirements for thermoregulation.

1.6 Seasonal adaptations of other physiological systems.

Adaptation to winter conditions are observed in the reproductive system, but do not occur simultaneously with changes in the body mass or the pelt. Duncan *et al.* (1985) studied under artificial conditions a range of light /dark cycles to find the critical daylength that triggered testicular regression (measured by palpation, under anaesthetic) and body mass reduction in *P. sungorus*, they found these changes began on a 14 h light /10 h dark cycle.

Testicular development in *P. sungorus*, was found to be strongly influenced by changes in photoperiod. Hamsters kept on long days (16 h light /8 h dark) have large testes in full spermatogenesis (approximately 400 mg /testis), and hamsters kept on short days (8 h L /16 h D) have small testes in regression (approximately 15 mg /testis) (Yellon and Goldman, 1984; Hoffmann, 1978; Hoffmann and Illernova, 1986). Hoffmann *et al.* (1973) observed that in *P. sungorus,* the increase in testes mass (900%) in animals transferred from short to long photoperiod was disproportionate to the increase in their body mass (30%). Mercer *et al.* (1994) found that *P. campbelli* also had substantial testicular regression at 22 ^{o}C when transferred from long photoperiod (testes mass = 1670 mg) to short photoperiod for up to 16 weeks (testes mass = 20 mg), however the majority of the weight loss in the testes occurred in the first 8 weeks (testes mass = 24 mg).

It was found that testicular regression was necessary for the occurrence of daily torpor. Castrated hamsters had an extended torpor season and testosterone implants immediately inhibited torpor even, on short photoperiod. From these results, Ouarour *et al.* (1991) and Vitale *et al.* (1985) concluded that there was a relationship between gonadal activity and torpor.

However, the winter moult into light-coloured pelage began when hamsters were kept on a 12 h light /12 h dark cycle, indicating that under natural conditions this process would occur later in the autumn than testicular regression and body mass reduction.

Puchalski *et al.* (1987) whilst studying the effects of organ blood flow and oxygen consumption produced a table of organ and body mass, as shown in Table 1.3, in *P. sungorus* when kept under natural photoperiod, either indoors at 23 ^{o}C or outdoors (at

an ambient temperature that fluctuated between -2 and +12 °C). They only stated the outdoor hamsters were cold acclimated and it can probably be assumed from the outside temperature that it was around the winter season and therefore the hamsters were on a fairly short light /dark cycle, so they are equivilent to CSD and WSD. The sex of these hamsters was not mentioned.

Table 1.3 Body and organ mass (g) of *P. sungorus* (Puchalski *et al.*, 1987).

Acclimation	Indoors (23 °C)	Outdoors (-2 to +12 °C)
Body mass (g)	29.90±2.30	35.60±2.20
Total BAT mass (g)	1.02±0.08	1.21±0.08
Heart mass (g)	0.21±0.01	0.23±0.01
Liver mass (g)	1.21±0.05	1.51±0.08
Kidneys mass (g)	0.15±0.01	0.19±0.01

1.7 Adipose tissue.

Adipose tissue accounts for between 5% (in lean mammals) and 30% (in obese mammals) of total body mass (Pond *et al.*, 1987). In adults, WAT accounts for at least 95% of the total adipose tissue (Pond *et al.*, 1987).

1.7.1 Function of brown (BAT) and white adipose tissue (WAT).

The surface/volume ratio decreases with increasing body size of organisms of similar shape. Also in large mammals the fur (which reduces heat loss at the surface) can increase in density and grow longer, under cold stress, without causing major weight problems for the carrier. A well insulated large mammal can easily maintain thermal homeostasis under normal or very cold conditions.

The function of WAT is the storage of fuel (triacylglycerol), ready for controlled release as required, for use almost entirely by other tissues. BAT can also act as a fuel store when dormant, however its main function is thermogenesis under cold-stress. In cold-acclimated mammals BAT elevates the body's production of heat by NST. It is located mainly in the thoracic region and is highly vascularised (Cannon and Nedergaard, 1985). Brown adipocytes are highly specialised cells, found only in mammals, particulariy neonates and small species that require supplementary heat

production because they have a high surface /volume ratio and therefore high heat loss. BAT is retained into adulthood in hibernators, where warming up is necessary. Heat is produced by non-shivering thermogenesis (NST). The oxidation of lipids or glucose is uncoupled from the synthesis of adenosine triphosphate (ATP) in mitochondria, with the activation of uncoupling protein (UCP), found only in the inner membrane of BAT mitochondria. A much greater proportion of the energy from the breakdown of glucose or fatty acids is liberated as heat and so released for maintaining thermal homeostasis or regaining thermal homeostasis during arousal from hibernation and torpor.

Nedergaard and Cannon (1984) studied golden hamsters (*Mesocricetus auratus*) which are known to be 'poor' hibernators, and acclimated them to cold on a long photoperiod. They found an increase in the gross mass of BAT, and that its measured protein content doubled. Total BAT mass was found to be even higher during hibernation. The lipid content of BAT during hibernation was nearly double that of non-hibernating hamsters. Each BAT depot lost 31-51% of its lipid on arousal from hibernation. Nedergaard and Cannon (1984) concluded from these data that BAT lipids were utilized for arousal and calculated that breakdown of these lipids would provide 90-100% of the heat required and supply energy-rich substrates for heat evolving processes in other organs, especially muscular shivering. When not involved in thermogenesis BAT can act as a storage tissue.

Pygmy shrews (*Sorex minutus*) have a similar body mass in summer and winter. In spring however, they are significantly heavier. In spring, interscapular BAT increases in relative and absolute mass, the measured lipid droplet size within this depot increases, due to triacylglycerol deposition. McDevitt and Andrews (1995) concluded that as the BAT becomes thermogenically dormant in spring, it assumes a fat storage role, possibly in preparation for the high energy demands of breeding which reduces foraging times, especially in males.

BAT is used for thermogenesis in many newborn mammals (Trayhurn, 1989), but most BAT changes to white adipose tissue (WAT) after the first few weeks of life.

1.7.2 Identification of brown (BAT) and white adipose tissue (WAT).

It is now known that histological (Table 1.4) and anatomical appearance are not enough to determine whether or not adipose tissue is functionally thermogenic, especially since when BAT is not activated, it closely resembles WAT in appearance (Cannon and Nedergaard, 1985). Although not obvious histologically, BAT may be widely distributed in many adult mammals (Trayhurn, 1989) and therefore histological appearance does, in some cases, lead to an incorrect conclusion as to whether a depot is functionally brown or white adipose tissue. The most relevant criterion for determination of BAT is the presence of uncoupling protein (UCP) (Trayhurn, 1989), which has been found to be present only in the mitochondrial protein of brown adipocytes, and increases in concentration with cold acclimation. It is only when a radioimmunoassay for UCP is performed that BAT adipocytes can truly be identified (Trayhurn, 1989).

Table 1.4 Basic histological characteristics of brown and white adipocytes (Cordell and Tedstone, 1994).

Characters	Brown Adipocytes	White Adipocytes
Cell Size	Smaller	Larger
Mitochondria	Large and numerous with a complex structure of cristae	Sparse and small with a simpler structure of cristae
Number and size of lipid vesicles	Numerous small vesicles, dispersed throughout the cytoplasm (multilocular)	Single large vesicle, taking up most of the cytoplasmic space in the cell (unilocular)
Vascularization	High vascularization, hence its brown colour	Sparse vascularization, hence its white colour

1.7.3 Anatomical distribution of white adipose tissue (WAT).

It is obvious from the research papers, WAT depots have not been widely studied in *P. sungorus*. No work appears to have been done on *P. campbelli*.

Mattacks and Pond (1988) reported that the mean body mass of female *P. sungorus* was significantly smaller than that of males on continuous warm/long day, with no sex

differences in fatness (Table 1.5). The body size of *P. sungorus* also varied greatly between similarly maintained animals of the same age.

Below Table 1.5 shows that when the ventral groin, behind forelimb, interscapular and dorsal wall of abdomen depots were expressed as a percentage of the total dissectable adipose tissue in WLD, there were significant sex differences. These depots were smaller in males than females (Mattacks and Pond, 1988).

Table 1.5 Mean (±SD) of body mass (g), total dissectable adipose tissue (fatness) as a percentage of final body mass and gross mass of depots of white adipose tissue as a percentage of the total dissectable adipose tissue in *P. sungorus* (Mattacks and Pond, 1988) kept on WLD.

sex + n	Males (8)	Females (8)
Body mass (g)	51.1±8.9	43.9±5.7
Fatness (%)	29.3±5.8	27.0±2.9
WAT depots as % total dissectable adipose tissue		
SUPERFICIAL		
In front of forelimb and shoulder	23.3±3.1	25.7±3.5
Behind forelimb	23.1±3.1	27.0±3.5
Groin side	14.7±2.1	15.5±3.0
Groin ventral	3.9±0.9	7.7±1.7
INTERMUSCULAR		
Medial to trapezius muscle of neck	3.5±0.5	4.1±0.8
Popliteal	0.9±0.2	1.0±0.3
INTRA-ABDOMINAL		
Mesenteric	3.2±1.2	4.5±1.6
Around kidney and dorsal wall of abdomen	5.6±1.6	10.4±2.2
Epididymal	18.3±6.0	-

Pond *et al.* (1987) reported that female *P. sungorus* had proportionally more superficial adipose tissue than males of a similar fatness (Table 1.5) and that the sex differences became greater with increasing obesity. The ventral groin and dorsal wall of abdomen depots had the greatest differences, and there were no sex differences in the relative masses of the mesenteric or intermuscular depots. These sex differences occurred at all levels of fatness (Mattacks and Pond, 1988). In suggesting a functional basis for these sex differences, Pond *et al.* (1987) pointed out that the ventral groin

depot in rodents is closely associated with the mammary glands, and that the reduction of intra-abdominal adipose tissue in females gives more room to the foetuses in utero.

Lean *et al.* (1983) tested for UCP in the parametrial (WAT) depot of hooded rats and found no detectable amounts in animals kept at 4 °C. They suggested that other WAT depots might be more likely to have BAT-like properties. Cousins *et al.* (1992) investigated the presence of UCP protein in several white adipose tissue (WAT) depots of 8-11 week old Wistar rats. The rats were housed with 12 h light /12 h dark, at a constant 24 °C or 4 °C for 24 h, 3 days or 10 days. At 24 °C, the periovarian and retroperitoneal depots both had detectable UCP revealed by Northern blotting, though to a lesser extent than the interscapular BAT depot of the same rats. The mesenteric and inguinal were the two other depots investigated and there was no detectable signal. They found that 24 h exposure to 4 °C led to an increase in detectable UCP in interscapular BAT, as expected, and in the periovarian and retroperitoneal depots. But there were still no detectable signals from the mesenteric and inguinal depots. Electron microscope photographs of the periovarian depot in rats kept at 4 °C for 10 days show mitochondria (although not densely populated) packed with cristae. The results demonstrate the presence of brown adipocytes in two of the four white adipose depots studied, and suggested that adipocytes with thermogenic properties may be present in several other of the adipose depots that are generally regarded as white and UCP increases on cold exposure.

The epididymal depot of Sprague Dawley rats that had been cold exposed (4 °C) for one week, and -20 °C for 1h twice daily the following week, after cold stress became dark brown, due to increased vascularization, a 95% reduction in adipocyte volume (Loncar *et al.*, 1988a) and mitochondrial enlargement. These changes resulted in the tissue morphologically resembling BAT, but the presence of UCP was not detected by immunoelectron microscopical reactivity or slot blot (Loncar *et al.*, 1988b).

1.7.4 Anatomical distribution of brown adipose tissue (BAT).

Table 1.6 The total mass of the main BAT depots and percent of total BAT of the depots of *P. sungorus*, data from Rafael *et al.* (1985b).

DEPOT	SIMULATED SUMMER (indoors)	NATURAL WINTER (outdoors)
Total mass of BAT	2.19 g	0.92 g
Interscapular	12.6%	11.6%
Dorsal-cervical	6.4%	6.7%
Subscapular	27.8%	30.0%
Axillary	46.1%	42.1%
Thoracic	7.1%	9.6%

As the data in Table 1.6. show, there were no substantial changes in the distribution of BAT, between hamsters kept in simulated summer and natural winter, just changes in its total mass. Rafael *et al.* (1985b) found the absolute amount of BAT lipid to be 1.81 g in simulated summer and 0.65 g in natural winter. Cannon and Nedergaard (1985) also listed the most common depots of BAT (as above, plus intercostal, periaortic and perirenal), pointing out that not all those depots are found in all animals.

Obese (*ob/ob*) mice, when kept at 20 ^{o}C undergo daily episodes of torpor (below this ambient temperature irreversible hypothermia occurs). EM studies on interscapular BAT from these 'cold' exposed mice show the characteristic increase in number and size of mitochondria and increase in numbers of cristae within the mitochondria, the lipid droplets become multilocular, but McBennett *et al.* (1993) were uncertain as to whether they actually had increased thermogenic capacity. However, other cells from the same depot appear to remain inactive, unilocular and have few mitochondria (McBennett *et al.*, 1993)

1.7.5 Conversion of brown adipose tissue to white adipose tissue.

Newborn reindeer (*Rangifer tarandus*) are able to generate heat by NST, which is essential for survival of the young in the first few days after birth, especially under severe weather conditions. Most of their adipose tissue has the typical appearance and biochemical and histological characteristics of BAT, both macroscopically and

under an electron microscope. During the first few postnatal weeks, however, the adipose tissue changes completely. By the second month of life, all depots show all the characteristics of WAT (Soppela *et al.*, 1992).

BAT does not reappear in adult reindeer, even after prolonged exposure to severe weather conditions. The reduction in the capacity for NST during the first two months of life may be due to the decreased need for NST heat production, as thermal insulation improves and growth of the reindeer results in a proportionally lower rate of heat loss as the ratio of surface area to body mass declines. Stronger, more active muscles and increasing rumination both create heat, again reducing the need for NST (Soppela *et al.*, 1991)

1.7.6 Changes in white adipose tissue (WAT) with temperature and photoperiod.

Bartness and Wade (1985) suggested that photoperiod in *P. sungorus* appears to affect the relative mass of adipose depots selectively. *P. sungorus* on short photoperiod was found on dissection to have almost no intra-abdominal adipose tissue. Wade and Bartness (1984) concluded that much of the remaining lipid was probably in the subcutaneous depots. Bartness and Wade (1985) proposed that the subcutaneous adipose tissue in *P. sungorus* was maintained or even increased for insulation purposes, due to there being no observable abdominal adipose tissue in winter acclimatized hamsters.

All white adipose tissue (WAT) depots in cold acclimated golden hamsters (*Mesocricetus auratus*) contained less lipid than during warm acclimation, with the wet weight of the epididymal depot half that of the warm acclimated hamsters (Nedergaard and Cannon, 1984).

Wade and Bartness (1984) found that the weight loss in *P. sungorus* on short photoperiod was almost entirely due to changes in total carcass lipid (35-40% decrease), and the heaviest hamsters lost the most weight. They could not account for the differences in their results from those of Heldmaier and Hoffmann (1974). Bartness *et al.* (1987) reported similar results when studying the effects of short photoperiod on *P. sungorus* and the Syrian hamster (*Mesocricetus auratus*), with the latter increasing

body mass. In both species these changes were attributable mainly to carcass lipid content.

1.7.7 Changes in brown adipose tissue (BAT) with temperature and photoperiod.

Rafael *et al.* (1985b) found that BAT constituted up to 5% of the total body mass in *P. sungorus* on long photoperiod at a constant 23 °C (indoor) and 3.7% of the total body mass in outdoor (ambient temperature) summer acclimatized *P. sungorus*. The total mass of BAT was greatly reduced in winter, in hamsters either kept outdoors (40% of the original mass) or indoors on natural photoperiod (54% of the original mass). Nonetheless the amount in proportion to total body mass (approximately 3.4%) only dropped slightly in winter because the total body mass fell, with virtually no difference found between outdoor (natural) and indoor (artificial) winter conditions. Rafael *et al.* (1985b) concluded that reduction of mass in BAT was triggered by changing photoperiod. The 40% reduction in total BAT mass in *P. sungorus* was achieved within 2-4 days and was due to depletion of lipids, from 80% (extractable lipids as percent of the total mass) in warm acclimatized, to 55% in cold acclimatized hamsters. Protein represented 4% of the total BAT tissue in warm acclimatized hamsters and 10% after 4 days cold exposure. The increase in protein was mainly due to the rapid proliferation of mitochondria. Total mitochondrial protein doubled in 24 h and increased by 3-fold after 4 days (Rafael *et al.*, 1985a).

Heldmaier and Hoffmann (1974) calculated that as a percentage of body mass, *P. sungorus* in cold /short photoperiod, contained 50% more BAT than those in warm /long photoperiod. Wade and Bartness (1984) also found that short photoperiod significantly decreased interscapular BAT mass (14-20%) in *P. sungorus*, but when they expressed their data, relative to body mass, the differences were not significant. Mercer *et al.* (1994) found that *P. campbelli* after 8 weeks exposure to short photoperiod at 22 °C showed no significant changes in the mass of interscapular BAT. However after 16 weeks exposure to short photoperiod, they found a significant reduction in interscapular BAT mass (0.17±0.03 g), when compared to the hamsters that had been kept on long photoperiod (0.28±0.02 g).

Similar measurements from other species of rodents point to quite different conclusions. The rat is native to tropical S. E. Asia and does not undergo marked seasonal changes in body mass or metabolism. Trayhurn *et al.* (1987) kept Norwegian hooded rats on a 12 h light /12 h dark cycle, at temperatures ranging from 4-29 ^{o}C. He found that interscapular BAT mass and protein content increased with decreasing temperature. The cytochrome oxidase (an index of mitochondrial mass) activity was measured and an 11-fold increase was found in tissue, between rats kept at 29 ^{o}C and those kept at 4 ^{o}C, clearly indicating that an increase in NST followed a reduction in ambient temperature, even on constant photoperiod.

1.7.8 The importance of white adipose tissue (WAT).

The main functions of WAT are the uptake, storage and release of lipids (Pond, 1992). There are site-specific differences, where the large depots, in response to lipid uptake and release, are generally the plodders and their main function is long-term storage of vast quantities of lipids. In contrast the small depots are generally rapid responders to uptake and release of lipids for routine local requirements (Pond, 1992). Generally, the size of each depot is related to the animals habits, thus the gross anatomy creates the body shape appropriate for the life style (Pond, 1998), for example, it would not be good if a hunter, needing to be lithe, had a large paunch depot.

1.7.9 Physiological responses in white adipose tissue (WAT).

The *ob* (obese) gene with a molecular mass of 18 K (Zhang *et al.*, 1994), now named leptin (Halaas *et al.*, 1995), is expressed in WAT and BAT (Klingenspor *et al.*, 1996) and the placenta (Hoggard *et al.*, 1997).

Klingenspor *et al.* (1996) studied leptin gene expression in the white and brown adipose tissue of *P. sungorus,* a hamster with natural seasonally fluctuations in body mass. Leptin is a gene recognised as the product of the mutant obese gene and studies had only been conducted on genetically obese laboratory animals. Klingenspor *et al.* (1996) found that levels of leptin mRNA expression in both WAT and BAT correlated with the naturally fluctuating body mass.

The WAT depots of lean Aston mice were studied by Trayhurn *et al.* (1995b) for the expression of leptin, by Northern blotting. Of the WAT depots studied, all expressed leptin, but with large inter-depot variation. The epididymal depot produced the strongest signal, followed by the perirenal. The faintest signals were produced by subcutaneous depots from around the rear and fore limbs, and the omental depot.

Trayhurn *et al.* (1995b) also compared the epididymal depots of lean and *ob/ob* littermates for levels of leptin, they found that the signal was much stronger in the *ob/ob* mice. Trayhurn *et al.* (1995b) reasoned that site-specific differences in leptin levels might relate to size of adipocytes in the depot, where large adipocytes had the highest levels of leptin and small adipocytes had the lowest levels of leptin. This would signal the state of energy stores, at adipocyte level, to be incorporated into an overall signal of the total fat for the whole body. They also discussed that since leptin levels in lean mice are affected by fasting and refeeding, the main function of leptin was in regulating the energy balance in the short term and not as a saiety factor, especially since leptin levels in *ob/ob* mice were unaffected by fasting. Trayhurn *et al.* (1995a) also studied the effects of cold exposure (4 ^{o}C) on levels of leptin in the epididymal depots of lean Aston mice. They found that leptin expression became undetectable following just 2 h exposure to 4 ^{o}C, a much stronger reaction than was observed from fasting. Trayhurn *et al.* (1995a) also found that returning cold-acclimated mice to the warm (24 ^{o}C) for 2.5 h, increased leptin levels back to that of the controls maintained in the warm. They concluded that these observations confirmed the link between energy expenditure and leptin levels.

Montague *et al.* (1997) obtained adipose tissue biopsies from abdominal subcutaneous and omental depots of non-obese and mildly obese humans, that had been fasted for 6 h before the operation. Levels of leptin were consistently higher in adipocytes expressed from the abdominal subcutaneous depot.

Moinet *et al.* (1995) looked for and found leptin expression in the inter-scapular and epididymal depots of Sprague-Dawley rats by Northern blots. Food restriction, both acute (36 h fasting) and chronic (intake reduced to 50% for 10 days), reduced levels of leptin significantly (-62% to -68%), when compared a*d lib.* fed rats. Cold acclimation (6±1 ^{o}C) for 24 h also significantly reduced levels of leptin (-84%) in BAT,

but had no effect on that of WAT. However, after three weeks of cold acclimation, leptin levels did not change in BAT or WAT, when compared to the warm acclimated controls. Moinet *et al.* (1995) concluded that the regulation of leptin by food restriction or cold exposure in both BAT and WAT was more closely linked with changes in the lipid content of the tissues than with changes in the sympathetic activity.

1.7.10 The importance of brown adipose tissue (BAT) in small mammals.

In small mammals the surface /volume ratio is high, creating problems for maintaining core temperature. Also the maximum fur density and length is limited by what the animal can sustain. Therefore under cold stress, small mammals increase heat production, either by muscular movement, i.e. physical exercise or shivering to produce heat, which is energetically very expensive, or by raising the metabolic rate (no metabolic processes are 100% efficient and heat is produced as a by-product). A specialised mechanism is found in small mammals and the neonates of larger mammals, NST is activated by cold stress: as the ambient temperature decreases, so heat production increases.

1.7.11 Physiological thermogenic responses: Non-shivering thermogenesis (NST).

In order for a mammal to maintain thermal homeostasis, the rate of heat loss must be equalled by the rate of heat production. Non-shivering thermogenesis (NST) helps to maintain this balance in times of cold stress. NST also plays a very important role in arousal from torpor and hibernation.

Rafael *et al.* (1981) and Heldmaier *et al.* (1981a) showed experimentally that extended exposure to short photoperiod (8 h light /16 h dark) in natural winter acclimatized *P. sungorus* was not effective in maximising seasonal capacity for NST. This conclusion was either checked by the use of a temperature-controlled chamber where oxygen consumption could be monitored at set ambient temperatures from +26 ^{O}C to -20 ^{O}C or by measuring the total mitochondrial protein content of brown adipose tissue (BAT). They concluded that the hamsters need to go through a set photoperiodic cycle of a minimum duration to maximise capacity for NST.

Significantly elevated NST capacity occurs after 3 weeks of exposure to short photoperiod (Heldmaier *et al.*, 1982a, 1985). Simulating the cold tolerance required for foraging in natural winter by *P. sungorus* with 13 days of 2 hours exposure to 5 °C twice a day (with a 12 h interval) or 4 hours exposure /day to 5 °C (Wiesinger *et al.*, 1989) and 12 days exposure for 0.5 h/day to -5 °C (Heldmaier and Jablonka, 1985). The increase in NST relative to body mass is significantly higher in winter acclimatized *P. sungorus* (162%), than in those exposed to cold on a long photoperiod (67%). NST was calculated from oxygen consumption in response to noradrenaline, and the data suggest that exposure to short photoperiod is essential to establish maximum NST (Rafael *et al.*, 1985a)

Heldmaier *et al.*, (1982a, 1982b) compared the effects on *P. sungorus* when housed at a constant 23 °C on long and short photoperiod and found that short photoperiod improved NST capacity by 55%. NST capacity was found to be improved by a further 45% by exposure to decreasing temperatures on short photoperiod, when comparing *P. sungorus* housed inside at 23 °C or outside at ambient temperature. The authors found that the hamsters cold limit was improved by over 20 °C, from -47 °C in hamsters that wintered inside (constant 23 °C), to -68 °C in hamsters that lived outside in the cold fluctuating temperatures of winter. These experiments were performed in a controlled temperature chamber, where the ambient temperature was gradually reduced until a decline in body temperature and increase in oxygen consumption confirmed that the hamsters' cold limit had been reached.

To study body temperature changes during arousal, Milner *et al.* (1989) induced hibernation in Richardson's ground squirrel (*Spermophilus richardsonii*). They found that the axillary depot is this animal's main BAT depot and when the rectal temperature had risen 3.6 °C, the temperature of the axillary BAT depot was 11 °C higher. Heldmaier (1975) implanted a thermocouple subcutaneously in the interscapular BAT depot of *P. sungorus* to give a continuous record during cold exposure and also measured rectal temperature. A temperature controlled chamber was used for exposure down to -35 °C. The rectal temperature rose slightly after prolonged cold exposure (36.1 °C at 24 °C to 37 °C at -35 °C). After each reduction in

environmental temperature the interscapular depot was 0.5-2.0 ^{o}C higher than the rectal temperature, showing that it is the main source of additional heat.

Studies were performed on long and short photoperiod and 'warm' and 'cold' ambient temperatures to see if NST capacity could be increased on a long photoperiod. Winter acclimatized short-tailed field voles (*Microtus agrestis*) housed at 5 ^{o}C, transferred to a long photoperiod (14 h light /10 h dark), produced results suggesting that the animals did indeed enhance their thermogenic capacity during cold-acclimation on a long photoperiod (McDevitt and Speakman, 1994). Similar studies by Heldmaier and Buchberger (1985) compared warm (23 ^{o}C) and cold (5 ^{o}C) acclimated *P. sungorus*, housed on a long photoperiod for 4 weeks. First they found the BMR for each hamster. Then they stimulated maximum NST capacity by subcutaneous administration of noradrenaline, and measured the increase in oxygen consumption and carbon dioxide production using an oxygen and carbon dioxide analyzer. Results revealed that although total mass of BAT was reduced because it contained less lipid, cold acclimated hamsters had a greater capacity for NST after only 4 weeks.

1.8 Pelage.

1.8.1 Seasonal changes in the pelt.

The structure of the pelt changes in *P. sungorus* when it is transferred from a long to a short photoperiod. On short photoperiod the pelt as a whole thickens and the hairs lengthen by nearly 2 mm (Table 1.7) even at a constant ambient temperature (23 ^{o}C). These differences were more exaggerated in hamsters kept outside in seasonally changing temperatures (Heldmaier and Steinlechner 1981b).

Tab. 1.7 Seasonal differences in length of fur (mm) in *P. sungorus* when kept on natural photoperiod, either inside or outside (Heldmaier and Steinlechner 1981b).

SEASON	OUTSIDE (seasonally changing temperature)	INSIDE (constant 23±1.5 °C)
SUMMER	8.05±0.20 mm	7.84±0.18 mm
WINTER	10.20±0.17 mm	9.58±0.15 mm

Fur forms on the walking surfaces of their paws, producing 'furry feet'. These changes are assumed to improve insulation (Heldmaier and Steinlechner, 1981b; Heldmaier, 1989). The pelt also changes colour, through to the virtually white pelage in *P. sungorus* after 8-14 weeks exposure to short photoperiod (Duncan and Goldman, 1984). Heldmaier (1989) found that the improved insulation of winter acclimatized *P. sungorus* was so effective that they had a much lower rate of heat loss than summer acclimatized hamsters. This heat loss was calculated from simultaneous measurements of body temperature and heat production at thermoneutrality and below.

Scholander *et al.* (1950) and Hart and Heroux (1953) both concluded that under similar climatic conditions there was an inverse relationship between basal metabolic rate (BMR) and fur insulation. Hart (1956) pointed out that even the limited seasonal changes in fur density and length in small mammals would enable them to maintain their body temperature at lower ambient temperatures for the same energy expenditure.

Sealander (1972) studied the seasonal pelage changes in the northern red-backed vole (*Clethrionomys rutilus dawsoni*), a small rodent native to Alaska. He found the seasonal changes in hair mass to be inversely related to the seasonal changes in thickness of the skin (it was noted that the skin became parchment-like in the winter) and that there was a correlation between mean hair mass (mg) /cm^2 of skin and the mean relative amount of interscapular BAT in summer and winter. Hair mass was found to be 10.6 mg /cm^2 in July and 18.9 mg /cm^2 in December, a seasonal increase of 78%. Sealander (1972) suggested that the reduction of hair mass in summer could be

due to hair follicles becoming inactive. Hair that is an effective insulator from the cold could lead to hyperthermia in the warm weather.

Al-Khateeb and Johnson (1971) studied the seasonal changes of the pelage in the vole (*Microtus agrestis*). Detailed measurements were taken histologically from the mid-dorsal region. They found that the density of hairs per unit area of skin was greater in winter than in summer, the increased number of hairs in the winter was mainly due to more follicles becoming active at the autumn moult than at the spring moult. A count showed that the increase was mainly in the number of fine hairs. They also found a sex difference in the width of the summer hairs: the guard hairs were coarser in males than females.

Heldmaier (1975) noted that at temperatures above 30 ^{o}C, *P. sungorus* lay flat out with limbs extended and did not have their fur fluffed evenly, but displayed several 'ventilation gaps' dorsally (the fur divides leaving 'gaps' where the skin can be seen). However as the temperature dropped below 20 ^{o}C, the fur became evenly fluffed with the 'ventilation gaps' disappearing.

1.8.2 Lipids on the pelt.

The lipids found on the hair of mammals are produced mainly by sebaceous glands. They assist the function of the pelt as a barrier against water loss and water repellancy over a large temperature range (Downing *et al.*, 1983). Sebum production is stimulated by hormones produced by the pituitary gland and by androgens (produced by the gonads and adrenal glands). Therefore sebaceous gland activity increases greatly at sexual maturity and may contain pheromones.

P. campbelli and *P. sungorus* have a sexually dimorphic sebaceous gland that is located midventrally and used for scent marking and grooming. It is larger in males than females and has an annual cycle in activity (Figala *et al.*, 1973) These glands also differ morphologically. The male gland consists of a pocket of skin opening posteriorly containing sebaceous material. The female gland consists of a small superficial area of sebaceous material (Reasner and Johnston, 1987).

Harlow (1984) studied the roles of pelt lipids in muskrats, *Ondotra zibethicus* for: (1) heat loss by forced submergence in cold water (4 ^{o}C), (2) evaporative water loss

by the use of a temperature control chamber set at 36 ^{o}C, where the air pumped in was scrubbed of water. The outgoing air was measured for water content, oxygen consumption and temperature. With this information, the water in the exhaled air could be calculated by subtraction. They compared animals that had been shampooed to remove most lipids from the pelt, with those that had had their Hardarian gland removed (an organ situated behind the eye, rich in lipids which are spread over the entire body during grooming) and a sham-operated control group.

His results showed that:

(1) Pelt lipids make the fur non-wettable, which increases the body's buoyancy and keeps water away from the skin, thereby assisting in cold resistance. The control and Hardarianectomized group did not undergo a drop in body temperature until after 6 minutes exposure to cold water, but the shampooed group became hypothermic after only 4 minutes in the cold water. So lipids produced by the skin itself must be as important for thermal insulation in water as lipids produced by the Hardarian gland.

(2) Pelt lipids retard fluid loss by evaporation, thereby improving survival time due to reduced loss of body water. It was estimated that cutaneous water loss normally accounted for 41% of the total evaporative water loss, which was increased to 52% by Hardarianectomy and 66% by shampooing. However, reducing evaporative water loss makes sweating less efficient. The animals are not be able to dissipate so much heat and risk becoming hyperthermic. Therefore the thermoregulatory behaviour of licking and saliva spreading becomes important.

Although *Phodopus* sp. do not swim as muskrats do, they are exposed to snow.

1.9 Aims.

The project investigates the effects of photoperiod and ambient temperature on body mass and composition, pelage and properties of adipose tissue in *P. campbelli*. In particular, it focuses on the distribution and chemical composition of adipose tissue.

The questions that were asked were: since *P. sungorus*, responds to the extreme environmental changes of daylength and temperature that occur between summer and winter conditions, with changes in body mass, testicular activity, reproduction, torpidity and pelt colour change (Figala *et al.*, 1973), (a) were these physiological

changes also found in *P. campbelli*? and (b) if so, which environmental conditions had the strongest effects on the metabolism and body composition of *P. campbelli*?

The hypothesis to be tested was that photoperiod (daylength) was a stronger determinant of adaptation to extreme seasonal conditions on *P. campbelli* than ambient temperature. This hypothesis was tested by controlling, under artificial conditions, two essential components of the seasons: ambient temperature and daylength. The animals were studied under four experimental conditions: cold short day (CSD) environment (simulated winter conditions), warm short day (WSD) environment, cold long day (CLD) environment; (both being unnatural combinations of conditions) and warm long day (WLD) environment (simulated summer conditions). In order to test my hypothesis I studied changes in body mass and pelleted diet intake and incidences of torpor, during the experimental regime. The organs, adipose tissue (mass, lipid, protein and UCP), insulin and pelt were studied for physiological differences that had occurred after 102 days in their experimental regime, in order to accept or reject my hypothesis.

2.0 MATERIALS AND METHODS.

2.1 Basic care and breeding.

The small colony of Dungarian hamsters, *Phodopus campbelli,* was derived from 6 males and 6 females purchased at weaning age on 26.2.91 from Wright's of Essex whose colony had been 'closed' for 3 years. On arrival at the Open University (latitude 52 ^{o}N) they were caged in monogamous pairs, in a conventional animal room that was also occupied by other small rodents. The temperature was maintained at 22±2 ^{o}C with a constant 14 h light /10 h dark cycle (lights on at 07.00 h), and the relative humidity was kept at approximately 50%.

The hamsters were housed in polypropylene rat cages fitted with high dome stainless steel wire tops with an external food hopper (North Kent Plastics), internal dimensions: L 41 cm x W 25 cm x H 20 cm. High grade sawdust (Gold Chips - Special Diet Services) and hay were provided for bedding and nesting material.

The hamsters were fed *ad lib.* standard rat breeding chow, PRD yielding a digestible energy of 12.6 MJ/kg (approximate analysis stated by the manufacturers, Special Diet Services). Tap water was continually available in bottles. In addition, slices of apple or carrot were given to each pair twice weekly. A small handful of sunflower seeds were put in the cages initially and at weekly intervals. Fresh hay was added whenever it needed replenishing.

The colony was slow to start producing offspring, but once started, 5 of the 6 pairs bred several times. The gestation period is 18-19 days with a fertile post-partum oestrus (Wynne-Edwards and Lisk, 1983). The litter sizes varied from 2-11 offspring, although 4-5 was normal, with a very low pre-weaning mortality rate. Most breeding females appear to rest for about a month after every few litters, and all breeding ceased from December through to March, even though the temperature and light regime in the laboratory remained constant. Females that did not have regular rests from breeding rapidly declined in general health and breeding performance and often died at less than 6 months old, compared to longevity of 1 year for those that did not breed continuously.

For the breeding of hamsters for the 1992-3 and 1993-4 runs, the photoperiod was lengthened to 16 h light /8 h dark. Accidental tampering with the time clocks confirmed that 14 h light /10 h dark was a borderline photoperiod, that resulted in a greatly reduced numbers of births. This breeding failure was probably due to the testes regression that occurs on photoperiodic regimes below 14 h light /10 h dark, as reported in *P. sungorus* by Duncan *et al.* (1985).

The pups were weaned at 21 days, as opposed to 18 days as recommended by Universities Federation for Animal Welfare for *P. sungorus* (Hobbs, 1987). This 3 day overlap with the newborns benefited the weaners, without appearing to cause any distress to the newborn pups of the following litter. The weaners were then kept in their sibling groups for a further 1-2 weeks, before separation of the sexes. Any replacement breeders were placed together at this time to establish compatability.

2.2 Experimental environmental conditions.

Two rooms were selected where the environment (i.e. photoperiod and temperature) could be controlled. They were prepared 2 weeks in advance to allow for any adjustments to the environmental controls which may have been necessary.

Before introducing the livestock, one room was regulated at ~10 ^{o}C, the 'cold room', and the other at about 24 ^{o}C the 'warm room'. The photoperiods were controlled at 8 h light /16 h dark or 16 h light /8 h dark, by time clocks that were checked very regularly.

Tab. 2.1 Summary of the experimental environmental regimes studied.

Photoperiod	Room Temp.	Environment	Abbr.
8 h light /16 h dark	cold ~10 ^{o}C	cold /short day	CSD
8 h light /16 h dark	warm 24±2 ^{o}C	warm /short day	WSD
16 h light /8 h dark	cold ~10 ^{o}C	cold /long day	CLD
16 h light /8 h dark	warm 24±2 ^{o}C	warm /long day	WLD

Table 2.1. shows the four conditions studied. The short photoperiod (8 h light /16 h dark) had lights on at 08.00 h and the long photoperiod (16 h light /8 h dark) had lights on at 04.00 h.

All experiments were conducted between mid November and late February, around the winter solstice, during the natural short photoperiod and the coldest part of the year. Keeping the temperature down reliably in the cold room was the biggest problem, and the low outside temperature minimised this problem. The cold room faced north with an outside wall, but with no obvious exposure to natural photoperiod and was cooled by the main ventilation system. When the lights went off in the room it was also dark outside and the outside air was rarely above 12 °C. There was also a back up chiller unit mounted on the outside wall that further cooled the air when necessary. The warm room had no outside wall and was warmed by the main ventilation system. A digital max. and min. thermometer was used to record the temperature range in both rooms for the duration of the experiments.

The WLD regime had conditions that were similar but not identical to those of the breeding/stock room. The 'warm' room needed no temperature adjustment relative to the rest of the unit. A fluorescent fitting was installed with a 'True-lite' tube to match the lighting in the 'cold' room. 'True-lite' produces a full-spectrum natural light that simulates the visible wavelength of sunlight, including ultra-violet.

CSD thus imposed the biggest change on the experimental hamsters.

The photoperiods were chosen because Heldmaier *et al.* (1985) showed that with *P. sungorus*, 8 weeks exposure to short photoperiod (8 h light /16 h dark) increased the cold response of non-shivering thermogenesis (NST) significantly, compared to long photoperiod (16 h light /8 h dark). Also Duncan *et al.* (1985) concluded that below 14 h light /10 h dark in *P. sungorus,* produced significant loss of body mass.

The temperature regimes were chosen because they could be maintained reliably with the facilities available. The chiller was not designed to work at below 10 °C. Heldmaier *et al.* (1982b) reported that hamsters would develop their maximum potential for NST after prolonged exposure to 10 °C, but that at lower ambient temperatures neither their metabolic rate changed nor the capacity for NST improved.

2.3 Experimental Procedures.

2.3.1 Run 1 = 1991-1992 experimental season.

On the 14th November 1991, 26 hamsters aged 100±2 days were weighed, the means for each sex were calculated and the specimens were then divided into two groups that were similar in body mass and sex. Each group consisted of 7 males and 6 females. This first experimental run was 8 h light /16 h dark 'cold' or CSD (lights on at 08.00 h) and 16 h light /8 h dark 'warm' or WLD (lights on at 04:00 h).

Each experimental animal was placed separately in a high dome rat cage in their new environment by 11.30 h. The pelleted chow (PRD) was provided *ad lib.* It was weighed and recorded, giving initially approximately 100 g /cage. Tap water was continually available in bottles. In addition, one slice of apple (~5 g) or carrot (~2 g) was given to each hamster twice weekly. Sunflower seeds (5 g whole, ~2.9 g kernel) were placed directly into the cages initially and then weekly. The hamsters relish such food and it helped them settle into their new cages, especially when they were young. Fresh hay was added whenever it needed replenishing.

Throughout the experimental period, the hamsters were weighed twice weekly on a portable Ohaus balance model CT-600, accurate to 0.1 g, that was calibrated between uses and after being moved between rooms. The pelleted diet in each animal's hopper was weighed weekly and the amount eaten recorded. Each hopper was refilled periodically to approximately 100 g. All procedures with the hamsters were carried out between 10.00-11.00 h. The daily range of temperature was recorded, between 09.00-10.00 h, using a digital max. and min. thermometer ±1 ^{o}C (with the sensor placed over the cages), along with a measurement taken using a whirling hygrometer (relative humidity). The hamsters were also checked for signs of torpor at this time each day.

On Mondays, a small slice of apple (~5 g) was given to each hamster after weighing. On Fridays, they were given a small slice of carrot (~2 g) each and on Thursdays they were placed in clean cages, given sunflower seeds, weighed and the pelleted diet checked.

Several hamsters on all the regimes died during the experiment. There were also occasional sudden deaths in the breeding colony. In the CSD experiment, 2 males died,

the first on 12.12.91. It was immediately replaced with another male and closely matched in weight and age. The second male died on 16.2.92, 93 days after the start of the experiment (only 9 days short of completion). Data from it were included in the results, because a replacement would have extended the data collection period to June '92. Neither hamster had shown any obvious signs of illness before death.

In the WLD experiment, 3 males and 1 female died. All were replaced, again closely matched for sex, weight and age. This substitution caused no problem as it was possible to maintain the warm conditions throughout the seasons. All these animals lost weight over a period of approximately two weeks before death.

Post-mortem examination revealed;

1) no obvious obstructions to the alimentary tract,

2) no physical damage (i.e. broken limbs),

3) some cheek pouches containing food, others empty,

4) some large bowel containing faeces, others empty,

5) some stomachs containing food, others empty,

6) little or no adipose tissue in any depots.

7) there was reduction in the amount of pelleted diet eaten during the final recorded period, by any of the hamsters.

2.3.2 Run 2 = 1992-1993 experimental season.

On the 19th November 1992, the second run of experiments was set up in exactly the same way as the first run with hamsters aged 102±3 days, again with two groups, this time, each consisting of 15 males and 13 females, matched for sex, body mass and age. All procedures were carried out in the same way as previously stated, except that procedures on Thursdays for cleaning out, weighing of the hamsters and the pelleted diet were performed between 10.00-11.45 h, taking longer due to the larger numbers involved. This second experimental run used the unnatural regimes of 8 h light /16 h dark 'warm' or WSD (lights on at 08.00 h) and 16 h light /8 h dark 'cold' or CLD (lights on at 04.00 h).

