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ENERGETICS OF PREGNANCY IN THE SYRIAN HAMSTER

BY

VIVIEN S.H.QUEK (C)

A THESIS

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submitted by Vivien S.H. Quek

in partial fulfilment of the requirements for the degree of Master of Science in Nutrition

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Abstract

Epidemiological studies have suggested that the efficiency of energy utilization may be increased during pregnancy, as an adaptive response to the energy requirements for fetal growth. The Syrian hamster (*Mesocricetus auratus*) was used as an animal model in which to examine the energetics of pregnancy, since it does not increase its food consumption during gestation, paralleling the human situation.

Energy expenditure during pregnancy in the hamster was measured continuously by indirect calorimetry. The results showed that total energy expenditure was increased by 14% above that of virgin control animals. However, daily expenditure did not increase significantly until Day 9 of gestation, suggesting that substantial energy utilization occurs during the last third of pregnancy (16-day pregnancy). Importantly, the increase in expenditure was followed by a substantial decrease in the respiratory quotient from Day 10 onwards, implying that maternal fat stores are utilized to fund the costs of pregnancy.

Carcass analysis was performed at different times throughout pregnancy. The results were in agreement with the indirect calorimetry data, showing substantial utilization of maternal body fat during the last third

of pregnancy. From the loss in maternal fat stores (42%), together with energy balance calculations, it is apparent that energetic efficiency is not increased during pregnancy in the Syrian hamster.

Diet manipulation studies were also performed, using low fat, high fat and "stock" diets. An adjustment period was not possible, so that initial decreases in energy consumption in animals fed new formulated diets resulted in differences in total energy intake between groups over the study period. Nevertheless, the results suggest that pregnancy per se does not lead to increased food consumption, and that high fat diets induce an increase in the efficiency of energy utilization. Fetal parameters were, however, unaltered by diet. Brown adipose tissue thermogenesis was reduced during pregnancy in hamsters, but this was less evident with a high fat diet. Fetal fat content remained unaffected by differences in maternal diet and body fat reserves.

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1 INTRODUCTION

1.1 Whole-body Energetics

(i) Energy Balance

In terms of whole-body energetics, to achieve energy balance energy intake and energy expenditure must be equal. Deposition or depletion of fat depends on whether intake exceeds expenditure or, correspondingly, if expenditure exceeds intake. Energy intake generally implies metabolizable energy. This is the amount that is actually absorbed and utilized by the body. Metabolizable energy can be determined by subtracting the fecal and urinary energy losses from the initial "gross energy" in the food. Total energy expenditure is the sum of the basal metabolic rate, the physical activity plus the thermogenic activity of the organism (Garrow 1988). Under certain physiological conditions, such as pregnancy and lactation, there is obviously an added element in the expenditure component of the equation. This extra cost in total energy expenditure has to be met by either an increase in intake, a decrease in body fat reserves or a decrease in one or more of the components of energy expenditure.

(ii) Energetic Efficiency

The idea of energetic efficiency can be difficult to conceptualize, mainly because although it suggests efficiency due to metabolic processes, it takes into account the energy balance equation as a whole, including the level of physical activity. In animal studies it is often described in terms of the yield in animal tissue in relation to the amount of feed consumed (Flatt 1978), as shown by carcass analysis. Efficiency of energy retention can therefore be calculated as body energy gain divided by energy intake (Richard 1986; Romsos 1989). However, this notion does not seem feasible in situations where carcass analysis is experimentally impractical and the difference between energy intake and expenditure is comparatively small, as in adult humans. In this case the concept of metabolic efficiency pertains primarily to the fact that energy balance can be maintained on energy intakes which are below or above predicted requirements (Flatt 1978). This is clearly difficult to determine in human studies. In the case of experimental animals, the genetically obese ob/ob mouse is an excellent example of an animal exhibiting increased efficiency in that expenditure is

decreased as a consequence of a decrease in thermogenesis, without a concurrent decrease in food intake, resulting in the deposition of fat (Trayhurn 1986).

1.2 Energy Requirements During Pregnancy

(i) Epidemiological Studies

During pregnancy, total energy requirement is generally assumed to be increased due to the requirements imposed upon the maternal body by the growth and development of the product of conception, and by the increase in maternal metabolism due to the expansion of blood volume, and the enlargement of breast/mammary glands and uterus (Hytten and Chamberlain 1980).

Recommendations on the extra energy required during pregnancy by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and United Nations Universities (UNU), have traditionally been based on theoretically calculated values by Hytten and Leitch due to the lack of direct available data (Hytten and Leitch 1971). As such, these recommendations vary slightly among different countries. Studies have indicated that the actual energy consumed by most

pregnant women, especially in developing countries, is substantially below these estimated values (Whitehead et al 1986; Lawrence et al 1987; Whitehead 1986; Whitehead and Paul 1982). This has led to the suggestion that a physiological adaptation may occur during pregnancy which increases the efficiency of energy utilization (Baird et al 1984; Whitehead 1986; Illingworth et al 1987).

Although the outcome of pregnancy in women from lower socio-economic groups and underdeveloped countries is generally associated with adversities such as higher perinatal morbidity and mortality, epidemiological studies are influenced by numerous confounding factors which make definite conclusions difficult (Hytten and Chamberlain 1980). Retrospective studies by Stein and Susser (Stein and Susser 1975a; Stein and Susser 1975b) on the effects of famine on pregnancy in Holland during World War II showed some striking results. During the severe winter of 1944 and 1945, a blockade of food supplies caused a drastic decrease in food consumption in an otherwise basically well-nourished and healthy population. There was a massive fall in birth rate nine months following the famine with an average decrease in birth weight of 350 grams. However, linear growth was little affected which raises the possibility that the babies were not stunted

but merely thin. Furthermore, follow-up studies indicated that subsequent growth and intellectual development were apparently not affected. Thus it would seem that the fetus of a basically healthy pregnant woman is remarkably unaffected, within a wide range, by overall dietary inadequacy probably due to metabolic readjustments (Hytten and Chamberlain 1980).

Knowledge and understanding of pregnancy and the possible physiological adaptations which may occur is still incomplete and controversial. The controversies arise primarily due to the lack of adequate longitudinal epidemiological studies, the conflicting results of supplemental studies associating fetal outcome with energy intake (Blackwell et al 1973; Lechtig et al 1975; Naismith 1981; Prentice et al 1987) and the numerous confounding factors and inherent errors encountered in such studies. Some of these inherent errors include the fact that food intake and energy expenditure are difficult to measure with sufficient precision in a free living population. Another major problem is the fact that during pregnancy body fluid is increased rendering the standard methods of body fat measurements questionable.

Recently, in an attempt to overcome some of these problems and determine the energy requirements of pregnancy, an extensive longitudinal study has been

conducted in five different countries (Durnin 1987a; Durnin 1987b; Durnin et al 1987; Tuazon et al 1987; Lawrence et al 1987; Thongprasert et al 1987). From these studies it would appear that pregnant women in the developed countries require very little extra energy intake to satisfy requirements. The data from the developing countries, however, are conflicting and as such inconclusive (Durnin 1987b).