In the CLD experiment 6 males and 2 females died between 24.12.92 and 22.2.93. Post-mortem examination revealed;

1) no obvious obstructions to the alimentary tract,

2) no physical damage (i.e. broken limbs),

3) some cheek pouches containing food, others empty,

4) some large bowel containing faeces, others empty,

5) some stomachs containing food, others empty,

6) one male had visible white adipose tissue in six depots, but no BAT. The remaining hamsters had little or no adipose tissue in any depots.

7) two males lost weight steadily over a month, the remaining hamsters lost weight quickly within a few days.

8) one male died from 'wet tail', a common bacterial infection of hamsters.

9) there was reduction in the amount of pelleted diet eaten during the final recorded period, by any of the hamsters.

In the WSD experiment two males and two females died, one male was found dead on the final day of the experiment and the other male died on 22.12.92. One female died within 11 days of the start, as she had not been under the environmental conditions long enough to show any effect, data from her were not included in the experiment. The other female died on 14.1.93.

Post-mortem examination revealed;

1) as in the CLD hamsters, no obvious obstructions or physical damage,

2) no adipose tissue in the female, but small amounts in all depots in one male,

3) cheek pouches in the female were empty, the cheek pouches of one male contained a small amount of food,

4) the small intestines of the female contained food and the large bowel contained faeces and the large bowel of the male had bloody faeces,

5) one male decreased the amount of pelleted diet eaten and lost weight just before death. The female showed no change in the amount of pelleted diet eaten during the final recorded period, but lost weight steadily over one month, then a rapid decline, before death,

6) the females ovaries were very dark.

No hamsters were replaced during this second run of experiments.

2.3.3 Run 3 = 1993-1994 experimental season.

On the 18th November 1993, a third experimental run was set up with hamsters aged 106±1.5 days. In exactly the same way as previous runs, again with two groups, each consisted of 12 males and 12 females, matched for sex, body mass and age. All procedures were carried out in the same way as previously stated for Run 2. This third experimental run was to confirm the data from Run 1 and increase the sample sizes.

Thus Run 3 was CSD, 8 h light /16 h dark 'cold' (lights on at 08.00 h) and WLD, 16 h light /8 h dark 'warm' (lights on at 04.00 h).

One female died within the first 2 weeks of Run 3, after steady weight loss from the start of the experiment. She was immediately replaced with another, as closely matched as possible for body mass and age.

2.4 Termination of experiments.

After 102 days (i.e. towards the end of February) under the controlled climatic conditions, the hamsters were weighed and given a terminal overdose of anaesthetic, with 0.08-0.17 ml of sodium pentobarbitone (Sagatal - 60mg /ml) by intraperitoneal injection. The dosage was adjusted according to body weight.

The animal was held until the effects of the anaesthetic were deep enough to allow up to 1 ml of blood to be withdrawn by cardiac puncture into a pre-heparinised syringe. The blood was left to separate overnight in a pre-heparinised vial, then spun slowly in an Eppendorf minifuge for 30 seconds. The serum was decanted into fresh labelled vials and stored at approx. -30 ^{o}C.

When dead, the hamsters were laid out flat for ease of dissection, stored in labelled, resealable plastic bags and placed in the freezer. Between two and four hours prior to dissection, the bodies were removed from the freezer and left to thaw at room temperature.

2.5 Dissection.

Each hamster was weighed again, placed on its back and the pelt dampened lightly with phosphate buffered saline (PBS) before being carefully slit from the near hind foot along the side, following the natural line which divides the ventral (white) and dorsal

(grey) hair to the lower jaw. Then the pelt was gently peeled away from the body and the adipose tissue with a scapel blade. The small tail was severed at its base and taken with the pelt, the paws of the hind- and fore-limbs were left with the body. The cheek pouches, which invariably contained small amounts of partially masticated chow were removed. Dissection continued over the head, and the ear lobes were removed with the pelt, then around the eyes, finishing on the upper jaw. The pelt was then weighed and stored at approx. -30 ^{o}C.

Figs 2.5.1-2 show the adipose tissue depots found in *P. campbelli* when the pelt is removed. This male hamster had been kept in the breeding room, hence the size of the depots, with the overlapping of some.

Fig. 2.5.1 General distibution of adipose depots studied in *P. campbelli.*

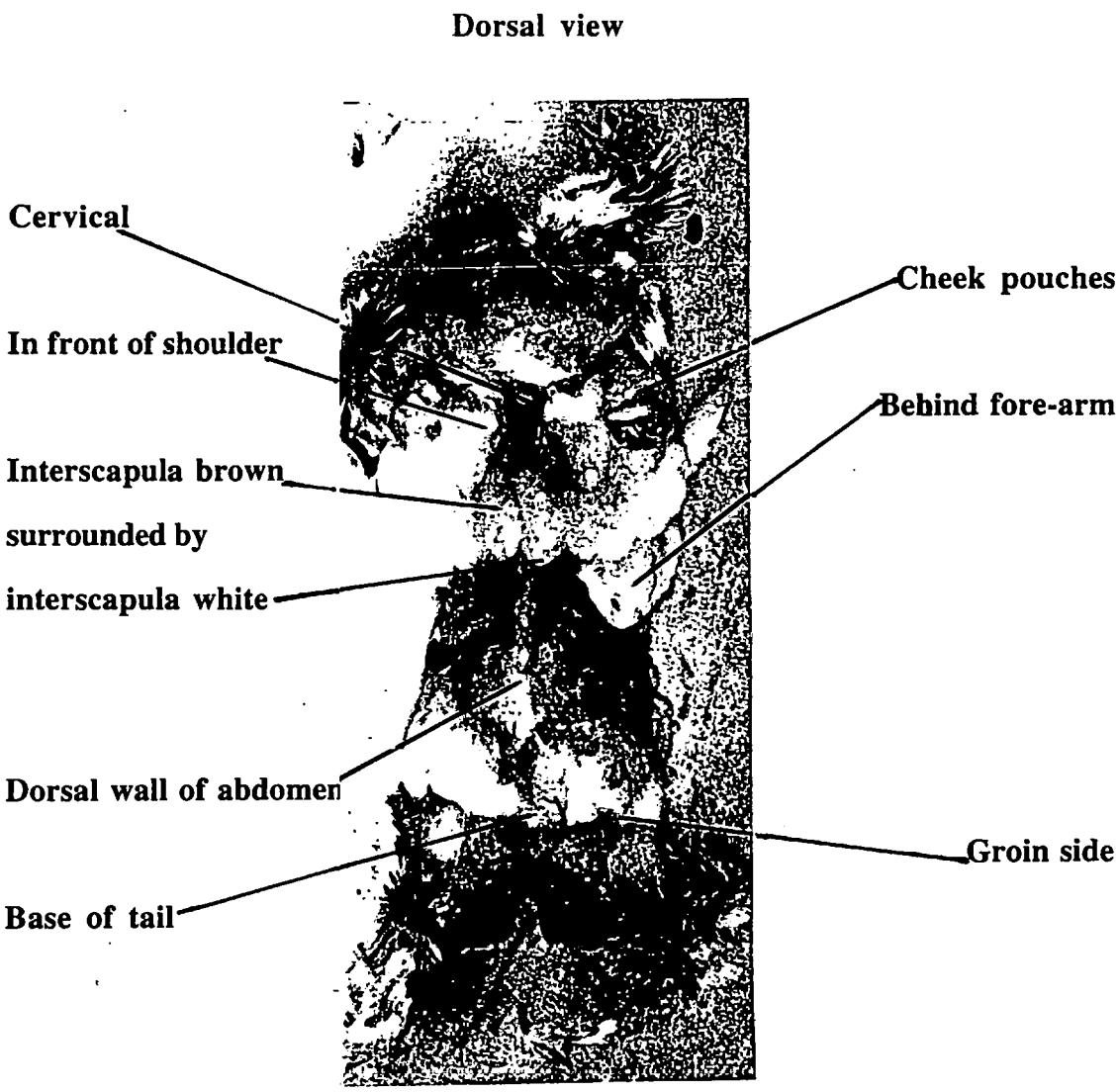
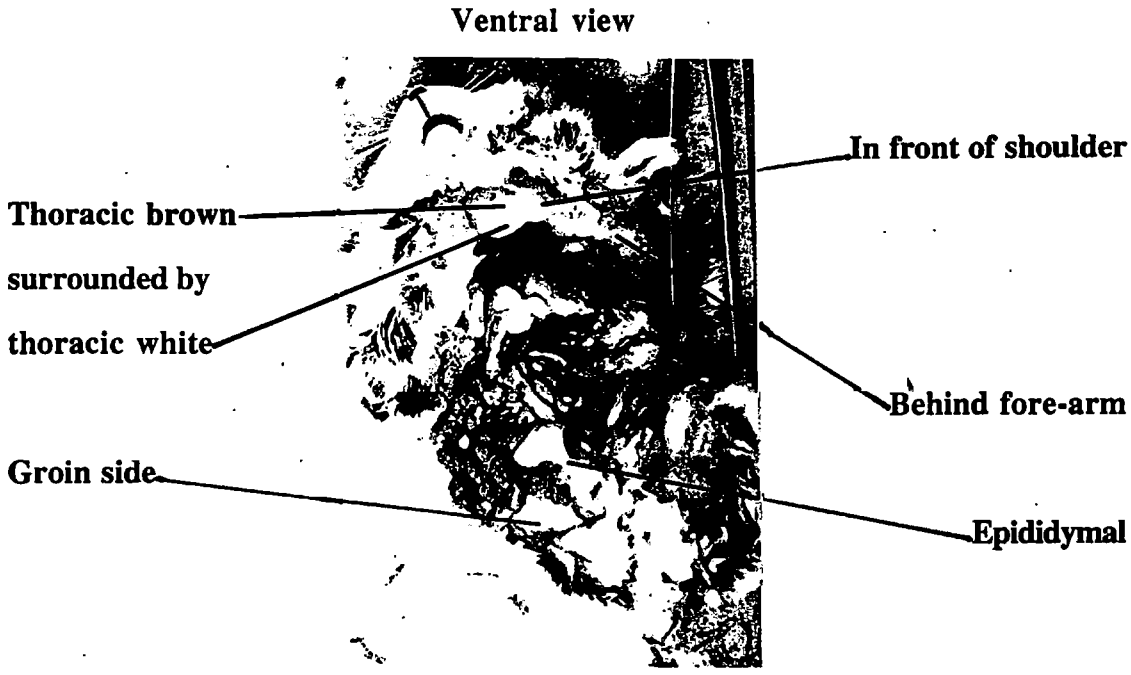


Fig. 2.5.2 General distibution of adipose depots studied in *P. campbelli.*



All the adipose tissue depots in these hamsters (Mattacks and Pond, 1988; Pond *et al.*, 1984a + 1984b) are easy to recognise and separate, especially in the hamsters on short photoperiod, when the depots are small and discrete. However with increasing fatness (Figs. 2.5.1-2), the depots both become thicker and cover a far greater area. Adjacent depots overlap in obese specimens, but can still be separated at dissection.

Table 2.2 Description of the intra-abdominal,superficial and intermuscular adipose depots studied.

SITE (abbreviation)	DESCRIPTION
INTRA-ABDOMINAL;	
Dorsal wall of abdomen (DWA)	White adipose tissue attached to or adjacent to dorsal wall of abdomen, including perirenal and along musculature ventral to the spinal column.
Attached to intestines (ATG)	Omental and mesenteric adipose tissue. Not easily distinguished in small rodents.
Epididymal (EP)	Male gonadal white adipose tissue attached to testes.
Ovarian (OV)	Female gonadal white adipose tissue attached to ovaries
SUPERFICIAL;	
Groin side (GS)	White adipose tissue anterior to the thigh progressing dorsally left and right and laterally to the abdominal wall.
Groin ventral (GV)	White adipose tissue found on the lower abdominal wall/pelvic region, left and right between inner thighs.
Base of tail (BOT)	White adipose tissue found surrounding the tail and ano-genital region.
Interscapular (HB & HW)	Covering the thoracic vertebrae, between the crests of the scapulae, separated into brown and white adipose tissue.
Thoracic (TB & TW)	Superficially covering an anterior section of the thoracic cavity, between the fore-arms, terminating at IFS and BA. The brown adipose tissue is covered by a pad of connective tissue, which in turn is surrounded by white adipose tissue.
In front of shoulder (IFS)	White adipose tissue continuing laterally in front of shoulder, (left and right) radiating posteriorly over scapulae, terminating at the interscapular depot.
Behind arm (BA)	White adipose tissue found posterior to forelimb, laterally left and right towards the scapulae.

INTERMUSCULAR;	
Popliteal (POP)	White adipose tissue at the rear of the lower thigh, posterior to left and right the knee and dorsal to the gastrocnemius muscle (between the major flexor muscles of the knee).
Cervical (UNM)	Brown adipose tissue found medial to trapezius muscle. It is a triangular site found on the left and right of the vertebrae, that goes surprisingly deep.
Axillary (AX)	Brown adipose tissue found in the left and right axilla, extending under the scapulae.

The superficial depots (Table 2.2) were dissected first, because once the pelt was removed, they tend to dry out very fast, so speed was essential. These depots were then weighed, sealed in bags and stored in the freezer.

The next stage was to open the peritoneal cavity for the intra-abdominal depots. In the males the epididymal depot (EP) was dissected out first, along with the testes, which were weighed and their condition recorded. In the females, in Run 2 and 3 the ovarian depot (OV) was dissected out, along with the ovaries that were weighed separately.

All the intestines between the stomach and the rectum (GUTS) were then removed, and separated from ATG the mesenteric and omentum adipose depot. The omentum and mesentary were not extensive enough to study be studied as two separate depots. The stomach, spleen and pancreas were discarded in run 1 making it easier to remove the liver for weighing. In run 2 and 3, however, these organs were weighed. The kidneys were found embedded in the adipose tissue depot that was found along the dorsal wall of abdomen and extended into the pelvis (DWA). During dissection care was taken to exclude the adrenals and ureters, plus the ovaries and horns of the uterus in the females.

The thoracic cavity was then opened and the heart removed.

The *vastus lateralis* muscle, found on the upper thigh, being small was dissected penultimately. The intermuscular depots were dissected last, since, although well protected between muscles, they were small and once exposed must be handled

quickly, to avoid desiccation. All depots were weighed, sealed in plastic bags and placed in the freezer as quickly as possible.

All weighings from the dissection were performed on a calibrated Mettler H54AR balance, accurate to 3 decimal places.

2.6 Lipid and Protein assays.

The adipose tissue from hamsters in each treatment group differed greatly in total mass and proportion of lipid and protein. So adjustments to the dilution of the samples were necessary, to obtain accurate measurements over the whole range of values!

For the lipid and protein extraction assay (Mattacks *et al.*, 1987), duplicate samples of 10 mg of tissue from each depot were accurately weighed and placed into clean labelled plastic tubes, avoiding hair and blood vessels. 1 ml of Edsons' mixture (2 g Tween 80, 1 litre acetone A.R) was added to each tube. Lids were put on the tubes and they were left in the fridge overnight.

There was over 1.5 g of adipose tissue in the larger depots (BA and IFS) of the obese animals, but only 10 mg of adipose tissue in the smaller depots (eg. POP) of CSD animals, barely sufficient tissue to perform both assays. So, in several cases, (especially CSD animals) I had to use all the tissue from a depot and there was not enough for a duplicate.

Each sample was homogenised for a minimum of 2X 30 s or until the tissue was broken down into suspension. For the samples from the WLD hamsters it was necessary to add a further 1 ml of Edsons' mixture to each tube because they contained so much lipid. The CSD hamster samples did not require this dilution. All tubes were vortexed, then centrifuged in tan MSE Coolspin at 4 ^{o}C for 10 minutes at 1500 rpm. The upper lipid layer was removed for assaying.

The protein precipitate was retained in the tubes and placed in a water bath at 60 ^{o}C for 20 min. to evaporate the acetone and then stored sealed at -30 ^{o}C.

2.6.1 Lipid assay.

Table 2.3 Standards for the lipid assay were made up from 100% corn oil stock (500 mg corn oil, 100 ml Edsons' mixture).

Concentration (%)	Edson's mixture (µl)	+ 100% corn oil stock (µl)
0	200	0
20	160	40
40	120	80
60	80	120
80	40	160
100	0	200

200 µl of sample extract was added to labelled tubes in triplicate, containing 2 ml Edsons' mixture. Dilutions were made as follows;

50% dilution = 100 µl sample+100 µl Edsons' mixture, in 2 ml Edsons' mixture, OR

100% dilution = 200 µl sample only, in 2 ml Edsons' mixture, OR

200% dilution = 200 µl sample only, in 1 ml Edsons' mixture.

After 400 µl of 2 M sodium acetate was added (to both the standards and samples) the tubes were vortexed, a further 3 X 400 µl of 2 M sodium acetate was added, and vortexed between each step (Mattacks *et al.*, 1987).

Readings were taken using cuvettes in a SP6-550 UV/visible spectrophotometer (Pye Unicam) at wavelength 440 nm against the reagent blank. The experimental samples were taken from the standard curve and the results calculated according to the dilutions.

2.6.2 Protein assay.

The protein assay was a micro Bradford method (Bradford, 1976) using a Titertek Multiskan plate reader.

1 ml of 1 M NaOH was added to the precipitate, obtained by centrifugation, and placed in a water bath for 1-2 hours at 60 ^{o}C. The tubes were then removed and vortexed to resuspend the precipitate, and allowed to cool.

Table 2.4 Standards for the protein assay were made up from protein stock solution, using bovine serum albumin (BSA) made up into a 1 mg/ml protein stock solution,

protein stock soln. (µl)	+ distilled water (ml)	Conc. /ml (µg /ml)	quantity /well (µg /10 µl)
0	1000	0	0
50	950	50	0.5
100	900	100	1.0
150	850	150	1.5
200	800	200	2.0
250	750	250	2.5
300	700	300	3.0
350	650	350	3.5
400	600	400	4.0
500	500	500	5.0

10 µl of sample or standard was placed in each well in triplicate. Dilutions of samples were made as necessary.

100% = 10 µl aliquot of sample taken, OR

50% dilution = 100 µl sample + 100 µl distilled water, vortexed, 10 µl aliquot taken, OR

20% dilution = 100 µl sample + 400 µl distilled water, vortexed, 10 µl aliquot taken, OR

10% dilution = 100 µl sample + 900 µl distilled water, vortexed, 10 µl aliquot taken.

Then 30 µl of 10 mM NaOH was added to each well, follwed by 250 µl of Bradford dye (50.0 mg coomassie blue 9250, 54.0 ml orthophosphoric acid, 46.7 ml ethanol (95%), in 1.0 litre deionised water) and stood at room temperature for a minimum of 10 min, but no longer than 60 min.

The readings were taken using a plate reader set at 595 nm against the reagent blank. The results were calculated from the standard curve and adjusted according to the dilutions.

2.7 Insulin radioimmunoassay (RIA).

An Insulin RIA diagnostic test kit was purchased from Kabi Pharmacia Diagnostics and the test procedure directions were followed.

100 µl of either standard (human) or unknown sample was added to labelled tubes, 50 µl insulin-^{125}I -colour coded blue, was then added to all the tubes, followed by 50 µl antibody (antiserum raised in the guinea pig) -colour coded yellow. All the tubes were shaken to ensure complete mixing and the contents checked to ensure that they had turned green. The tubes were then covered and incubated at room temperature for two hours. Next 2 ml of decanting suspension (Sepharose -anti-guinea pig IgG antibodies raised in sheep) was added to each tube and again incubated at room temperature for 30 min, before centrifuging at 1500 x g for 10 min. The tubes were then decanted straight away in one movement, and left upside down for 30 sec on absorbent paper, to remove as much liquid as possible from the tube, leaving just the precipitate. Finally the level of radioactivity was determined using a gamma counter. Standard curves were drawn for each assay and concentrations of the unknown samples calculated.

2.8 Isolation of mitochondrial proteins from brown adipose tissue.

Adipose tissue from whole BAT depots was required in order to collect enough mitochondrial proteins at the end of the isolation procedure to use on Western blots. With BAT depots, a minimum of 200 mg of tissue was needed, so in the case of the smaller BAT depots, the depots were pooled from up to 18 hamsters that had been kept under identical experimental conditions. The samples were weighed, finely minced with scissors and placed in ice-cold BAT buffer (Trayhurn *et al.*, 1993) (250 mM sucrose, 1 mM Hepes, 0.2 mM EDTA) at 5% (w/v) (Cannon and Lindberg, 1979).

The tissue was then homogenised in 30 s bursts, as previously described for the lipid and protein assay. The homogenate was centrifuged in a Beckman J2-21 at 8 500 g for 10 min to separate the surface fat layer, from the cell solids, which formed a pellet. The pellet was resuspended by vortexing in the original volume of BAT buffer and centrifuged at 4 ^{o}C, in an MSE Coolspin at 700 g for 10 min to separate the mitochondria (which remained in suspension from the rest of the cell debris which

formed the pellet). The supernatant was centrifuged in the Beckman J2-21 at 8 500 g for 10 min, forming the pellet, which was now made up of mitochondria. The pellet was washed 3 times by re-centrifuging in 2 ml of BAT buffer at 8 500 g for 10 min. The final suspension was attained by homogenising in 1 ml of BAT buffer. An aliquot was taken to determine levels of protein (Cannon and Lindberg, 1979), using the microplate method with bovine serum albumin (BSA) as the standard (Bradford, 1976). The sample suspension was aliquoted, labelled and then frozen at -20 °C.

2.9 Isolation of mitochondria from white adipose tissue.

Adipose tissue from the whole of the WAT depots was required (i.e. at least 1 g of tissue). So that at the end of the isolation procedure there was enough mitochondrial protein in suspension for Western blotting. Again, with the small depots, individual depots were pooled from several, sometimes all of the hamsters kept under identical environmental conditions, in order to get enough tissue. In some depots there was no tissue left after having performed the lipid and protein assays (i.e. the ovarian (OV) and popliteal (POP) depots) in either the CSD or WLD hamsters.

The tissue was weighed, minced finely with scissors, added to ice-cold WAT buffer (Rafael *et al.*, 1970) (10 mM triethanolamine-HCl (pH 7.5), 2 mM EDTA, 0.3 M sucrose, 2% BSA (fraction V)) at 5% (w/v) and homogenised in 30 s bursts, as previously described. The homogenate was centrifuged following the same procedure as that stated in the isolation of BAT mitochondria, until the washing stage, when the pellet was resuspended in BAT buffer. This buffer was necessary because the WAT buffer contained BSA which would give false readings with the protein assay and overload the blot. The final suspension was in 1 ml BAT buffer, an aliquot was taken to ascertain levels of protein, again following the same procedure as that stated in isolation of BAT mitochondria. The final suspension was then aliquoted, labelled and frozen at -20 °C.

2.10 Western blotting for uncoupling protein.

Western blots are the only recognised method to confirm, by the presence of uncoupling protein (UCP), which depots contain brown adipocytes and which only

contain white adipocytes (Trayhurn, 1989). For electrophoresis, each lane of the polyacrylamide gel was loaded with 25 µl of sample, containing the mitochondrial protein, plus 25 µl of gel sample buffer. I found that 5 µg of mitochondrial protein produced a good blot from BAT depots. Mitochondrial protein was heavily loaded (25 µg) from the WAT depots of both the CSD and WLD hamsters, in order to maximise any chance of there being UCP and it showing on the blot (Trayhurn *et al.*, 1989).

The mitochondria were solubilised by heating to 95 °C for 5 min after diluting 50/50 with a gel sample buffer (for recipe, see section 6.2). The samples were then added to a 10% polyacrylamide gel (for recipes, see section 6.1) and run on a minigel Mighty Small (Hoefer Scientific Instruments) system with gel running buffer (for recipe, see section 6.2) for 60-90 min, with settings of 150 V constant, polarity normal and current minimum, set on ice.

After electrophoresis, the proteins were transferred to nitrocellulose membrane (Biotrace NT) by electroblotting using a Biorad mini trans-blot, with transfer buffer (for recipe, see section 6.2) overnight (14 h) at 30 V and 90 mA.

To check that the proteins had transferred satisfactorily (to reduce the waste of anti-bodies), the nitrocellulose membrane was removed from the Biorad, washed briefly with distilled water, placed in a staining jar and covered with Ponsceau S staining solution (0.2% in 1% acetic acid) for approx. 5 min. The stain was returned to its' bottle and the blot washed 2/3 times with 1% acetic acid, to clear the background, leaving the protein bands stained red. The acetic acid was replaced with 20mM NaOH (which washes out the stain from the blot), then washed with distilled water 2/3 times and covered with Blotto (1 litre 1X TBS- for recipe see section 6.2, 1 ml 50% Tween 20, 5% DSMP (Marvel) (w/v)) for 3-4 h at room temperature, to block the spare protein binding sites. After which the liquid was poured off and the first antibody added, rabbit anti-ground squirrel UCP serum (which was kindly donated by Prof. P. Trayhurn) diluted 1/1000 with Blotto (5% Marvel), overnight, on a rocker, in the cold room at 4 °C.

The liquid containing the first antibody was poured off the following morning and the nitrocellulose membrane washed 3X with Blotto at approximately 30 min /wash. The second antibody (goat anti-rabbit IgG serum conjugated to horseradish peroxidase

(Sigma) diluted 1/500 with Blotto) was again added overnight, in the cold room at 4 ^{o}C.

The next morning the liquid was poured off and the nitrocellulose membrane washed 3X with Blotto (5% Marvel) and 2X TTBS (400 ml 1X TBS, 0.4 ml 50% Tween 20) at room temperature. Detection of the UCP protein was with DAB (Diaminobenzidine tetrahydrochloride) in 1X TBS (for recipe, see section 6.2), which was left on for 5-30 min until required depth of colour was attained. Finally several washings with distilled water removed the surplus DAB, the standards were marked with a pencil and the nitrocellulose membrane was hung out to dry.

To check that the transfer of proteins had been complete, the gel was placed in a staining jar and stained with Coomassie Brilliant Blue (0.25 g Coomassie Brilliant Blue R250, 90.0 ml methanol, 90.0 ml distilled water, 10.0 ml glacial acetic acid) overnight at room temperature on a rocking platform. The staining solution was returned to its' bottle and destaining solution added (65.0 ml methanol, 30.0 ml distilled water, 5.0 ml acetic acid), destaining was also done at room temperature overnight on a rocker, the destaining solution was changed 3-4 times. A sponge was placed at the side of the jar to absorb the stain as it came out from the gel.

2.11 Pelt lipid assay.

When dissecting of the hamsters from the first run of CSD and WLD, I noticed a difference in the 'wetability' of the pelt (when damping it down for ease of dissection), so I decided to see if there were differences in the lipid content of the pelt of hamster kept within the 4 environmental conditions.

During the development of an assay to extract and quantify lipids in the pelt, I tried several sizes of pelt samples and compared samples from several different regions of the pelt. The sample area was restricted by the small size of the hamsters. I found that there were three regions of the pelt (dorsal-fore, ventral-fore, dorsal-rear) that showed differences in the lipid content and that results from these 3 regions were consistent with other hamsters from the same environmental conditions.

To find the quantity of lipid that could be extracted from the hair of the pelt samples, with care being taken to avoid skin cells and sebaceous secretions, I developed the

following assay using Edsons' mixture and the solvent 2 M sodium acetate, as in the standard lipid assay.

Each sample was carefully placed hair down in 1 ml of Edsons' mixture in a glass petri dish and allowed to soak for 10 s (when most of the Edsons' was absorbed onto the hair). A further 1 ml was added to the petri dish and the pelt swirled gently for a further 10 s, the sample was then carefully stripped with forceps to remove the surplus solvent from the hair. Approximately 1 ml in total was normally recovered, enough for assaying the lipid extracted from each pelt sample in triplicate. The extract was then pipetted into a test tube, set on ice and covered. When all the extracts had been collected, the standards were made up as in the case of the assay for adipose tissue lipids. The sample extracts were then vortexed, and 200 µl was added to labelled tubes in triplicate. The remainder of the pelt assay was as for the assay of adipose tissue lipids (Section 2. 6.1).

The total volume of the extraction solution was multiplied by the fraction of the lipid recovered and divided by the mass of the hair from the sample, multiplied by 1000 to give µg lipid per mg hair.

The pelt samples were laid out to dry after the surplus solvent had been removed. At the end of the assay, the hair length, for Run 3 only, was measured whilst the fur was still attached to the skin, using a vernier calliper. Finally the hair was shaved from the skin, weighed and stored for further study.

At a later date, using a light microscope with a line graticule attachment (1 division = 0.1 mm), the width of 50 hairs from over the rump of each hamster was measured.

2.12 Pelage colour changes.

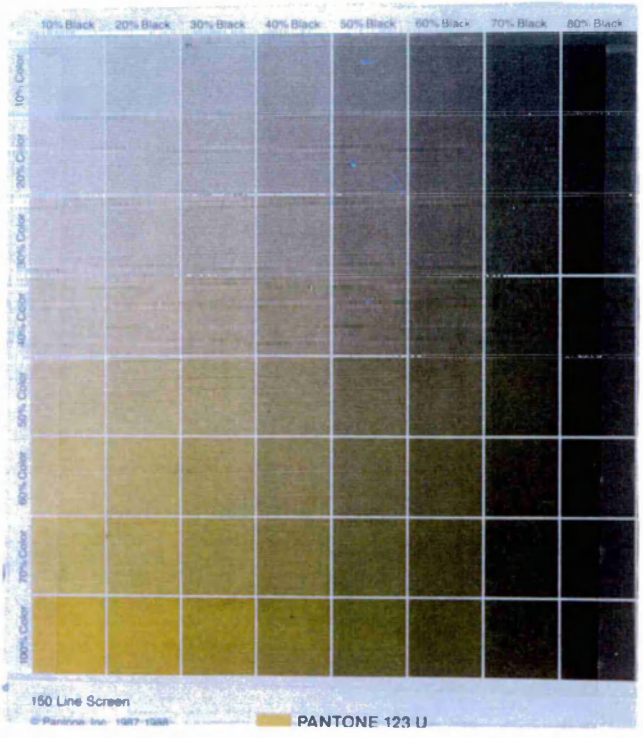
As explained in Section 1.1 it has been well documented that the pelage of *P. sungorus* undergoes extensive colour change in winter conditions, while that of *P. campbelli* is believed to change much less. Figala *et al.* (1973) developed a scale of changes for *P. sungorus*, where stage 1 was dark (summer coat), through to a virtually white pelage (winter coat) at stage 6. Duncan and Goldman (1984) had developed a similar scale with only 4 stages.

I found that there were pelage changes in *P. campbelli* on short photoperiod, which, though not as extreme as *P. sungorus*, were detectable as a definite lightening of the grey pelage. The scale of changes developed for *P. sungorus* could not be used since the changes in *P. campbelli* were far more subtle so I developed my own scale of colour changes using a Pantone Colour and Black Selector 747XR, Page 123U (Fig. 2.12.1) as shown in Table 2.5.

Table 2.5. Scale of pelage changes that were defined using colour on the Pantone Colour and Black Selector card.

Stage (1)	30 % colour /40 % black (summer pelage)
Stage (2)	30 % colour /30 % black
Stage (3)	20 % colour /20 % black
Stage (4)	10 % colour /20 % black (winter pelage)

Fig. 2.12.1 Chart used for changes in pelt colour that was developed for *P. campbelli* using a Pantone colour and black selector 747XR, page 123U (4U).



2.12 Statistics.

All measurements of means and shown including ± SEs.

One-way and three-way ANOVA were performed using an SPSS package. The Least Significant Differences (LSD, $p<0.05$) were recorded in the Statistics Appendix.

Students *T*-test.

3.0. RESULTS

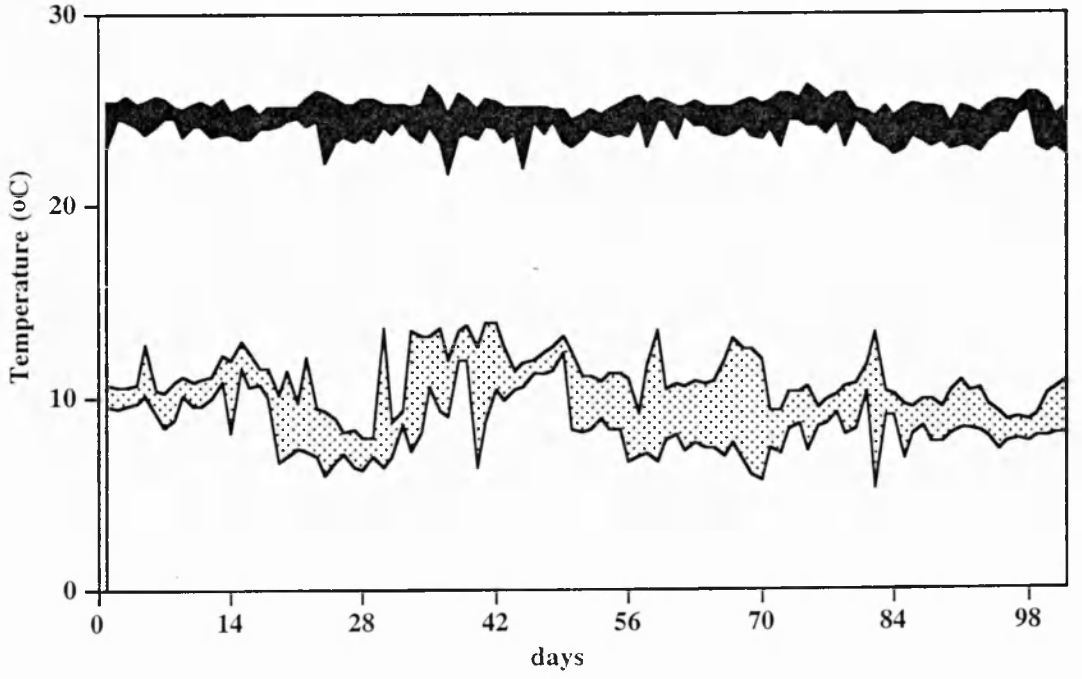
A preliminary report of some of my data appear in an abstract (Sadler and Pond, 1996).

3.1 Environmental conditions.

3.1.1 1991-1992 experimental season (Run 1).

Fig 3.1.1 shows the maximum and minimum ambient temperaures for the warm and cold room in Run 1 (winter 1991-1992 spring).

Fig. 3.1.1 Environmental temperatures for Run 1 (1991-1992). Upper dark band = the warm room and the lower light band = the cold room.



The temperature controls in the 'cold' room for Run 1 did not function reliably, due mainly to faults on the back-up chiller unit. The cooling of the air was via the main unit in the ventilation system, backed up by a smaller chiller unit situated on the north facing outside wall. The chiller was controlled by a computerised environmental control unit which was set at 10 ^{o}C, but unfortunately at intervals the chiller went into 'reverse' and warmed the air. During this period, it was closed down and the room temperature closely monitored, relying on the main system only. The mean temperature for the cold room was 9.7±0.14 ^{o}C. Problems with the chiller caused the

large overall temperature range, 5.3-13.9 ^{o}C, but these extremes lasted no longer than overnight. These temperature variations also had an effect on the stability of the relative humidity, RH = 75.62±0.72. The problem with the chiller was rectified on 6th February 1992 and the temperature then remained more stable for the duration of Run 1 (as shown in Fig. 3.1.1).

However, the chiller and main system could not maintain the room reliably at <10 ^{o}C when the outside air temperature was higher than 12 ^{o}C, which strengthened the decision for the experiments to only be run around the winter solstice.

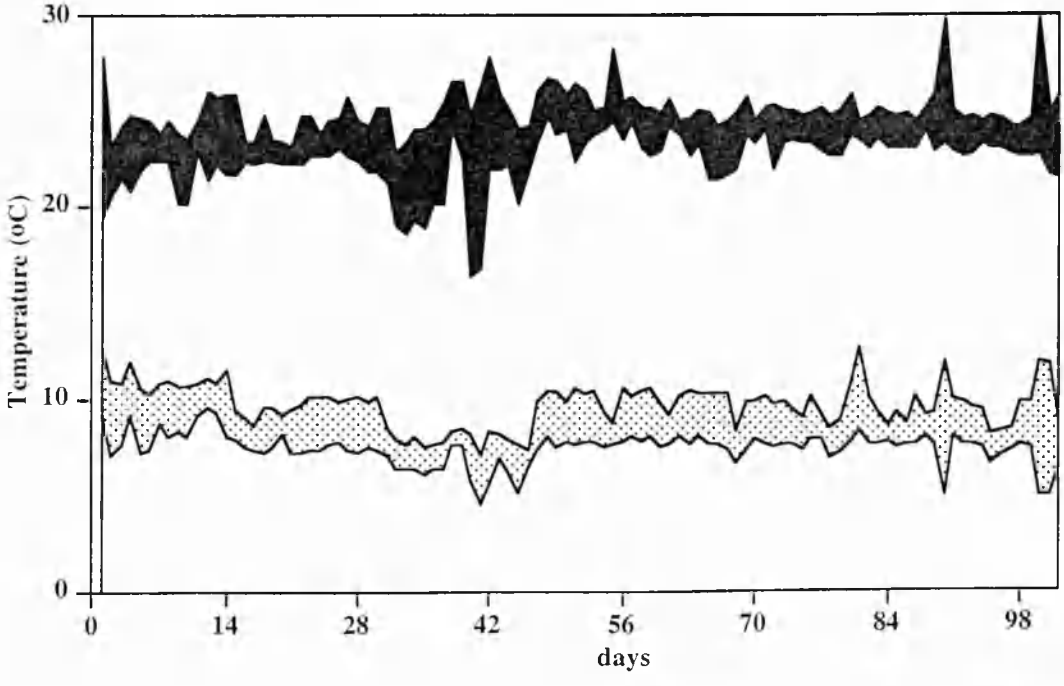
The warm room remained relatively stable for the duration of Run 1 at a mean of 24.5±0.07 ^{o}C, with an overall range of 21.9-26.3 ^{o}C. The large variation in the relative humidity could only be due to fluctuations in the air conditioning system, RH = 46.34±0.87.

For all three runs the imposed light /dark cycles were checked regularly, and found to be satisfactory.

3.1.2 1992-1993 experimental season (Run 2).

Fig. 3.1.2 shows the maximum and minimum ambient temperatures for the warm and cold room in Run 2 (winter 1992-1993 spring).

Fig. 3.1.2 Environmental temperatures for Run 2 (1992-1993). Upper dark band = the warm room and the lower light band = the cold room.



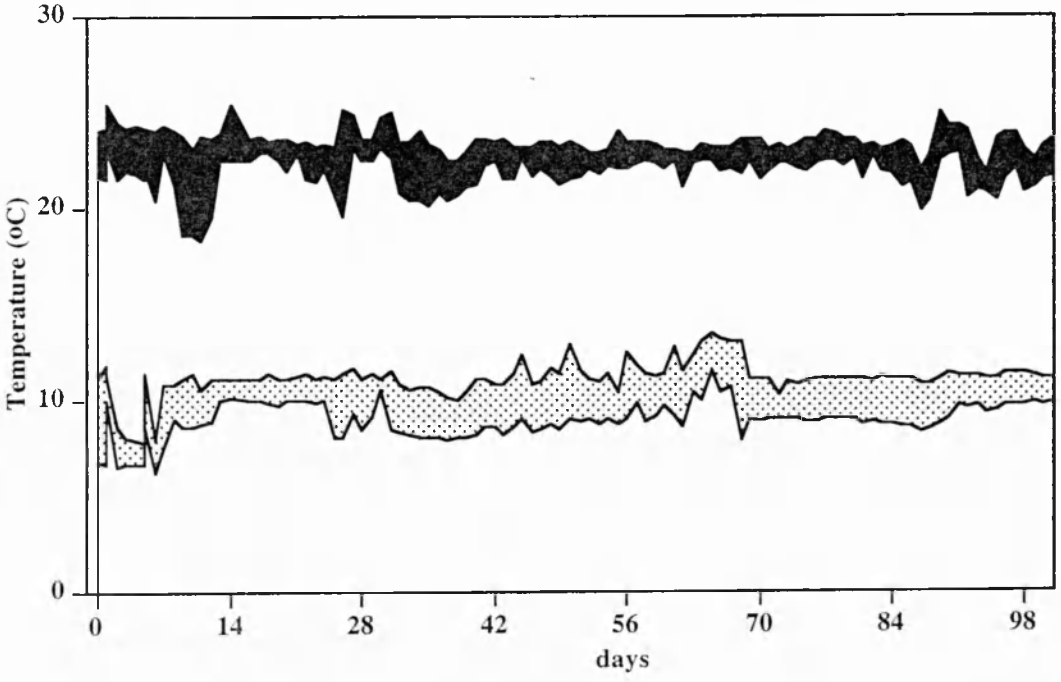
For Run 2, the temperature in the cold room was cooler at a mean of 8.6±0.11°C and remained more stable than for Run 1 for the duration of the experiment. The overall range was 4.6-12.7 °C. RH = 73.64±0.61.

The mean temperature for the warm room was also cooler at 23.7±0.13 °C. In 1992-93, it was the warm room that had the greater temperature variation (16.4-29.9 °C). These variations occurred throughout the whole unit, due to fluctuations in the air conditioning system. It also affected the relative humidity, RH = 43.01±0.61, which was lower than it should have been.

3.1.3 1993-1994 experimental season (Run 3).

Fig. 3.1.3 shows the maximum and minimum ambient temperatures for the warm and cold room in Run 3 (winter 1993-1994 spring).

Fig. 3.1.3 Environmental temperatures for Run 3 (1993-1994). Upper dark band = the warm room and the lower light band = the cold room.



in 1993-94, the warm room was over 1 °C cooler than the previous runs at 22.6±0.09 °C, with an overall range of 18.2-25.4 °C, but remained relatively stable after an initial problem with temperature differences. The cold room worked comparatively well at 10.0±0.10 °C, with an overall range of 6.2-13.4 °C. The relative

humidity continued to fluctuate, warm room RH = 41.83±0.59, cold room RH = 68.97±0.63.

3.2 Behavioural observations.

P. campbelli are very active little creatures in captivity, resembling wind-up toys, that run around and sit up on their short hind-legs (own observations).

'Winter' acclimatized hamsters (CSD) spent most of their time curled up in compact nests that they constructed of hay, appearing to leave the nest only for short foraging and exploration trips. The hamsters in WSD also built nests, but the nests were not so tightly constructed. The hamsters on this regime were seen more frequently outside the nest. By touching the hamsters when curled up in their nests, I was able to confirm that they felt warm suggesting that their nests did create a microclimate that conserved heat.