The inherent errors involved in body fat, food intake and energy expenditure measurements have still not been fully overcome and the question of whether there is an increase in the efficiency of energy utilization is uncertain. Nevertheless, recent studies appear to indicate that a major review of the current recommendations is required (Durnin 1987b), and that under adverse conditions such as those found in Gambia (Lawrence et al 1987), physiological adaptations in the energy costs of pregnancy may well exist.

(ii) Animal Studies

For obvious ethical reasons, studies on pregnant women generally rely on indirect measurements and are thus limited. However, in animal studies more direct and invasive measurements can be undertaken, such

as carcass analysis, in energy balance studies. The several energy balance studies done on laboratory rodents, however, in general do not indicate any overall increase in energy efficiency during pregnancy. In laboratory rodents such as rats and mice, an increase in food consumption occurs during the second half of pregnancy (Richard and Trayhurn 1985; Steingrimsdottir et al 1980; Andrews et al 1986). This increase in food consumption does not, however, stimulate an increase in diet-induced thermogenesis. Normally, adaptive diet-induced thermogenesis, though highly variable depending on genotype, age etc., is stimulated by hyperphagia (Stock and Rothwell 1989). During pregnancy, however, diet-induced thermogenesis is suppressed. Thus, the increase in energy intake during pregnancy is stored with increased efficiency, relative to that of the non-pregnant rodent.

In order to evaluate the <u>overall</u> energy efficiency, and overcome the problem of normal hyperphagia during pregnancy, a study was done in which pregnant mice were pair-fed to the intake of ad libitum fed virgin controls (Richard and Trayhurn 1985). The results indicated that there was no increase in overall energetic efficiency in the pregnant mice. Energy balance measurements of the pregnant mice fed ad libitum showed that the increase in energy consumed was totally

accounted for by the expected increase in fat and protein deposition compared to that of the pair-fed pregnant mice. The pair-fed mice were fully able to sustain pregnancy without any apparent detrimental effect on fetal growth. It should be noted that the "restriction" was modest, with the mean consumption being 85% of that of the ad libitum fed pregnant mice, which is in agreement with other published data indicating that modest restriction does not affect fetal growth (Naismith 1969). The hyperphagia seen in the pregnant mouse, however, is necessary for the deposition of additional maternal fat reserves.

Differing from rats and mice, the Syrian, or golden, hamster (Mesocricetus auratus) does not increase its food consumption during pregnancy (Fleming et al 1983; Wade et al 1986). This makes the species a good animal model in which to study energetic efficiency and energy utilization during pregnancy, particularly since no dietary intervention is required. The Syrian hamster does not appear to store energy as body fat during pregnancy (Wade et al 1986), unlike other rodent species, but instead loses fat. The Djungarian hamster (Phodopus sungorus sungorus), despite a significant increase in food intake during the latter half of gestation, also loses a significant amount of body fat in pregnancy (Schneider and Wade 1987).

In contrast to both rats and mice where no major changes occur in the mitochondrial content of brown adipose tissue during pregnancy (Andrews et al 1986), both Djungarian and Syrian hamsters show a significant decrease in brown adipose mass and cytochrome oxidase activity during pregnancy (Wade et al 1986; Schneider and Wade 1987). This decrease in brown adipose tissue thermogenesis, thus, results in a "saving" of maternal energy expenditure. Nevertheless, this adaptive suppression of brown adipose tissue thermogenesis during pregnancy surprisingly does not appear to lead to an increase in overall energetic efficiency. From the energy balance study done on Syrian hamsters (Wade et al 1986), it would appear that in order to compensate for the energy costs of pregnancy without any increase in food intake, fat deposits are mobilized and used even with the suppression of brown adipose tissue thermogenesis.

1.3 Energy Intake

(i) Human Studies

It is well recognized that highly precise and accurate measurements of energy intake in a free-living human population are difficult to accomplish. This is

based on three factors. Firstly, it is almost impossible not to introduce biases and errors into dietary records of any kind (Block 1982; Garrow 1988). The validity of the 24-hour dietary recall method, assessed by Acheson et al (1980), showed that even subjects who had to keep a dietary record, when asked to recall their recorded intake demonstrated large discrepancies between what they actually recorded and what they can recall. However, comparisons of methods cannot reveal which method is "best" (Dwyer 1988), since prospective studies such as record keeping also involve errors. The 7-day weighed diet inventory method, considered to be the most accurate available method, also has its flaws. It is tedious to perform, and may not be practical for large scale studies. Prentice et al (1986) found that the results from this method may not always agree with total energy expenditure which was concurrently measured using the doubly-labelled water technique. Although the results for lean subjects appear to be only slightly under-estimated, obese subjects in the same study demonstrated that their actual intake was systematically under-reported, even though the reported intake was already less than their habitual intake, i.e. they were actually in negative energy balance during the study. This is not surprising since the act of weighing and recording the diet may tend to inhibit food intake for

most subjects. Secondly, spontaneous intake is to fluctuate widely from day to day and this can thus limit the validity of extrapolation. In other words, within and between-subject variability can be large (Garrow 1988). Thirdly, although the gross energy content of a given amount of food is easily determined by bomb calorimetry, only a portion of this is available to the body, i.e. the metabolizable energy. Protein is not completely combusted to oxides of nitrogen, as it is in the bomb calorimeter, but is excreted as urea. Hence, approximately 1.25 kcal/g is lost as urinary urea (Garrow 1988). The main difficulty in calculating metabolizable energy, however, is the general disagreement regarding the "availability" of complex carbohydrates such as starch and dietary fiber. A high fiber content tends to decrease the amount of fat absorbed, while the availability of dietary carbohydrate is highly dependent on various factors; such as the way the food is processed and cooked, the degree of ripeness (i.e. bananas) and the type of starch ingested. All these factors thus limit the conveniently calculated "available" energy (Garrow 1988).

(ii) Animal Studies

In contrast to human studies, food intake of animals can be very well controlled and accurately measured. Apart from the problems of measuring metabolizable energy, which can be partially overcome by using metabolic chambers, food intale is not subject to bias and is remarkably constant when the regular chow diet is fed, probably due to the monotony of the diet. The diets can be manipulated to induce obesity as shown by studies using "cafeteria" and high fat diets. In the Syrian hamster high fat diets have been shown to induce obesity in the absence of hyperphagia (Wade 1982). The weight gain is mainly due to increases in body lipid, as revealed by terminal carcass analysis, although there is also a small increase in lean body mass (Wade and Bartness 1983a). The development of obesity in the absence of hyperphagia results from a decrease in energy expenditure, as demonstrated by measurements of resting oxygen consumption (Wade 1982). The energy cost of fat synthesis from dietary lipid is, however, less than that from dietary carbohydrate. High fat diets therefore mean that more energy is available for storage compared to high carbohydrate diets, on an equivalent energy basis (Trayhurn 1986).

The effects of high fat diets on body weight and body fat are more marked in the female than in the male (Wade 1983), but the fatty acid composition of the fat source does not seem to have a bearing on the resulting obesity (Wade and Bartness 1983a; Wade and Bartness 1983b). In contrast, studies on ob/ob mice have shown that a beef tallow diet results in a higher energy gain compared to a corn oil diet, even though both groups show isoenergetic intakes of the two diets (Mercer and Trayhurn 1987).

The feeding patterns of Syrian hamsters are relatively consistent and rigid. They feed approximately every two hours (Borer 1979) and when subjected to a variety of regimens of food deprivation, they do not alter the amount nor the pattern of feeding from that observed in a non-deprived state (Borer 1985). In fact when subjected to 1 hour exposures to food separated by 5 hour inter-meal intervals, Syrian hamsters will keep losing weight and eventually die of excessive weight loss (Borer et al 1979). Furthermore, 24 hour deprivation followed by refeeding does not result in any compensatory increase in consumption (Borer et al 1979) in the short term, which is in contrast to studies done on rats and mice. When allowed free access to food, recovery from weight deficit does not include hyperphagia. Hamsters will consume the "normal" amount

of food and gain weight at rates that are proportional to the magnitude of their weight deficit evidently by reducing their rate of energy expenditure (Borer et al 1979; Borer 1985). Like other rodents, Syrian hamsters also demonstrate the ability to maintain constant caloric intake when presented with food stuffs of different caloric density (Silverman and Zucker 1976; Wade 1982).

Increases in energy consumption do occur during cold exposure (Minor et al 1973; Bartness et al 1984) and increased exercise, after an adjustment period (Borer 1985; Tsai et al 1981). Nevertheless, it seems that this compensatory mechanism is still not adequate enough to counteract the loss of body lipid (Borer 1985). The general response of the hamster to energy shortage thus appears to be a reduction in energy expenditure and an increase in the efficiency of energy storage rather than increased food intake (Borer 1985).

1.4 Energy Expenditure

(i) Basal Metabolic Rate/ Resting Metabolic Rate

Total energy expenditure can be divided into three major components: basal metabolic rate, physical

activity and thermogenesis. The physical activity component is fairly self-explanatory. The terms basal metabolic rate (BMR) and resting metabolic rate (RMR) are concerned with similar processes. Basal metabolic rate is defined as the energy expended when an individual (or animal) is physically and mentally at rest in a thermal neutral environment, and 12 to 18 hours after a meal. It is an attempt to measure the basic energetic processes in the cells which constitute the "active tissue mass" of the body (Grande and Keys 1980) i.e. the basal energy required by the body. In practice, this is often difficult to obtain, thus the term resting metabolic rate appears more appropriate as it is defined as the metabolic rate at rest, measured after 30 minutes of rest and at least several hours after a meal (Sjostrom 1983).

(ii) Thermogenesis

The term thermogenesis is relatively ill defined. In the strict sense, thermogenesis simply means the generation of heat, which would include all metabolic processes including physical activity (Trayhurn and Milner 1987). Nevertheless, the term is

generally divided into either obligatory or facultative components.

Obligatory thermogenic processes are those essential for the maintenance of the living state, i.e. the basal metabolic processes of life, and for endothermy (Himms-Hagen et al 1989). Under certain physiological or pathological conditions such as pregnancy, growth, lactation, hyperthyroidism etc. the source of increased heat production would also be classified under obligatory thermogenesis. The thermic effect of food, i.e. the energy expended in the essential ingestion, digestion and processing of food, is also included in this category (Himms-Hagen et al 1989).

The thermic effect of food is different from the term diet-induced thermogenesis, which is an adaptive dissipation of heat. Confusion arises when these and other terms such as specific dynamic action, post-prandial thermogenesis, heat increment of feeding and luxusconsumption are used interchangeably. Currently the most commonly used terms describing the adaptive process of heat production induced by over-feeding are diet-induced thermogenesis and luxusconsumption (Trayhurn and Milner 1987).

Facultative thermogenesis, also known as adaptive thermogenesis, or simply as thermogenesis, is

generally described as a metabolic process in which heat is the principle product. It includes shivering, non-shivering and diet-induced thermogenesis (Trayhurn 1986; Himms-Hagen et al 1989). It is rapidly switched on and off, adaptive to changes, and is the potential source of a considerable amount of heat. The term facultative thermogenesis usually implies thermogenesis of the non-shivering and diet-induced type, i.e. excluding shivering thermogenesis (Trayhurn and Milner 1987).

(iii) Brown Adipose Tissue

Brown adipose tissue thermogenesis plays a major role in the energy balance of small rodents mainly through the process of facultative thermogenesis (Trayhurn 1986; Himms-Hagen 1986; Rothwell and Stock 1986). In some mammalian species, mainly neonatal mammals, rodents and hibernators, exposure to cold or food consumption result in the activation of brown adipose tissue thermogenesis. This tissue produces energy as heat mainly through a mitochondrial proton conductance pathway. The thermogenic capacity of brown adipose tissue is dependent on the level of a tissue specific 32,000 M_r uncoupling protein which acts by controlling the permeability of the inner mitochondrial

membrane to protons. This protein therefore regulates the uncoupling of respiration from ATP synthesis by short-circuiting the proton electrochemical gradient (Nicholls and Locke 1984).

The amount of uncoupling protein present is dependent on two factors. One, the specific concentration of uncoupling protein in the mitochondria, and two, the number of mitochondria present in the tissue. The amount of mitochondria can be determined, in arbitrary units, by measurement of the activity of a mitochondrial marker enzyme, e.g. cytochrome oxidase. Thus, the changes in the level of cytochrome oxidase activity gives an indication of the relative thermogenic capacity of the brown adipose tissue by virtue of the amount of mitochondria present. Since brown adipose tissue thermogenesis is variable, depending on environmental and nutritional influences, and is a significant component of total energy expenditure it can thus have a major effect on the efficiency of energy utilization. Though the role of brown adipose tissue in adult humans is still speculative and unclear, the tissue specific mitochondrial uncoupling protein necessary for nonshivering thermogenesis has been found, in both newborn and adult, human adipose tissue (Lean et al 1986).

1.5 Measurements of Energy Expenditure

Energy expenditure can be measured several ways. Heat production can be determined directly by direct calorimetry, or indirectly by indirect calorimetry, activity diary or heart rate; the last two methods are, however, not accurate enough for energy balance studies. In animal studies, carcass analysis is another available option. Recently another method, the doubly-labelled water ($^2H_2{}^{18}O$) technique, introduced by Lifson and McClintock in 1966 (Garrow 1988; Coward 1988), has been developed for both animal and human studies. Although this technique appears very promising, it is expensive and relatively untested.