The 'summer' acclimatized hamsters (WLD) did not use their hay to make nests, but left it scattered around the cage. These hamsters did not appear to be very comfortable in this environment; they were lethargic, even when compared to WSD hamsters, who were kept at the same ambient temperature. Their pelts looked stark and greasy. The ventilation gaps in the fur and the changes in behavioural posture mentioned by Heldmaier (1975) from his observations of *P. sungorus* in the warm environment, were clearly visible in *P. campbelli* on WLD. The CLD hamsters appeared to override the long photoperiod stimulus and made nests, behaving like those kept on WSD.

3.2.1 Torpor observations.

For the duration of all three experimental periods, all hamsters were checked at approximately 10.00 h and 15.00 h daily for signs of torpor. At no time were the hamsters on WLD (simulated summer), CLD or WSD, found to be in torpor, or going into/ coming out of torpor. The only hamsters that were ever observed to show signs of torpor were those kept on CSD (simulated winter) and then it was only a small group of males that were predisposed to torpor. No hamsters were observed to be in torpor before the 6th week of the experimental regimes.

It is possible that torpor in some hamsters was missed, because (1) they had come out of torpor before the morning check, (2) they went into and came out of torpor between the morning and afternoon checks, or (3) *P. campbelli* predominantly become torpid during the hours of darkness, unlike *P. sungorus* (Ruf *et al.*, 1993). Hamsters that were awake at 10.00 h were never found to have become torpid at the 15.00 h check and hamsters that were torpid at the morning check were always awake and active when checked again in the afternoon.

In total, there were only 7 males that were ever found to either be torpid or going into /coming out of torpor. In my results I have classified those hamsters that were found to be either torpid or in the sleepy state around torpor, as torpid.

Of the 7 males :

2 males were found torpid only once,

2 males were found torpid twice,

1 male (H91) was found torpid 8 times,

1 male (H89) was found torpid 9 times and

1 male (H92) was found torpid 29 times.

Fig. 3.2.1 Incidence of male hamsters found torpid in CSD, both total and for individuals H89, H91 and H92. Pooled data from 1991-92 and 1993-94 experimental periods.

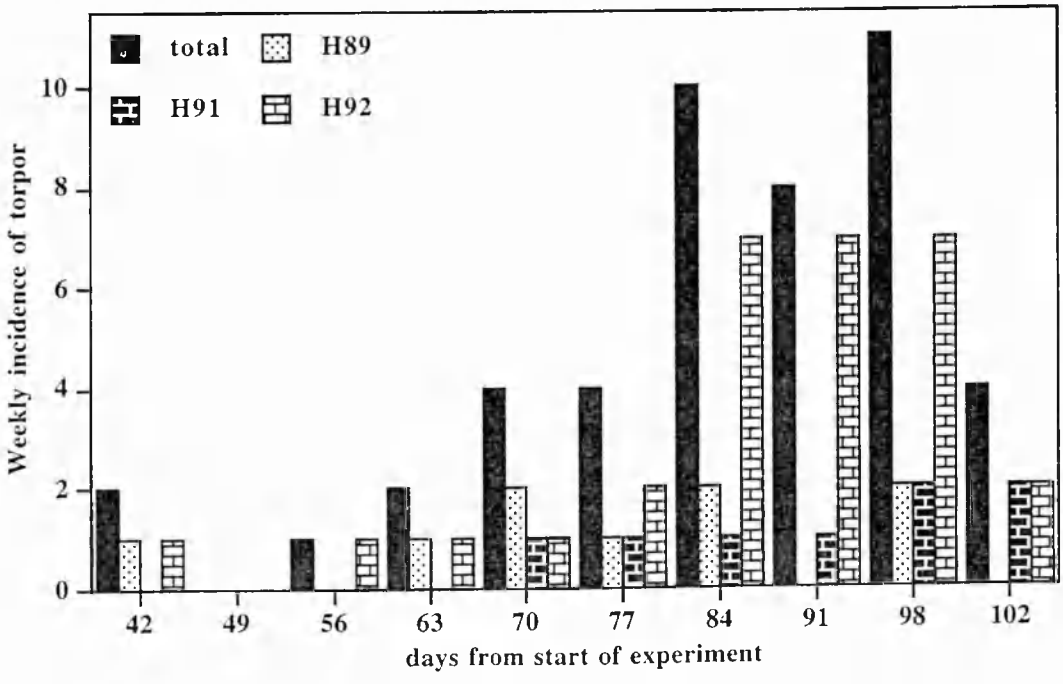


Fig. 3.2.1 shows that the total observed incidence of torpor per week in male hamsters and for the individual males H89, H91, and H92 that had a very high predisposition to torpor. Although a few bouts of torpor were observed around the 36th-42nd days (week 6), it was not until the 64th day (week 10) onwards that hamsters regularly began to be found torpid.

Fig. 3.2.2 Mean (± SEs) body mass of all CSD male hamsters and the three individuals with a high incidence of torpor, H89, H91 and H92.

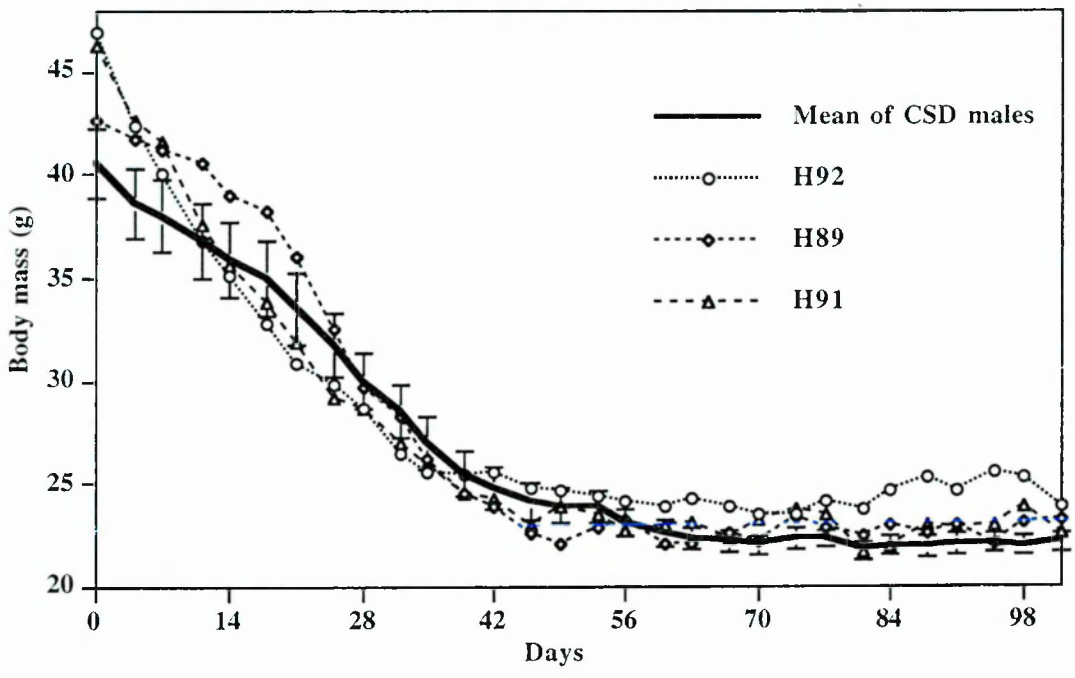
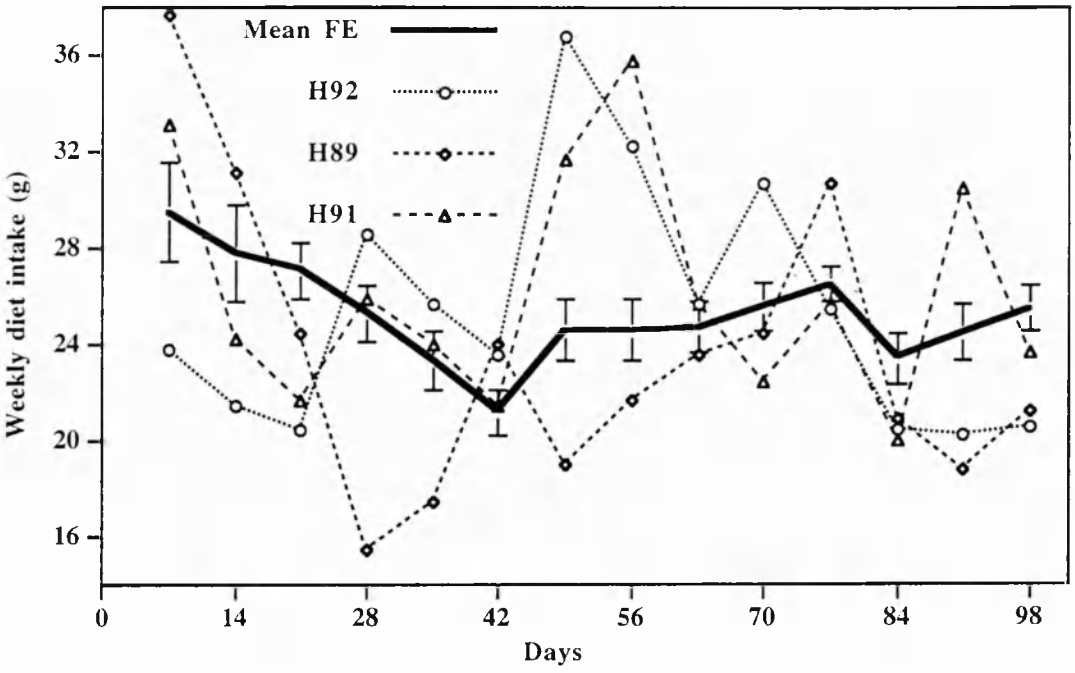


Fig. 3.2.2 shows that the body mass of individuals H89, H91 followed a similar pattern of changes for the mean of all the male hamsters on the same CSD regime, and H92 (whose incidence of torpor was extreme) became heavier than the average with increasing incidence of torpor.

Fig. 3.2.3 Mean (± SEs) weekly pelleted diet intake (FE) of all CSD male hamsters and the three individuals with a high incidence of torpor, H89, H91 and H92.



The hamsters that spent some of their time torpid did not eat vastly different quantities of pelleted diet from the mean of the male CSD hamsters. The data for H92 shows that he ate more pelleted diet than average from his first occasional incidence of torpor until he was found torpid almost every day (Fig. 3.2.3), when his food intake dropped below average.

Although H92 did not eat a hugely different quantity of pelleted diet from the average, yet his body mass was greater than the average for male CSD hamsters, suggesting that torpor, at least in his case, appeared to save energy.

3.3 Body mass.

Table 3.1 The numbers, initial age, body mass (mean ± SEs) and range from all regimes.

Environment	CSD 'simulated winter'	CLD	WSD	WLD 'simulated summer'
MALES				
N	17	9	14	19
Age (days)	105.2±2.1	102.0±6.8	109.0±5.4	106.4±2.0
Initial body mass (g)	40.5±1.7	37.7±1.5	38.2±1.1	39.9±1.6
Initial body mass range (g)	25.4-46.9	29.1-43.1	29.1-45.8	26.8-47.7
FEMALES				
N	18	11	11	20
Age (days)	104.0±3.3	96.0 ±5.2	100.1±6.2	100.9±2.8
Initial body mass (g)	31.7±0.9	29.4 ±1.2	30.4±1.5	31.7±1.0
Initial body mass range (g)	24.0-38.7	24.3-33.8	21.4-37.1	19.1-38.3

The only significant differences in the initial body mass of the hamsters were between the males and the females in each group ($f=11.3$, $p<0.0000$, one-way ANOVA, for the Least Significant Differences (LSD) see statistics table, S1 (p. 140)).

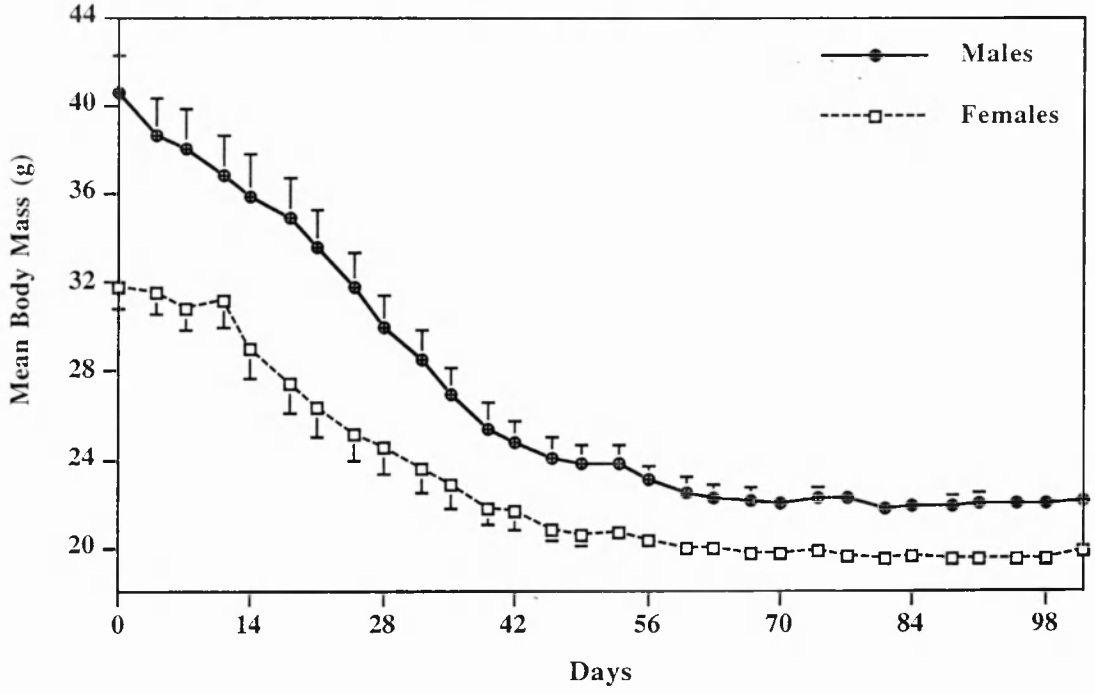
Even amongst siblings, the colony of *P. campbelli* had a large range of body masses. The mean body masses of each group were matched as nearly as possible, as shown in Table 3.1. The variations in the initial mean body mass occurred where the replacement hamsters were not able to be matched weight for weight.

All body mass changes were monitored closely, confirming that the hamsters were not distressed. I also used Run 1 (1991-92) to find when the hamsters' body mass would plateau at their maximum or minimum values. This information enabled me to estimate how long each experimental treatment should last to achieve maximum changes in body mass. No significant changes in body mass occurred after 80 days (Figs. 3.3.1-3.3.4), so an experimental period of 102 days was more than enough to observe the effects under investigation.

Heldmaier *et al.* (1985) had already reported that in *P. sungorus*, 8 weeks exposure to short photoperiod increased NST significantly. Figala *et al.* (1973) found that when *P. sungorus* were left for too long in any given constant photoperiod, they progress

physiologically to the next season. For example, winter acclimatized hamsters would begin to gain weight again as though spring had arrived.

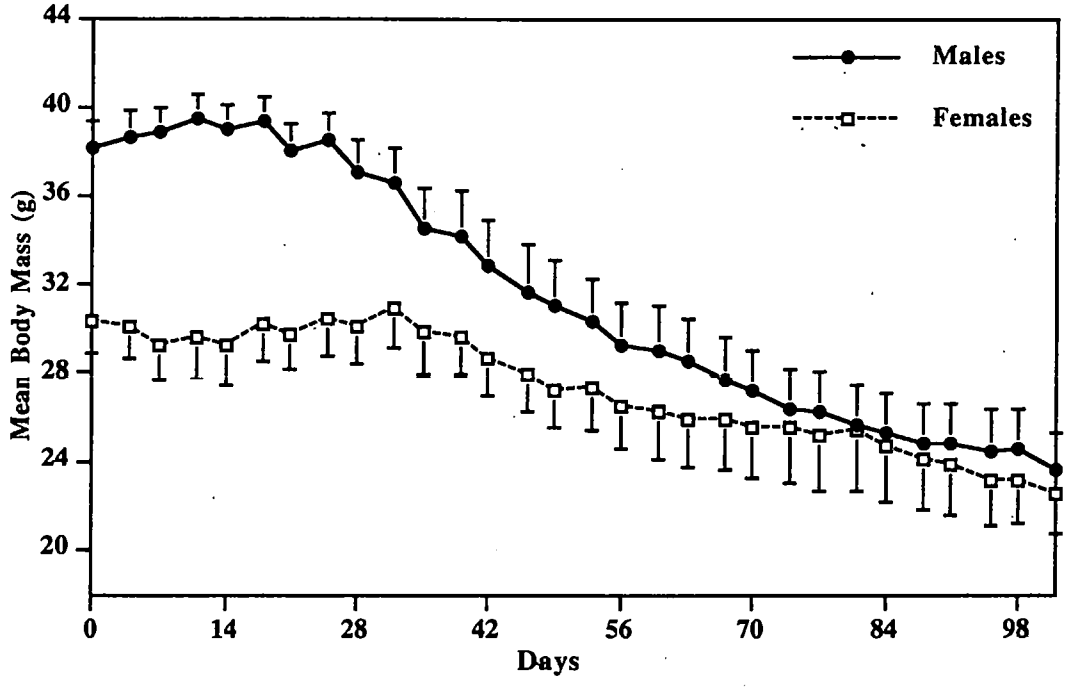
Fig. 3.3.1 Changes in body mass (mean ± SEs) of male and female *P. campbelli* in CSD environment (n = m 17, f 18). For clarity, the SEs are shown in one direction only. The SEs reduce and disappear with time because they become too small to be seen.



On the short photoperiod, in both the warm and cold environment, systematic weight loss occurred. This weight loss began immediately in the cold environment (Fig. 3.3.1), and continued for about 63 days in the males and 67 days in the females after which body mass was constant. The mean body mass of the males dropped to 22.1±0.40 g, which was 55.8±2.0% of their original body mass (BM0). Although the mean body mass of the females dropped to 19.8±0.4 g, this weight loss was smaller than that of the males at 63.2±1.8% of BM0 at 102 days. The loss of body mass in both the males (t=10.7, p<0.001, d.f. 33) and females (t=11.8, p<0.001, d.f. 35) was significant. The total changes in body mass between males and females were significantly different (f=20.6, p<0.0000, one way ANOVA, LSD p<0.05), because the males began the experiment heavier. The differences in body mass between the male and female hamsters kept on CSD were significant until the end of week 5 (f=15.8, p<0.0000, one way ANOVA, for LSD see S1 (p. 140)), but then became statistically

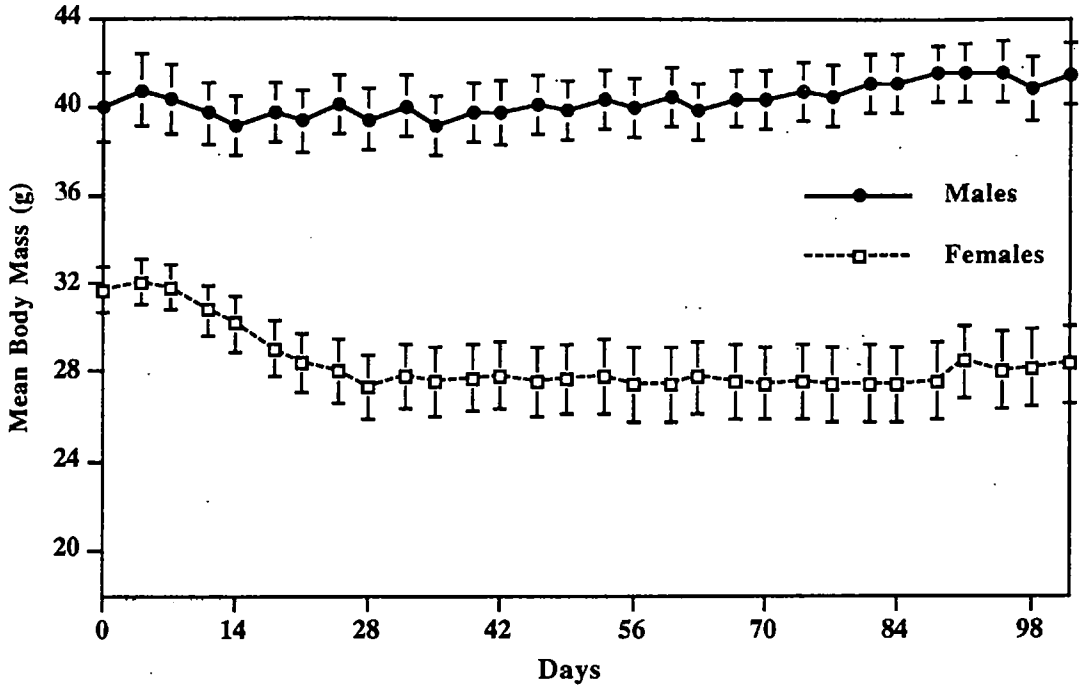
indistinguishable. The SEs get smaller for both sexes on this natural combination of environmental conditions.

Fig. 3.3.2 Changes in body mass (mean ± SEs) of male and female *P. campbelli* in WSD environment (n = m 14, f 11). For clarity, SEs are shown in one direction only.



The differences in body mass between the male and female hamsters kept on WSD (Fig. 3.3.2) were significant only until week 6 ($f=19.5$, $p<0.0000$, one way ANOVA, for LSD see S1 (p. 140)), but then become statistically indistinguishable. The weight loss in the warm environment did not begin until after day 21 in the males and day 31 in the females, and they were still losing weight at the end of the experiment, although slowly. The mean body mass of the males dropped significantly to 23.6±1.7 g (61.9±4.2% of BM0), (t= 7.0, $p<0.001$, d.f.27). That of the females dropped to 22.5±1.8 g, this reduction in body mass was smaller than that of the males at 75.6±6.4% (of BM0), but still significant (t= 3.3, $p<0.01$, d.f.21). The total change in body mass between males and females was significantly different ($f=20.6$, $p<0.0000$, one way ANOVA, for LSD see S1 (p. 140)). In contrast to simulated winter (Fig. 3.3.1) the SEs became larger as the experiment progressed, indicating variable responses to this unnatural combination of environmental conditions.

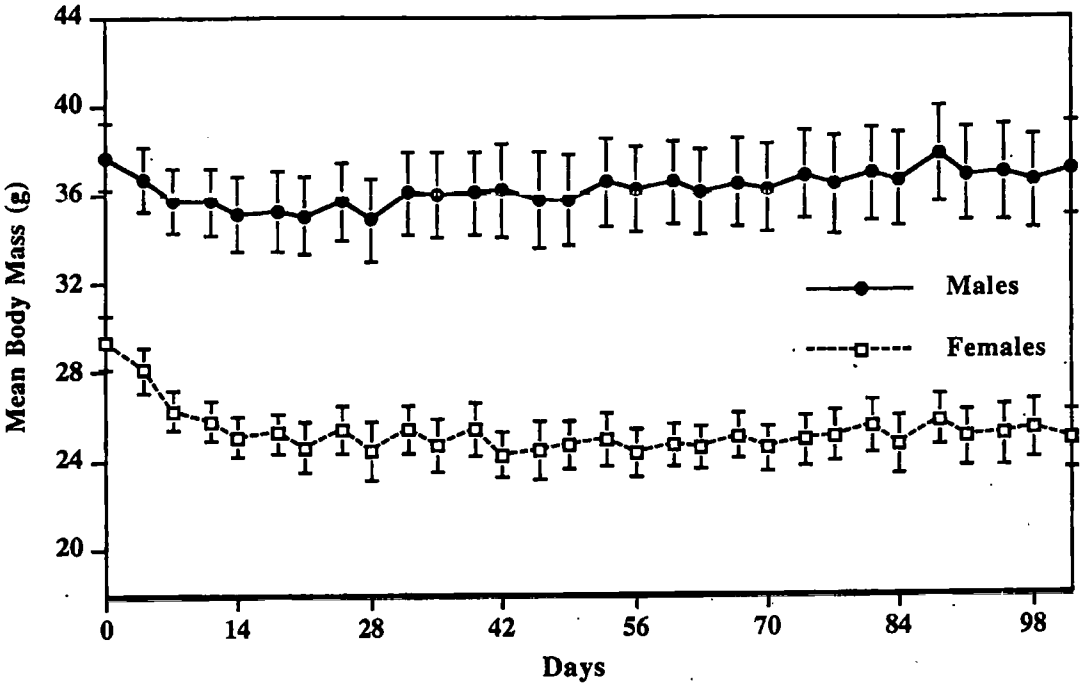
Fig. 3.3.3 Changes in body mass (mean ± SEs) of male and female *P. campbelli* in WLD environment (n = m 19, f 20).



On the long photoperiod, similar body mass changes occurred in both the warm and cold environment (Figs. 3.3.3 and 3.3.4). The differences in body mass between the male and female hamsters kept on WLD (Fig. 3.3.3) were significant for the whole experimental period ($f=11.3$, $p<0.0000$, one way ANOVA, LSD $p<0.05$).

On the WLD environment, the mean body mass of the males remained constant. The mean body mass of the females dropped significantly to 27.3±1.4 g (87.1±4.3% of BM0) by day 28 ($t= 2.5$, $p<0.02$, d.f 39), and then remained constant. The total changes in body mass between males and females were significantly different ($f=20.6$, $p<0.0000$, one way ANOVA, for LSD see S1 (p. 140)). The SEs remained large for the duration of this experiment.

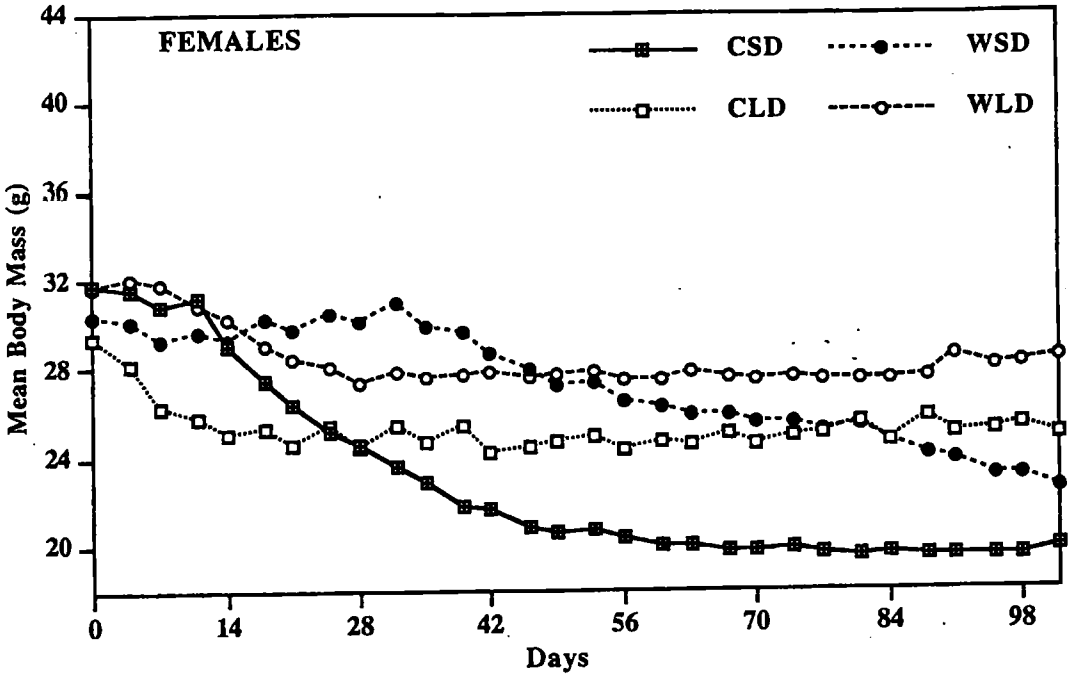
Fig. 3.3.4 Changes in body mass (mean ± SEs) of male and female *P. campbelli* in CLD environment (n = m 9, f 11).



The mean body mass of the females dropped significantly during the first two weeks to 24.5±1.34 g (83.4±2.72% of BM0), (t= 2.7, p<0.02, d.f. 21). After this time, the body mass of the female hamsters remained constant. The differences in body mass between the male and female hamsters kept on CLD (Fig. 3.3.4) were significant for the whole of the experimental period (f=11.3, p<0.0000, one way ANOVA, for LSD see S1 (p. 140)). The total changes in body mass between males and females CLD hamsters were not significantly different. The SEs were large throughout the experimental period on this unnatural combination of environmental conditions.

Figs. 3.3.1-3.3.4, show that the mean changes in body mass followed a similar pattern in both males and females when under the same environmental conditions, even though at the beginning of each experiment, the males were significantly heavier at 21.2±0.43% (f=11.3 p<0.0000, one way ANOVA, for LSD see S1 (p. 140)).

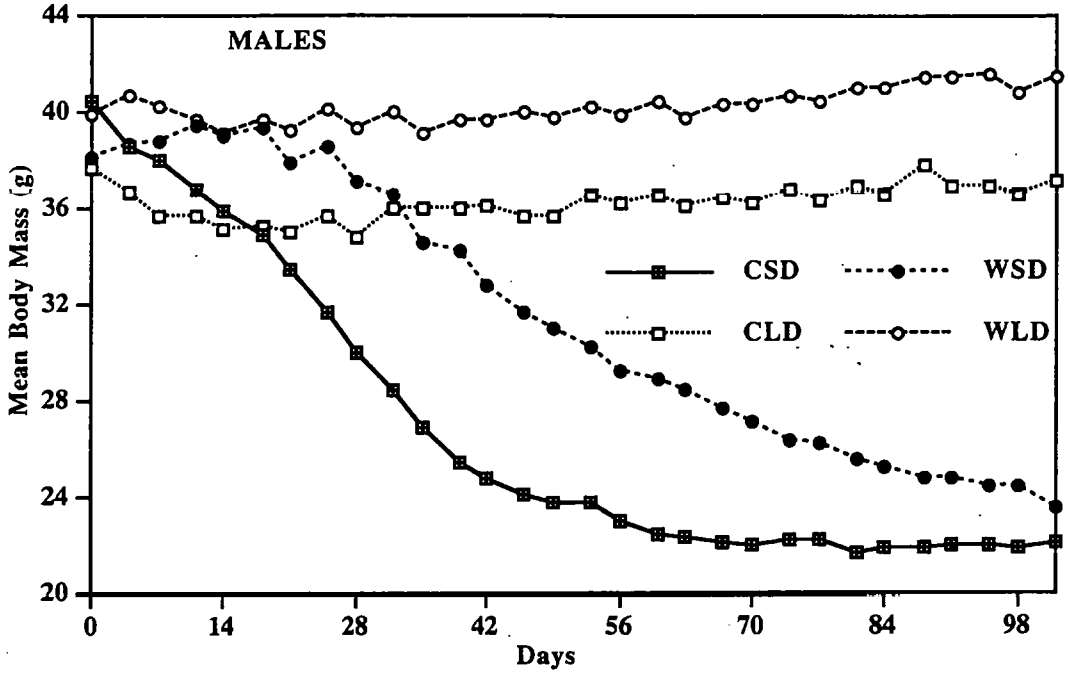
Fig. 3.3.5 Summary of changes in mean body mass of the females from all four regimess (the SEs are shown in Figs. 3.3.1-3.3.4).



There were significant differences in the body mass of the female hamsters on the different regimes from day 18 onwards ($f=14.3$, $p<0.0000$, one way ANOVA, for LSD see S1 (p. 140)).

Significant differences were found between the four regimes in the changes in body mass (mass lost or gained from the original body mass) from day 3 ($f=8.5$, $p<0.0000$, one way ANOVA, for LSD see S1 (p. 140)) onwards.

Fig. 3.3.6 Summary of changes in mean body mass of the males from all four regimes (the SEs are shown in Figs. 3.3.1-4).



In Figs. 3.3.5-6 the mean initial body masses in both the males and females show slight separation, due to deaths in the initial groups and it being impossible to match the replacements weight for weight.

There were significant differences in the body mass of the male hamsters on the different regimes from day 3 onwards ($f=8.5$, $p<0.0000$, one way ANOVA, for LSD see S1 (p. 140)). The hamsters on short photoperiod whether in the warm or cold environment reduced their body mass and those kept on long photoperiod again regardless of whether in the warm or cold environment maintained their body mass.

The interaction of photoperiod and sex had significant differences from day 28 onwards ($f=5.5$, $p<0.021$, three way ANOVA).

Table 3.2 Percentage changes in body mass at the end of the experiment.

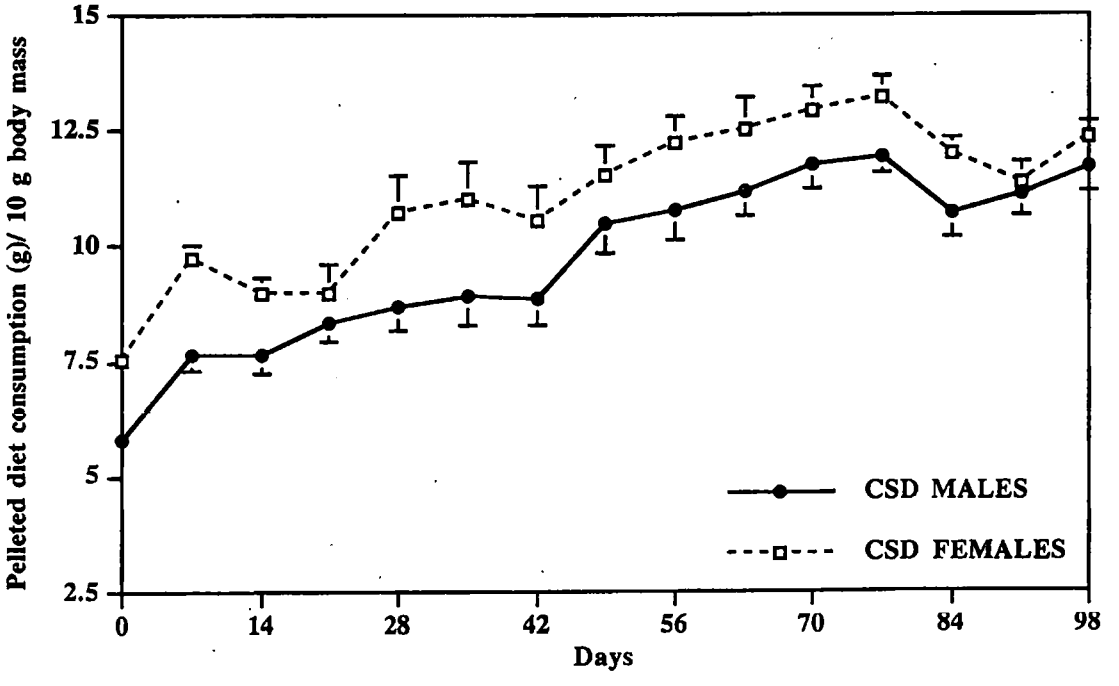
Environment	**CSD 'simulated winter'**		**CLD**		**WSD**		**WLD 'simulated summer'**	
sex	M	F	M	F	M	F	M	F
Body mass (%) lost or gained at end of expt.	-44.2 ±2.0	-36.8 ±1.8	-1.6 ±3.6	-14.5 ±3.6	-38.1 ±4.2	-24.4 ±6.4	+6.1 ±4.4	-9.6 ±5.3

As Table 3.2, shows there were significant differences in the percentage lost or gained final body mass of the hamsters (f=20.7, p<0.0000, oneway ANOVA, for LSD see S1 (p. 140)). The percentage losses of body mass in hamsters kept on short photoperiod, both in the warm and the cold conditions were significantly different between the males and females in CSD and between the females of WSD and CSD.

3.4 Pelleted diet intake normalised for body mass.

The pelleted diet remaining in the hoppers was weighed weekly and from these measurements the food intake for each hamster was calculated. Unfortunately the baseline data for food consumption was not recorded before the experimental regimes began. So, retrospectively data for food intakes of 12 male and 12 female stock hamsters, that were housed individually, were recorded for several weeks. The mean from both sexes was calculated and these data were used as the baseline for these results.

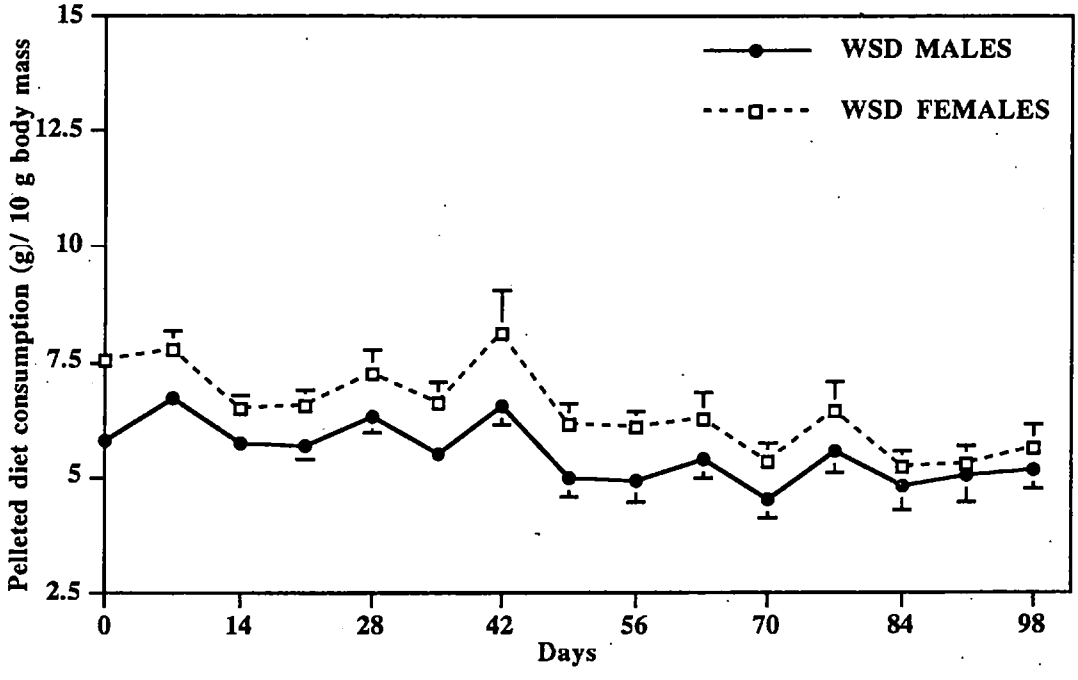
Fig. 3.4.1 Pelleted diet (mean ± SEs) consumption in male and female CSD hamsters normalised for body mass. The SEs are shown in one direction only for clarity. (n = m, 17, f, 18)



When food consumption was normalised for body mass, the female hamsters kept in CSD environment (Fig. 3.4.1), ate significantly more than the males for days 0-14,

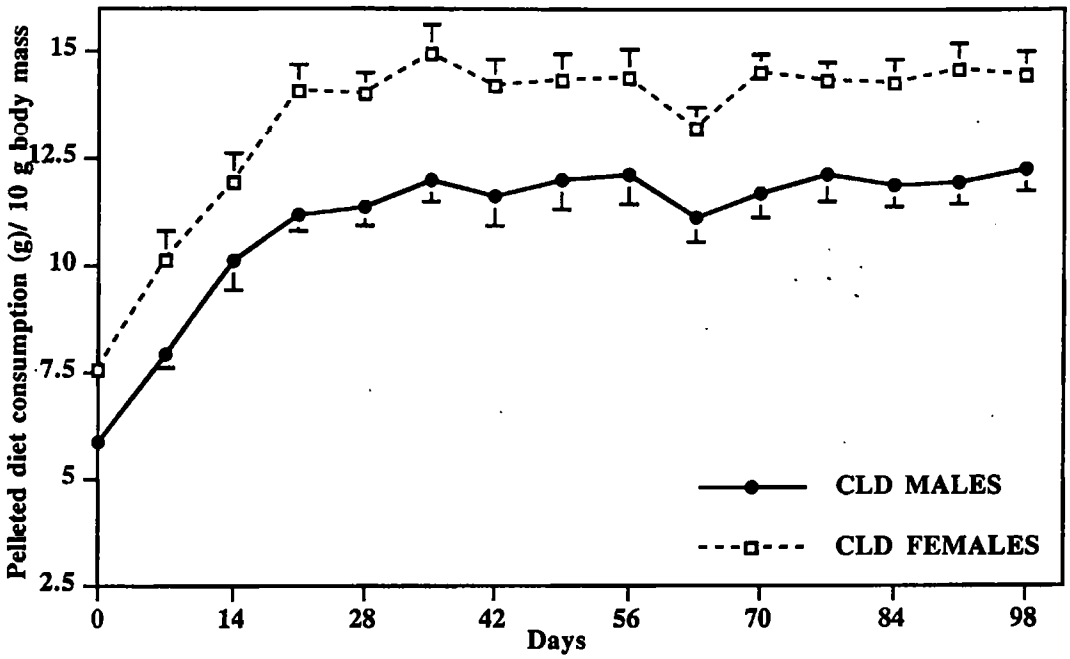
28-42, and 56-84 (f=25.3, p<0.0000, one way ANOVA, for LSD see S2 (p. 141)). The pelleted diet intake /10 g body mass changed significantly from the start to the finish of the experiment, in both the males (t=41.1, p<0.001, df 33) and the females (t=41.0, p<0.001, df 35), increasing almost steadily for 10 weeks.

Fig. 3.4.2 Pelleted diet (mean ± SEs) consumption in male and female WSD hamsters normalised for body mass. Shown on the same scale as Fig. 3.4.1. The SEs for clarity, are shown in one direction only. (n = m, 14, f, 11).