Direct calorimetry is generally considered the most accurate method of assessing energy expenditure. This method, however, is also very expensive and requires the subject to be confined to a closed calorimeter chamber. Another method of measuring energy expenditure is by indirect calorimetry, which gives results very similar to those obtained from direct calorimetry (Atwater and Benedict 1905; Lusk 1924). The principles of indirect calorimetry for estimating energy expenditure and substrate utilization have been known and used extensively since the turn of the century. The

method is based on the fact that energy expenditure is proportional to the rate of oxygen consumption and carbon dioxide production. The relationship between heat released per liter of oxygen consumed is relatively constant as shown by classical calorimetric studies (Kleiber 1961). Theoretically, the oxidation of 1 mole (180 grams) of glucose requires the consumption of 6 moles (134.4 liters at STP) of oxygen and the production of 6 moles (134.4 liters) of carbon dioxide and 6 moles (108 grams) of water, with the liberation of 2.78 MJ of heat (Garrow 1988). Thus, in this simplistic model of glucose oxidation, the 2.78 MJ of heat produced can be measured directly by direct calorimetry or indirectly by measuring the amount of oxygen consumed and carbon dioxide produced. From the ratio of carbon dioxide produced to oxygen consumed the respiratory quotient (RQ) can be calculated. This ratio gives an indication of the substrate being utilized as when the three main fuel sources, carbohydrate, fat and protein, are oxidized they produce and consume different amounts of carbon dioxide and oxygen, respectively. The utilization of a carbohydrate source produces an RQ of 1, whereas protein gives a value of approximately 0.81. Fat gives an approximate value of 0.71 (Garrow 1988), while ethanol has a value of 0.67 (Livesey and Elia 1988).

In practice, however, the above situation is far more complicated since the energy value of oxygen varies according to the fuel used. In other words, the amount of energy produced per liter of oxygen consumed varies slightly depending of the substrate being utilized thus affecting the calculation of energy expenditure. For every liter of oxygen consumed the energy produced is 5.1 Kcal if the substrate is glucose, if the substrate is palmitic acid the energy is approximately 4.6 Kcal, and if the substrate is glycine the energy expenditure is 4.6 Kcal (Livesey and Elia 1988). Nevertheless, it is generally agreed that the assessment of energy expenditure is highly accurate relative to RQ measurements (Livesey et al 1988; Jequier 1980), even during net lipid synthesis (Jequier 1980; Flatt 1978).

Open-circuit indirect calorimetry should be able to yield measurements of oxygen consumption and carbon dioxide production within a precision of $\pm$2% (Jequier and Schutz 1988). Apart from the very expensive direct calorimetry, indirect calorimetry appears to be the most accurate technique available for the measurement of energy expenditure in vivo.

1.6 The Syrian hamster: A model for the study on the energetics of pregnancy.

The absence of hyperphagia coupled with the adaptive changes seen in the brown adipose tissue during pregnancy in the Syrian hamster is interesting in two ways. First, parallel to human studies which seem to indicate that there is very little increase in food consumption during pregnancy, the Syrian hamster appears to be a good model for evaluating energy expenditure during pregnancy without the concurrent increase in food consumption. Second, despite the fact that BAT thermogenesis is suppressed during pregnancy, as demonstrated by a significant decrease in interscapular BAT weight, protein content and cytochrome oxidase activity (Wade et al 1986), the overall energetic efficiency is not increased. This nevertheless is an adaptive response indicative of energy balance changes during pregnancy.

Energy expenditure throughout pregnancy has, so far, not been monitored continuously. Since carcass analysis can only evaluate energy balance at point in time, energy expenditure during pregnancy can be extrapolated from the two data points, in the end. Hence, continuous monitoring of energy is

by indirect calorimetry may reveal some important information.

Accordingly, the first study in this thesis involves the continuous measurement of energy expenditure during pregnancy, utilizing an indirect calorimeter. The aims of the study were to (i) compare the amount of food consumed by the pregnant group to that of the virgin controls, (ii) determine if there is an increase in energy utilization during pregnancy and if so at what stage, (iii) determine the changes, if any, in the type of substrate being utilized by means of the RQ value, (iv) evaluate the possibility of an increase in energetic efficiency during pregnancy, by virtue of the amount consumed in relation to the amount expended and the substrate utilized.

The second study consists of performing carcass analysis at the different stages of pregnancy. The first objective was to complement the findings obtained from the indirect calorimetry study. By employing carcass analysis, the change in energy and fat content during pregnancy is directly determined at different points throughout pregnancy. The second objective is to evaluate the changes seen in brown adipose tissue thermogenesis during pregnancy as this tissue has been shown to play a major role in the energy balance of small rodents (Trayhurn 1986; Himms-Hagen

1986). Since brown adipose tissue thermogenesis is evidently suppressed during pregnancy (Wade et al 1986), the degree of this suppression in relation to the stage of pregnancy and carcass energy content may provide some important clues as to the possible mechanisms involved in energy balance regulation.

The third part of the present work evaluates the effects of diet manipulation during pregnancy. High fat diets apparently increase the energetic efficiency of the Syrian hamster by reducing energy expenditure (Wade 1982) without a concurrent decrease in food intake resulting in a significant increase in body fat. In spite of this decrease in expenditure, brown adipose tissue (BAT) thermogenic capacity is increased, as suggested by an increase in BAT mass, DNA and protein content (Wade 1982; Wade and Bartness 1983a). On the other hand, pregnancy significantly decreases brown adipose tissue thermogenesis despite a major loss of body fat (Wade et al 1986). Although it appears that a high fat diet minimizes weight loss during lactation in the Syrian hamster (Fleming and Miceli 1983), the effect of a high fat diet on body lipid, carcass energy content and brown adipose tissue thermogenesis during pregnancy and lactation is not known. The effects of a high fat diet during pregnancy should in theory reduce the suppression of brown adipose tissue thermogenesis and

minimize body fat loss. By the same token, a low fat diet would be expected to enhance fat loss since the cost of lipid synthesis is more from a carbohydrate source than from a lipid source (Trayhurn 1986). The ability of the hamster to replenish its fat stores from a high carbohydrate diet, in view of lipid mobilization during pregnancy, would probably be drastically curtailed. The aim of this last study is, thus, to determine when dietary fat influences energetic efficiency and energy balance during pregnancy and the effect on fetal outcome.

2 INDIRECT CALORIMETRY: CONTINUOUS MEASUREMENT OF ENERGY EXPENDITURE DURING PREGNANCY IN THE SYRIAN HAMSTER.

2.1 Introduction

Indirect calorimetry has been used to estimate energy expenditure and metabolic mixtures of fuels since the turn of the century. In an open-circuit indirect calorimeter, oxygen consumption and carbon dioxide production can be measured with a precision of ± 2%, and as such energy expenditure can be measured with a high degree of accuracy, even for short-term measurements under steady-state conditions (Jequier and Schutz 1988). Although RQ measurements may not be a highly accurate method of assessing substrate utilization during lipogenesis, they nevertheless serve to provide a more than adequate general indicator of the intermediary metabolism taking place in vivo, which is its main function in this study.

Theoretically the RQ, which is the ratio of carbon dioxide production to oxygen consumption, represents body tissue metabolism. The measurement of this ratio at any point in time has been termed the

respiratory exchange ratio (RER). When the entire respiratory pathway is in equilibrium, this measurement accurately reflects the average of the total body metabolic respiratory quotient. The respiratory exchange ratio (RER) can be altered in conditions such as transient hypoventilation or hyperventilation, resulting in a value which does not reflect tissue metabolism. However, under continuous monitoring, these errors are avoided since they would be balanced out in the long term (Jequier 1980). Therefore, with continuous monitoring, total RER averages should accurately reflect tissue metabolism.