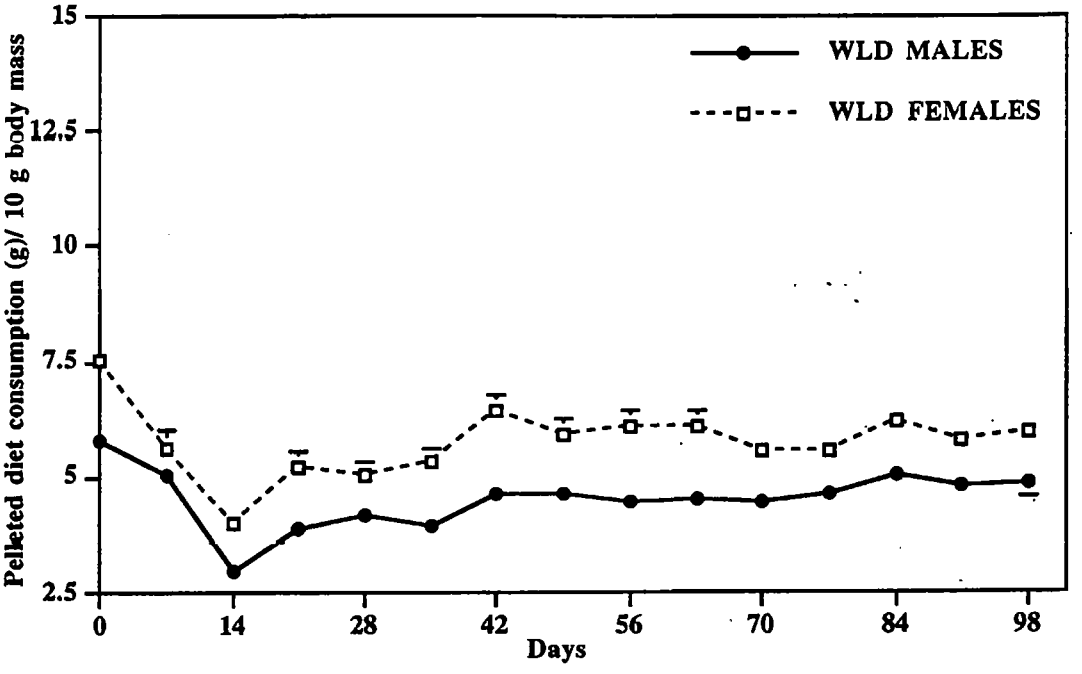
When food consumption was normalised for body mass, the male and female hamsters kept on WSD environment (Fig. 3.4.2), did not eat significantly different quantities, at any time point during the experiment. The males and females followed the same fluctuations in consumption, there were large variations in environmental temperature between week five and six that might have caused the sharp rise in food intake around week six.

Fig. 3.4.3 Pelleted diet (mean ± SEs) consumption in male and female CLD hamsters normalised for body mass. Shown on the same scale as Fig. 3.4.1. The SEs for clarity are shown in one direction only. (n = m, 9, f, 11).



When food consumption was normalised for body mass, the female hamsters kept on CLD environment (Fig. 3.4.3) ate significantly more than similarly treated males, for the duration of the experiment, ($f=25.3$, $p<0.0000$, one way ANOVA, for LSD see S2 (p. 141)). The pelleted diet intake /10 g body mass increased significantly from the start to the finish of the experiment in both the males ($t=31.5$, $p<0.001$, df 17) and the females ($t=24.4$, $p<0.001$, df 21), but food intake levelled out after 3 weeks and thereafter remained almost constant.

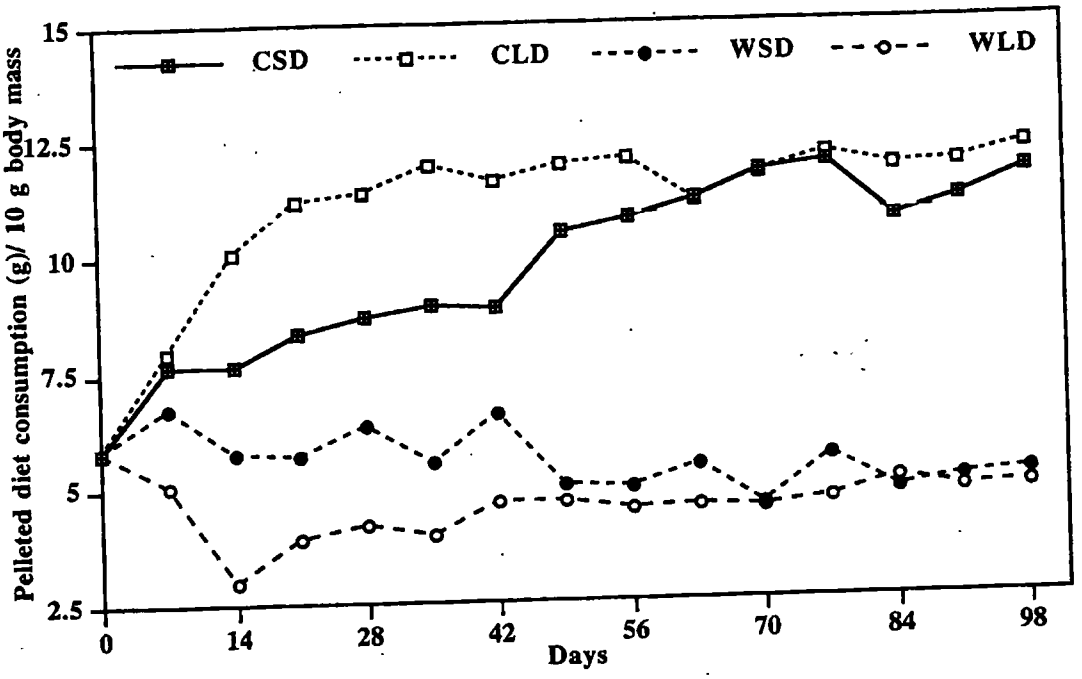
Fig. 3.4.4 Pelleted diet (mean ± SEs) consumption in male and female WLD hamsters normalised for body mass. Shown on the same scale as Fig. 3.4.1. The SEs for clarity are shown in one direction only. (n = m, 19, f, 20).



When food consumption was normalised for body mass, the female hamsters kept on WLD environment (Fig. 3.4.4) ate significantly more than the males, for days 14-21, 35-70 and for the final week, ($f=28.1$, $p<0.0000$, one way ANOVA, for LSD see S2 (p. 141)). The pelleted diet intake /10 g body mass decreased significantly from the start to the finish of the experiment in both the males ($t=11.9$, $p<0.001$, df 37) and the females ($t=15.3$, $p<0.001$, df 39). Food intake dropped abruptly in the first two weeks, then increased and remained almost constant after 6 weeks.

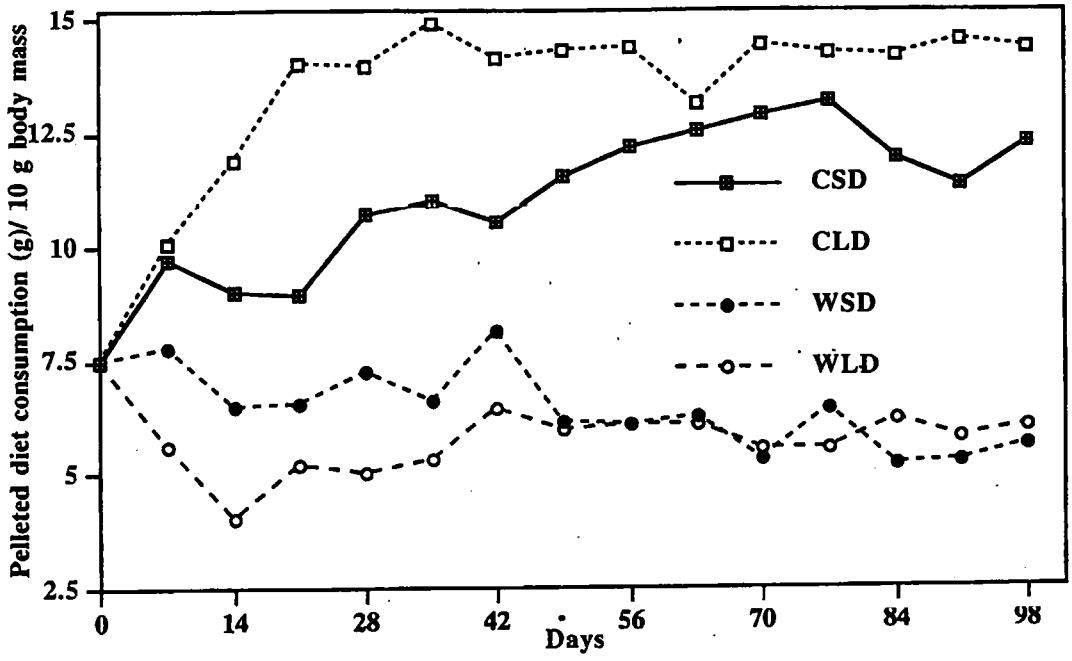
A significant interaction between photoperiod and temperature was demonstrated from week 2 onwards ($f=43.9$, $p<0.000$, three-way ANOVA) and the interaction between photoperiod and sex was significant from week 5 onwards ($f=5.0$, $p<0.027$, three-way ANOVA).

Fig 3.4.5 Summary of pelleted diet consumption normalised for body mass in male hamsters from all four regimes. The SEs are shown in Figs. 3.4.1-3.4.4.



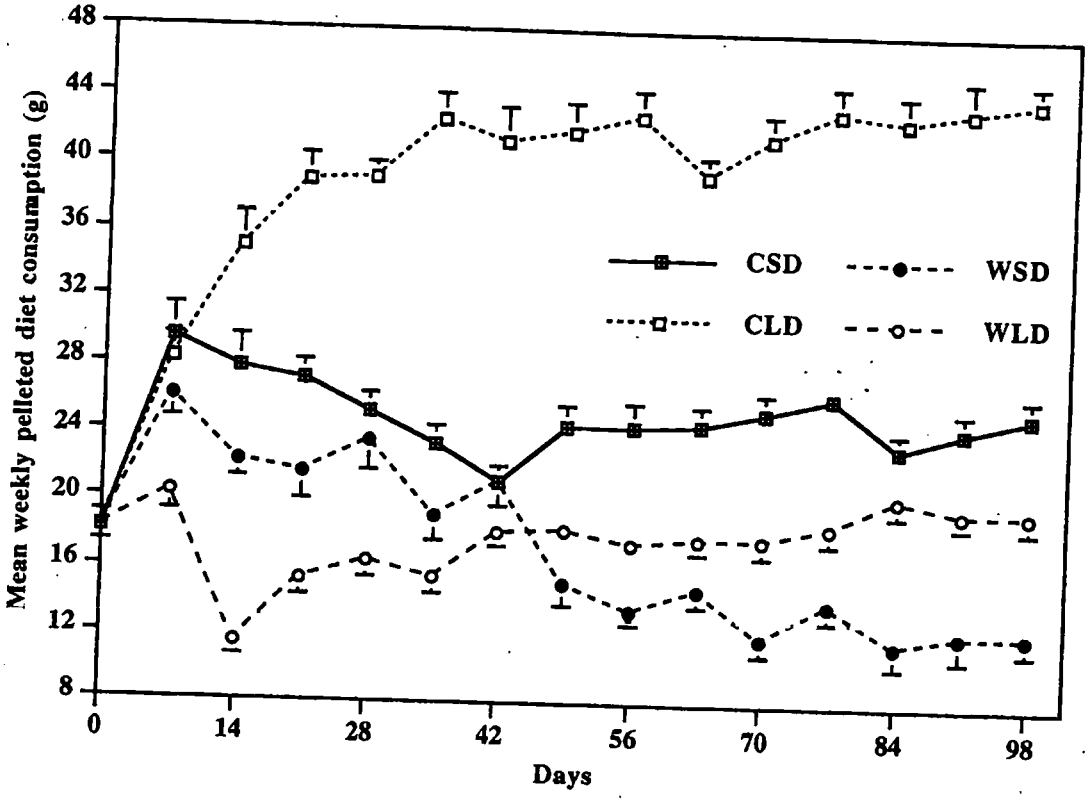
When food consumption was normalised for body mass, the males kept in the cold environment, on either long or short photoperiod, ate up to three times as much food, for the duration of the experiments ($f=25.3$, $p<0.0000$, one way ANOVA, for LSD see S2 (p. 141)). The food intake for hamsters of the two cold and the two warm regimes ended up very similar, but took over six weeks to get there, in spite of large differences in body mass (see Fig. 3.3.6).

Fig 3.4.6 Summary of pelleted diet consumption normalised for body mass in female hamsters from all four regimes. The SEs are shown in Figs. 3.4.1-3.4.4.



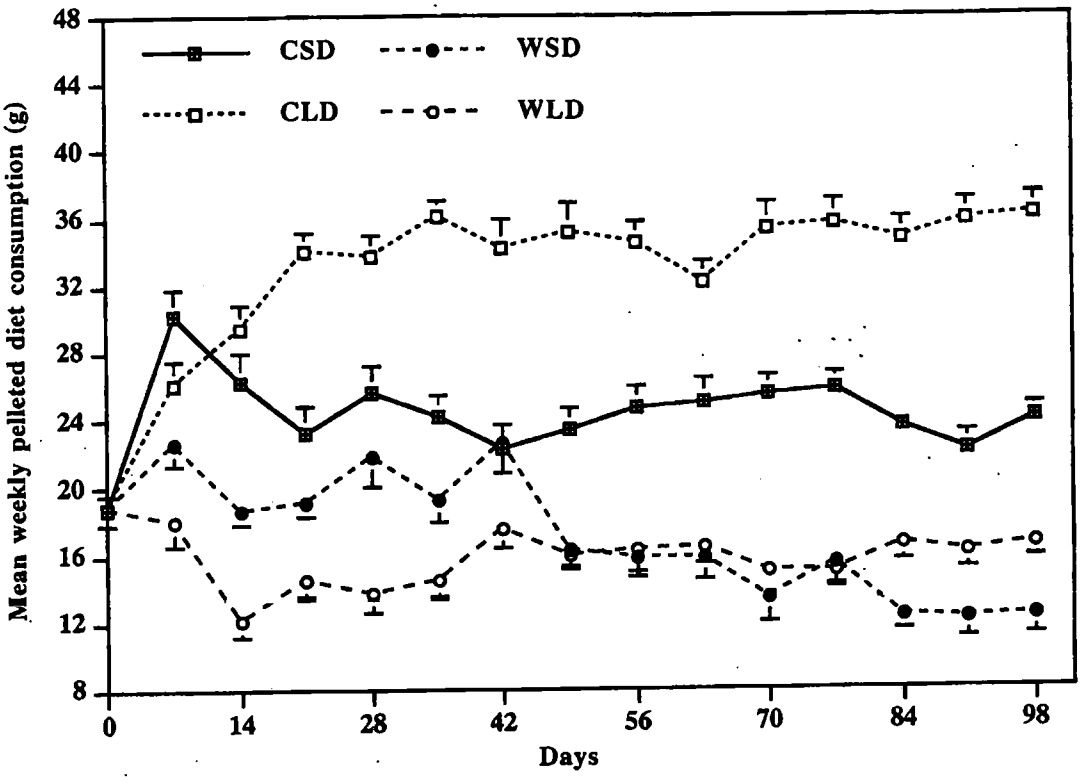
When food consumption was normalised for body mass, the females kept in the cold environment, either on long or short photoperiod, ate up to three times more food than those in the warm, for the duration of the experiments ($f=25.3$, $p<0.0000$, one way ANOVA, for LSD see S2 (p. 141)), significant sex differences occurred in CSD, CLD and WLD. The interaction of photoperiod and temperature was significant for the whole experiment ($f=4.3$, $p<0.040$, three way ANOVA). The patterns of food intake for female hamsters on the two cold and two warm regimes ended up very similar to that of the males and took similar lengths of time to get there, although differences in absolute body size were smaller (see Fig. 3.3.5).

Fig. 3.4.7 Mean (±SEs) pelleted diet consumption in male hamsters from all four regimes.



When the males were kept in the cold environments on either long or short photoperiod, food consumption was up to twice that of their counterparts in the warm. Significant differences occurred from week two onwards between all four regimes in the males ($f= 34.4$, $p<0.0000$, one way ANOVA, for LSD see S3 (p. 141), apart from week six (Fig. 3.4.7).

Fig. 3.4.8 Mean (±SEs) pelleted diet consumption of the female hamsters in all four regimes.



When the females were kept in the cold environments on either long or short photoperiod, food consumption was also up to twice that of their counterparts in the warm. Significant differences also occurred from week two onwards between all four regimes in the females ($f= 34.4$, $p<0.0000$, one way ANOVA, for LSD see S3 (p. 141), again apart from week six (Fig. 3.4.8).

Fig. 3.4.9 Mean (± SEs) consumption of pelleted diet (g) /10 g body mass /day. n= (m 17, f 18; CSD), (m 14, f 11; WSD), (m 9, f 11; CLD), (m 19, f 20; WLD). Calculated from the whole of the experimental period.

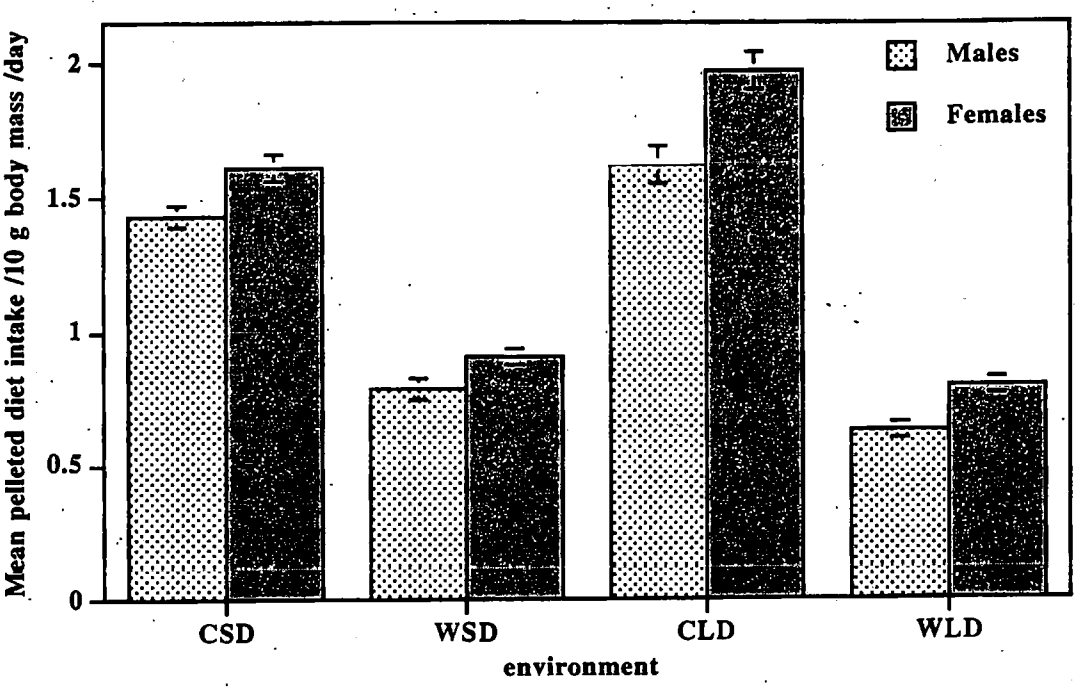
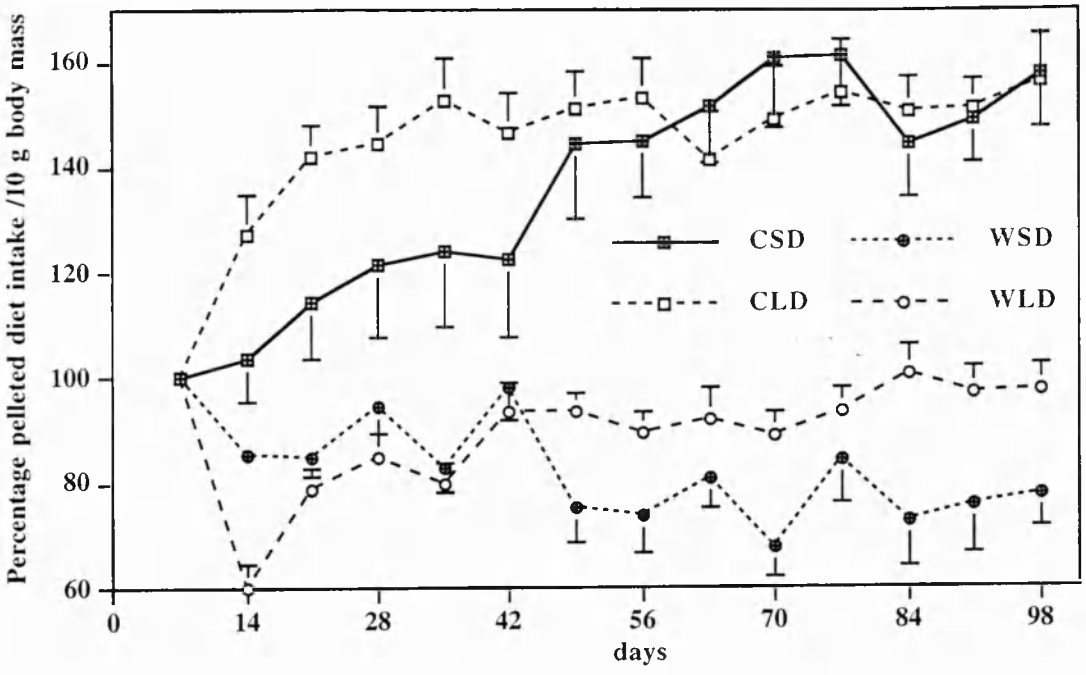


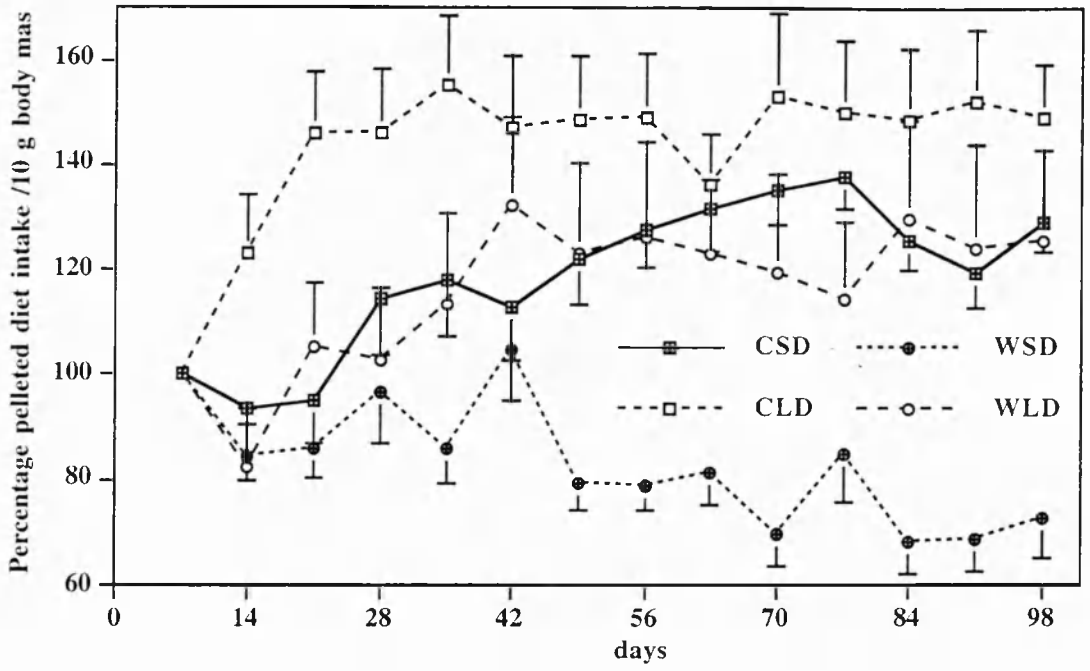
Fig. 3.4.9 shows that when the mean daily food intake was normalised for body mass, the females ate more than the males on all four regimes. These sex differences were significant in CSD, CLD and WLD and there were significant differences in the mean pelleted diet intake /10 g body mass /day ($f=125.1$, $p<0.0000$, one way ANOVA, for LSD see S4 (p. 141)) between groups.

Fig. 3.4.10 Changes in pelleted diet (mean ± SEs) intake /10 g body mass from original (diet consumed during week 1 = 100%), of the male hamsters.



When food intake during the experimental runs (Fig. 3.4.10) was taken as a percentage of the food intake during week 1 /unit (10 g) body mass in male hamsters, the hamsters from the cold environments (CSD and CLD) increased their food intake significantly. The male hamsters kept in WSD reduced their food intake significantly and that of the male hamsters kept in the WLD environment after an initial dip, remained remarkably close to the original. From week 2 onwards there were significant differences ($f>2.5$, $p<0.0192$, one way ANOVA, for LSD see S5 (p. 141)) in changes in food intake.

Fig. 3.4.11 Changes in pelleted diet (mean ± SEs) intake /10 g body mass from original (diet consumed during week 1 = 100%), of the female hamsters.



When food intake during the experimental runs (Fig. 3.4.11) was taken as a percentage of the food intake during week 1 /unit (10 g) body mass, the food intake significantly increased in the female hamsters from CLD and CSD. WLD, after an initial dip was the only regime significantly different from the males. The female hamsters kept in WSD reduced their food intake significantly, similar to the males (Fig. 3.4.10). From week 2 onwards there were significant differences ($f>2.5$, $p<0.0192$, one way ANOVA, for LSD see S5 (p. 141)) in changes in food intake.

3.5 Dissection observations.

The thoracic white adipose depot (TW) was found superficially (innermost, next to the ribs) on the upper thorax, with a 'pad' of connective tissue embedded in it and thoracic brown adipose tissue (TB) was found embedded in the connective tissue (outermost) next to the skin. All the BAT depots became very dark when active, as did the muscles in the cold and/or short photoperiod, resulting in it being difficult to distinguish intermuscular adipose tissue depots from the muscle itself. All BAT depots became paler when inactive, i.e. in the warm and/or long photoperiod, making it hard to distinguish from the connective tissue pad in the case of TB.

In *P. campbelli,* the midventral sebaceous gland changed in size in hamsters that had been kept under different environmental conditions. In male hamsters kept on long photoperiod, regardless of environmental temperature, the sebaceous gland was large and swollen, it was smaller in the females. In the hamsters kept on short photoperiod, regardless of environmental temperature, the sebaceous gland was always small and shrunken.

3.6 Mass of pelt and major organs.

The following Tables 3.3.1-2 detail the masses of the pelt and major organs of male and female *P. campbelli*, weighed on removal at dissection.

Table 3.3.1 Mean (± SEs) mass of the pelt and the major organs in male *P. campbelli*. Intestine = large and small intestines inclusive.

Regime	CSD	WSD	CLD	WLD	Statistics
n	17	14	9	19	one way ANOVA
Pelt	3.29±0.08	3.70±0.36	4.76±0.31	6.11±0.22	f=19.4, p<0.0000
Intestine	2.48±0.07	1.71±0.09	3.88±0.09	2.56±0.07	f=27.8, p<0.0000
Liver	1.05±0.03	1.02±0.10	1.88±0.09	1.77±0.08	f=18.3, p<0.0000
Testes	0.14±0.02	0.15±0.02	1.52±0.07	1.76±0.03	f=661.5, p<0.0000
Kidneys	0.30±0.01	0.24±0.01	0.41±0.05	0.41±0.01	f=19.1, p<0.0000
Heart	0.18±0.01	0.16±0.01	0.27±0.02	0.21±0.01	f=21.9, p<0.0000
Pancreas	0.19±0.01	0.13±0.01	0.17±0.01	0.18±0.01	f=3.0, p<0.0073
Muscle	0.05±0.00	0.06±0.00	0.07±0.00	0.07±0.00	f=23.2, p<0.0000
Spleen	0.03±0.00	0.03±0.00	0.04±0.00	0.04±0.00	f=6.5, p<0.0000

Table 3.3.2 Mean (± SEs) mass of the pelt and the major organs in female *P. campbelli*. Intestine = large and small intestines inclusive.

Regime	CSD	WSD	CLD	WLD	Statistics
n	18	11	11	20	one way ANOVA
Pelt	2.96±0.07	3.44±0.04	3.44±0.25	4.40±0.29	f=19.4, p<0.0000
Intestine	2.39±0.07	1.63±0.14	3.38±0.25	2.49±0.15	f=27.8, p<0.0000
Liver	0.92±0.06	0.91±0.11	1.44±0.09	1.56±0.11	f=18.3, p<0.0000
Kidneys	0.27±0.01	0.23±0.01	0.33±0.01	0.29±0.01	f=19.1, p<0.0000
Heart	0.17±0.00	0.15±0.01	0.19±0.01	0.16±0.01	f=21.9, p<0.0000
Pancreas	0.17±0.01	0.14±0.01	0.18±0.01	0.16±0.01	f=3.0, p<0.0073
Muscle	0.05±0.00	0.05±0.00	0.05±0.00	0.06±0.00	f=23.2, p<0.0000
Spleen	0.02±0.00	0.03±0.00	0.03±0.00	0.05±0.01	f=6.5, p<0.0000
Ovaries	0.008±0.00	0.008±0.00	0.008±0.00	0.013±0.00	f=8.9, p<0.0001

The masses of the pelt and major organs (Tables 3.3.1-2) were significantly different between experimental groups. The sizes of the pelt, the muscle *vastus lateralis* and the spleen were proportional to the final body mass of the hamsters (see Figs. 3.3.5-6). The masses of the intestines (the high SEs were probably due to the variable gut content), the liver, the kidneys, the heart and the pancreas were probably related to food intake, which was much higher in the cold (see Figs. 3.4.5-6), although the kidneys in the WLD males were probably also large because they were serving large body mass (Fig. 3.3.6). The ovaries appeared only to respond to the combination of photoperiod and temperature. The testes responded to photoperiod and were much larger in hamsters on both long day regimes. There were significant sex differences in the masses of the PELT, INTESTINES and LIVER in the hamsters that were kept in CLD, in the PELT with WLD, in the KIDNEYS, HEART and in the MUSCLE with WLD and CLD, and in WSD with the MUSCLE. These sex differences were probably due to differences in the size of the hamsters, where the males were larger in all cases. For all the LSD see S6 (p. 142).

The interaction of photoperiod and temperature was significant in the pancreas ($f=5.1$, $p<0.027$, three way ANOVA), the muscle *vastus lateralis* ($f=6.0$, $p<0.016$, three way ANOVA), the testes ($f=10.2$, $p<0.002$, two way ANOVA) and the ovaries ($f=7.2$, $p<0.010$, two way ANOVA). The interaction of photoperiod and sex was significant in the kidneys ($f=12.4$, $p<0.001$, three way ANOVA), the heart ($f=25.2$, $p<0.000$, three way ANOVA), the muscle *vastus lateralis* ($f=8.4$, $p<0.004$, three way ANOVA) and the pelt ($f=11.3$, $p<0.001$, three way ANOVA). Finally there was a significant three-way interaction with the muscle *vastus lateralis* ($f=4.4$, $p<0.038$, three way ANOVA).

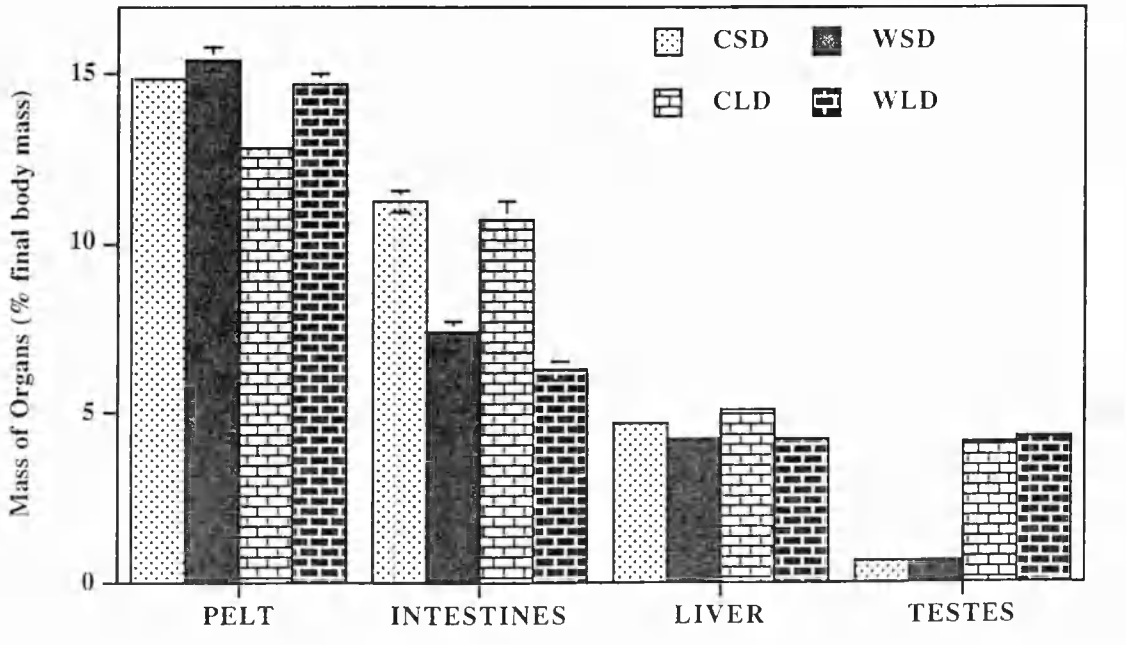
To find out whether the masses of the viscera were determined by environmental regimes or were secondary to the metabolic requirements of maintaining the body under the various conditions, I compared food intake (data from Figs. 3.4.5-6) with the mass of the intestines (data from Tables 3.3.1-2).

Table 3.4 The mean (g/day) pelleted diet intake (data from the final week) expressed as a fraction of the mean mass (g) of the alimentary tract (INTESTINES), large and small inclusive, complete with contents.

	Final daily intake (g/g of intestines+contents)	
Environment	MALES	FEMALES
CSD	1.49±0.07	1.46±0.06
CLD	1.62±0.05	1.58±0.09
WSD	1.05±0.09	1.09±0.07
WLD	1.10±0.05	0.97±0.03

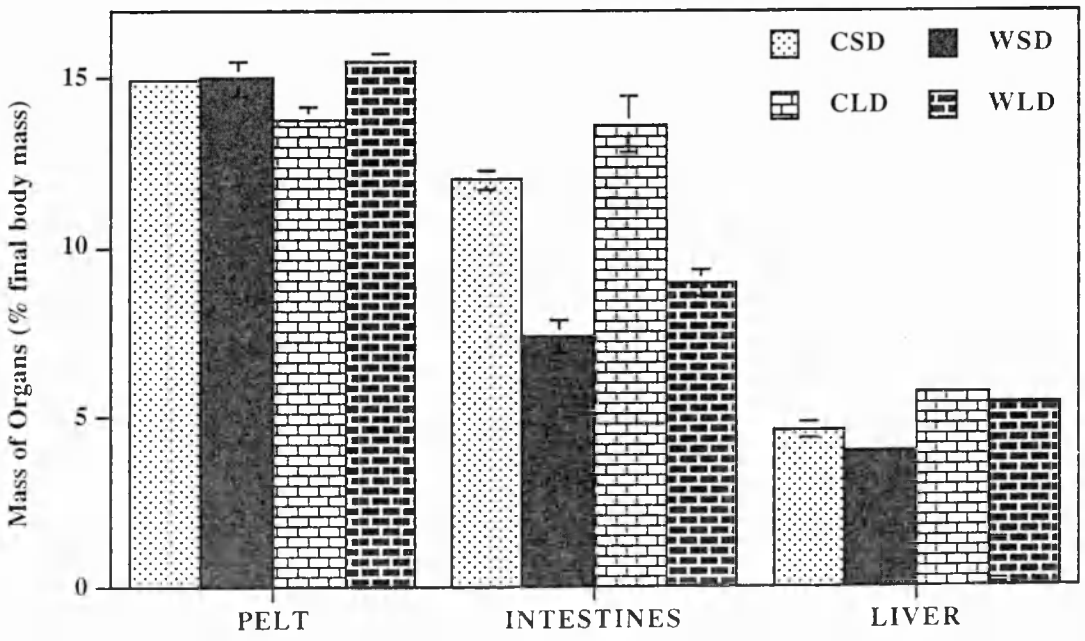
The results in Table 3.4. show that the mean values were similar between the males and females kept in the cold environment on long or short photoperiod and the males and females kept in the warm environment on long or short photoperiod. These differences were significant between the warm and cold environment ($f=16.5$, $p<0.0000$, one way ANOVA, for LSD see S6 (p. 142)), but there were no significant sex differences. These results show that the mass of the intestines was determined by food intake.

Fig. 3.6.1 Mass of the large organs (mean ± SEs) when expressed as a percentage of the final body mass in males. The SEs were too small in several cases to show on the graph.



When the organs were expressed as a percentage of final body mass in the males (Fig. 3.6.1), there were still significant differences. The masses of the pelt ($f=7.1$, $p<0.0000$, one way ANOVA) were very similar in all except CLD, which possibly could not increase in size and hence in insulative capacity on this unnatural regime. None the less most of the hamsters survived, possibly by increased thermogenesis. The intestines ($f=39.0$, $p<0.0000$, one way ANOVA) adjusted to food intake, which was related to temperature because more food was needed in the cold, the mass of the intestines included contents and the hamsters appeared to have been feeding. The liver ($f=11.4$, $p<0.0000$, one way ANOVA) had very little differences. The testes ($f=270.4$, $p<0.0000$, one way ANOVA) obviously responded to photoperiod. For all the LSD see S7 (p. 142). The interaction of photoperiod and temperature was significant in the pelt ($f=12.4$, $p<0.001$, three-way ANOVA), and between photoperiod and sex in the pelt ($f=5.9$, $p<0.016$, three-way ANOVA), the intestines ($f=16.8$, $p<0.000$, three-way ANOVA) and the liver ($f=19.3$, $p<0.000$, three-way ANOVA).

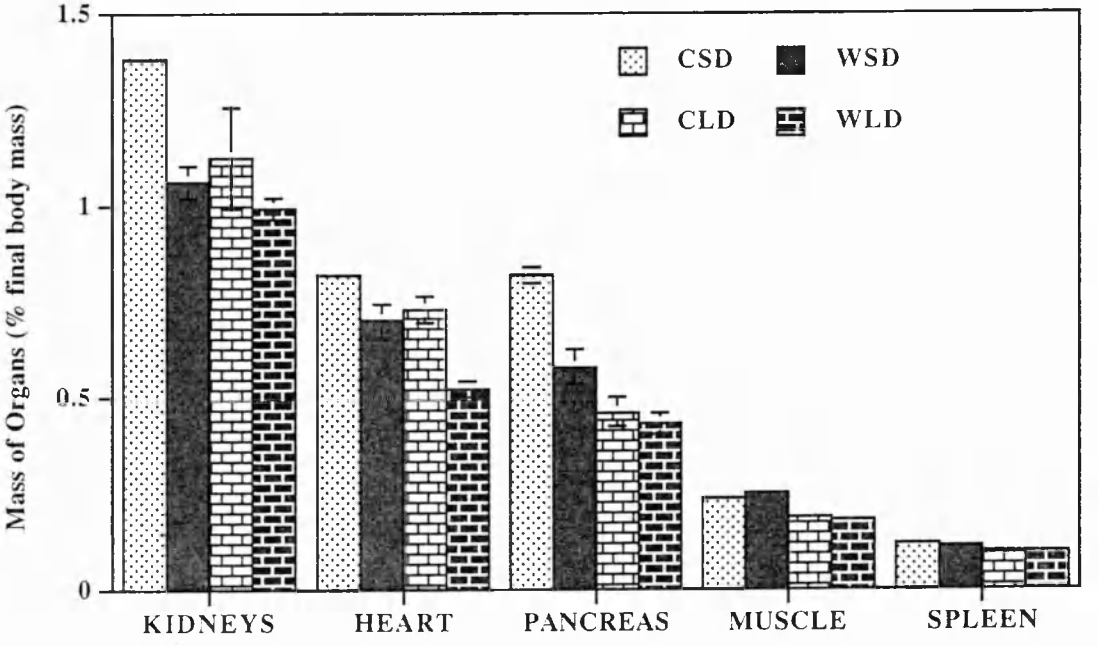
Fig. 3.6.2 Mass of the large organs (mean ± SEs) when expressed as a percentage of the final body mass in females. The SEs were too small in several cases to show on the graph.



When the organs were expressed as percentage of final body mass in the females (Fig. 3.6.2) there were some significant differences. The masses of the pelt ($f=7.1$, $p<0.0000$, one way ANOVA) were again similar in all except CLD, which possibly

could not increase in size and therefore insulative capacity on this unnatural regime, as appeared to be the case in the males. The intestines ($f=39.0$, $p<0.0000$, one way ANOVA) responded to temperature and adjusted to food intake. The liver ($f=11.4$, $p<0.0000$, one way ANOVA) showed smaller changes. Comparing these data to those in Fig. 3.6.1 revealed that there were sex differences in all three large organs in WLD and in the LIVER and INTESTINES in CLD, where in all cases the females were larger. For all the LSD see S7 (p. 142).

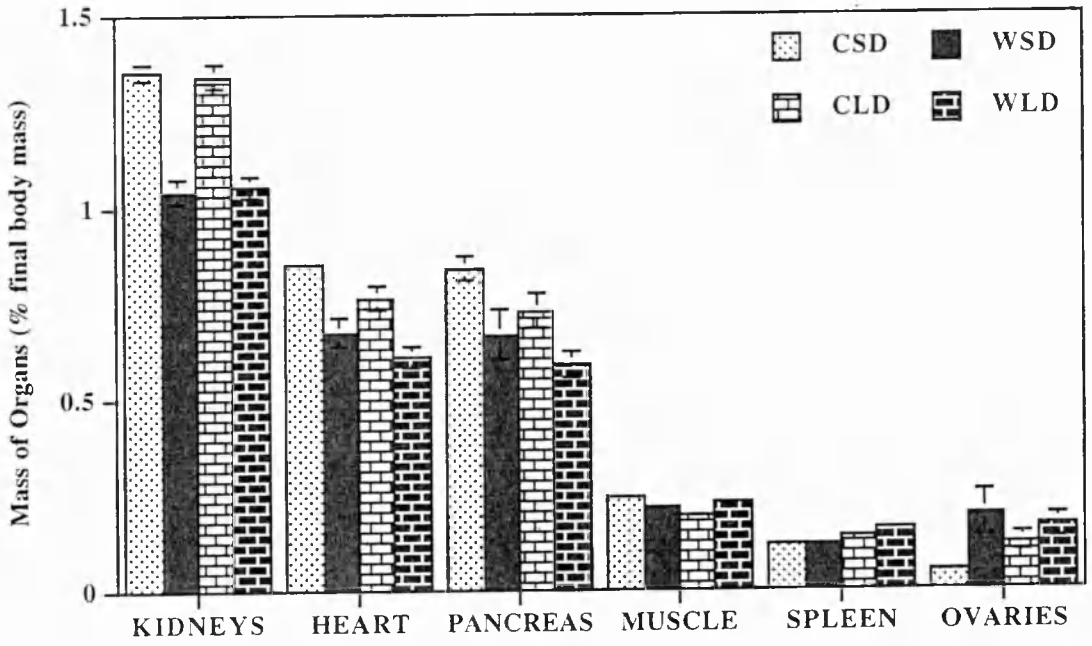
Fig. 3.6.3 Mass of the small organs (mean ± SEs) when expressed as a percentage of the final body mass in males. In several cases the SEs were too small to show on this graph.



When expressed as a percentage of the final body mass of the males (Fig.3.6.3), there were significant differences in the masses of the smaller organs. The kidneys ($f=17.5$, $p<0.0000$, one way ANOVA) were needed to process the end-products of metabolism and like the intestines (Figs. 3.6.1-2), were larger, in hamsters kept in the cold. The large SEs of the data from the CLD males suggested the hamsters' bodies were in a confused state and their kidneys were 'trying' to enlarge because of the extra workload. The hearts of the WLD males ($f=16.5$, $p<0.0000$, one way ANOVA) might have been proportionally smaller because they did not have to work so hard, because a greater proportion of the body mass was adipose tissue (see Fig. 3.7.1), which required less blood perfusion. The pancreas ($f=12.9$, $p<0.0000$, one way ANOVA) is

very important for digestion, yet in the males, when expressed as %BM, the smaller their body mass the larger, proportionally, the pancreas and not relating to food intake. The muscle ($f=8.3$, $p<0.0000$, one way ANOVA) and the spleen ($f=7.8$, $p<0.0000$, one way ANOVA) responded to body mass. For all the LSD see S7 (p. 142).

Fig. 3.6.4 Mass of the small organs (mean ± SEs) when expressed as a percentage of the final body mass in females. The SEs in several cases were too small to show up on this graph.



When expressed as a percentage of the final body mass of the females (Fig. 3.6.4), there were significant differences in the masses of the smaller organs. The relative masses of the kidneys of the females were ($f=17.5$, $p<0.0000$, one way ANOVA) similar to those of the males, except that those of the CLD females were more uniform and the data did not have the large SEs. The heart ($f=16.5$, $p<0.0000$, one way ANOVA) would also need to be large in hamsters kept in the cold due to the higher metabolic rate which would be sustained by increased blood flow. The pancreas ($f=12.9$, $p<0.0000$, one way ANOVA) is very important for digestion and thus responds to food intake. The muscle ($f=8.3$, $p<0.0000$, one way ANOVA) and the spleen ($f=7.8$, $p<0.0000$, one way ANOVA) responded to body mass and finally the ovaries ($f=3.7$, $p<0.0197$, one way ANOVA) were significantly reduced only in CSD, ie the females were out of breeding condition, although the absolute mass of the ovaries implied that the females would only breed on WLD. These data contrast with those for the testes

(see Fig. 3.6.1 and Table 3.3.1) which responded strictly to photoperiod. For all the LSD see S7 (p. 142).

Comparing these data to those in Fig. 3.6.3 revealed that there were sex differences in the KIDNEYS, PANCREAS and SPLEEN with CLD, in the HEART, PANCREAS, MUSCLE and SPLEEN with WLD, and in the MUSCLE with WSD. The interaction of photoperiod and temperature was significant in the pancreas ($f=4.064$, $p<0.047$, three way ANOVA), and the interaction of photoperiod and sex was significant in the kidneys ($f=6.9$, $p<0.010$, three way ANOVA), the muscle ($f=11.6$, $p<0.001$, three way ANOVA), the pancreas ($f=6.4$, $p<0.013$, three way ANOVA), and the spleen ($f=21.2$, $p<0.000$, three way ANOVA). Finally the three way interaction was significant in the muscle ($f=9.3$, $p<0.003$, three way ANOVA),

3.7 Adipose tissue.

3.7.1 Gross mass of adipose tissue.

Fig. 3.7.1 Total dissectable adipose tissue (**TDAT**) expressed as a percentage of the final body mass, n= (m 17 f 18, CSD), (m 14 f 11, WSD), (m 9 f 11, CLD), (m 19 f 20, WLD).

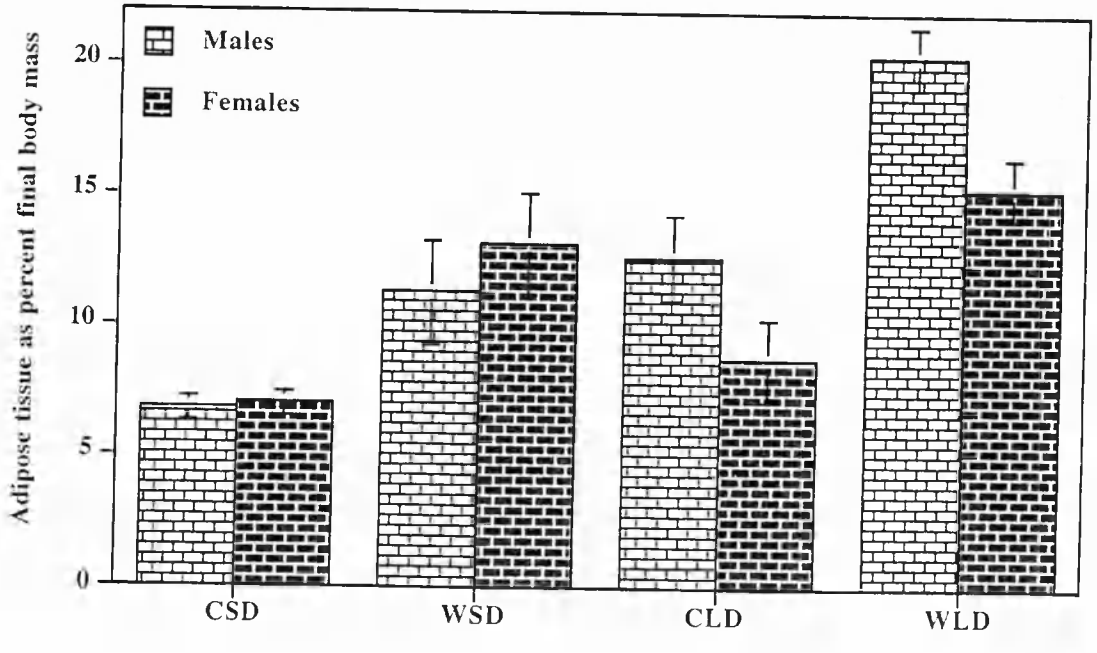


Fig. 3.7.1 shows the total dissectable adipose tissue expressed as a percentage of the final body mass. The only difference between the sexes was found in the WLD group, where the males were significantly fatter ($t= 2.59$, $p<0.02$, df 38). When

expressed as a percentage of the final body mass there were significant differences in the total dissectable adipose tissue (TDAT), where CSD hamsters had the least and WLD hamsters had the most TDAT (f=13.5, p<0.0000, one way ANOVA, for LSD see S8 (p. 142)). The TDAT for the WSD and CLD hamsters was surprisingly similar considering how dissimilar their final body mass was (Figs. 3.3.5-6). The interaction of photoperiod and sex was also significant in this case (f=8.1, p<0.005, three way ANOVA).

Fig. 3.7.2 Mass (mean ±SEs) of total dissected adipose tissue (g), n= (m 17 f 18, CSD), (m 14 f 11, WSD), (m 9 f 11, CLD), (m 19 f 20, WLD). In two cases the SEs were too small to show up on this graph.

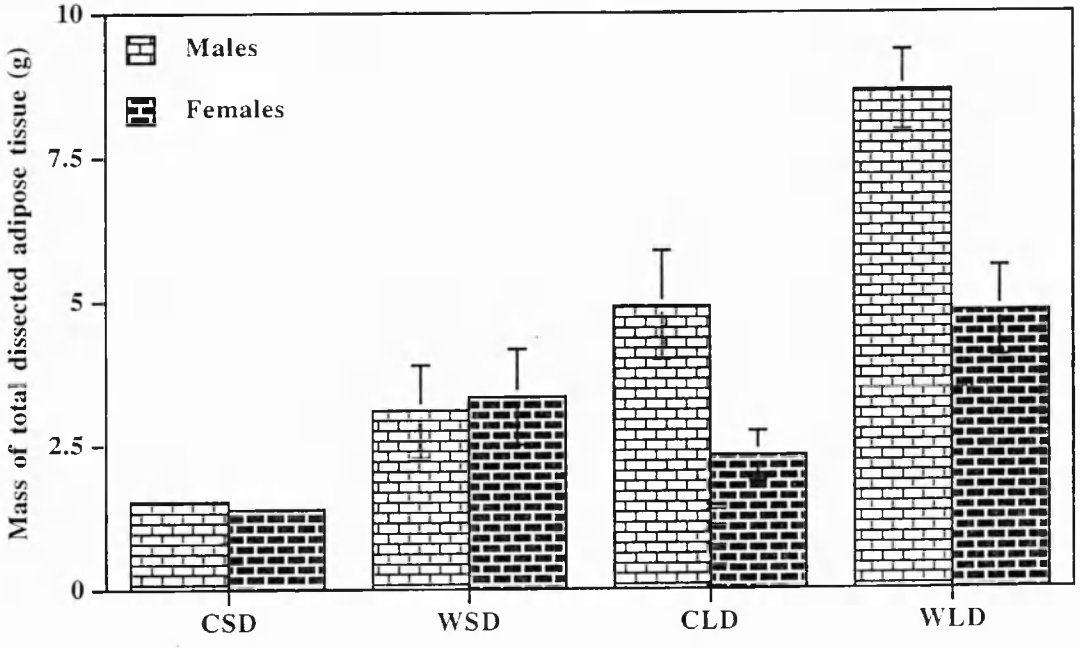
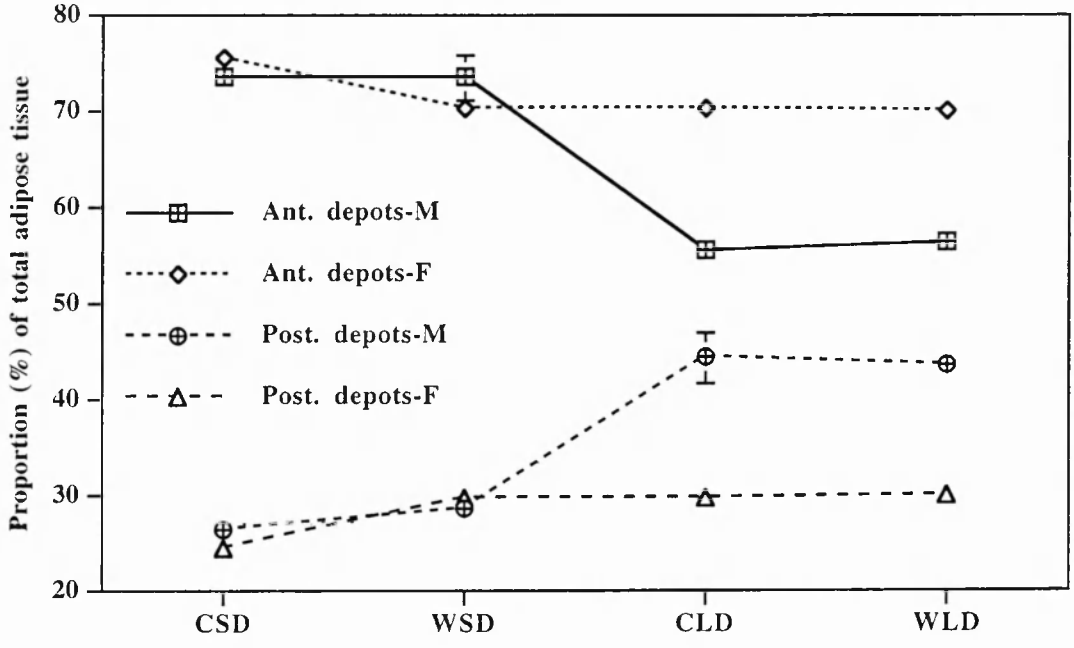


Fig. 3.7.2 shows the total dissected adipose tissue. Significant differences between the sexes occurred in the CLD and WLD groups, where in both cases the males were fattest. There were significant differences between regimes, where CSD hamsters had the least total adipose tissue and WLD had the most total adipose tissue (f=16.2, p<0.0000, one way ANOVA). For all the LSD see S8 (p. 142). The body mass of WSD hamsters was not significantly different from the CSD hamsters, and the body mass of the CLD hamsters was not significantly different from the WLD hamsters, yet in both cases the warm hamsters had approximately twice the amount of dissectable adipose tissue.

The adipose tissue of the hamsters appeared to naturally divide into anterior and posterior depots. The anterior depots were; AX, BA, HW, HB, IFS, TW, TB and UNM, and the posterior depots were; ATG, BOT, DWA, GS, GV, POP and OV /EP. See Table 2.2 where the depots are described and Figs. 2.1.1-2 where the depots are shown.

Fig. 3.7.3 Comparison of the proportion of adipose tissue in the anterior (ant.) and posterior depots (post.) in males and females under the four regimes. In most cases the SEs were too small to show on this graph.



When expressed relative to the total dissectable adipose tissue there were significant differences between the anterior ($f=28.9785$, $p<0.0000$, one way ANOVA) and posterior depots ($f=35.7769$, $p<0.0000$, one way ANOVA) under the four environmental conditions (Fig. 3.7.3), but there were significant sex differences only in CLD and WLD. For all the LSD see S8 (p. 142). The distribution of adipose tissue remained remarkably similar in the females for all of the four conditions and in the males on short photoperiod. The distribution of adipose tissue in the males on long photoperiod however changed significantly which was due almost entirely to the greatly increased size of the epididymal depot on long photoperiod. The testes also undergo large changes in mass (Table 3.3.1) on long photoperiod. So, to find out whether the masses of the two tissues were correlated, the data were compared in Fig. 3.7.4.

Fig. 3.7.4 Relationship between the mass of the testes (data from Table 3.3.1) and the epididymal depot (data from Table 3.5.1) in hamsters kept on either long or short photoperiod. (n= 32, SHORT and 28, LONG)

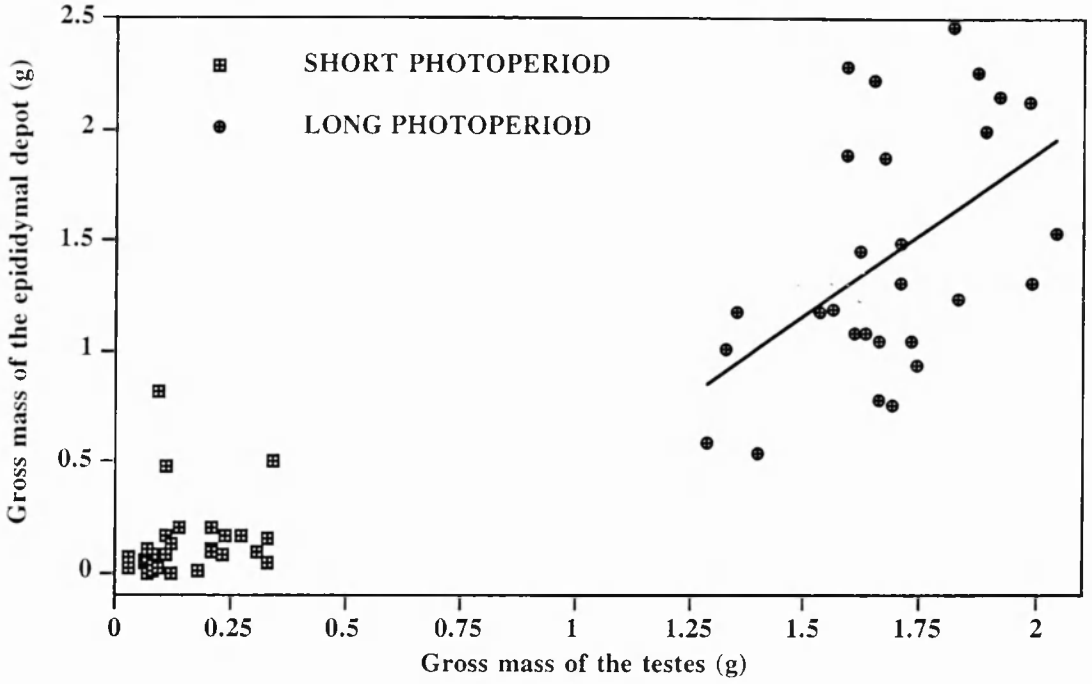


Fig. 3.7.3 above shows the relationship between the mass of the testes and the mass of the epididymal depot in hamsters that had been kept on similar photoperiods, regardless of environmental temperature. Only the data from hamsters that had been kept on long photoperiod had a significant regression, LONG ($r= 0.513$, $p<0.01$, solid line), and SHORT ($r= 0.168$, n.s.).

The results were very obviously divided by the photoperiod, as expected. The large testes, on long photoperiod, in full spermatogenesis had a large epididymal depot attached and the small testes that were dormant, on short photoperiod, had a small epididymal attached. The correlation between the testes and epididymal depot during the most active spermatogenesis on LONG photoperiod suggested that it was likely that EP contributed specifically to the nutrition of the testes, OR they might quite simply share a blood supply, where, as the testes became active and enlarged, the EP depot cashed in on the improved blood supply and enlarged as well. The correlation could quite easily come from either suggestion or possibly a combination of both ideas. So, these data help to explain why murid rodents have such large EP adipose depots.

The following Tables 3.5.1-2 detail the masses of the all the adipose depots studied in male and female *P. campbelli*, weighed at dissection.

Table 3.5.1 Gross mass (mean ± SEs) of all the adipose depots (g) in male *P. campbelli*. AX was only studied for Run 3 (1993-4), consequently there were only results for CSD and WLD. For abbrevations see Table 2.2.

	CSD	WSD	CLD	WLD	Statistics
n	17	14	9	19	one way ANOVA
UNM	0.097±0.013	0.093±0.015	0.173±0.028	0.208±0.017	f=8.0, p>0.0000
TW	0.024±0.004	0.100±0.027	0.097±0.024	0.298±0.054	f=6.2, p>0.0000
TB	0.118±0.012	0.146±0.032	0.190±0.022	0.234±0.029	f=2.5 p>0.0210
HW	0.014±0.001	0.061±0.019	0.075±0.016	0.112±0.015	f=6.6, p>0.0000
HB	0.054±0.005	0.097±0.021	0.098±0.015	0.204±0.021	f=7.1, p>0.0000
AX	0.133±0.009	-	-	0.297±0.048	f=6.6, p>0.0000
BA	0.387±0.029	0.899±0.230	1.108±0.232	2.031±0.205	f=10.3, p>0.0000
IFS	0.348±0.041	0.809±0.197	1.011±0.248	1.714±0.141	f=11.2, p>0.0000
ATG	0.018±0.001	0.025±0.005	0.043±0.013	0.068±0.007	f=7.2, p<0.0000
DWA	0.021±0.002	0.093±0.032	0.129±0.034	0.296±0.048	f=10.7, p<0.0000
EP	0.078±0.011	0.225±0.065	1.071±0.157	1.595±0.121	f=59.7, p<0.0000
POP	0.016±0.005	0.019±0.004	0.030±0.005	0.054±0.005	f=10.2, p<0.0000
GS	0.159±0.015	0.392±0.130	0.519±0.133	1.094±0.117	f=11.2, p<0.0000
GV	0.079±0.008	0.177±0.039	0.209±0.043	0.330±0.037	f=9.2, p<0.0000
BOT	0.028±0.003	0.100±0.034	0.175±0.054	0.280±0.036	f=8.6, p<0.0000

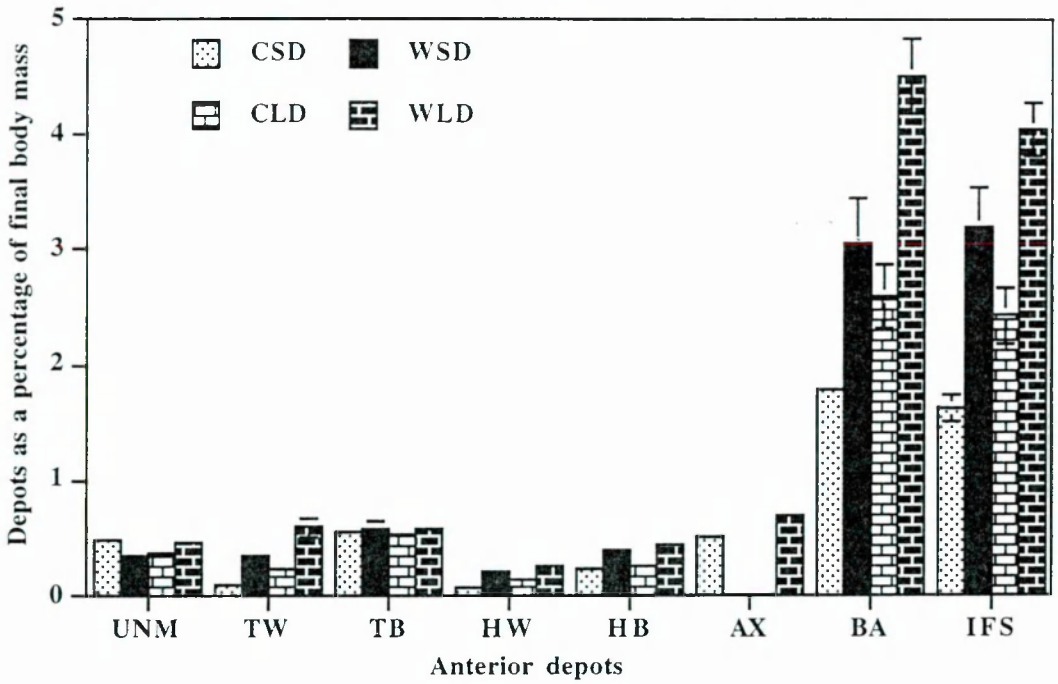
Table 3.5.2 Gross mass (mean ± SEs) of all the adipose depots (g) studied in female *P. campbelli*. AX was only studied for Run 3 (1993-4), consequently there were only results for CSD and WLD. For abbrevations see Table 2.2.

	CSD	WSD	CLD	WLD	Statistics
n	18	11	11	20	one way ANOVA
UNM	0.102±0.012	0.066±0.010	0.073±0.007	0.137±0.024	f=8.0, p>0.0000
TW	0.020±0.003	0.079±0.025	0.050±0.011	0.170±0.048	f=6.2, p>0.0000
TB	0.116±0.009	0.145±0.036	0.143±0.014	0.174±0.036	f=2.5 p>0.0210
HW	0.019±0.003	0.049±0.011	0.029±0.007	0.084±0.020	f=6.6, p>0.0000
HB	0.047±0.003	0.103±0.021	0.072±0.019	0.133±0.031	f=7.1, p>0.0000
AX	0.090±0.008	-	-	0.212±0.045	f=6.6, p>0.0000
BA	0.358±0.017	0.938±0.251	0.605±0.126	1.282±0.235	f=10.3, p>0.0000
IFS	0.340±0.032	0.870±0.219	0.608±0.100	1.261±0.191	f=11.2, p>0.0000
ATG	0.022±0.007	0.029±0.008	0.028±0.003	0.047±0.007	f=7.2, p<0.0000
DWA	0.018±0.002	0.093±0.026	0.049±0.016	0.155±0.031	f=10.7, p<0.0000
OV	0.010±0.001	0.052±0.017	0.032±0.009	0.049±0.011	f=3.2, p<0.0308
POP	0.011±0.001	0.022±0.005	0.024±0.005	0.034±0.005	f=10.2, p<0.0000
GS	0.160±0.011	0.468±0.138	0.348±0.097	0.661±0.110	f=11.2, p<0.0000
GV	0.064±0.004	0.181±0.049	0.106±0.022	0.175±0.030	f=9.2, p<0.0000
BOT	0.062±0.008	0.223±0.059	0.141±0.031	0.332±0.057	f=8.6, p<0.0000

There were significant differences in the gross mass (Tables 3.5.1-2) of all the anterior depots between the experimental groups. Comparing these data with Table 3.5.1 reveals that sex differences occurred in the anterior depots of animals on WLD and CLD, where those of the males were larger in every case. Significant differences were also found in all the masses of the posterior adipose depots between experimental groups. Comparing these data with Table 3.5.1 reveals there were significant sex differences in the posterior depots in WLD only, where the males were also larger in every case. For all the LSD see S9 (p. 143).

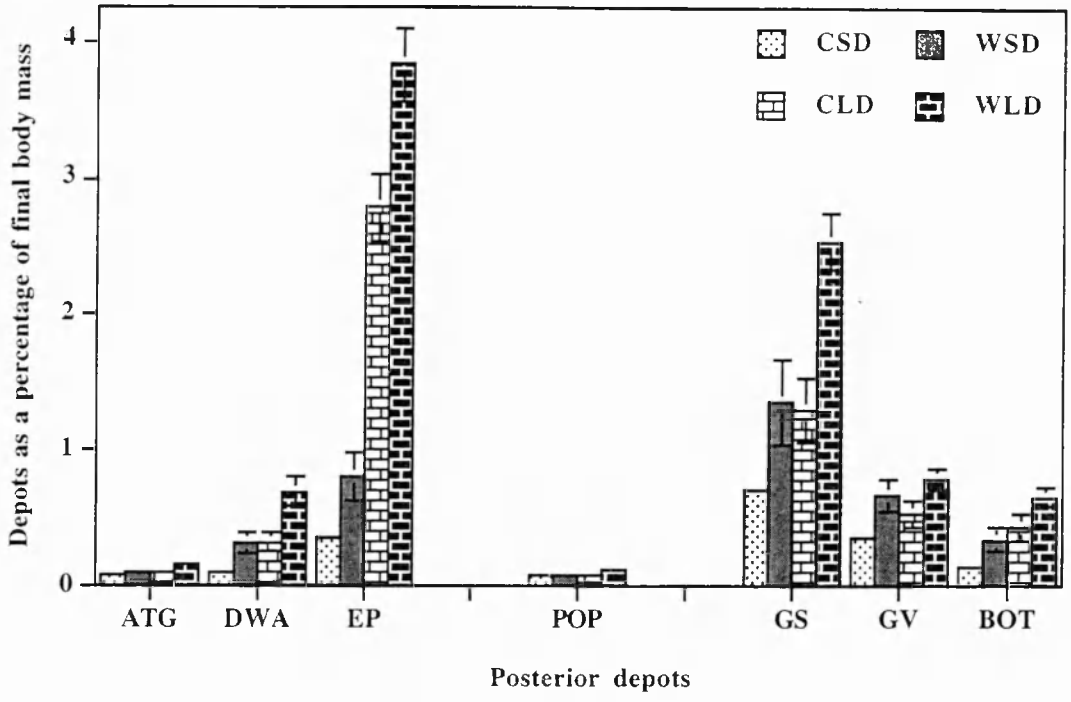
The gross mass of most depots shown in Tables 3.5.1-2 show differences between the summer and winter conditions, in keeping with the differences in body mass. The data from hamsters on WSD and CLD were invariably similar to each other and situated between the values for hamsters on CSD and WLD.

Fig. 3.7.5 Mass (mean ± SEs) of the anterior adipose depots expressed as a percentage of the final body mass. No significant sex differences were found, so the data were combined for the males and females. In several cases, the SEs were too small to show up on this graph.



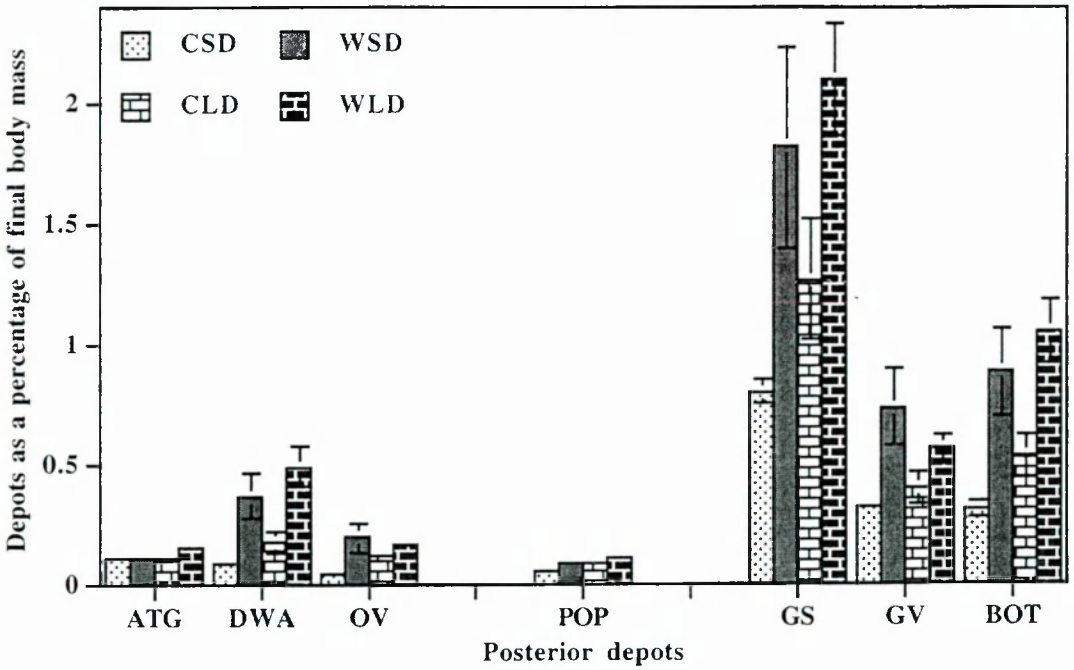
When the anterior depots were expressed as a percentage of the final body mass (Fig. 3.7.5), there were significant differences between treatment groups in UNM ($f=2.8$, $p<0.0081$, one way ANOVA), TW ($f=6.1$, $p<0.0000$, one way ANOVA), HW ($f=6.3$, $p<0.0000$, one way ANOVA), and HB ($f=4.7$, $p<0.0001$, one way ANOVA), but these differences were reduced when the data were expressed in this way. BA ($f=8.5$, $p<0.0000$, one way ANOVA) and IFS ($f=10.2$, $p<0.0000$, one way ANOVA) kept the large differences of the gross mass, with values from hamsters on WSD and CLD still similar. For all the LSD see S10 (p. 143).

Fig. 3.7.6 Mass (mean ± SEs) of the posterior adipose depots expressed as a percentage of the final body mass in the males. In several cases, the SEs were too small to show up on this graph.



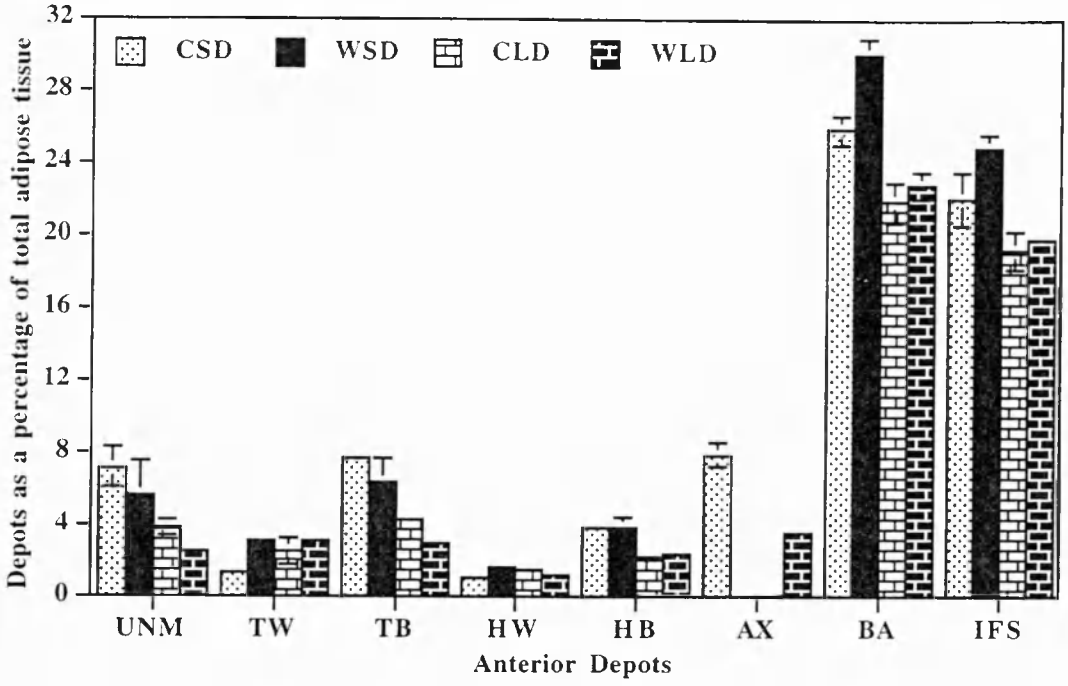
There were significant differences in all the posterior depots of the males (Fig. 3.7.6), when expressed in proportion to the final body mass, ATG ($f=2.3$, $p<0.0302$, one way ANOVA) and POP ($f=3.9$, $p<0.0007$, one way ANOVA) responded to changes in body mass. In DWA ($f=8.7$, $p<0.0000$, one way ANOVA), GS ($f=9.3$, $p<0.0000$, one way ANOVA), and to a lesser extent, BOT ($f=11.4$, $p<0.0000$, one way ANOVA) and GV ($f=5.3100$, $p<0.0000$, one way ANOVA) were disproportionate. These depots were much smaller in hamsters on CSD and much larger in those kept on WLD. Even when expressed as a percentage of body mass, EP was up to eight times larger on long photoperiod, even in the cold, far more than any of the other depots. For all the LSD see S10 (p. 143). These depots may be the principal whole-body energy stores that enlarge disproportionally in fatter hamsters (see Fig. 3.7.1).

Fig. 3.7.7 Mass (mean ± SEs) of posterior depots in the females expressed as a percentage of the final body mass. In several cases the SEs were too small to show on this graph.



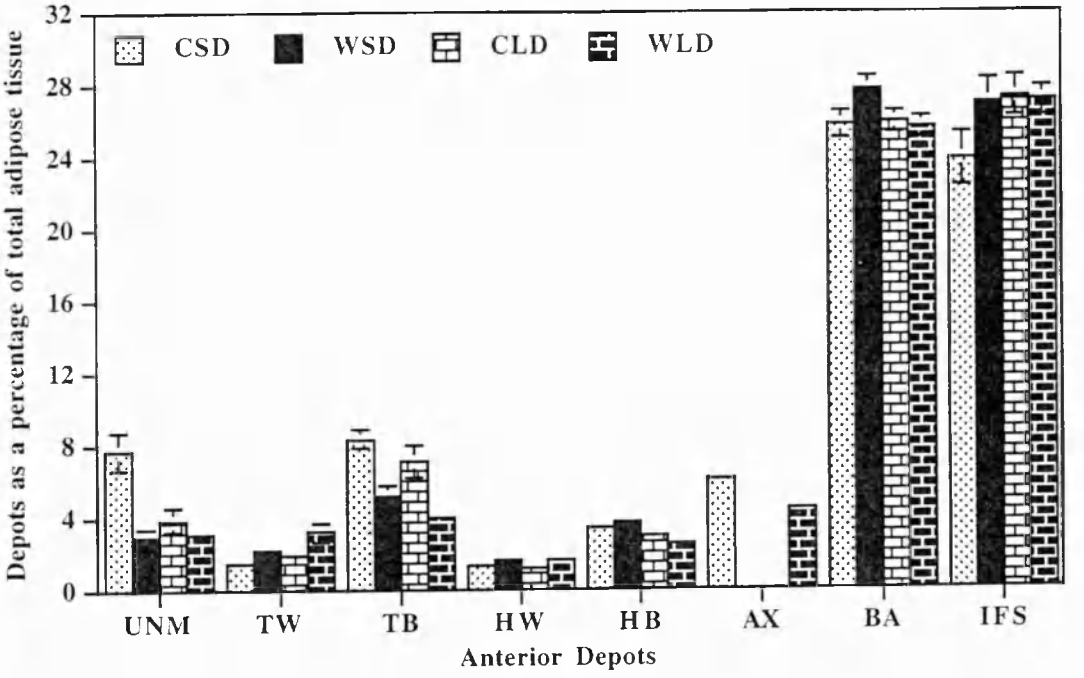
In the females, when expressed as a percentage of final body mass (Fig. 3.7.7), there were significant differences in OV (f=3.6, p<0.0197, one way ANOVA) where CSD was disproportionately smaller, for all the others see Fig. 3.7.6. Comparing these data with those of Fig. 3.7.6 revealed that there were sex differences with DWA, GV (males larger) and BOT (females larger) in WLD and with BOT (females larger) in CLD. For all the LSD see S10 (p. 143). The females made up slightly for the lack of EP by having more BOT, but it was tiny compared to EP (Fig. 3.7.7). The intra-abdominal depots (ATG and DWA) need to remain relatively small to allow plenty of room for growing foetuses. The specialised depots like ATG and POP remained almost constant as a %BM. As suggested with the males these depots may be the principal whole-body energy stores that enlarged disproportionately in fatter hamsters (Fig. 3.7.1).

Fig. 3.7.8 Mass (mean ± SEs) of the anterior depots expressed as a percentage of the total dissectable adipose tissue in males. In several cases the SEs were too small to show up on this graph.



When the anterior depots of the males were expressed as a percentage of the total dissectable adipose tissue (Fig. 3.7.8), there were significant differences between regimes in UNM ($f=5.0$, $p<0.0001$, one way ANOVA), TW ($f=3.3$, $p<0.0030$, one way ANOVA), TB ($f=9.8$, $p<0.0000$, one way ANOVA), HW ($f=2.0$, $p<0.0589$, one way ANOVA), HB ($f=5.2$, $p<0.0000$, one way ANOVA), BA ($f=9.8$, $p<0.0000$, one way ANOVA), IFS ($f=7.5$, $p<0.0000$, one way ANOVA). For all the LSD see S11 (p. 144). In the hamsters on CSD there was more UNM, TB, and AX, BAT depots, and less TW and HW, both of which were attached to the BAT depots.

Fig. 3.7.9 Mass (mean ± SEs) of the anterior depots expressed as a percentage of the total dissectable adipose tissue in females. In several cases the SEs were too small to show up on this graph.



When the anterior depots of the females (Fig. 3.7.9) were expressed as a percentage of the total dissectable adipose tissue, values for BA and IFS remained amazingly constant. There were significant differences in nearly all the anterior depots, see Fig 3.7.8 for details. Comparing these data to those of Fig. 3.7.8 reveals that there were significant sex differences in the four regimes, in several of the depots. For all the LSD see S11 (p. 144).

The following Table 3.6 details any changes in the total BAT and the individual BAT depots in the simulated 'summer' and simulated 'winter' environments.

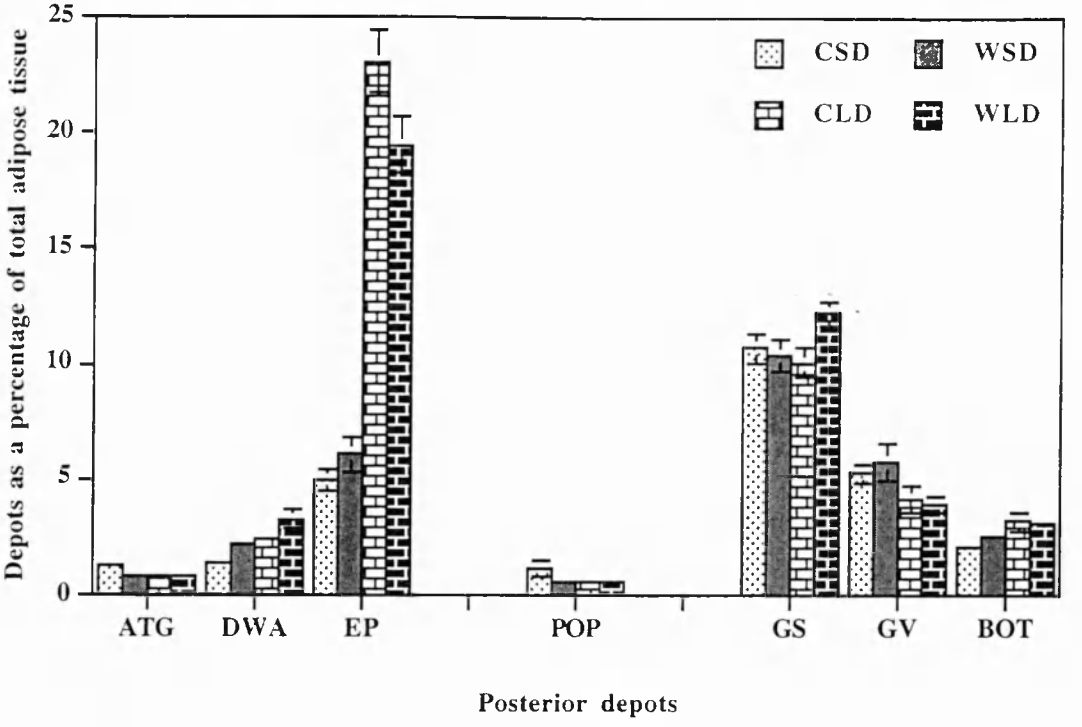
Table 3.6 Relative quantities of BAT (mean ± SEs), in male and female hamsters living in the two 'natural' environments.

Environment + sex (N)	'simulated winter' males (11)	'simulated summer' males (11)	'simulated winter' females (12)	'simulated summer' females (14)
Total BAT (g)	0.4±0.03	1.0±0.12	0.3±0.02	0.7±0.15
BAT (% BM)	1.8±0.10	2.3±0.24	1.6±0.07	2.1±0.31
BAT (% total AT)	23.6±1.36	11.8±0.93	23.4±1.12	13.9±0.71
BAT depots as a percentage of total BAT.				
HB (%)	12.3±0.76	21.4±1.25	14.3±0.82	16.5±0.97
TB (%)	33.6±2.06	27.2±1.45	36.0±2.56	29.8±1.38
UNM (%)	20.2±2.43	22.2±2.00	22.9±3.23	21.3±1.28
AX (%)	33.9±2.51	29.2±1.86	26.8±1.93	32.4±1.37

As Table 3.6. shows there was in fact significantly more of BAT (gross mass) in the WLD (simulated summer) hamsters ($f=7.5$, $p<0.0003$, one way ANOVA, for all the LSD see S8 (p. 142)) and significant differences were found between the sexes only in WLD, but there was no significant difference in BAT between the two regimes, when the mass was expressed as a percentage of the final body mass.