The fuel oxidized over a 24 hour period has been demonstrated to be closely associated with the composition of the ingested diet (Jequier and Schutz 1988). A consistent decline in the RQ indicates that more catabolism of fat and less of carbohydrate has occurred (Kleiber 1961). Thus, in the present study, given the fact that the food source is high in carbohydrate, an RQ close to 1 would be expected. A decrease in this value would signify that fat is being utilized, thus indicating that maternal body fat is being catabolized. An RQ of 0.8 does not necessary indicate the exclusive catabolism of protein as substrate utilization in a living organism is not solely dependent on one source, it is representative of the

average substrate utilized. Furthermore, values for specific amino acids can differ by a substantial amount (Livesey and Elia 1988). Thus, in general terms, it is the declining trend which indicates that more fat than carbohydrate is being catabolized (Kleiber 1961).

The purpose of this investigation is to hence establish, (i), the precise amount of energy being utilized daily throughout the course of pregnancy, (ii), whether, and at what point, an increase in energy expenditure occurs, (iii), the amount of food consumed during pregnancy relative to the virgin control, (iv), the change, if any, in fuel utilization during pregnancy and at what stage of pregnancy does this change occur.

2.2 Materials and Methods

Animals and Housing

Female Syrian hamsters weighing between 80 to 90 grams were purchased from Charles River Breeding Laboratory (Quebec, Canada), and housed singly in plastic cages. Room temperature was controlled at 25 $\pm$ 2^{o} C and on a 14 hr :10 hr light-dark cycle (lights on at 600 hour and lights off at 2000 hour). Tap water and

TABLE 2.1 COMPOSITION OF STOCK DIET

Stock Diet (4% fat w/w):

Protein	24%
Fat (mainly Soybean oil)	4%
Fiber	4.5%
Carbohydrate	62%
Vitamins	1%
Minerals	4.5%

Ingredients:
Corn and wheat flakes, ground corn, soybean meal, fish meal, wheat middlings, wheat red dog, dried whey, brewers dried yeast, soybean oil, animal liver meal, cane molasses, vitamin A supplement, D-activated animal sterol (source of vitamin D_3), vitamin B_{12} supplement, vitamin E supplement, menadione sodium bisulfite complex (source of vitamin K activity), riboflavin supplement, niacin supplement, calcium pantothenate, choline chloride, folic acid, thiamine, ground limestone, calcium phosphate, salt, manganous oxide, copper oxide, iron carbonate, ethylenediamine dihydriodide, cobalt carbonate and zinc oxide.

food pellets (Rodent Blox, Wayne Research Animal Diets, Chicago, Illinois) were available ad libitum. The crude composition of the diet was (min.) 24.0% protein, (min.) 4.0% fat, (max.) 4.5% fiber, carbohydrate 62% (refer to Table 2.1).

Timing of Estrous Cycle and Mating

The vaginal discharge was examined and noted each day to determine the day of estrus. The es ous cycle in the hamster is 4 days in length and is highly regular (Carter 1985). On day 4, a clear sticky secretion may be noted. This is followed by day 1, when the vaginal discharge can be observed as a mucus plug or as a thick, sticky yellow secretion. It is this observation which usually characterizes the onset of the cycle. Ovulation occurs just after the midpoint of the dark phase, sometime between the night of day 4 and the early part of day 1. The female is sexually receptive on the evening of day 4 to noon of day 1 (Lisk 1985; Carter 1985).

At least 2 cycles were observed in order to ensure regula and to establish that the timing was correct before the hamsters were used in the experiments. Mating occurred on the evening of day 4 at 1800 hour. Each female was placed with either one or two sexually active males from 1800 to approximately 2400 hour. Lordosis (the posture assumed by the female in the

presence of a male 1 to 2 hours before dark prior to the day of estrus) by the female hamster was observed, as a confirmation of sexual receptivity (Kent 1968).

Materials

Oxymax-85: Description and principle of operation (Figure 1)

The indirect calorimeter used here was the "OXYMAX-85"(TM) purchased from Columbus Instruments International Corporation, Columbus, Ohio, USA. This system consists of a software and hardware component. The software component is the computer program which functions as the controller, operating the system and recording the data input from the sensors.

The hardware component consists of a dual disc drive IBM-PC compatible computer system, an oxygen sensor, a carbon dioxide sensor, a system junction cabinet with 2 mass flow meters, 4 cage supply pump cabinets, 4 cage sampling pump cabinets and 4 acrylic animal cages. Commands issued from the computer are converted to control signals in the system junction cabinet and sent to the various electronic or pneumatic controlling devices in the system. The system junction cabinet also formats sensor signals for conversion by the computer. Two Mass Flow meters on this cabinet

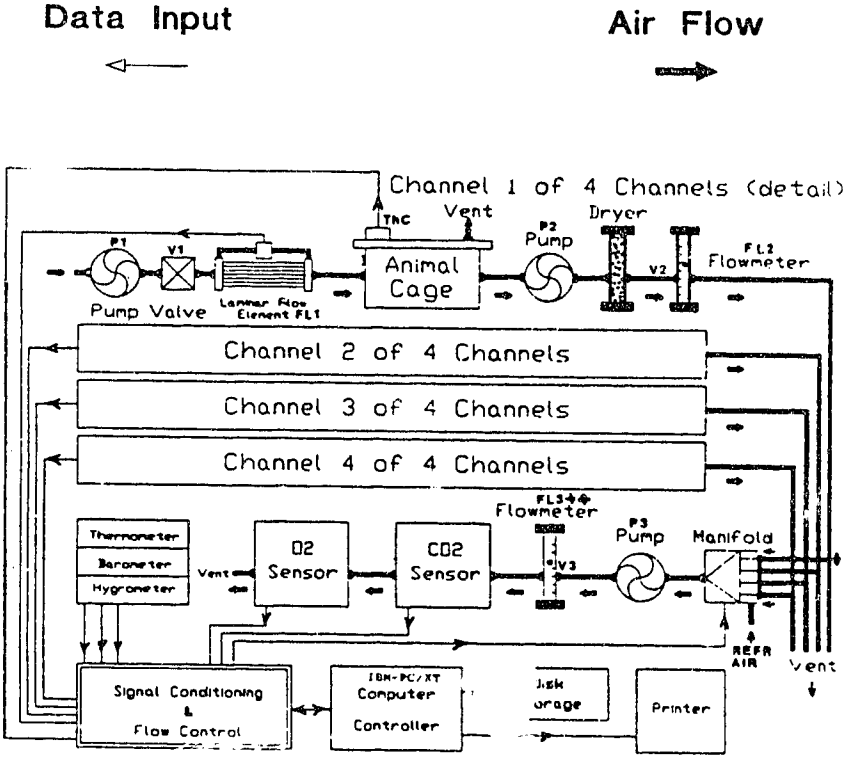


Figure 1

Oxymax-85: Flow Chart

regulate the sample gases and reference gases going to the sensors (Figure 1).

The Oxymax system has 4 "channels" or sets of pumps and animal cages. Each set consists of a supply pump, a sample pump and an acrylic cage. The supply pump (P1) cabinet draws room air from a glass container which acts as a mixer since room air is drawn into this receptacle through a small aperture and is then channelled to the 4 supply pump cabinets. This is to ensure that any slight variations in gas concentration across the room do not affect the system. The room air, forced by pump P1 through a controlled valve V1 and regulated via a laminar flow pressure transducer flow meter (FL1) which provides the computer with a precise measurement of the volume of air passing through it in the supply pump cabinet, is then supplied to the cage (see Figure 1). The volumetric measurement from FL1 is converted to a mass measurement using an STP correction factor and the room air characteristics (temperature, humidity and barometric pressure).

The acrylic cage, in which the experimental animal resides, has an air tight seal cover with 3 vents and a thermocouple. One vent is sealed with a plug, the other is for the ventilation of excess air since the air supplied is more than that sampled. The last vent is for the sampling of gases. The gases are sampled by the

sample pump (P2) and are passed through a column filled with a drying agent, silica gel.

Initially, Drierite was used but was proven impractical as anhydrous calcium sulfate tends to dissolve when moist which causes the granules to adhere to each other. This makes removal from the column difficult and reduces the size of the relatively uniform granules into powder which in turn increases resistance and impedes air flow. Thus, silica gel was used instead.

The sampled gas is then regulated by the flow meter FL2, which is used to visually monitor the flow rate. From the output of the flow meter FL2 the air sample is passed through a "T" junction. A third pump, system sample pump (P3), located in the system junction carinate then removes a portion of the now dried sample gas from the "T" junction while the rest is exhausted into the room. The air sampled from here is regulated by the system sample flow meter FL3 and is first channeled to the carbon dioxide and then the oxygen sensor where the respective gases are measured and results collected by the computer. Reference room air is drawn from the same mixer container where the animals' air supplies are drawn. This air regulated via the reference mass flow meter FL4, situated on the system junction cabinet, is also channeled to the sensors in the same fashion. Figure 1 shows the general flow chart of the system.

This chart does not include the various cabinets nor the room air mixer due to simplification.

Carbon dioxide sensor

The sensor used in this system utilizes an infrared CO_2 analyzer (personal communication, Ken Kober, Design engineer, Columbus Instruments Corp.). The principle is based on the phenomenon that various gas molecules absorb energy from different portions of the infrared (IR) spectrum. Figure 2 shows a diagram of an infrared gas analyzer. Basically the infrared sources emit beams that pass through parallel cells, one containing a reference gas and the other the sample gas. A rotating blade alternates the infrared beams in a rhythmic fashion so that when both the reference and sample cells are comprised of the same concentration of the gas, there is no variation in the radiation reaching either half of the detector cell. When sample gas is introduced, varying amounts of radiation reach the two halves of the detector cell, causing the diaphragm separating the compartments of the detector to oscillate. This oscillation is then transformed into a signal proportional to the difference in gas concentrations. The signal is then transmitted to the computer via the system junction cabinet.

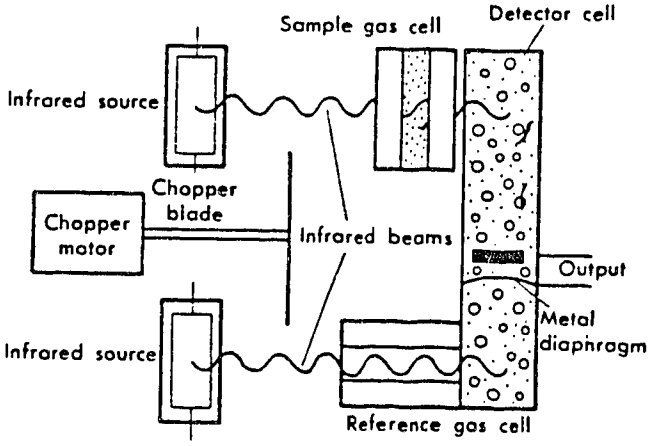


Figure 2

Basic Components of an Infrared Gas Analyzer

Oxygen sensor

This system utilizes a polarographic oxygen sensor. This sensor consists of 2 electrodes, a gold cathode and a silver anode, which are immersed in potassium chloride gel and mounted behind a permeable membrane. The special membrane permits diffusion of oxygen into the cell. During operation, a polarizing voltage is applied between the two electrodes and the resultant current flow is directly proportional to the oxygen partial pressure present. This polarizing voltage has been selected to make the sensor insensitive to other normally associated gases i.e. nitrogen, carbon dioxide, helium, water vapor etc. The sensor measures oxygen partial pressure, which is then converted by the computer to percentages etc. for calculations.

Calibration with Primary Standard Gases

The system was calibrated with primary standard calibration gases. Two types of gases were used for calibration. One was "Ultra High Purity Nitrogen" (99.999%) and the other was "Specialty Gas Mixtures" of oxygen (20.50%, usable range 20.40 to 20.60%), carbon dioxide (0.496%, usable range 0.40 to 0.60%) with the balance as nitrogen, purchased from Medigas Alberta Ltd. (Linde Division, Union Carbide). The system was calibrated prior to the start of every experiment and recalibrated prior to each and every "run".

Experimental Procedure

Experiment #1: Trial Run 4 female hamsters on the same estrous cycle were used. A timer was used to provide a light-dark cycle of 14:10 hours respectively, equivalent to that of the animal room where they were housed. Room temperature was approximately 25 ± 3°C (averages from computer data). On day 4 of their cycle, 2 females were mated while the other 2 were left undisturbed on the evening of estrus. Just prior to 2400 hours (Day 1), all 4 were weighed and placed in their individual Oxymax-85 acrylic cages with access to tap water and regular laboratory chow pellets. The Oxymax system, calibrated with commercial gas standards, was then programmed to supply each of the 4 cages with approximately 1250 ml/min. of room air; the precise flow for each cage was measured via a pressure transducer and documented by the computer. The amount sampled from the cages was 1 L/min. and from this 750 ml/min. was channelled to the sensors. Excess air, approximately 250 ml/min. in the first instance, was thus vented out from the cage through one of the vents on top of the cage. Another 250 ml/min. was vented out at the "T" junction just before reaching the system junction cabinet. Due to the excess air supplied, a slightly positive pressure in the cage was created. This is to ensure that all incoming air had come from

the supply pump and through the controlled flow meter. Information on the weight, number of animals and the length of the run was then entered into the program. The length of the run was determined by the amount of samples requested. The system takes a sample approximately once every 1.5 minutes (since all 4 cages are in operation). The system was then programmed to ru for approximately 43 hours. Data was stored in a floppy disk. At the end of the run, the system was stopped. The animals were weighed and fresh water and food provided. The drying columns, containing silica gel, were changed and the system recalibrated. The drying agent was regenerated in the oven overnight and reused in the next run. The system was then reprogrammed to run for a further 43 hours. This procedure was repeated until parturition, which occurred on the morning of day 16.

Experiment #2: As previously stated, 4 female hamsters on the same estrous cycle were used. In this and subsequent runs, an adjustment period of 6 days was included. Thus the hamsters were placed in the Oxymax system 6 days prior to mating. The Oxymax system was calibrated and run as before, stopping after every 46 hours. On the evening of the 6th day, just prior to ovulation, the system was stopped and 2 of the hamsters were removed and housed with sexually active males from

1800 to approximately 2400 hour. After mating, they were returned to their respective cages and the system started up again as before. The other 2 virgin controls were left undisturbed. The system was programmed to run for approximately 46 hours, after which the program would automatically stop. The animals and food were weighed. Fresh water and food were given and the drying columns changed as previously stated. This procedure was repeated until parturition, at day 16.