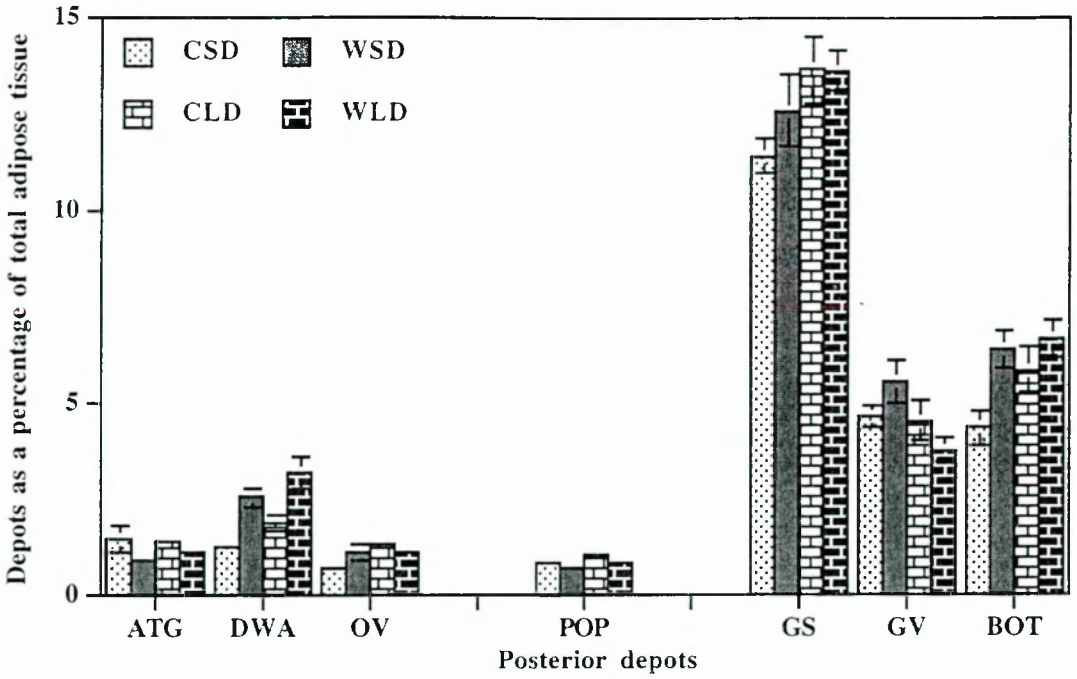
When expressed as a percentage of the total adipose tissue there was significantly more total BAT ($f=35.6$, $p<0.0000$, one way ANOVA) in the CSD group. When the individual BAT depots were expressed as a percentage of total BAT, HB ($f=14.9$, $p<0.0000$, one way ANOVA) in the WLD males had significantly more than the CSD males and the WLD males had more than the WLD females. TB ($f=4.1$, $p<0.0119$, one way ANOVA) in the CSD hamsters had significantly more than the WLD hamsters. UNM and AX had no significant differences between the two regimes or sexes. For all the LSD see S8 (p. 142).

Fig. 3.7.10 Mass (mean ± SEs) of the posterior depots expressed as a percentage of the total dissectable adipose tissue in males. In several cases the SEs were too small to show up on this graph.



When the masses of posterior depots of the males were expressed as a percentage of total dissectable adipose tissue (Fig. 3.7.10) significant differences were found in DWA ($f=6.6$, $p<0.0000$, one way ANOVA) and GS ($f=4.4$, $p<0.0002$, one way ANOVA) which were a bit bigger in the very fat WLD hamsters. GV ($f=2.7$, $p<0.0105$, one way ANOVA) and BOT ($f=18.3808$, $p<0.0000$, one way ANOVA) hardly had any differences. EP ($f=77.5413$, $p<0.0000$, one way ANOVA) was still much larger on long photoperiod even in the cold. ATG remained remarkably constant. For all the LSD see S11 (p. 144).

Fig. 3.7.11 Mass (mean ± SEs) of the posterior depots expressed as a percentage of the total dissectable adipose tissue in the females. In several cases the SEs were too small to show up on this graph.

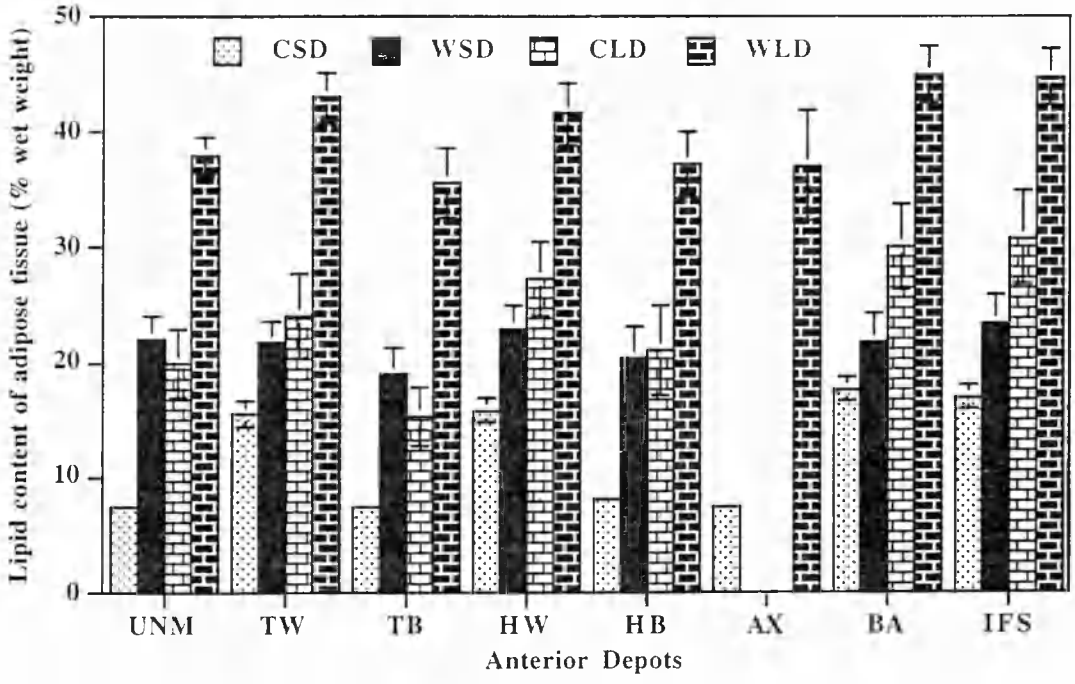


In the females (Fig. 3.7.11) there were significant differences, although these differences were evened out when expressed as a percentage of the total adipose tissue, OV ($f=3.4$, $p<0.0242$, one way ANOVA) and see Fig. 3.14.3 for details. Comparing these data with those in Fig. 3.7.10 revealed that there were significant sex differences in CSD, WSD, CLD and WLD. For all the LSD see S11 (p. 144).

3.7.2 Lipid in adipose tissue.

Results from the lipid assays performed on all adipose tissue depots are shown in the following graphs.

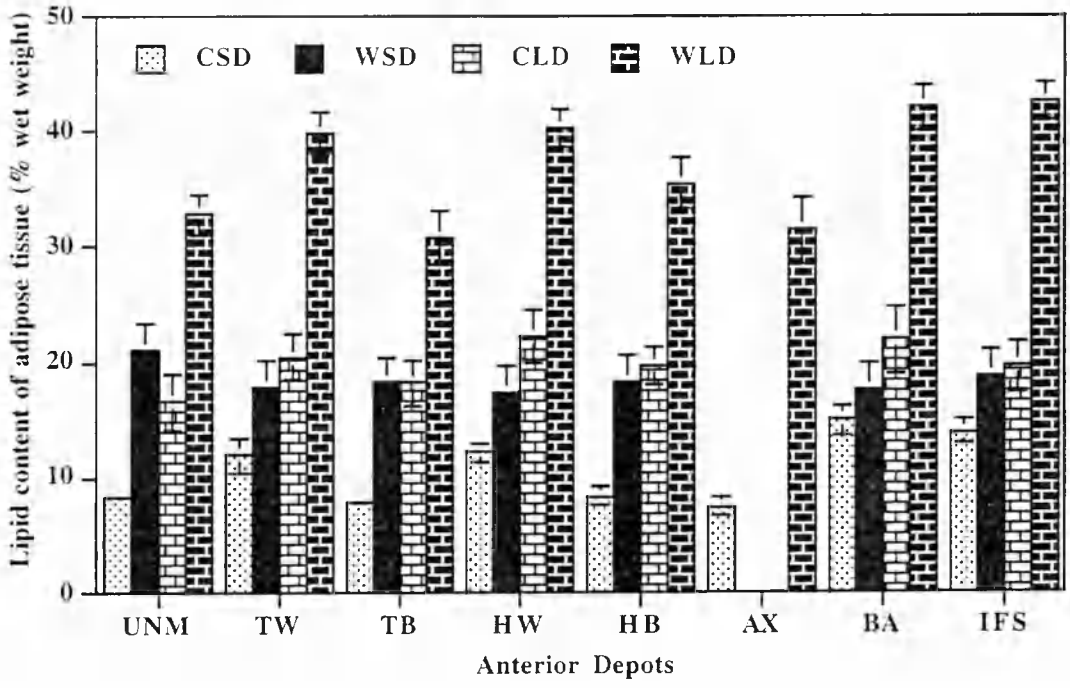
Fig. 3.7.12 Calculated (mean ± SEs) lipid content (% wet weight) found in anterior adipose tissue depots of male *P. campbelli*, n = 17 (CSD), 14 (WSD), 9 (CLD), 19 (WLD). In several cases the SEs were too small to show up on this graph.



In all cases the depots in CSD had the lowest range of lipid content (7.3±0.4%-17.7±1.1%), WSD (18.9±2.4%-23.4±2.6%) and CLD (15.2± 2.5%-30.8±4.0%) were surprisingly similar in the mid-range and WLD (35.7±3.1%-45.0±2.4%) had the highest range of lipid content (Fig. 3.7.12). The lipid content of the BAT depots was consistently less than that of the other anterior depots in the CSD hamsters. There were also slight reductions in the lipid content of the BAT depots in the other regimes. The lipid content of the adipose depots was very different across the four regimes, especially when compared to Fig. 3.7.5 which show that the gross masses of the depots were remarkably similar with the exception of BA and IFS. There were significant differences between regimes in the percentage lipid content of all the anterior depots, UNM ($f=45.9$, $p<0.0000$, one way ANOVA), TW ($f=34.9$, $p<0.0000$, one way ANOVA), TB ($f=25.3$, $p<0.0000$, one way ANOVA), HW ($f=37.3$, $p<0.0000$,

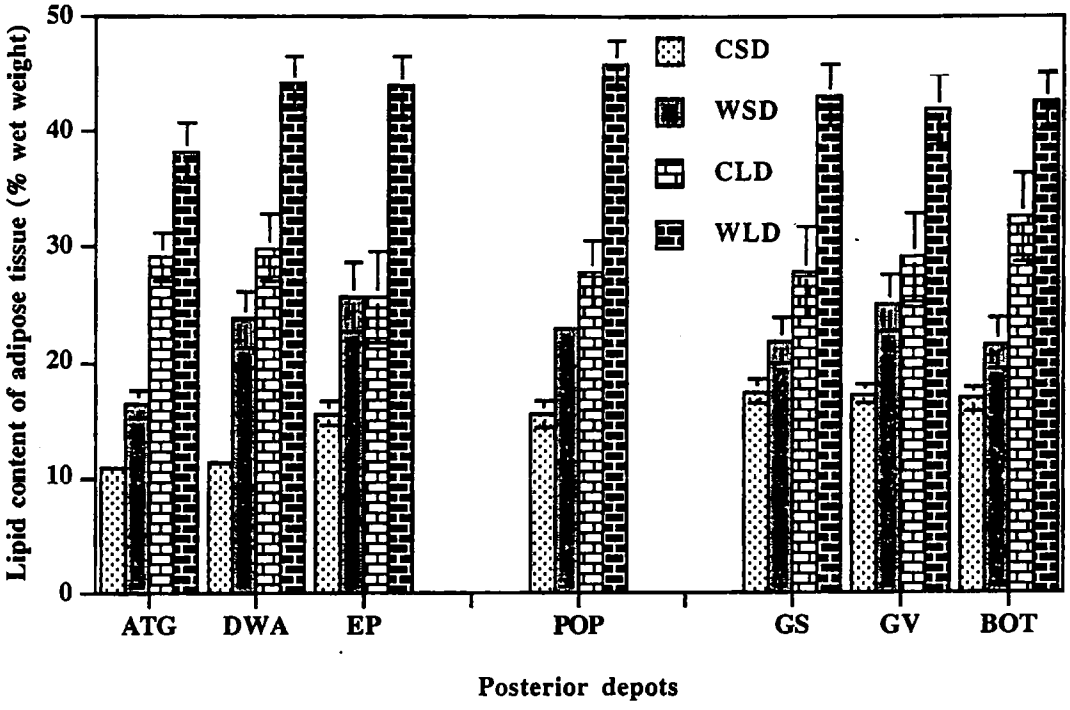
one way ANOVA), HB (f=29.4, p<0.0000, one way ANOVA), AX, (f=30.6, p<0.0000, one way ANOVA), BA (f=33.8, p<0.0000, one way ANOVA) and IFS (f=37.4, p<0.0000, one way ANOVA). For all the LSD see S12 (p. 144).

Fig. 3.7.13 Calculated (mean ± SEs) lipid content (% wet weight) found in anterior adipose tissue depots of female *P. campbelli*. n = 18 (CSD), 11 (WSD), 11 (CLD), 20 (WLD). In several cases the SEs were too small to show up on this graph.



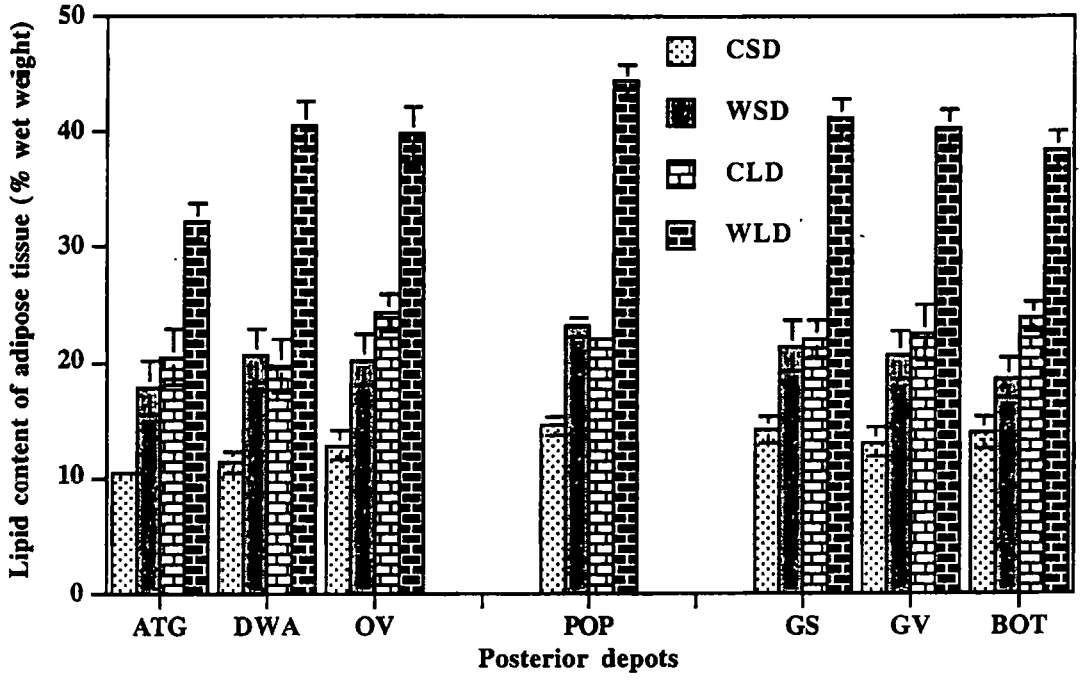
The overall picture for the lipid content of BAT and WAT in female hamsters on different regimes was similar to that of the males. In all cases, the depots of the female hamsters in CSD (Fig. 3.7.13) had the lowest range of lipid (7.4±0.8%-14.9±1.3%), WSD (17.4±2.3%-21.1±2.3%) and CLD (16.6±2.5%-22.2±2.2%) had similar mid-ranges and WLD (30.8±2.4%-42.6±1.7%) had the highest range of lipid. The BAT depots (UNM, TB, HB and AX) in CSD and WLD had consistently less lipid than the other anterior depots, yet all the values of the anterior depots of hamsters from WSD and CLD remained very similar. There were significant differences between regimes in the percentage lipid content of the anterior depots of the females. See males Fig.3.7.12 for the statistical results. Comparing these data with those from Fig. 3.7.12 revealed that there were sex differences in several depots from WLD and CLD. For all the LSD see S12 (p. 144).

Fig. 3.7.14 Calculated (mean ± SEs) lipid content (% wet weight) found in posterior adipose tissue depots of male *P. campbelli*, n = 17 (CSD), 14 (WSD), 9 (CLD), 19 (WLD). In several cases the SEs were too small to show up on this graph.



In all cases, the WAT posterior depots of CSD hamsters (Fig. 3.7.14) had the lowest range of lipid (11.0±0.6%-17.4±1.0%), WSD (16.5±1.1%-25.7±3.0%) and CLD (25.7±4.0%-32.6±3.8%) had similar mid-ranges and WLD (38.2±2.5%-45.9±2.0%) had the highest range of lipid and all the posterior depots had remarkably similar levels of lipid for each environmental regime. Although the masses of ATG and POP (Table 3.5.1) were similar in hamsters on all regimes, the differences in the lipid content was in keeping with the other depots, whereas EP and GS (Table 3.5.1) differed a lot in mass, but no more than others in lipid content. There were significant differences in the percentage lipid content in all the posterior depots of males, ATG ($f=37.9$, $p<0.0000$, one way ANOVA), DWA ($f=46.3$, $p<0.0000$, one way ANOVA), POP ($f=82.8$, $p<0.0000$, one way ANOVA), GS ($f=32.6$, $p<0.0000$, one way ANOVA), GV ($f=27.4$, $p<0.0000$, one way ANOVA), BOT ($f=33.5$, $p<0.0000$, one way ANOVA) and EP ($f=25.7$, $p<0.0000$, oneway ANOVA). For all the LSD see S12 (p. 144).

Fig. 3.7.15 Calculated (mean ± SEs) lipid content (% wet weight) found in the posterior adipose tissue depots of female *P. campbelli*, n = 18 (CSD), 11 (WSD), 11 (CLD), 20 (WLD). In several cases the SEs were too small to show up on this graph.



There were significant differences between regimes in the percentage lipid content of the posterior depots in the females (Fig. 3.7.15), OV ($f=36.8$, $p<0.0000$, one-way ANOVA) and see the males Fig. 3.7.14. Although the masses of ATG and POP (Table 3.5.2) were similar in hamsters on all regimes, the differences in the lipid content was in keeping with the other depots, whereas GS (Table 3.5.2) differed a lot in mass, but no more than others in lipid content. Comparing these data to those in Fig. 3.7.14 revealed that there were significant sex differences in several of the posterior depots in WLD and CLD only. For all the LSD see S12 (p. 144). In all cases the depots in CSD contained the least amount of lipid (10.5±0.6%-14.6±0.8%), WSD (17.8±2.2%-23.0±0.9%) and CLD (19.8±2.3%-24.3±1.6%) had a similar range in the middle and WLD (32.1±1.8%-44.4±1.4%) contained the highest amount of lipid. The ranges for both CLD and WLD were lower for the males, although the males were only significantly fatter on WLD.

The posterior depots shown in Figs. 3.7.14-15 had very similar proportions of lipid to the anterior depots, very low levels in CSD and much higher levels in WLD, though still low compared to rat or guinea-pig values.

Table 3.7 The mean (± SEs) lipid content (% wet weight) overall for the adipose tissue depots, showing the significant sex differences between male and female *P. campbelli.*

Environment	Males (n)	Females (n)	one way ANOVA
CLD	23.2±2.2% (9)	18.2±0.8% (11)	f= 64.7, p<0.0000
WLD	37.9±2.1% (19)	34.4±1.3% (20)	f= 61.7, p<0.0000

When the lipid content (% wet weight adipose tissue) was calculated as an overall mean for the male and female hamsters (Table 3.7) on all four regimes, the only significant differences in the sexes were found to be in the long photoperiods. Sex differences had occurred regularly in CLD and WLD individual adipose depots. However as Fig. 3.7.1 shows, the only significant sex differences in total dissectable adipose tissue occurred in WLD where the males were fatter. In CLD, the males showed a trend towards being fatter, but they had large variations, hence the effect was not significant. So, in both cases, they not only have more adipose tissue, but a greater proportion of the adipose tissue is lipid.

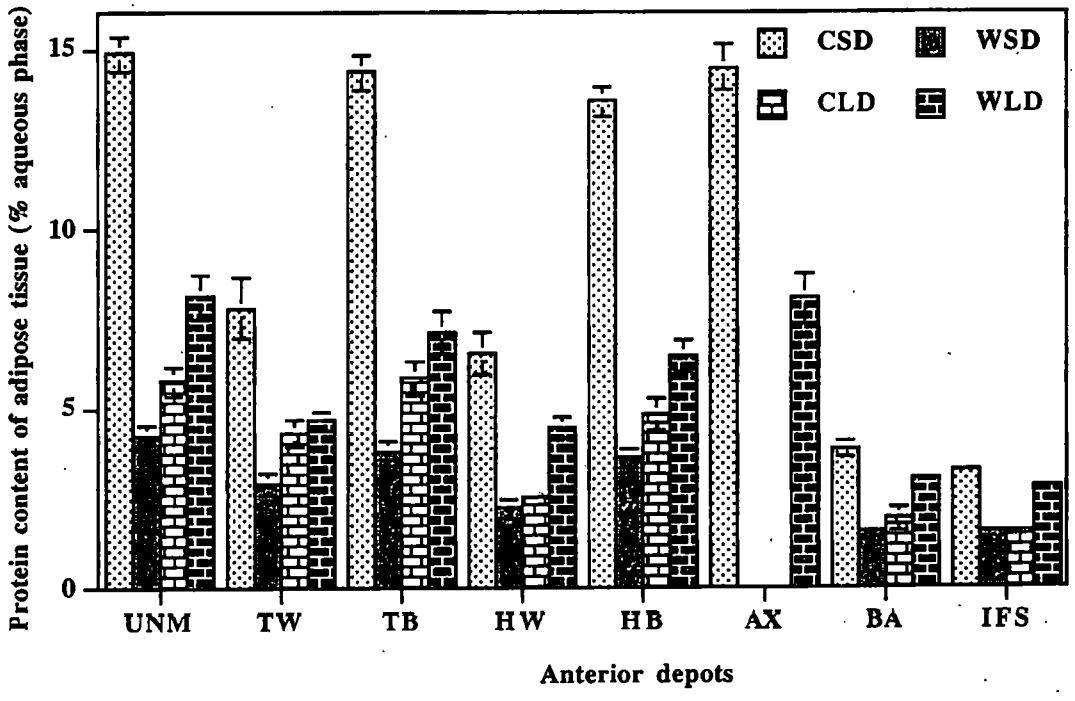
In general, in hamsters in regimes in which total fatness (dissectable adipose tissue) was greater (Fig. 3.7.1), the lipid content of their adipose tissue was high (Figs. 3.7.12-15).

3.7.3 Protein in adipose tissue.

The BAT in the WLD hamsters was very pale by comparison to that of those kept in the cold and not easy to distinguish from WAT. Yet, even the WAT depots of animals kept under CSD had higher levels of protein, than those kept on the other conditions. The BA and IFS depots went brown in places in the hamsters kept on CSD, probably due to increased vascularisation. The sample taken from these two depots for the lipid and protein assays was always from as near the same site as possible and was always white in colour.

Results from the protein assays performed on all adipose tissue depots are shown in the following graphs.

Fig. 3.7.16 Calculated (mean ± SEs) protein content of the anterior adipose depots (% aqueous phase) of *P. campbelli*. In several cases the SEs were too small to show up on this graph. (n = 35 (CSD), 25 (WSD), 20 (CLD), 39(WLD)), AX was not studied until Run 3 (1993-4) consequently only CSD (n = 23) and WLD (n = 25) were studied.

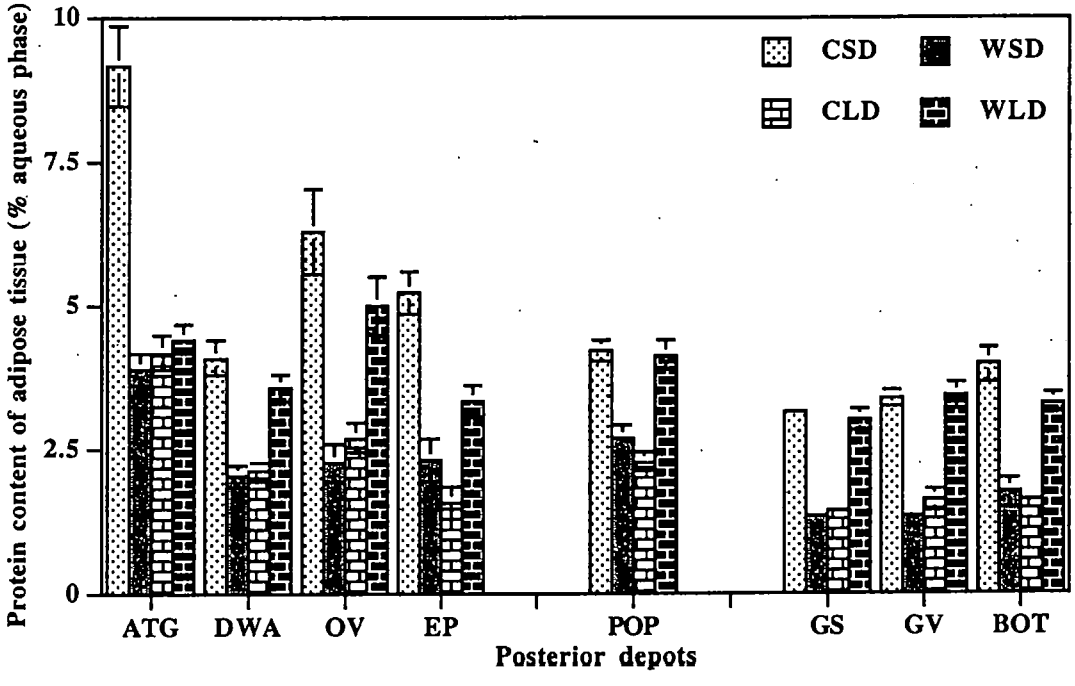


In all cases the anterior depots (Fig. 3.7.16) of hamsters on CSD had the highest protein content (3.3±0.2%-14.9±0.5%), WSD (1.6±0.2%-4.2±0.3%) and CLD (1.5±0.1%-5.8±0.4%) had again similar low ranges in several depots and WLD (2.8±0.2%-8.1±0.6%) had a mid-range protein content. The BAT depots of all the regimes had the highest protein (% aqueous phase) content of the anterior depots, also on CSD TW and HW had more protein than IFS, BA and most of the posterior depots. Since there was only one sex difference in HW (CSD), the results from both sexes, for all the anterior depots, were pooled.

There were significant differences in the protein content of all depots in hamsters on different regimes, UNM ($f=32.2$, $p<0.0000$, one way ANOVA), TW ($f=8.9$, $p<0.0000$, one way ANOVA), TB ($f=37.5$, $p<0.0000$, one way ANOVA), HW ($f=12.5$,

p<0.0000, one way ANOVA), HB (f=47.8, p<0.0000, one way ANOVA), AX (f=16.6, p<0.0000, one way ANOVA), BA (f=10.4, p<0.0000, one way ANOVA) and IFS (f=13.0, p<0.0000, one way ANOVA). For all the LSD see S13 (p. 145). There was a significant interaction between photoperiod and temperature (p<0.001, three-way ANOVA) in the values from all the anterior depots. Samples from BA and IFS depots were always taken from the same areas of the depots and they remained visibly white. Hence, probably, the reason in the low protein content, but they were very similar to each other and DWA, GS, GV and BOT.

Fig. 3.7.17 Calculated (mean ± SEs) protein content of the posterior depots (% aqueous phase) in *P. campbelli*. In several cases SEs were too small to show on this graph. (n = 35 (CSD), 25 (WSD), 20 (CLD), 39(WLD)), with the exception of EP (n = 17, 14, 9 and 19 respectively) and OV (n = 12, 11, 11 and 14 respectively).



All the posterior depots (Fig. 3.7.17), in all the regimes, had lower levels of (% aqueous) protein than the anterior depots. Although most of the values for samples from hamsters on WLD were surprisingly high, sometimes (in the case of WAT depots) as high as those from CSD hamsters and in hamsters on WLD these depots also had lots of lipid (Figs. 3.7.14-15). Of the posterior depots, ATG in hamsters on CSD had the highest protein content (9.1±0.7%). The other posterior depots in CSD

hamsters had a similar range of protein (3.2±0.1%-6.3±0.7%) to those of hamsters on WLD (3.0±0.2%-5.0±0.5%). Values from WSD (1.3±0.1%-3.9±0.3%) and CLD hamsters (1.4±0.1%-4.2±0.3%) had a similar low range. Since there was only one significant sex difference in GV (WLD) the results from both sexes, for all the posterior depots, were pooled. ATG and POP both contain lymph nodes, which would contribute to their higher protein content and both depots may have specialised roles.

There were significant differences between measurements from different regimes in all the posterior depots, ATG ($f=14.6$, $p<0.0000$, one way ANOVA), DWA ($f=7.7$, $p<0.0000$, one way ANOVA), POP ($f=9.3$, $p<0.0000$, one way ANOVA), OV ($f=12.7$, $p<0.0000$, one way ANOVA), GS ($f=16.2$, $p<0.0000$, one way ANOVA), GV($f=16.3$, $p<0.0000$, one way ANOVA), BOT ($f=10.0$, $p<0.0000$, one way ANOVA) and EP ($f=20.4$, $p<0.0000$, one way ANOVA). For all the LSD see S13 (p. 145). There was a significant interaction between photoperiod and temperature in all the posterior depots ($p<0.0.001$, three-way ANOVA) and between photoperiod and sex ($p<0.025$, three-way ANOVA) in GV.

Fig. 3.7.18 Relationship between lipid content (Figs. 3.7.12-15) and protein content (Figs. 3.7.16-17) of adipose depots. n= 30 (CSD), 28 (CLD), 28 (WSD), 30 (WLD).

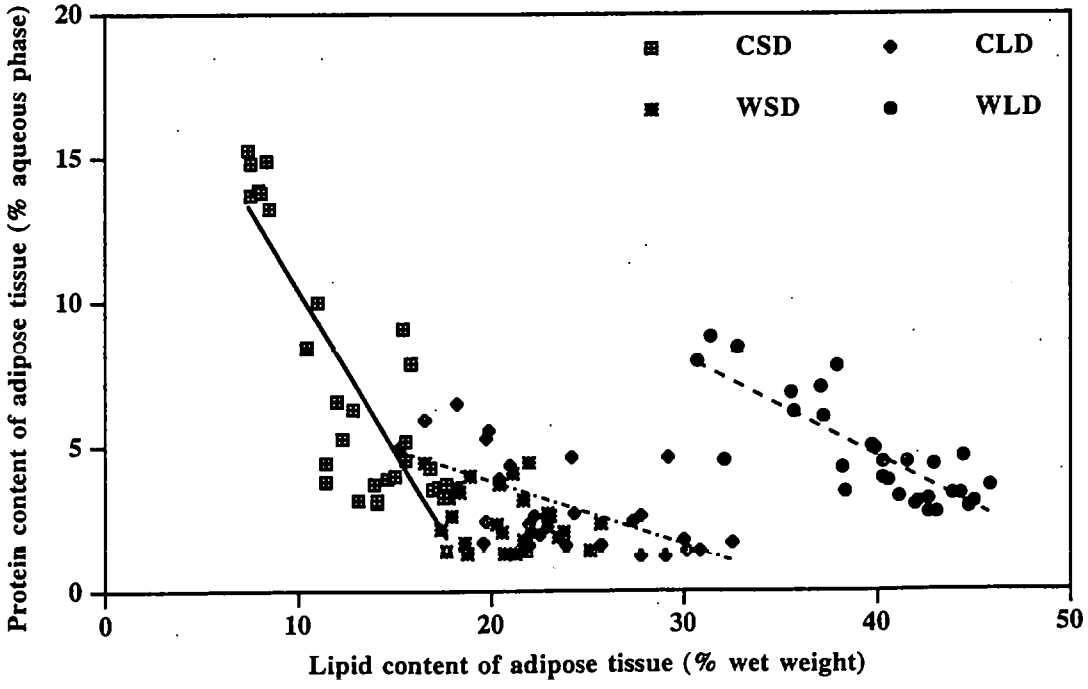
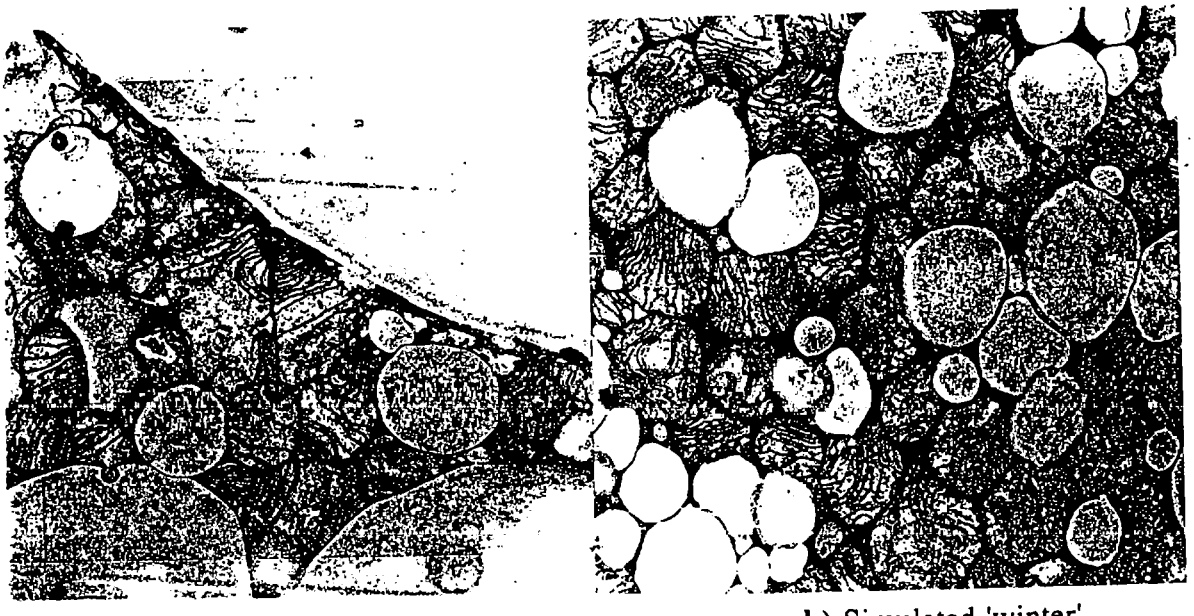


Fig. 3.7.18 shows the regressions between the lipid and protein content of each adipose tissue depot. CSD (unbroken line, r= 0.849, p< 0.001), CLD (short hatched line, r= 0.620, p< 0.001), WSD (r= 0.272, n.s.), WLD (long hatched line, r=0.811, p< 0.001). In all cases, as expected, the lipid content decreased as the protein content increased, but the relationships between protein and lipid values were quite different for data from hamsters on CSD and WLD. The regression line for the CLD values was virtually parallel to that of the WLD data, but the values for lipid and protein were lower.

3.8 Mitochondria in brown and white adipose tissue.

Electron micrographs of three depots UNM, HB and GS in both hamsters kept on 'simulated summer' (Figs 3.8.1a-3.8.3a) and 'simulated winter' (Figs. 3.8.1b-3b) clearly demonstrate the differences between 'pure' BAT and 'pure' WAT depots, especially the large tightly packed mitochondria of the two BAT depots, UNM and HB, and the occassional small mitochondria of the WAT depot, GS. The increase in the size of the lipid droplets can clearly be seen in all three depots, between hamsters on CSD and those on WLD, in keeping with the theory that all the depots become lipid stores when kept in simulated 'summer'.

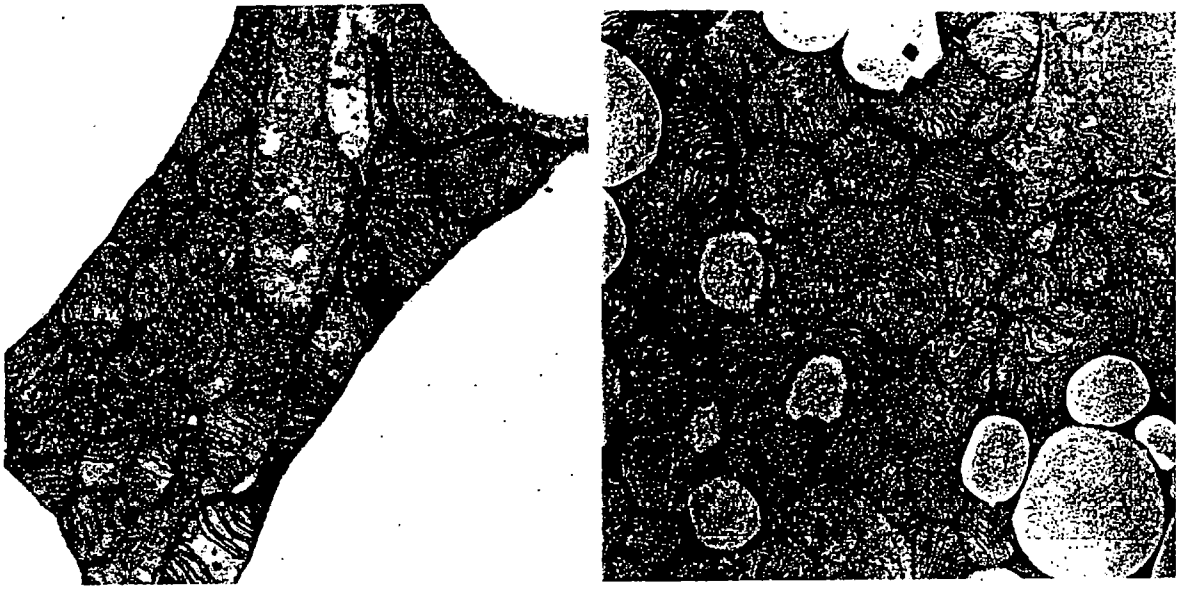
Fig. 3.8.1a + b Electron micrographs (X9000) of the interscapular (HB) BAT depot in simulated 'summer' and simulated 'winter' conditions. With grateful thanks to Heather Davies for the electron micrographs.



a) Simulated 'summer'

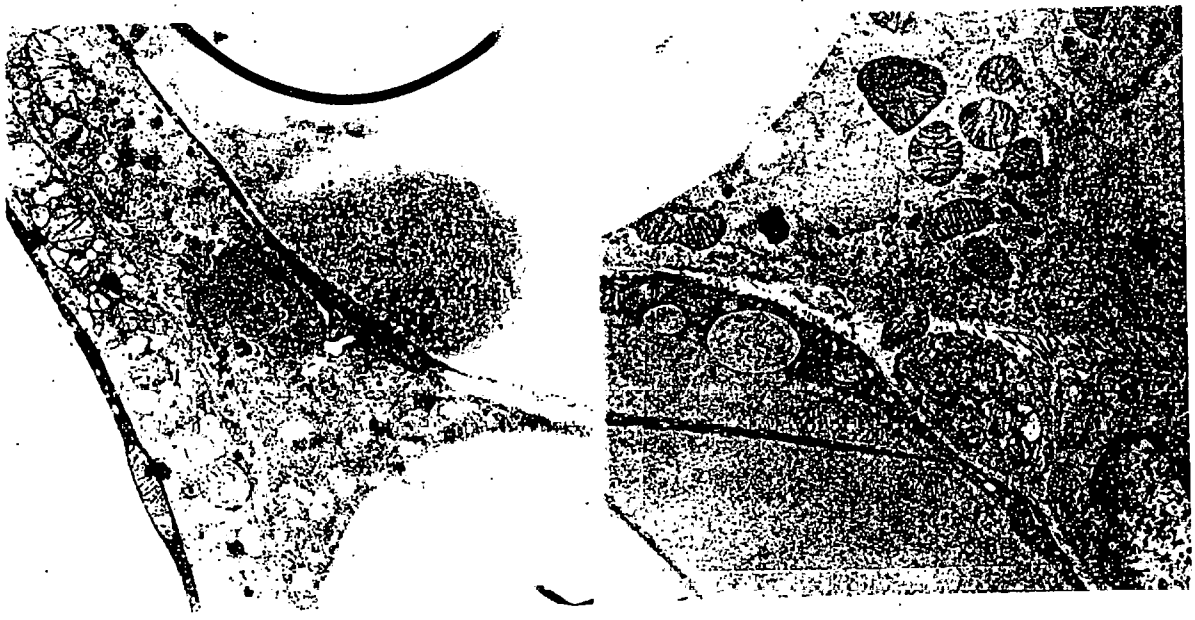
b) Simulated 'winter'

Fig. 3.8.2a + b Electron micrographs (X9000) of the cervical (UNM) BAT depot in simulated 'summer' and simulated 'winter' conditions. With grateful thanks to Heather Davies for the electron micrographs.



a) Simulated 'summer' **b)** Simulated 'winter'

Fig. 3.8.3a + b Electron micrographs (X9000) of the side groin (GS) WAT depot in simulated 'summer' and simulated 'winter' conditions. With grateful thanks to Heather Davies for the electron micrographs.