Each experiment consisted of alternate 24 hour and approximately 21 hour days. In other words, data was collected continuously for 24 hours on one day and due to the necessity of interrupting the system to weigh animals and food, replace drying agent and recalibrating the system, data was collected for only approximately 21 hours the next day. The exception to this is on the day of mating in which only 18 hours were monitored.

Experiment #3 to #5: Same as Experiment #2, giving a total of 4 experiments with an adjustment period of 6 days prior to mating. In 3 of the 4 experiments, the run was continued until day 17 postpartum but due to an unexpected disk format error some of the data was lost. The limited data on days 16 and 17 are thus not presented. In the last experiment, #5, one of the 2

mated hamsters apparently aborted resulting in a total sample size of 7 pregnant and 8 virgin controls.

Calculation of Data: The Oxymax data was translated into a format that can be "imported" by Lotus 1,2,3 (TM). This translation was done by animal, i.e. hamster A, B, C, etc was translated individually per run. This was then imported into the Lotus 1,2,3 format and the data grouped and calculated as blocks of 6 hour averages and then daily averages for each individual hamster. The initial trial, Experiment #1, consisted of the averages of 2 pregnant and 2 virgin controls. The final averages are those of all the pregnant and all the virgin controls from Experiment #2 to #5. Daily averages were calculated up to and including Day 15, 2400 hour. Day 16 was not included as parturition usually occurred in the morning, at between approximately 600 to 1000 hour.

Statistical Analysis

The statistical significance of differences between groups was assessed using Student's t-test in this and in subsequent chapters.

2.3 Results

The initial trial run, Experiment #1, was done without an adjustment period. Figure 3 shows the average

daily respiratory quotient throughout pregnancy during this run. The sample size in this run was only 2 pregnant and 2 virgin controls. Both food intake and body weight are shown in Figure 4. It would appear from these two graphs that an adjustment period was required as initial food intake for both groups was substantially below average (Figure 4) and the daily RQ (Figure 3) was identically low for both groups perhaps suggesting that body fat was being utilized in the presence of a decrease in food consumption. Both graphs appear to indicate that approximately 6 days were required for the animals to stabilize to the new conditions.

In Experiment #2 to #5 an adjustment period of 6 days was included before mating. The results presented here will be the final averages of these 4 experiments.

Figure 5 shows the average body weight of the hamsters in all 4 experiments. As can be seen from the graph, the virgin control group is also gaining weight as the hamsters are still in their growing phase. By the 11th day of pregnancy, there was a significant increase in weight gain compared to that of virgin controls. Figure 6 shows the food intake. there was no increase in food consumption in the pregnant group, which is in agreement with previous studies which showed no increase in consumption during pregnancy (Wade et al 1986;

Fig 3: Daily RQ Averages

Initial Trial Run: Exp. 1

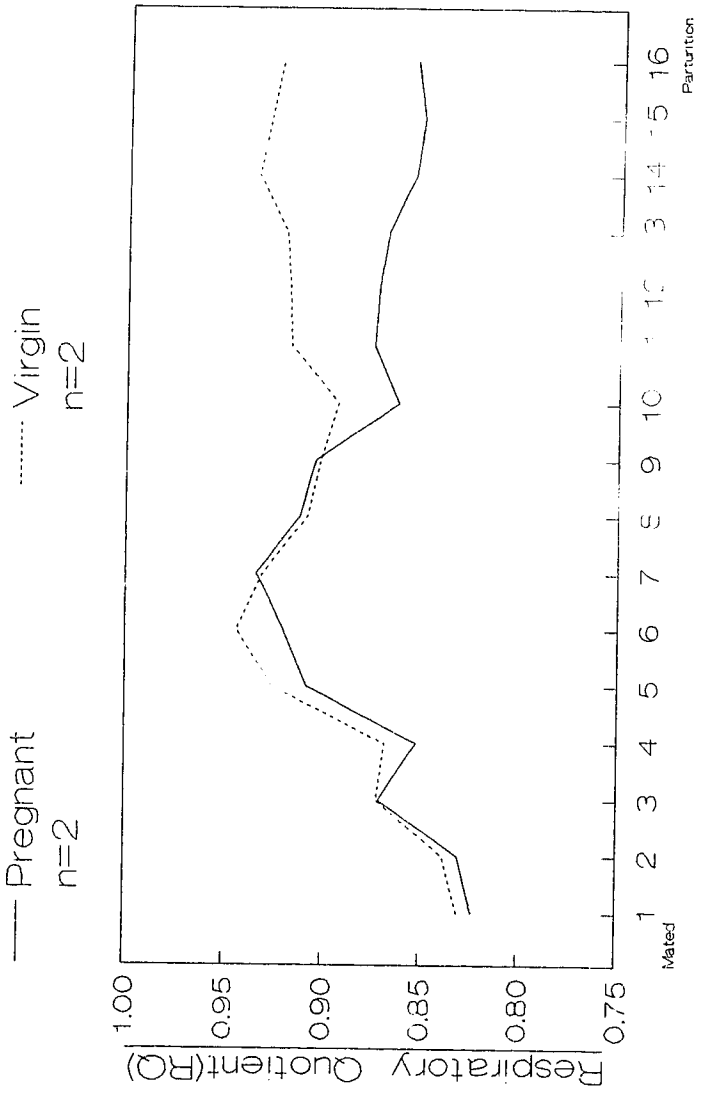


Fig 4: Body Weight and Food Intake (Exp. 1)