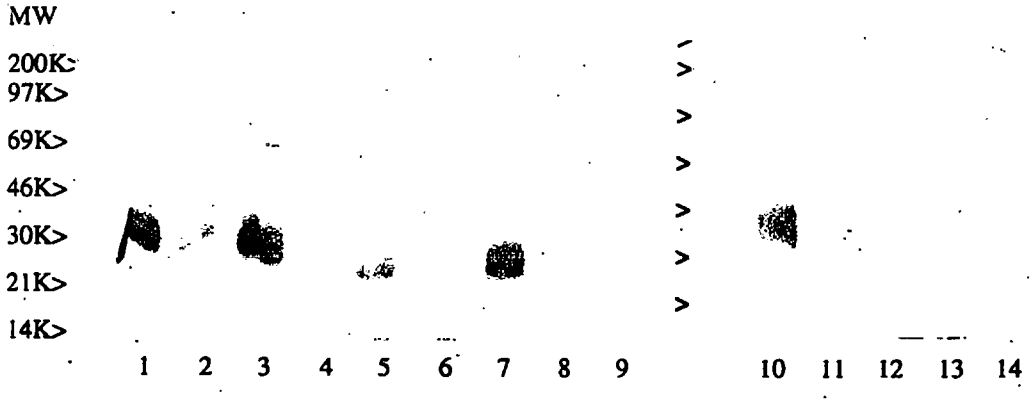


a) Simulated 'summer' **b)** Simulated 'winter'

3.9 Western blots for uncoupling protein.

The technique of Western blotting can be used to specifically identify UCP (with a molecular weight of 32K), that is found only in brown adipocytes and defines them as such. This assay is very sensitive and even when only 5 μg of protein from the mitochondria isolated from BAT are loaded into a lane, a clear staining can be seen (Figs. 3.9.1-2). The anterior WAT depots, TW, HW, IFS and BA (Figs. 3.9.1-2), show clearly the presence of UCP. All the WAT depots were very heavily loaded to give the maximum chance of any UCP, if present, showing up on the blot, UCP was not detected in any of the posterior depots.

Fig. 3.9.1 Western blots of mitochondrial protein to test for the presence of uncoupling protein in the adipose tissue of *P. campbelli* kept in simulated 'summer' and simulated 'winter'.



Lane 1 CSD UNM Lane 2 WLD UNM Lane 3 CSD BA Lane 4 WLD BA

Lane 5 CSD IFS Lane 6 WLD IFS Lane 7 CSD HW Lane 8 WLD HW

Lane 9 WLD EP Lane 10 CSD AX Lane 11 WLD AX Lane 12 CSD BOT

Lane 13 WLD BOT Lane 14 CSD EP

Fig. 3.9.2 Western blots of mitochondrial protein to test for the presence of uncoupling protein in the adipose tissue of *P. campbelli* kept in simulated 'summer' and simulated 'winter'.

Lane 15 CSD HB Lane 16 WLD HB Lane 17 CSD TW Lane 18 WLD TW

Lane 19 CSD ATG Lane 20 WLD ATG Lane 21 CSD DWA Lane 22 WLD DWA

Lane 23 CSD TB Lane 24 WLD TB Lane 25 CSD GS Lane 26 WLD GS

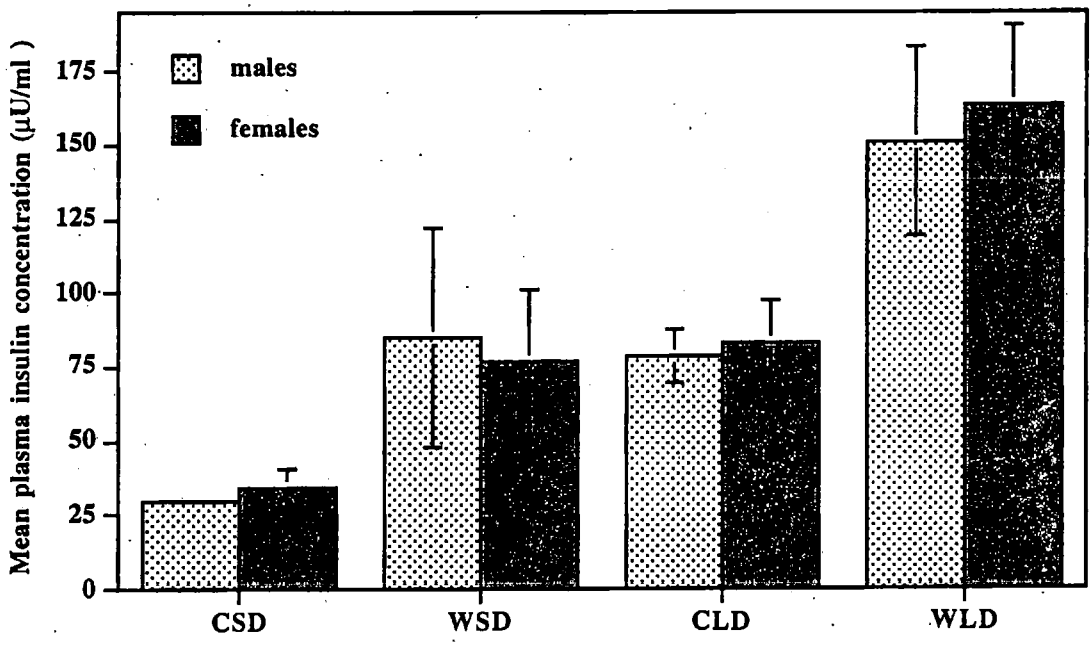
Lane 27 CSD GV Lane 28 WLD GV

In Figs. 3.9.1-2, the lanes containing BAT from CSD hamsters clearly demonstrate the presence of UCP. The same was so for BAT from WLD hamsters, although the concentration appeared lower. The detection of UCP in the remaining anterior depots that were recognised as being WAT (BA, IFS, HW and TW), albeit at lower levels than from the BAT depots in either regime, suggested the main function of the all anterior depots as being thermogenic in nature. No UCP was detected in any of the posterior depots tested, confirming the main function of the posterior depots as being whole-body stores.

3.10 Insulin.

When the blood taken from the hamsters at death was separated by centrifuging, there was not always 100 µl of plasma available, consequently these data were based on reduced numbers of samples.

Fig. 3.10.1 Mean (± SEs) plasma insulin concentration (µU/ml) in male and female hamsters at death. CSD (m=9, f=10), WSD (m=7, f=8), CLD (m=8, f=8) and WLD (m=9, f=14). In one case the SEs were too small to show on this graph.



There were significant differences between data from hamsters on different regimes in the mean plasma insulin (Fig. 3.10.1) concentrations (µU/ml) between the four regimes ($f=5.1$, $p<0.0001$, one way ANOVA, for all the LSD see S14 (p. 145)). CSD hamsters had the lowest levels, WLD the highest and the two unnatural combinations of photoperiod and temperature were once again very similar mid-level. There were no significant sex differences at all, in spite of the males being significantly fatter than the females on WLD (Fig. 3.7.1).

Fig. 3.10.2 Comparison of plasma insulin concentration (μU/ml) (Fig. 3.10.1) and the food intake (g) during the final week of the experiments (data from Figs. 3.4.1-4). (n= 35 COLD and 38 WARM).

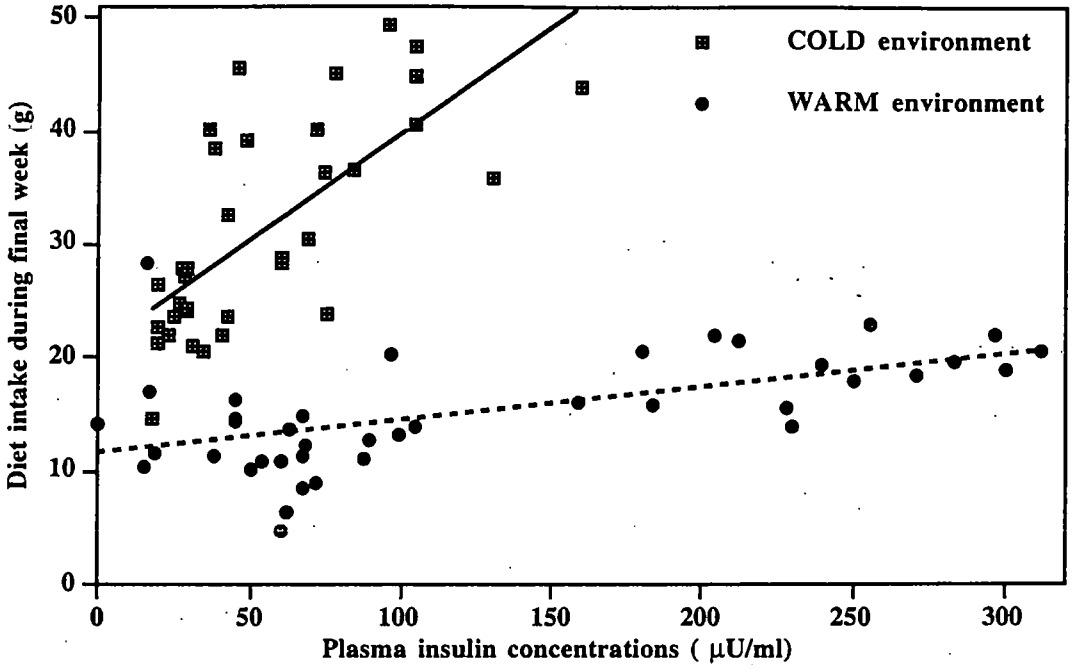


Fig. 3.10.2 compared the relationship between the levels of plasma insulin (μU/ml) at death and the final weeks food intake, for both sexes. These results were clearly divided by environmental temperature, the hamsters from the COLD short and long day ($r= 0.711$, $p<0.001$, solid line) had high food intake, but low plasma insulin concentrations. The hamsters from the WARM short and long day ($r=0.560$, $p<0.001$, hatched line) had low food intake, but high plasma insulin concentrations. ($f=10.11$, $p<0.0000$, oneway ANOVA, for the LSD see S14 (p. 145). So, in spite of eating much more in the cold, the hamsters still had low plasma insulin, signalling fasting physiologically. While the warm hamsters had low food intake, yet high levels of plasma insulin, signalling over-eating physiologically.

3.11 Pelage.

3.11.1 Colour and texture changes in the pelt.

At the beginning of all experiments, all the hamsters had the dark grey pelage (designated stage 1) of simulated summer (14 h light /10 h dark at 22±2 ^{o}C). It was only the hamsters that were placed in a room on short photoperiod (either cold or

warm) that changed their coat colour through towards a light grey pelage by the end of the experimental period at 102 days.

Fig. 3.11.1 Mean (± SEs) changes in the pelt colour of male and female *P. campbelli* under the 4 regimes. In most cases the SEs were too small to show on this graph. (n= 35 CSD, 25 WSD, 20 CLD and 39 WLD).

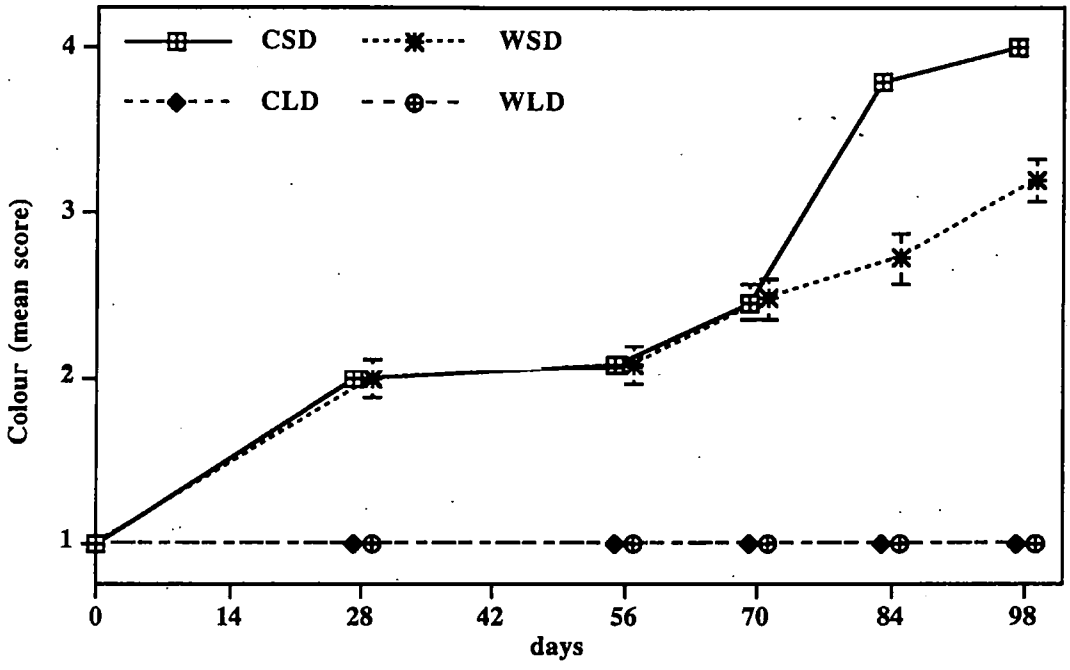


Fig. 3.11.1 shows that the colours of the coats of all the hamsters kept on WLD and CLD remained unchanged at stage 1. All the hamsters in CSD had changed through to stage 4 by the end of the experiment at 102 days. All CSD and nearly all WSD hamsters had reached stage 2 by week 4. After week 10, the pelt colour of the hamsters on WSD changed more slowly than that of the CSD hamsters, and not all the WSD hamsters had changed through to stage 4 by the end of the experimental period.

My studies of *P. campbelli* had shown that the pelt can be divided into three different areas in hamsters kept under all the environmental conditions studied, Table 3.8 describes the three areas.

Table 3.8 Showing the 3 areas of the pelt that were found to be most diverse.

SITE	DESCRIPTION
1) dorsal-thorax (**FORE**)	Behind the ears, covering the interscapular depot. Duplicate samples taken from on either side of the dorsal stripe.
2) rump (**REAR**)	Above the tail, covering the groin-side depots (in fat hamsters only). Duplicate samples taken from on either side of the dorsal stripe.
3) ventral thorax (**V/THOR**)	Between the fore-limbs, covering the thoracic depot. Unfortunately this area was not large enough for duplicate samples.

1) **FORE**, has short and fine hair and quite densely packed. This area is situated over the interscapular BAT depot and would need to be able to perform a dual role, heat conservation during cold weather and heat dissipation during thermal stress from attempted predation or during hot weather.

2) **REAR**, has the longest, coarsest, thickest and most densely packed hair on the body. This area of the pelt is exposed when the animal is sleeping curled up and would thus be a very important area in limiting heat loss under most environmental conditions.

3) **VENTRAL THORAX** (V/THOR), has short, fine, sparse hairs. As *P. campbelli* has short legs, this area is permanently near to the ground and would need the most lipid for waterproofing the very sparse hairs.

3.11.2 Lipid recovered from the pelage.

The Fig.3.11.2 below show the results from the assay that I developed for lipid extraction from the pelt

Fig. 3.11.2 Lipid (µg/ mg fur) in male and female hamsters (mean ± SEs). (n = 17, 18 CSD; 14, 11 WSD; 9, 11 CLD and 19, 20 WLD), with the exception of V/Thor where, n = 11, 12; 14, 11; 9, 11 and 11, 14, respectively. In two cases the SEs were too small to show on this graph.

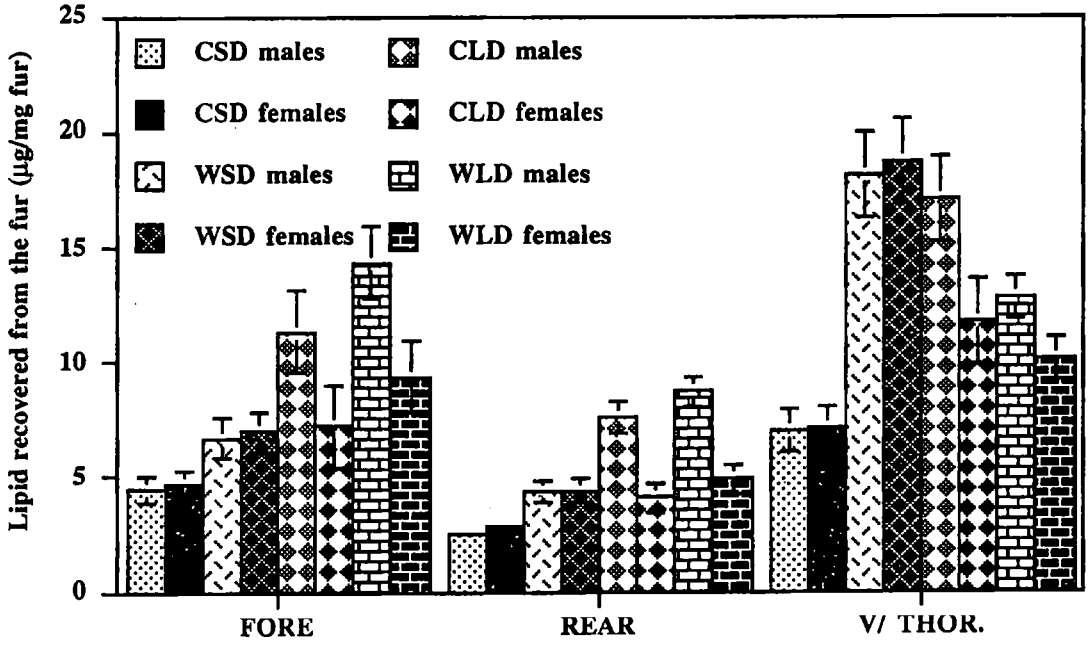


Fig. 3.11.2 show that there was always more lipid per mg of cut fur on the pelt behind the ears (FORE) than on the REAR and the largest quantity of lipid per mg of cut fur was found on the ventral fur (V/THOR) in both males and females. There were significant differences between hamsters on the four regimes in all three pelt areas, FORE ($f=10.6$, $p<0.0000$, one way ANOVA), REAR ($f=17.0$, $p<0.0000$, one way ANOVA), V/THOR ($F=8.6$, $p<0.0000$, one way ANOVA). The general pattern was similar for both sexes in all these areas of pelt. Surprisingly CSD hamsters had the lowest level of fur lipids in all three sites studied. For all the LSD see S15 (p. 146).

I could not assay the ventral thorax (V/Thor.) site from 1991-2 season (CSD and WLD) because of the way I had cut the pelt during dissections, but this procedure was changed for 1992-3 and 1993-4 seasons to permit such assays. Under all environmental conditions, the V/Thor area of hair was sparse in quantity, fine in

texture and has greatest quantity of lipid per mg of hair. The REAR area of pelt, which covered the largest area of superficial WAT always had the lowest lipid content. The V/THOR area in WSD and CLD hamsters was surprisingly high, perhaps again another adaptation to survival on unnatural combinations of photoperiod and temperature that confused their bodies.

3.11.3 Mass and linear dimensions of the hair.

Fig. 3.11.3 Mass of hair (mean ± SEs) shaved from 4 cm^2 samples of three regions of the pelt in male and female *P. campbelli*. In several cases the SEs were too small to show on this graph.

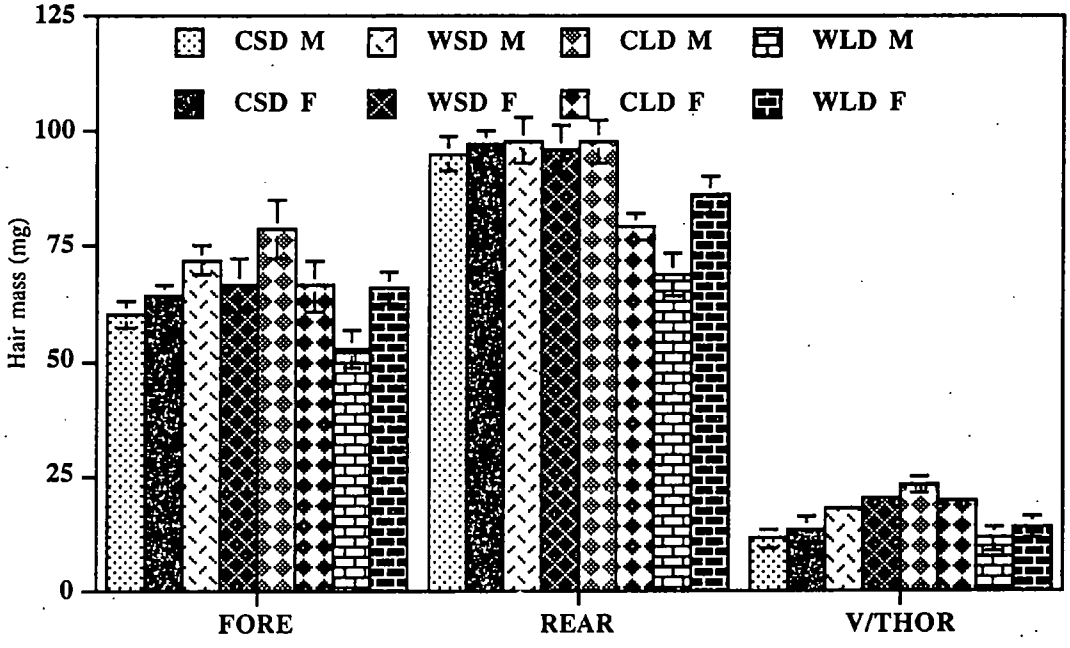


Fig. 3.11.3 shows the large differences in the mass of the hair from the three regions of the pelt. There were significant differences between regimes in the mass of the fur in two of the regions of the pelt. The mass of the FORE region of the pelt ($f=3.4$, $p<0.0021$, one way ANOVA) in the males increased from a minimum on WLD, CSD, WSD and through to CLD, which had the greatest mass of hair per unit area. The hair on the FORE region of the pelt in the females (Fig. 3.11.3) was very similar in all four regimes. The hair on the REAR region ($f=7.1$, $p<0.0000$, one way ANOVA) had the greatest mass of hair overall per unit area and values from male hamsters on CSD, WSD and CLD were very similar, but the hamsters on WLD had up to 24% less. The REAR region of the pelt in female hamsters also had the greatest mass of hair overall

and the data from hamsters on short photoperiods were very similar and heavier than in hamsters on the long photoperiods. There were no significant differences between treatment groups in the hair mass of the V/THOR region of the pelt (f=1.1. p<0.3194, one way ANOVA), for all the LSD see S16 (p. 146). The interaction of photoperiod and temperature had a significant effect (p<0.001, three-way ANOVA) on the mass of hair in the V/THOR region of the pelt. The three-way interaction had a significant effect (p<0.001, three-way ANOVA) on both the FORE and REAR region of the pelt. There were significant sex differences in WLD for both the FORE and REAR, where the females had the greatest and CLD for REAR, where the males had the greatest mass.

Photoperiod had the greatest influence, making data from hamsters on CSD and WSD almost identical, so without the use of the the 'ventilation gaps' and licking the warm hamsters could overheat. Males on CLD grow fur like those on CSD, but the females on CLD grow fur like WLD. Perhaps that was why the males managed to maintain their body mass closer to the original value (Figs. 3.3.5-6).

Table 3.9 Linear dimensions of the hair of some regions of the pelt.

Environment	CSD 'simulated winter'		WLD 'simulated summer'	
sex	M	F	M	F
N	11	12	11	14
LENGTH				
FORE (mm)	8.28±0.15	8.13±0.13	7.39±0.12	7.49±0.10
REAR (mm)	9.28±0.16	9.18±0.15	8.08±0.11	8.52±0.11
V/THOR. (mm)	6.86±0.16	7.01±0.16	6.22±0.10	6.16±0.13
WIDTH				
REAR (μm)	18.16±0.23	17.83±0.20	16.61±0.21	16.26±0.14

Table 3.9 shows the means (± SEs).of fur length (mm) and fur width (μm) in samples from hamsters on two of the four regimes: CSD and WLD. There were significant differences in all the measurements. The length of the FORE (f=12.6, p<0.0000, one way ANOVA), REAR (f=17.4, p<0.0000, one way ANOVA) and V/THOR (f=9.7, p<0.0000, one way ANOVA) hairs were significantly longer in CSD hamsters and the width of the REAR hairs (f=23.4, p<0.0000, one way ANOVA) were significantly greater in hamsters on CSD. Sex differences were detected only in the

REAR hair length in hamsters on WLD. Changes in fur mass between CSD and WLD regimes appeared to arise from both changes in mean hair length (7-13% decrease in 'summer') and hair width (11% decrease in 'summer'). For all the LSD see S17 (p. 146).

4.0 DISCUSSION

The hamsters tolerated the experimental conditions well and there were few losses. The regimes produced clear cut changes in body size and tissue composition. This chapter discusses the results in the order in which they are reported in chapter 3.

4.1 Torpor and hypothermia.

Torpor was not observed at all until the sixth week, two weeks after the moult into the paler and thicker pelage of winter had begun, which was after 4 weeks exposure to short photoperiod and about the time that food intake was stabilized (Figs. 3.4.5-6). Initially, only the occasional episode of torpor was observed. By the time that the period of maximum torpor frequency (for this colony of hamsters) was taking place, the hamsters had moulted through to their full winter pelage (Fig. 3.11.1) and body mass (Fig. 3.3.6) and food intake were stabilized (Fig. 3.4.5).

In my colony of *P. campbelli,* torpor was found to occur exclusively in hamsters kept on the combination of short photoperiod and cold environment and then only in a few males with a large inter-individual range. The hamsters that had the highest incidence of torpor had below average food intake (Fig. 3.2.3) during the periods of frequent torpor, yet their body mass was above average (Fig. 3.2.2). These observations support the concept that torpor is a survival mechanism for energy conservation. Perhaps on CSD, some male hamsters could not increase their food intake enough, so their bodies went into the survival mode of torpor.

The criteria listed by Bartness *et al.* (1989) for defining torpor in *P. sungorus* worked well for my observations on *P. campbelli.* Ruf *et al.* (1993) reported episodes of torpor in *P. sungorus* on short photoperiod in both the warm and the cold, mainly during the hours of daylight. There was however a greater incidence (up to 95%) and duration of torpor (up to 10.9±0.52 h) in the cold exposed hamsters than the warm exposed hamsters (up to 35%, for up to 7.2±0.75 h). Ouarour *et al.* (1991) also observed torpor in *P. sungorus* during exposure to short photoperiod in both warm and cold environment (week 13 onwards), but the incidence of torpor in my colony of *P. campbelli* began much earlier (week 6 onwards). In my colony, maximum torpor

frequency occurred from week 11 to the end of the experiments. Up to 3 (8.5%) male hamsters were torpid on any day, which was far lower than that observed (30%) by Heldmaier and Steinlechner (1981a) for *P. sungorus*, they did not state the sex of the animals that they observed, although the body mass data implies that it was males. However, they had the advantage of the use of thermocouple implants to record body temperature automatically.

Although many of the hamsters that had been kept on WSD moulted through to full winter pelage (Fig. 3.11.1), no incidences of torpor were observed on this regime. The interaction of short photoperiod and cold ambient temperature seems to be the cue for torpor in *P. campbelli* .

None of my female *P. campbelli* were observed to go into torpor and they ate significantly more diet than the males (Fig. 3.4.7), when the data was normalized for body mass. Female *P. campbelli* may adopt a strategy of more efficient foraging and perhaps the small females are faster or more efficient at getting the food, so the males save energy by going into torpor .

Torpor is regarded as a mechanism of energy economy that aids survival (Ruf *et al.*, 1991), so why only males were observed to be torpid is a mystery. It is possible that females in torpor were just missed, because they were torpid at a different time, i.e. overnight. This explanation could only be checked with the use of a thermocouple implant or by a reverse lighting system, where the hamsters could be observed with the use of light that they cannot see.

4.2 Body mass.

The changes in body mass of both male and female *P. campbelli* followed similar trends in specimens kept under the same environmental conditions. The body mass of both male and female hamsters kept on short photoperiod declined. In the cold, their body mass fell immediately and progressively until week ten, and then remained at the constant reduced level (Fig. 3.3.1). However, in the warm they did not begin to reduce body mass until around week four and in spite of access to food *ad lib.*, were still losing weight at the termination of the experiment (Fig. 3.3.2).

The male hamsters kept on long photoperiod maintained their body mass remarkably close to the original values. In the warm, they gained up to 6% of their mass from the start of the experiment (Fig. 3.3.3) and in the cold they lost up to 2% (Fig. 3.3.4). Although they followed this trend, the females did not maintain their body mass quite as close to the control values as the males; in the warm, they lost up to 10% (Fig. 3.3.3) and in the cold they lost up to 14.5% (Fig. 3.3.4).

Work by others on both *P. sungorus* and *P. campbelli* has produced similar patterns of changes in body mass to my data. The information available from Heldmaier and Steinlechner (1981b) and Steinlechner *et al.* (1983) on *P. sungorus* suggested that the hamsters on WLD gain on average 3 g, those on CSD lose weight, as do the hamsters on WSD. Rafael *et al.* (1985a) also found that *P. sungorus* on CLD lost weight within the first few weeks and then remained at constant body mass.

Flint (1966) and Figala *et al.* (1973) both reported that *P. sungorus* had more exaggerated seasonal changes in body mass than *P. campbelli.* My results produced discrepancies with this conclusion. My data (Fig. 3.3.3) show that *P. campbelli* had similar body mass on WLD to *P. sungorus* (Heldmaier and Steinlechner, 1981b). However, on CSD my colony of *P. campbelli* had a more extreme drop in body mass than that reported by Heldmaier and Steinlechner (1981b) for *P. sungorus,* and their hamsters were kept outside in natural environmental conditions, while mine were under artificial regimes.

My work with *P. campbelli* revealed a more gradual loss of body mass when the hamsters were placed on WSD than those on CSD. There was also a greater range of values in the final body mass of both male and female *P. campbelli* kept on WSD, as Ruf *et al.* (1993) found with *P. sungorus.*

Rafael *et al.* (1985a) moved *P. sungorus* (sex not stated) to a CLD regime and these hamsters lost 13% of their body mass within three weeks and then remained constant. My colony of *P. campbelli* followed the same pattern, but the females lost slightly more (14.5%). The males' losses were not significant at 1.6% of the original body mass.

With these minor exceptions, my results for *P. campbelli* agree with the published data and show that photoperiod must be the overriding cue for changes in body mass in

animals that are indigenous to a geographical region that has large annual changes in daylength and are relatively new to the laboratory.

Generally, hamsters such as *M. auratus,* which are physically much larger and hibernate, gain weight and increase energy stores for the winter (Bartness *et al.* 1987), but *Phodopus* sp. do the opposite. Instead of hibernating supported by fat stores, *Phodopus* sp. continue to forage but minimise their energy needs by reducing their body mass and stopping all sexual activity. *P. campbelli* then gain weight with the lengthening photoperiod and replenish depleted tissues. Then they accumulate lipid stores until they are in appropriate physiological condition for the energetically expensive process of reproduction.

4.3 Pelleted diet intake.

When food intake was expressed relative to body mass, after six weeks, the food intake for the males in the two warm regimes decreased to around 95% (WLD) and 75% (WSD) of their original intake. The amount females ate on WSD decreased to 79% of the initial value, but the WLD females were eating up to 123% of their original intake. The males in the two cold regimes and CLD females increased food intake to similar levels of around 145% of the baseline values, and the CSD females increased to 122% of their original intake (Figs. 3.4.8-9). The WLD females may have increased their food intake to maintain their energy stores at high levels in preparation for producing young. When normalised for body mass, the females in all four regimes had a higher food intake than the males (most of the time these differences were significant), although they were only significantly fatter on WLD (Fig. 3.7.1). Females must have a higher metabolic rate, perhaps due to their smaller body size.

Food intake was determined by temperature, almost independent of daylength. Both males and females on the two cold regimes ate significantly more than those on the two warm regimes for the duration of the experiments. When normalised for body mass (Figs. 3.4.5-6), and body mass is determined by daylength, they consume as much as they need to maintain this 'set' mass in the ambient temperature. In a cold environment, more food would be required to maintain the normal body (core) temperature for basic physiological functions.

Hamsters kept on CLD (Fig. 3.3.4) managed to maintain their original body mass remarkably well, whilst only increasing their diet intake by 68% over CSD hamsters. The CSD hamsters might not have eaten so much because of needing less energy to fuel a smaller body mass. Another factor could be that they only ate during the light phase of the day, and they simply had less time to eat. Those on WSD did not eat as much pelleted diet as those on CSD, probably due to lower energy requirements in a warm environment. In all cases, appetite (measured as food intake) adjusted to the energy balance produced by the unnatural combinations of environmental cues.

Bartness and Wade (1985) and Masuda and Oishi (1988) found that *P. sungorus* when kept on WLD ate more chow than those kept on WSD. My results differed from these reports for the effect of daylength on food intake: my data show no significant differences between *P. campbelli* kept on WSD (Fig. 3.4.2) and WLD (Fig. 3.4.4). Masuda and Oishi (1988) reported that when *P. sungorus* were kept in the cold on either long or short photoperiod, they had doubled their food intake by week 2 and it continued to increase. On the two cold regimes, CSD (Fig. 3.4.1) and CLD (Fig. 3.4.3) *P. campbelli* increased their food intake significantly, but the increase was not as much as twofold even after 98 days on the experimental regimes. Finally, the WLD (Fig. 3.4.4) hamsters significantly decreased their intake during the first part of the experiment and then remained fairly constant. It took the hamsters on the unnatural regimes longer to adjust and acclimatise to their new environment, but they managed it with surprisingly few deaths.

Previous investigators results are qualitatively similar to mine. The minor quantitative differences may be due to contrasts in exercise habits and torpor frequency between colonies, etc.

4.4 Organs.

There were significant differences between the experimental groups in the masses of all of the organs examined and sex differences were recorded in most (Table 3.3.1-2). These differences remained when expressed relative to the final body mass (Figs. 3.6.1-4), although reduced.

Most of the organ masses of *P. campbelli* listed in Table 3.3.1-2 were smaller than those quoted by Puchalski *et al.* (1987, their Table 1.3) for *P. sungorus,* probably because the body masses of their hamsters were greater. I assumed that their *P. sungorus* were on natural short photoperiod, as the ambient temperature was quoted as cold. However if their data were expressed relative to the final body mass, as mine were in Figs. 3.6.1-4, any discrepances in the masses of the heart and liver disappear. The kidneys in both WSD and CSD were 62% larger in *P. campbelli,* perhaps because the climate in the natural range of *P. campbelli* is much drier than the native climate of *P. sungorus* and water retention is thus more important.

The size of most organs of *P. campbelli* appeared to adapt to the functional demand imposed by the environmental conditions, i.e. the pelt grew when the hamster became fatter. Where food intake increased in the cold environments, the related organs, intestines, liver, kidneys and heart changed in size, relative to body mass, to cope with the increased work load. The intestines including contents, when expressed relative to the food intake, had an obvious significant correlation with temperature (Table 3.4). However, maintaining the internal organs is energetically very expensive and reducing their mass would make a substantial contribution to energy economy.

The data in Table 3.3.1-2 show that the mass of the testes was quite obviously responding to photoperiod, in contrast to the other organ measured. From their size, the testes of *P. campbelli* males on the long photoperiods were probably in full spermatogenesis. Even the testes of hamsters on CLD were probably mature enough for reproductive activity, but since they were housed individually we do not know! After 102 days on short photoperiod in both the warm and cold environment, all *P. campbelli* males had a small testicular mass (Table 3.3.1). Mercer *et al.* (1994) with *P. campbelli* and Duncan *et al.* (1985) with *P. sungorus* amongst many reported comparable results, although the *P. campbelli* studied by Mercer *et al.* (1994) had smaller testicular mass (20 mg) than hamsters from my colony of *P. campbelli* on a similar regime (145 mg) of CSD. The testicular mass decreased to a similar extent in male *P. campbelli* and *P. sungorus* hamsters kept on CSD. Although the decrease in total body mass was greater in *P. campbelli* than in *P. sungorus* (Hoffmann *et al.*, 1973), changes in the

mass of testes were still very disproportionate to those of other tissues in both species.

The ovaries (Table 3.3.2) responded to both warmer temperature and longer photoperiod, and were significantly larger in hamsters on what was probably the optimum regime for reproduction of WLD. Hamsters could not afford to start the energetically expensive process of reproduction in anything but the most favourable environmental conditions and with a body mass that contained plentiful energy stores (adipose tissue) i.e. in the 'summer'. Therefore in CLD, even though the long photoperiod would tell the females that it was 'summer', the food would most likely not be available naturally or they would not be able to increase their food consumption enough (because of the food intake being very high in CLD), for them to support pups! There would be the increased problem of keeping the pups warm if they bred in the cold simulated 'summer' of CLD.

4.5 Adipose tissue.

4.5.1 Gross mass as a percentage of body mass (fatness).

Photoperiod has the greater effect on the mass and composition of adipose depots in *Phodopus campbelli* (Table 3.5.1-2). The total dissectible adipose tissue (TDAT) as a percentage body mass (%BM) (Fig. 3.7.1) was, as expected, lowest for hamsters that had been living in the cold on short photoperiod (7%) and greatest in the male hamsters that had been living in the warm on long photoperiod (20%). The corresponding value for WLD females was 15%. It was the unnatural regimes that produced surprising results. There was very little difference in TDAT as %BM in male WSD (11.3%) or CLD (12.6%), considering that the final body masses of these males (Fig. 3.3.6) were so different. In the females, however, there were no significant differences (Fig. 3.3.5) in body mass, but there was significantly more TDAT as %BM in female WSD (13.2%) than in female CLD (8.7%).

My data fall within the range of measurements previously reported by Pond *et al.* (1987). They found that adipose tissue in *P. sungorus* kept on WLD constituted from 5% to 35% of total body mass, of which WAT represented at least 95% of the total. The

data reported for the total BAT mass in *P. sungorus* (no sex reported) by Puchalski *et al.* (1987) and Rafael *et al.* (1985b), were around 100% more than those found in the males from my colony of *P. campbelli*. When the masses of adipose tissue were expressed as a %BM, this difference remained.

It was very likely that because of the cue from long photoperiod, the CLD hamsters were trying to maintain their body mass and energy stores (total adipose tissue). Under the cold conditions, they needed to eat large quantities of pelleted chow (Fig. 3.4.3). They did manage to maintain the masses of their lean tissues (Tables 3.3.1-2) at close to WLD values, but they had small adipose stores, especially the females, probably due to the increased energy requirements of living in a low ambient temperature and adaptations of the pelt were incomplete on CLD (Tables 3.3.1-2), so insulation may have been less efficient than on CSD. With the cue from short photoperiod, the WSD hamsters adjusted their metabolism and reduced their body mass (Fig. 3.3.2). They ate similar quantities of chow to the WLD hamsters and the WSD hamsters were able to maintain significantly higher energy stores than the CSD hamsters, though the values were more variable, probably due to the lower energy requirements of living in a warm ambient temperature.

4.5.2 Identification of BAT and WAT.

Both visually and histologically, adipose tissue can be categorised as either BAT or WAT in cold adapted *P. campbelli,* but when the hamsters have been kept in WLD, which makes BAT less active, visual distinction was not so easy.

A good guide to the histological characteristics of brown and white adipocytes was reported by Cordell and Tedstone (1994, their Table 1.4). Trayhurn (1989) stated that the best criterion for determining as to whether adipose tissue was 'brown' or 'white' was to assay for the presence of UCP. This protein is unique to thermogenic mitochondria and so its presence identifies depots as BAT, typical WAT or WAT with thermogenic properties (Trayhurn, 1989).

Studying electron micrographs of a few depots clarified assigning depots as BAT or WAT. The electron microscope can only look at one or two cells at a time, which is very good for studying the internal structure of cells, but it is not so good for examining

the composition of mixtures of cells. However, after assaying for the presence of UCP, it became clear that, as stated by Trayhurn (1989), histological study was not enough to establish the presence of thermogenic adipocytes. The presence of UCP was detected by Western blotting in AX, HB, TB and UNM, indicating that they are all BAT depots, both when they were presumed to be fully functional thermogenically (CSD) and inactive thermogenically (WLD). Mattacks and Pond (1988) had classified UNM (Table 1.5) as a WAT depot, though these observations confirm that it is BAT. The presence of UCP was not detected in any of the posterior depots (ATG, BOT, DWA, GS, GV, POP and EP/OV), in keeping with my visual observations that they appeared to remain pure WAT under all conditions studied. The presence of UCP binding data from the anterior WAT depots (TW, HW, BA and IFS) were the surprise: although BA and IFS became 'brown in places', I had assumed the colour to be due to increased vascularisation. The presence of UCP in these anterior WAT depots meant that they contained brown adipocytes and therefore, in keeping with the other anterior depots, were functioning as heat-producing tissues.

Lean *et al.* (1983) tested for UCP in the parametrial adipose depot of hooded rats kept in the cold, but found none. Cousins *et al.* (1992) detected the presence of UCP in the periovarian and retroperitoneal depots of Wistar rats when they had been kept in the warm at 24 ^{o}C and found an increased signal after just 24 h at 4 ^{o}C. Cousins *et al.* (1992) suggested from their studies on Wistar rats that WAT depots, which contained unilocular cells that were positive to UCP, were really 'masked' brown adipocytes, which could be 'unmasked' by certain physiological or pharmacological stimuli. Both papers suggest that other WAT depots might contain a number of adipocytes that produce UCP and therefore have thermogenic properties.

Leptin is only expressed in WAT, not BAT (Trayhurn *et al.*, 1995b). So far it has only been studied in the large WAT superficial such as GS and GV and intra-abdominal depots such as DWA, that undergo large changes in size. Human and rat adipose depots, whose role is mainly storage, contribute most to determining apetite (Montague *et al.*, 1997).

Small mammals (body mass <100 g) have a proportionately larger surface area, therefore they lose more heat than large mammals and prospects for increasing thermal

insulation by lengthening the fur are restricted. The vital organs are situated mainly in or near the thoracic cavity and are 'surrounded' by the BAT depots (UNM, TB, HB and AX) most likely to aid in maintaining core temperature by NST. For TW, HW, BA and IFS to have thermogenic properties makes sense, as they are well placed to warm vital organs when required. The posterior WAT depots remain as vital storage for triacylglycerols, which are released on demand and fuel the BAT and other tissues.

4.5.3 Distribution and selective depletion of depots.

Changes in adipocyte type were site-specific. The posterior adipose depots remained white with no sign of an increase in vascularization. The anterior brown and white depots altered morphologically and increase in levels of UCP, the WAT depots probably assumed the thermogenic properties of BAT and assisted in maintaining the core temperature to protect the vital organs.

All the adipose depots of WLD *P. campbelli* were greater in mass than those on WSD and CLD (which were very similar to one another), and much greater in mass than those on CSD (Table 3.5.1-2). Therefore it appears that photoperiod is the main cue, but the cold ambient temperature increases the effect of short daylength, as it did for body mass as a whole.

With respect to adipose tissue, the body of *P. campbelli* appeared to be divided into an anterior and posterior section. All the posterior adipose depots were WAT and remained white in appearance under all the conditions studied. The anterior depots were a mixture of BAT and WAT. In CSD, the WAT became increasingly 'brown' in appearance and the BAT, as with the muscles, became a very dark brown, all due probably to increased vascularisation. Total BAT (only the recognised BAT depots were included UNM, TB, HB and AX, not the WAT depots that expressed UCP) made up 1.71% (CSD) and 2.22% (WLD) of the final body mass (Table 3.6).

Rafael *et al.* (1985a) found that in *P. sungorus,* BAT constituted up to 5% total body mass on WLD, 3.7% on CLD and 3.4% on WSD, although BAT mass was actually reduced to 40% of the original in CSD and 54% in WSD in *P. sungorus* (Rafael *et al.*, 1985b). The total BAT mass was much smaller in *P. campbelli* than that quoted for *P. sungorus* by Puchalski *et al.* (1987), and even when expressed relative to body mass,

BAT mass was less than half that of *P. sungorus*. But proportionally the reduction in BAT mass between WLD and CSD, was the same for *P. campbelli* and *P. sungorus*.

The masses of several of the adipose tissue depots when expressed as %BM were disproportionately large in the warm regimes and disproportionately small in the cold regimes, particularly BA and IFS in the anterior depots (Fig. 3.7.5) and DWA, GS, GV and BOT in the posterior depots (Figs. 3.7.6-7), i.e. these whole-body storage depots accumulate most as hamsters get fatter, but when expressed as a percent of total adipose tissue, these depots were remarkably constant (see Figs. 3.7.8-11). Although BOT did not undergo large changes in mass in all the four regimes studied, it was significantly larger in the females than the males. The relative masses of POP and ATG hardly changed at all. Although they look like typical WAT, these depots are believed to have specialised roles in relation to immune function (Pond, 1986). In *P. campbelli*, they do not enlarge with increasing fatness as GS, DWA and EP do. Mattacks and Pond (1988) studied fatty acid/ triacylglycerol cycling in *P. sungorus* that had been kept on WLD they found the highest levels to be in the two small intermuscular depots (UNM and POP), that had very small changes in mass. The lowest levels were in the large groin, BA, EP and DWA depots, that had large changes in mass. Suggesting the large depots are the lipid storage depots and the small depots have other functions.

The male gonadal depot, epididymal, had extreme differences in mass in the hamsters on long and short photoperiod at both temperatures, as did the testes, although there were significant correlations in the masses of the two tissues only in the cold environments (Fig. 3.7.4). The epididymal depot is probably a whole-body energy store, along with the other posterior depots of DWA, GS, GV and BOT, although in contrast to epididymal, their mass did not change disproportionately with daylength. The mass of the female gonadal depot, ovarian, was significantly smaller on CSD (Table 3.5.2) only, and was only a fraction of the size of EP.

Bartness and Wade (1985) suggested that photoperiod in *P. sungorus* affected the relative mass of adipose tissue depots selectively. They concluded that subcutaneous adipose tissue must be for insulation purposes because in winter acclimatized hamsters, it remained or even enlarged, while intra-abdominal adipose tissue

disappeared. They also found that *P. sungorus* on CSD had no visible intra-abdominal adipose tissue and that in males, the mass of the epididymal depot was 70% smaller when compared to WLD. The data from Mattacks and Pond (1988) showed significant sex differences in *P. sungorus* kept on WLD in BA, GV, DWA and inter-scapular, when expressed as a percentage of total dissectable adipose tissue. Also when Pond *et al.* (1987) normalised the mass of adipose tissue depots in *P. sungorus* for lean body mass, significant sex differences were found in IFS, BA, GS and GV. I found significant sex differences in DWA, GV and BOT in WLD.

These site-specific changes in adipose tissue mass and composition were not consistent with superficial adipose tissue acting as thermal insulation. The superficial depots were proportionally larger in both warm regimes.

4.5.4 Lipid content (% wet weight).

All my results for the lipid content of adipose tissue of *P. campbelli* were similar to the low levels found in naturally obese wild mammals such as arctic foxes (Pond *et al.*, 1995) and wolverines (Pond *et al.*, 1994). *P. campbelli* are naturally obese in the wild, and are a comparatively recent introduction into the laboratory (during the mid 1960s). Mattacks *et al.* (1987) found much higher levels of lipid in the corresponding depots of the adipose tissue of laboratory guinea-pigs, even in specimens that had lost weight by restricted feeding.

Mattacks *et al.* (1987) studied the lipid content of several adipose tissue depots in guinea pigs kept on WLD and found site-specific differences. It was lowest in UNM at 51%, as would be expected for a BAT depot, and highest in GS at 86.8%, closely followed by GV, both WAT posterior depots. Pond *et al.* (1995) found the lipid content of arctic foxes in the winter to be 42.3% in the UNM depot and 65.7% in the GS depot. Pond *et al.* (1994) found the lipid content of adipose tissue of wolverines, caught in northern Canada during the winter, to be 48.9% in the UNM depot and 68.0% in the groin depot. The results for the lipid content of the UNM depot in *P. campbelli* ranged from 8.0% in animals kept on CSD through to 35.3% in those on WLD and for the GS depots from 15.7% in CSD through to 42.1% on WLD. So the pattern of site-specific

differences was similar and values for the wild animals were like WLD hamsters, even though arctic foxes and wolverines were caught in winter.

High levels of lipid, at an average of 40% (wet weight tissue), were found in all the adipose depots (Figs. 3.7.12-15) in *P. campbelli* on WLD and lower levels of lipid (average 10.5%) were found in all depots in those on CSD. These animals were not starving: their body mass had been steady for at least 50 days (Figs. 3.3.5-6) and they were feeding *ad lib.* Lipid levels for hamsters on WSD were about 21% (double that of hamsters on CSD) and were remarkably similar to hamsters on CLD at 23%. This similarity was surprising considering that the CLD maintained their body mass, while WSD hamsters lost weight. The higher proportion of lipid showed that all the adipose depots appeared to become stores for triacylglycerol when thermogenically inactive (ie those from hamsters on WLD).

Nedergaard and Cannon (1984) stated that the lipid content of WAT depots in *M. auratus* on WLD was high (82%) and in active BAT of animals on CSD was low (30%), the same pattern as *P. campbelli,* but higher absolute values. Wade and Bartness (1984) stated that the weight loss on short photoperiod in *P. sungorus* was due to changes in total carcass lipid (35-40% decrease).

The lower proportion of adipose tissue from the CLD hamsters, when compared to CSD hamsters (Fig. 3.7.1), made it appear that they were responding to the long photoperiod by maintaining their body mass. However their adipose tissue had a lower lipid content (Figs. 3.7.12-15) indicating that their lipid stores had been used up in the expensive process of maintaining thermoneutrality in the cold ambient temperature. Likewise it appeared that the WSD hamsters were taking their cue from the short photoperiod with a reduction in body mass, but their energy stores would not have been in such great demand in the warm ambient temperature, so the lipid content of their adipose tissue remained higher than the hamsters on CSD. The very low lipid content (Figs. 3.7.12-15) of superficial WAT in CSD hamsters is also inconsistent with the hypothesis that adipose tissue acts as an insulator.

The variable and and sometimes very low lipid content of adipose tissue means that the mass of dissectable adipose tissue, by itself, is not a reliable indicator of lipid

stores. Both the mass of whole adipose tissue and its' lipid content need to be measured in order to ascertain the true state of an animals energy stores.

4.5.5 Protein content of adipose tissue.

The levels of protein in the aqueous phase of adipose tissue (Figs. 3.7.16-17) were highest in the BAT depots of CSD (136.8 mg/g), WLD (52.7 mg/g), CLD (47.8 mg/g) and WSD (33.3 mg/g) hamsters and lowest in the WAT posterior depots, of CSD (26.6 mg/g), WLD (17.0 mg/g), CLD (10.7 mg/g) and WSD (10.2 mg/g) hamsters. Rafael *et al.* (1981) stated that the greater proportion of protein in BAT compared to WAT was due to the increased amounts of mitochondrial enzymes needed for NST in hamsters. The low levels of protein detected in the posterior depots (Fig. 3.7.17) of hamsters from all four regimes is in keeping with the idea that they were storage depots and contain no brown adipocytes at all for thermogenic purposes, but they still need quite a lot of protein for the rapid mobilization and deposition of lipids.

The tissues in hamsters on WSD did not have the high levels of protein expected from being on short photoperiod or high levels of lipid expected for being kept in the warm environment. Heldmaier and Steinlechner (1981b) found that BMR of *P. sungorus* was high in hamsters in 'winter' and low in 'summer'; so energy stores would be kept low in WSD hamsters because of their raised metabolism, but their BAT must be switched off or they would over heat. Rafael *et al.* (1981) found far more mitochondrial protein (mg/g BAT) in *P. sungorus* that were living outside subject to natural temperature variation, than those living inside at a constant 23 ^{o}C. Similar mechanisms may explain my findings that the adipose tissue of WSD hamsters contain lower levels of protein.

In CSD, significantly more protein was found in the ATG depot, probably due to the fact that it had been impossible to remove all the blood vessels and lymphoid tissues from this highly vascularised, tiny depot and because the hamsters were eating so much that the guts must have a rich blood supply.

The low protein of the storage depots in the two unnatural regimes may indicate that these depots were inert. Values for POP were always a bit higher, possibly

because of the lymph nodes they contain, as in the case in guinea pigs (Mattacks *et al.*, 1987) and all other eutherian mammals (Pond, 1996).

The protein content of BAT was reported to be 4% in WLD and 10% in CSD by Rafael *et al.* (1985a) in *P. sungorus*. Nedergaard and Cannon (1984) quoted similar levels, although they stated that the protein content of active BAT could go as high as 15% in *M. auratus*. Mattacks *et al.* (1987) studied the protein content of several adipose tissue depots in guinea pigs kept on a 12 h light/12 h dark cycle in the warm and found a pattern of site-specific differences similar to those reported in Figs. 3.7.15-16. Protein content was highest at 8.9 mg/g in the POP depot and lowest in the groin depots at 2.4 mg/g. The adipose tissue of *P. campbelli* had much higher levels of protein overall; the hamsters in the warm on long days had 22.1 mg/g in the POP depot and 17.0 mg/g in GS, but these levels were not the highest or lowest, UNM had 52.7 mg/g and IFS had 15.3 mg/g. The hamsters in the warm on short days had 20.7 mg/g in the POP depot and 10.2 mg/g in GV, the highest level recorded was UNM at 33.3 mg/g, but still the pattern was similar.

The guinea-pig is much larger and native to the temperate regions of South America and has been developed as a laboratory animal for many years. Under laboratory conditions they have not been subjected to the extremes of temperature experienced by *P. campbelli*, therefore guinea pigs have possibly never developed the capacity to form the numbers of mitochondria found in BAT of *P. campbelli*, reflected by their levels of protein, being up to five times higher than that of the equivalent depots in guinea pigs.

The high levels of protein in the adipose tissue of hamsters kept in the simulated winter conditions reflect the increased numbers of mitochondria that are required in order for thermogenesis to work, especially the case in pure BAT (Figs. 3.8.1a+b, 3.8.2a+b). The fact that the next highest levels of protein were found in the BAT of hamsters kept in the simulated summer conditions, and that the lowest levels were found in hamsters kept in the two unnatural conditions, suggests that the action of the cold environment or the short days by themselves did not stimulate an increase in the numbers of mitochondria because the normal metabolism of the hamsters could maintain the core temperature.

4.6 Insulin

The plasma insulin concentration (Fig. 3.10.1) in *P. campbelli* followed a very similar pattern to the lipid level (Figs. 3.7.12-15) of adipose tissue in hamsters on the four regimes (ie low in CSD hamsters through to high in WLD), probably because of the effect insulin has on lipid metabolism. Herberg *et al.* (1980) had suggested that *P. campbelli* was a hamster with 'inappropriate' hyperglycaemia, where those with high levels of plasma insulin excreted high levels of glucose in their urine. They also found a correlation between plasma triglyceride concentration and plasma insulin levels.

There was a significant correlation between plasma insulin and food intake (Fig. 3.10.2) in hamsters that had been kept in the cold environment. They had a high food intake, yet comparatively low levels of insulin, hence their bodies thought they were fasting, which would help to sustain feeding. Conversely hamsters that had been kept in the warm environment (Fig. 3.10.2) had a low food intake, yet high levels of insulin, hence their bodies thought they were overeating, so their appetite was suppressed.

4.7 Pelage.

Small mammals, when exposed to the harsh environmental conditions of winter have two choices, either to seek shelter or sit it out. The second choice was used by *P. sungorus* (Weiner and Heldemaier, 1987). They adopt a heat-saving posture when resting, with the head and upper thorax curled up under the body, leaving predominantly the rump region in contact with the cold air. Either way, they are totally dependent on their pelt being waterproof, to reduce chilling because of the body becoming wet and for warmth, to help maintain core temperature until shelter could be found.

4.7.1 Pelt colour change.

The pelage of the hamsters responded mainly to the photoperiod, changing into winter colouration only when they were placed on short photoperiod, regardless of temperature. This colour change through to winter pelage (Fig. 3.11.1) occurred in 100% of the hamsters that were placed in the cold on short photoperiod. The colour change was complete in less than 50% of the hamsters in the warm on short

photoperiod by the end of the experimental period. The remainder of these hamsters appeared to be changing slowly towards winter colouration. In all cases, the black dorsal stripe remained unchanged.

Duncan and Goldman (1984) found that the pelage of *P. sungorus* went virtually white after 8-14 weeks exposure to WSD. No colour change for *P. campbelli* has been reported anywhere.

The fact that the colour of the pelage did change in *P. campbelli,* although much less than *P. sungorus*, might be due to the winter climate of *P. campbelli's* natural range being much drier than that of *P. sungorus*. A moult through to pure white would not provide a suitable camouflage in dry tundra grasses. The pelage colour change for winter observed in *P. campbelli* contrasted with the observed greying of old age in elderly hamsters over 18 months old, which radiated dorsally and included the black dorsal stripe becoming grey.

4.7.2 Mass and length of hair.

As expected, the coats of both sexes thickened significantly in the short photoperiod in both the warm and cold environments. Although changes in superficial adipose tissue are not constant with it having a significant role as an insulator, those of the hair are. Hair colour and length change reliably with photoperiod. NST, etc. is used as a metabolically expensive interim to maintain body temperature until the fur has grown enough to insulate better. The dependence of fur growth on photoperiod may help to explain why the CLD hamsters ate much more food relative to body mass (Fig. 3.4.5-6) during the first 50 days of the experiment than CSD hamsters, they had to keep fuelling their BAT.

The maximum increase in length of just over 1 mm occurred in the hair over the rump in the males (Table 3.8). As the Fore and V/Thor regions surround the thoracic cavity, the ability for body heat to escape would also be important. So, fine but not too densely packed hair would allow heat to escape, especially through the 'ventilation gaps' (Heldmaier, 1975) that develop in hamsters kept at warmer environmental temperatures. The mass of the hair shaved from the fore region remained remarkably similar between the females and the males kept in the simulated summer and winter

(Fig. 3.11.3). On the two unnatural combinations of daylength and temperature, the males had significantly more hair in the fore region than their females (Fig. 3.11.3). At all times, hair was growing on the whole of the walking surfaces of the feet.

Heldmaier (1989) reported that in *P. sungorus* on short photoperiod, the pelt as a whole thickened and mean hair length increased by as much as 2 mm and fur grew on the walking surfaces of their feet in short photoperiod. When studying the pelt of the red-backed vole, Sealander (1972) found a 78% increase in hair mass (mg /cm^2 of pelt) between summer and winter.

4.7.3 Changes in lipid content of the pelt.

No measurements comparable to my data in Figs. 3.11.2 on lipid in the pelt in hamsters of any species have been found in the literature.

By far the largest proportion of lipid recovered from the pelt was from the thoracic ventral region (Fig. 3.11.2). In hamsters kept under all the regimes studied this area has relatively sparse and fine short hair (Table 3.8). The short legs of the hamsters mean that their undersurface would always be in close contact with the ground and therefore near to any moisture. So a high level of lipids in the ventral pelt would be necessary to keep the hamster dry and therefore warm in damp conditions.

The lipid recovered from the fore region of the pelt was next most abundant. This area of the pelt also had fine hair (Table 3.8), and the lipid would be vital in helping to keep the animal dry. Finally, the hair over the rump contained the least recoverable lipid. It is densely packed, long coarse hair which may not need much lipid to provide adequate insulation. These differences in the quantities of pelt lipids are probably related to differences in the fur. Lipids help to waterproof the more sparsely haired regions of the hamster, both serving to conserve and repel water to aid thermal insulation.

Harlow (1984) demonstrated experimentally the importance of pelt lipids to thermal insulation. He measured for a drop of body temperature in muskrats after submergence in water at 4 ^{o}C. The animals that had been shampooed to remove the pelt lipids became hypothermic within 4 min. Hardarianectomised (to eliminate the natural source of pelt lipid) and sham-operated animals had a drop in body temperature

after 6 min in the same conditions, proving the importance of pelt lipids for thermal insulation.

The experimental regimes only appear have had a real effect on the lipid recovered from the pelt (Fig. 3.11.2) in the V/Thor region, where the CSD hamsters had much less recoverable lipid than the other regimes, yet there were no significant differences in the mass of hair for this region. There is no recorded rainfall for winter (see Section 1.1) in the natural range of *P. campbelli*, but there is for the summer and that is when the pelt lipids would be of most use.

The natural progression of daylength through the year is unwavering and the safest signal for these small mammals to use as a cue for physiological adjustments to their body composition and metabolism to match the anticipated seasonal temperatures. Of course, the weather is not as reliable an indicator of season as photoperiod, but the hamster must be responding to temperature, or those in the unnatural environments would not have survived being put into cold conditions when the photoperiod cued them for summer, or being put in the warm when the photoperiod cued them for winter. However the detailed analysis of adipose tissue and fur showed that the hamsters metabolism was 'confused' by the unnatural regimes. What is surprising, is that they survived as well as they did! In the breeding colony the occasional death occurred. In the simulated 'summer' regime, 4 hamsters died (9%) and in the 'winter' regime 3 hamsters died (8%), but in the unnatural regime of CLD 8 hamsters died (29%), and in the unnatural regime of WSD 4 hamsters died (14%).

5.0 CONCLUSIONS

1) Photoperiod more than environmental temperature proved to be the overriding cue for the changes in body mass, although temperature played a subsidiary part. The hamsters had consistently lower body mass in the cold than in the warm on the same photoperiod.

2) Temperature determined food intake, as the hamsters' energy needs to maintain body temperature dictated appetite, and they ate roughly twice as much in the cold regimes.

3) Only the interaction of cold and short days produced any episodes of torpor and all of those observed were in the male hamsters. This mechanism for surviving the 'depths of winter' seems to be so strong in those hamster that it occurred even when food was available *ad lib.*

4) The hamsters that had the highest frequency of torpor maintained above average body mass and yet their food intake was below average, confirming it as an energy-saving survival mechanism.

5) Changes in the size of the guts, liver, heart, pancreas and kidneys were most closely related to food intake. The hamsters ate more food in the cold environments, so their organs were proportionately larger. The spleen and muscle remained at a constant proportion of the body mass.

6) The mass of the testes was strongly affected by photoperiod, but not by environmental temperatures. Long photoperiod meant summer to this species and irrespective of temperature, the males were in breeding condition with large active testes. The females' bodies were more cautious, their ovaries were significantly larger only on the warm long day regime. I have concluded that the only environmental regime under which the females would conceive in is WLD. Further experiments would clarify this hypothesis.

7) Photoperiod also determined the fatness (% adipose tissue) of the hamsters, although again environmental temperature played a subsidiary part. The hamsters were fat on the long photoperiod, especially in the warm environment and thin but healthy on the short photoperiod, especially in the cold environment.

8) All the anterior adipose depots expressed UCP and were either BAT, or WAT that contains brown adipocytes, suggesting that the main function of the anterior depots is thermogenic in nature. The posterior depots were WAT and expression of UCP was not detected. They appear to have the important role of whole-body energy stores. The WAT depots become depleted in the cold regimes, but increased food intake prevents them falling below 5% of total body mass.

9) The pattern of depletion and the chemical composition of the superficial and internal adipose depots were not consistent with the theory that superficial adipose tissue contributes to body insulation.

10) Site-specific differences in the lipid and protein content were maintained in all experimental regimes and corresponded to patterns reported in guinea-pigs.

11) The lipid content of adipose tissue seemed to be mainly under the control of photoperiod, but temperature played a very important part. Lipid content was always lower than that of humans and laboratory rodents and where adipose tissue was more massive it also contained a greater percentage lipid. The colder it was, the more the energy stores became depleted as if the hamsters could not eat enough to meet requirements, even though food was available *ad lib.*

12) The protein content of adipose tissue seemed to be under the control of both photoperiod and temperature. Maximum increase in protein content in all depots only occurred from the interaction of the cold environmental temperature and short days. Depots that were BAT had much more protein in all regimes. That of ATG and POP were also higher under all regimes, although they do not contain brown adipocytes.

13) The physiological changes found in *P. campbelli* were in the main similar to those reported in *P. sungorus*. Although *P. campbelli* were always lighter than *P. sungorus,* which was probably why the quoted masses for the organs and adipose tissue of *P. sungorus* were greater. The exception was the kidneys of *P. campbelli* (as already discussed), which could be due to *P. campbelli* being native to a drier climate. Torpor habits were also different. *P. sungorus* became torpid on short photoperiod, irrespective of temperature, but I only found male *P. campbelli* torpid in the cold and on short photoperiod. This survival mechanism for *P. sungorus* is wholly responsive to photoperiod, while in *P. campbelli* it depends upon both photoperiod and temperature.

14) Pelage change was cued by photoperiod, but it was only the interaction of cold and short days that produced 100% change through to the winter pelage.

15) The sparser the pelt, the more lipid was present, probably as insulation and waterproofing. The rump region of the pelt was the heaviest, had the longest hairs and the least lipid. Its structure changed most with regimes. The interaction of cold with short days stimulated the best defences against severe weather.

6.0 APPENDIX (materials and methods)

6.1 Gels for Western blots.

Polyacrylamide gel (10%) enough for 4 gels at 1.5 mm thick

double distilled water	16.59 ml
1.5 M Tris, pH 8.8	10.00 ml
Acrylamide solution (30%)	13.30 ml
TEMED (tetramethylethylenediamine)	10.00 µl
(Volatile and will evaporate, add in the fume cupboard, tip under liquid)	
10% APS (ammonium persulphate) (0.1 g /ml, made up fresh)	100.00 µl

Stir very gently.

Stacking gel (4-4.5%) enough for 4 gels

double distilled water	12.25 ml
0.5 M Tris, pH6.8	5.00 ml
Acrylamide solution (30%)	2.65 ml
TEMED	20.00 µl
10% APS (0.1 g /ml, made up fresh)	100.00 µl

Stir very gently.

6.2 Recipes for Western blots.

Gel sample buffer

distilled H_2O	4.0 ml
0.5 M Tris-HCl (pH 6.8)	1.0 ml
glycerol	0.8 ml
10% (w/v) SDS	1.6 ml
0.05% bromophenol blue	0.2 ml

Add 50 µl ß-mercaptoethanol to 950 µl of gel sample buffer immediately prior to use.

Gel running buffer stock (10X) (pH 8.3)

Tris base	30.3 g
glycine	144.0 g
SDS	10.0 g

Dissolve on a stirrer, adjust to 1000ml with double distilled water.

Gel running buffer (1X) (do not adjust pH)

10X gel running buffer stock	100.0 ml
double distilled water	900.0 ml

Transfer buffer

25 mM Tris	3.03 g
192 mM glycine	14.40 g
20% (v/v) methanol, pH8.3	200.00 ml

Dissolve Tris and glycine in 800 ml double distilled water on a stirrer, add methanol with a short twirl and place in a fridge or cold room.

10X TBS stock solution, ph 7.4

Tris	60.55 g
NaCl	85.20 g

made up to 1000 ml with double distilled water.

DAB (diaminobenzidine tetrahydrochloride) in 1X TBS (20 ml /blot)

1X TBS	20.0 ml
DAB (tablet), swirl carefully to dissolve the tablet	10.0 mg
hydrogen peroxide (add just before use)	10.0 µl

Procedure must be done in the hood, DAB is a known carcinogen.
All liquid containing DAB, must be neutralised with hyperchlorite.

7.0 STATISTICS APPENDIX

Analysis of Variance (one-way) + LSD

CODE for LSD (0.050) results

CSD males	**1**	**CSD females**	**5**
CLD males	**2**	**CLD females**	**6**
WSD males	**3**	**WSD females**	**7**
WLD males	**4**	**WLD females**	**8**

(S1) Body mass for *P. campbelli* (Figs. 3.3.1-6 + Tab. 3.2).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
wk 0	7	311.94	11.2521	.0000													*	*	*	*
wk1	7	367.13	12.9689	.0000					*		*				*		*	*	*	*
wk 2	7	384.43	12.0374	.0000											*		*	*	*	*
wk 3	7	446.09	13.5427	.0000		*	*							*			*	*	*	*
wk 4	7	502.83	15.8666	.0000	*	*	*		*			*		*			*	*	*	*
wk 5	7	529.26	15.8373	.0000	*	*	*			*		*	*	*			*	*	*	*
wk 6	7	627.57	19.9932	.0000	*	*	*			*		*	*					*		*
wk 7	7	667.11	22.2528	.0000	*	*	*	*		*		*	*					*		*
wk 8	7	712.22	23.9369	.0000	*	*	*	*		*		*	*					*		*
wk 9	7	732.26	24.9444	.0000	*	*	*	*		*	*	*	*					*		*
wk 10	7	797.44	27.4192	.0000	*	*	*	*		*	*	*	*					*		*
wk 11	7	802.58	24.8792	.0000	*		*	*		*	*	*	*					*		*
wk 12	7	872.05	27.1628	.0000	*		*	*		*	*	*	*					*		*
wk 13	7	931.57	30.3984	.0000	*		*	*	*	*	*	*	*			*		*		*
wk 14	7	880.98	28.2111	.0000	*		*	*		*	*		*			*		*		*
wk 14/ % BM0	7	5460.4	20.7208	.0000	*		*	*		*	*	*	*			*			*	*

(S2) Pelleted diet intake normalised for body mass (Figs. 3.4.1-6)

	DF	Mean Square	F	Sig. of F (p<)	1x2	1x3	1x4	2x3	2x4	3x4	5x6	5x7	5x8	6x7	6x8	7x8	1x5	2x6	3x7	4x8
wk 0	7	311.94	11.2521	.0000													*	*	*	*
wk 1	7	5046.1	25.3264	.0000			*	*	*	*		*	*	*	*	*	*	*		
wk 2	7	13264.	70.0612	.0000	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*
wk 3	7	14937	49.4665	.0000	*	*	*	*	*	*	*	*	*	*	*	*		*		*
wk 4	7	16109	41.9086	.0000	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
wk 5	7	20035	50.6714	.0000	*	*	*	*	*	*	*	*	*	*	*		*	*		*
wk 6	7	13374	28.0543	.0000	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*
wk 7	7	19292	49.6078	.0000		*	*	*	*		*	*	*	*	*			*		*
wk 8	7	21437	56.8356	.0000		*	*	*	*		*	*	*	*	*		*	*		*
wk 9	7	18825	52.6837	.0000		*	*	*	*			*	*	*	*		*	*		*
wk 10	7	26137	99.2268	.0000		*	*	*	*		*	*	*	*	*		*	*		*
wk 11	7	24017	96.9610	.0000		*	*	*	*			*	*	*	*		*	*		
wk 12	7	20054	83.0186	.0000		*	*	*	*		*	*	*	*	*		*	*		
wk 13	7	20812	72.3200	.0000		*	*	*	*		*	*	*	*	*			*		
wk 14	7	22292	88.0343	.0000		*	*	*	*		*	*	*	*	*			*		*

(S3) Mean pelleted diet consumption (g) (Fig. 3.4.7-8)

	DF	Mean Square	F	Sig. of F (p<)	1x2	1x3	1x4	2x3	2x4	3x4	5x6	5x7	5x8	6x7	6x8	7x8	1x5	2x6	3x7	4x8
wk 1	7	342.31	9.5381	.0000			*		*	*		*	*		*	*				
wk 2	7	1021.3	34.3816	.0000	*	*	*	*	*	*		*	*	*	*	*		*		
wk 3	7	950.05	38.8657	.0000	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
wk 4	7	907.08	33.8153	.0000	*		*	*	*	*	*		*	*	*	*		*		
wk 5	7	1179.2	56.6731	.0000	*	*	*	*	*	*	*	*	*	*	*	*		*		
wk 6	7	791.75	31.1014	.0000	*			*	*		*		*	*	*	*		*		
wk 7	7	1084.9	53.7192	.0000	*	*	*	*	*	*	*	*	*	*	*			*		
wk 8	7	1021.8	61.7628	.0000	*	*	*	*	*	*	*	*	*	*	*			*		
wk 9	7	890.05	46.9363	.0000	*	*	*	*	*		*	*	*	*	*			*		
wk 10	7	1350.3	77.9531	.0000	*	*	*	*	*	*	*	*	*	*	*			*		*
wk 11	7	1312.9	86.8495	.0000	*	*	*	*	*	*	*	*	*	*	*			*		*
wk 12	7	1271.1	78.3109	.0000	*	*	*	*	*	*	*	*	*	*	*			*		*
wk 13	7	1367.4	71.1091	.0000	*	*	*	*	*	*	*	*	*	*	*			*		*
wk 14	7	1404.7	94.6115	.0000	*	*	*	*	*	*	*	*	*	*	*			*		*

(S4) Total mean daily pelleted diet intake (Fig. 3.4.9).

	DF	Mean Square	F	Sig. of F (p<)	1x2	1x3	1x4	2x3	2x4	3x4	5x6	5x7	5x8	6x7	6x8	7x8	1x5	2x6	3x7	4x8
FE/Day	7	3.48	125.1179	.0000	*	*	*	*	*	*	*	*	*	*	*		*	*		*

(S5) Changes (%) in pelleted diet intake (Figs. 3.4.10-11).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
wk 2	7	6656.6	7.5500	.0000			*	*	*	*	*			*	*					*
wk 3	7	8043.1	6.3024	.0000		*	*	*	*		*			*	*					*
wk 4	7	6568.2	3.7214	.0012			*	*	*					*	*					
wk 5	7	11038	4.9772	.0001		*	*	*	*		*			*	*					*
wk 6	7	5710.8	2.5209	.0192				*	*					*						*
wk 7	7	12254	5.9718	.0000		*	*	*	*			*		*		*				*
wk 8	7	13839	7.2848	.0000		*	*	*	*			*		*		*				*
wk 9	7	11239	7.5670	.0000		*	*	*	*			*		*		*				*
wk 10	7	19450	8.7450	.0000		*	*	*	*			*		*		*				*
wk 11	7	14403	8.9409	.0000		*	*	*	*			*		*	*					
wk 12	7	13886	6.9618	.0000		*	*	*	*			*		*		*				*
wk 13	7	14225	6.6693	.0000		*	*	*	*			*		*		*				
wk 14	7	15202	8.5796	.0000		*	*	*	*			*		*		*	*			*

(S6) Mass of the organs (Tabs. 3.3.1-2 + 3.4).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
Pelt	7	18.40	19.4057	.0000	*		*	*	*	*			*		*	*		*		*
Intest.	7	6.16	27.7984	.0000	*	*		*	*	*	*	*		*	*	*		*		
Liver	7	2.15	18.3387	.0000	*		*	*		*	*		*	*		*		*		
Testes	3	11.66	666.5204	.0000	*		*	*	*	*	-	-	-	-	-	-	-	-	-	-
Kidneys	7	0.07	19.1170	.0000	*	*	*	*		*	*			*		*		*		*
Heart	7	0.02	21.9068	.0000	*	*	*	*	*	*		*		*	*			*		*
Panc.	7	0.004	2.9978	.0073		*		*		*				*						
Vaslat	7	0.002	23.1829	.0000	*		*	*		*			*		*	*		*	*	*
Spleen	7	0.001	6.4859	.0000	*		*	*		*	*		*		*	*				
Int./fin wk fe	7	1.01	16.5189	.0000		*	*	*	*			*	*	*	*					

(S7) Mass of organs, expressed as a percent of final BM (Figs 3.6.1-4).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
Pelt	7	18.40	19.4057	.0000	*		*	*	*	*			*		*	*		*		*
Intest.	7	6.162	27.7984	.0000	*	*		*	*	*	*	*		*	*	*		*		
Liver	7	2.15	18.3387	.0000	*		*	*		*	*		*	*		*		*		
Testes	3	11.66	666.5204	.0000	*		*	*	*	*	-	-	-	-	-	-	-	-	-	-
Kidneys	7	0.07	19.1170	.0000	*	*	*	*		*	*			*		*		*		*
Heart	7	0.02	21.9068	.0000	*	*	*	*	*	*		*		*	*			*		*
Panc.	7	0.004	2.9978	.0073		*		*		*				*						
Vaslat	7	0.002	23.1829	.0000	*		*	*		*			*		*	*		*	*	*
Spleen	7	0.001	6.4859	.0000	*		*	*		*	*		*		*	*				
Ovaries	3	0.0001	8.9017	.0001	-	-	-	-	-	-			*		*	*	-	-	-	-

(S8) Adipose tissue (Figs 3.7.1-3 + Tab. 3.6).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
Totfat (% bm)	7	369.48	13.4528	.0000	*	*	*		*	*		*	*		*					*
Totfat	7	100.79	16.2279	.0000	*		*		*	*		*	*		*			*		*
Ant. depots	7	864.09	28.9785	.0000	*		*	*		*	*	*	*					*		*
Post. depots	7	830.12	35.7769	.0000	*		*	*		*	*	*	*					*		*
TotBAT (gw)	3	0.98	7.5917	.0003	-	-	*	-	-	-	-	-	*	-	-	-		-	-	*
TotBAT (% bm)	3	1.12	1.9247	.1395																
TotBAT (% tfat)	3	449.47	35.6640	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
% total BAT																				
HB	3	168.47	14.9920	.0000	-	-	*	-	-	-	-	-		-	-	-		-	-	*
TB	3	177.98	4.1026	.0119	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
UNM	3	15.63	0.2478	.8624																
AX	3	120.08	2.7754	.0524	-	-		-	-	-	-	-	*	-	-	-	*	-	-	

(S9) Mass of adipose tissue depots (Tab. 3.5.1-2).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
UNM	7	0.04	8.0407	.0000	*		*	*		*					*	*		*		*
TW	7	0.14	6.2515	.0000			*		*	*			*		*					*
TB	7	0.02	2.4868	.0210			*			*										
HW	7	0.02	6.6277	.0000	*	*	*			*			*		*					
HB	7	0.05	7.0580	.0000			*		*	*			*		*					*
AX	3	0.10	6.5926	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
BA	7	5.36	10.3075	.0000	*		*		*	*		*	*		*					*
IFS	7	3.88	11.1655	.0000	*	*	*		*	*		*	*		*					*
ATG	7	0.005	7.1776	.0000	*		*		*	*			*							*
DWA	7	0.15	10.6739	.0000	*		*		*	*			*		*					*
POP	7	0.003	10.1974	.0000			*		*	*			*							*
OV	3	0.004	3.2507	.0308	-	-	-	-	-	-		*	*				-	-	-	-
GS	7	1.67	11.2117	.0000	*		*		*	*		*	*		*					*
GV	7	0.13	9.2080	.0000	*	*	*		*	*		*	*							*
BOT	7	0.20	8.6184	.0000	*		*			*		*	*		*					
EP	3	8.42	59.7258	.0000	*		*	*	*	*	-	-	-	-	-	-	-	-	-	-

(S10) Mass of adipose depots, as a % of final BM (Fig. 3.7.5-7).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
UNM	7	0.10	2.8933	.0081							*	*			*	*				
TW	7	0.61	6.1721	.0000		*	*		*	*			*		*					
TB	7	0.01	0.1965	.9856																
HW	7	0.09	6.3362	.0000	*	*	*					*	*		*					
HB	7	0.14	4.7527	.0001		*	*		*			*	*	*	*					
AX	3	0.17	2.3773	0.0827	-	-		-	-	-	-	-		-	-	-		-	-	
BA	7	20.06	8.5077	.0000		*	*		*	*		*	*	*	*					
IFS	7	16.65	10.2030	.0000		*	*		*	*		*	*	*	*					
ATG	7	0.01	2.3213	.0302			*			*										
DWA	7	0.75	8.7732	.0000		*	*		*	*		*	*		*					*
POP	7	0.01	3.9392	.0007			*		*	*			*							
OV	3	0.05	3.6531	.0197	-	-	-	-	-	-		*	*				-	-	-	-
GS	7	7.08	9.3474	.00000			*		*	*		*	*		*					
GV	7	0.50	5.3100	.0000		*	*		*			*	*	*						*
BOT	7	1.60	11.3983	.0000			*			*		*	*	*	*				*	*
EP	3	43.86	68.7061	.0000	*		*	*	*	*	-	-	-	-	-	-	-	-	-	-

(S11) Mass of adipose depots, as a % of totfat (Fig. 3.7.8-11).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
UNM	7	66.35	5.0073	.0001	*		*			*	*	*	*							
TW	7	8.02	3.3538	.0030		*	*			*			*		*					
TB	7	58.47	9.8198	.0000	*		*	*		*		*	*		*			*		
HW	7	0.90	2.0192	.0589	*	*														
HB	7	6.88	5.2691	.0000	*		*	*		*			*			*				
AX	3	42.09	20.6763	.0000	-	-	*	-	-	-	-	-	*	-	-	-	*	-	-	
BA	7	86.54	9.8252	.0000	*	*	*	*		*								*	*	*
IFS	7	143.09	7.5871	.0000				*		*	*		*					*		*
ATG	7	1.00	1.9382	.0702																
DWA	7	10.01	6.6071	.0000	*	*	*			*		*	*		*					
POP	7	0.54	1.5017	.1714																
OV	3	0.87	3.4656	.0242	-	-	-	-	-	-	*	*	*				-	-	-	-
GS	7	26.63	4.4309	.0002					*	*	*		*					*	*	
GV	7	8.42	2.7844	.0105			*	*		*						*				
BOT	7	50.13	18.3808	.0000			*				*	*	*				*	*	*	*
EP	3	11122	77.5413	.0000	*		*	*	*	*	-	-	-	-	-	-	-	-	-	-

(S12) Lipid content of adipose tissue (Figs. 3.7.12-15).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
UNM	7	2057.7	45.9461	.0000	*	*	*		*	*	*	*	*		*	*				*
TW	7	2011.1	34.9324	.0000	*	*	*		*	*	*		*		*	*				
TB	7	1792.3	25.3141	.0000	*	*	*		*	*	*	*	*		*	*				
HW	7	2140.3	37.3206	.0000	*	*	*		*	*	*		*		*	*				
HB	7	2128.5	29.4939	.0000	*	*	*		*	*	*	*	*		*	*				
AX	3	2883.7	30.6847	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
BA	7	2342.0	33.8924	.0000	*		*	*	*	*	*		*		*	*		*		
IFS	7	2459.0	37.4005	.0000	*	*	*	*	*	*			*		*	*		*		
ATG	7	1792.7	37.9196	.0000	*	*	*	*	*	*	*	*	*		*	*		*		*
DWA	7	2692.3	46.3744	.0000	*	*	*		*	*	*	*	*		*	*		*		
POP	7	2618.9	82.8500	.0000	*	*	*	*	*	*	*	*	*		*	*		*		
OV	3	1680.9	36.8732	.0000	-	-	-	-	-	-	*	*	*		*	*	-	-	-	-
GS	7	2064.3	32.6614	.0000	*		*		*	*	*	*	*		*	*				
GV	7	1952.0	27.4519	.0000	*	*	*		*	*	*	*	*		*	*				
BOT	7	1945.2	33.5188	.0000	*		*	*	*	*	*		*		*	*		*		
EP	3	2506.9	25.7398	.0000	*	*	*		*	*	-	-	-	-	-	-	-	-	-	-

(S13) Aqueous protein in adipose depots (Figs. 3.7.16-17).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
UNM	7	284.12	32.2650	.0000	*	*	*			*	*	*	*			*				
TW	7	48.97	8.9698	.0000	*	*	*				*	*								
TB	7	284.64	37.5742	.0000	*	*	*				*	*	*			*				
HW	7	57.33	12.5457	.0000	*	*	*			*	*	*				*	*			
HB	7	261.56	47.8782	.0000	*	*	*			*	*	*	*			*				
AX	3	175.55	16.6289	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
BA	7	14.22	10.4175	.0000	*	*			*	*	*	*				*				
IFS	7	9.68	13.0016	.0000	*	*			*	*	*	*			*	*				
ATG	7	89.88	14.6027	.0000	*	*	*				*	*	*							
DWA	7	13.67	7.7971	.0000	*	*			*		*	*			*	*				
POP	7	13.08	9.3819	.0000	*	*					*	*			*	*				
OV	3	41.31	12.7580	.0000	-	-	-	-	-	-	*	*			*	*	-	-	-	-
GS	7	11.89	16.2059	.0000	*	*			*	*	*	*			*	*				
GV	7	17.03	16.3418	.0000	*	*			*	*	*	*			*	*				*
BOT	7	16.41	10.0024	.0000	*	*			*	*	*	*			*	*				
EP	3	32.36	20.4630	.0000	*	*	*		*		-	-	-	-	-	-	-	-	-	-

(S14) Plasma insulin (PI) levels (Figs. 3.10.1-2).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
(μU/ml)	7	25075	5.1891	.0001			*		*				*		*	*				
PI/fin fe	7	118.06	10.1077	.0000		*	*	*	*			*	*	*	*	*				

(S15) Pelt (lipid (µg/mg fur)) (Fig. 3.11.2).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
FORE	7	191.54	10.6743	.0000	*		*	*		*			*					*		*
REAR	7	75.65	17.0440	.0000	*	*	*	*		*			*					*		*
V/Thor	7	260.11	8.6905	.0000	*	*	*			*	*	*		*		*		*		

(S16) Pelt (mg of fur) (Fig. 3.11.3)

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
FORE	7	803.18	3.4684	.0021	*	*			*	*										*
REAR	7	1895.8	7.1728	.0000			*		*	*	*		*	*				*		*
V/Thor	7	23.89	1.1863	.3194																

(S17) Pelt (length (l) and width (w)) (Tab. 3.9).

	DF	Mean Square	F	Sig. of F (p<)	1 x 2	1 x 3	1 x 4	2 x 3	2 x 4	3 x 4	5 x 6	5 x 7	5 x 8	6 x 7	6 x 8	7 x 8	1 x 5	2 x 6	3 x 7	4 x 8
F (l)	3	2.37	12.6981	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
R (l)	3	3.67	17.4011	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	*
V/T (l)	3	2.30	9.7607	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	
R (w)	3	10.37	23.4727	.0000	-	-	*	-	-	-	-	-	*	-	-	-		-	-	

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