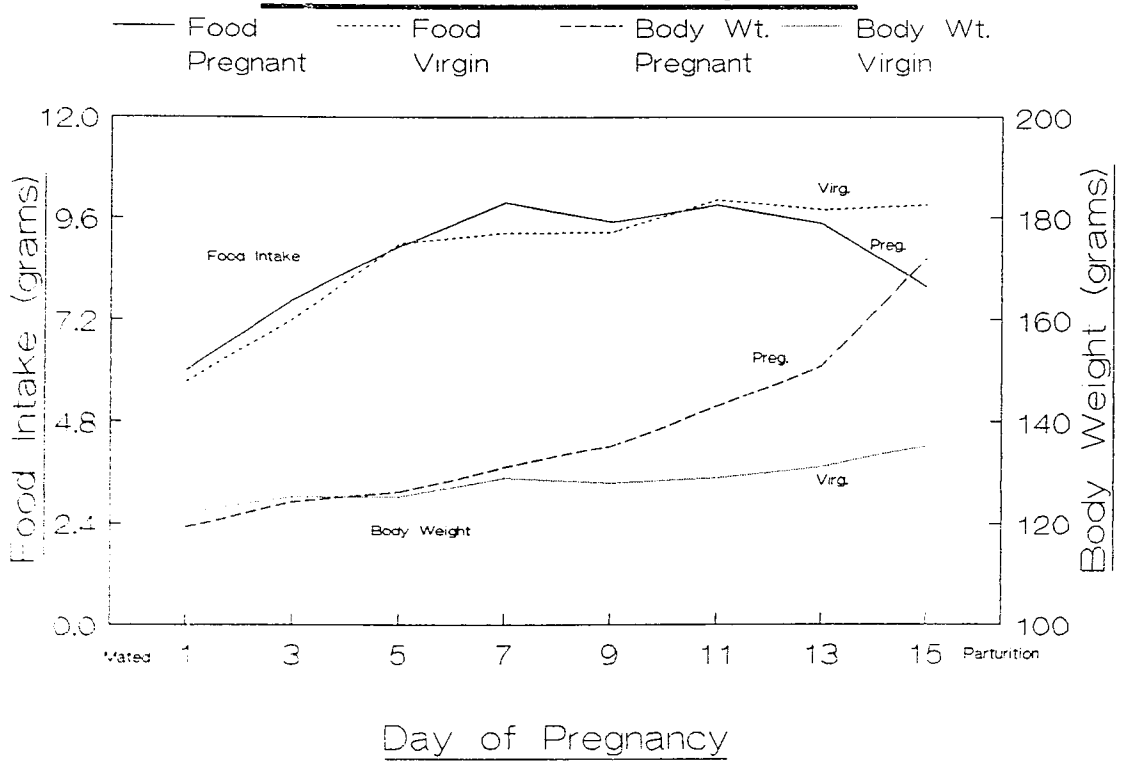
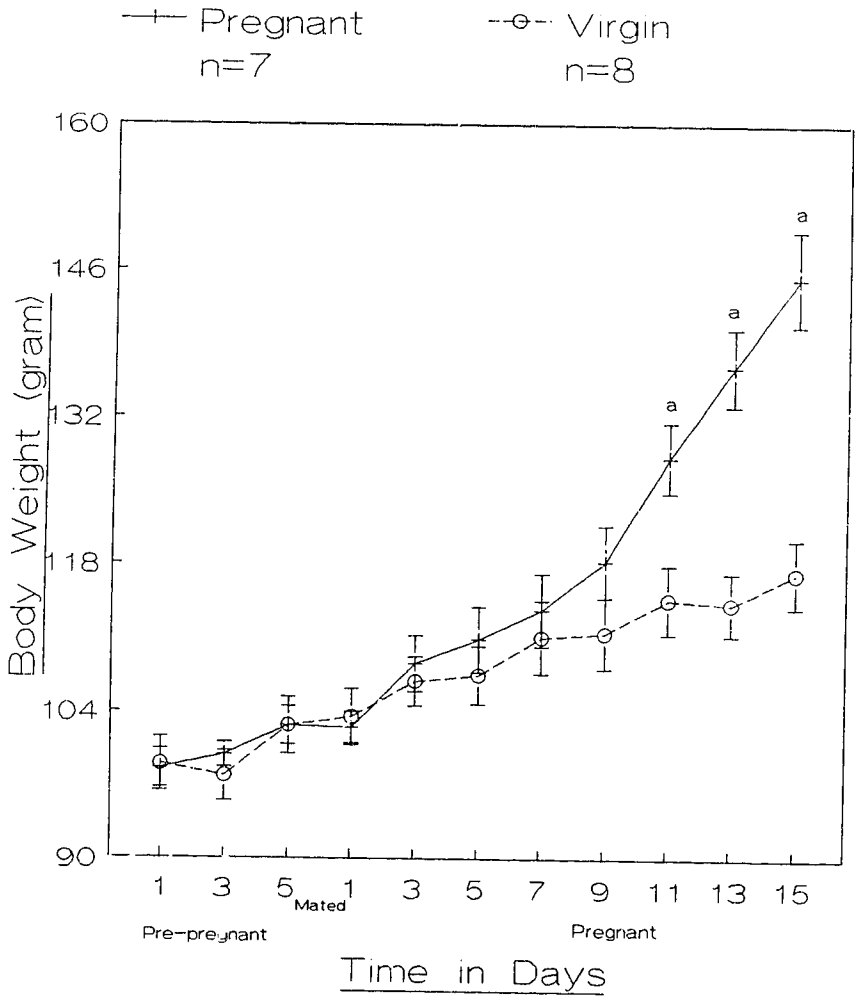
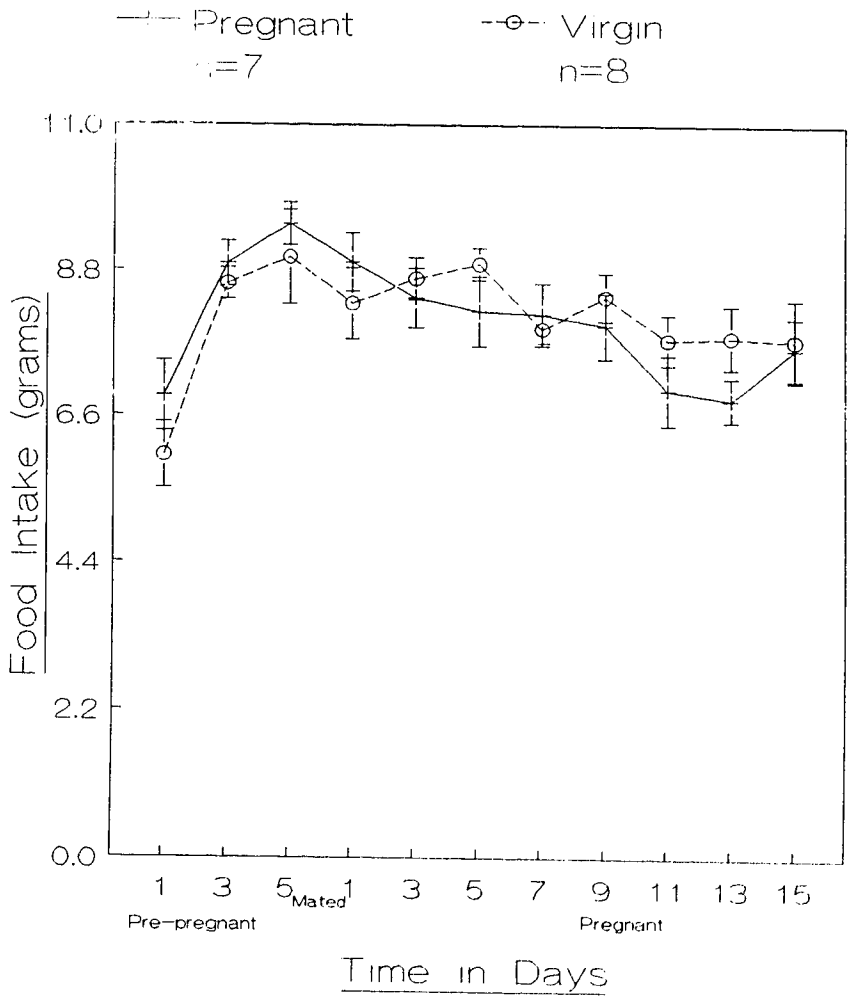


Fig 5: Average Body Weight (g)



Values are means ± SE (bars)
n = number of animals
a : $p < 0.05$ vs. virgin controls

Fig 6: Average Food Intake (g)

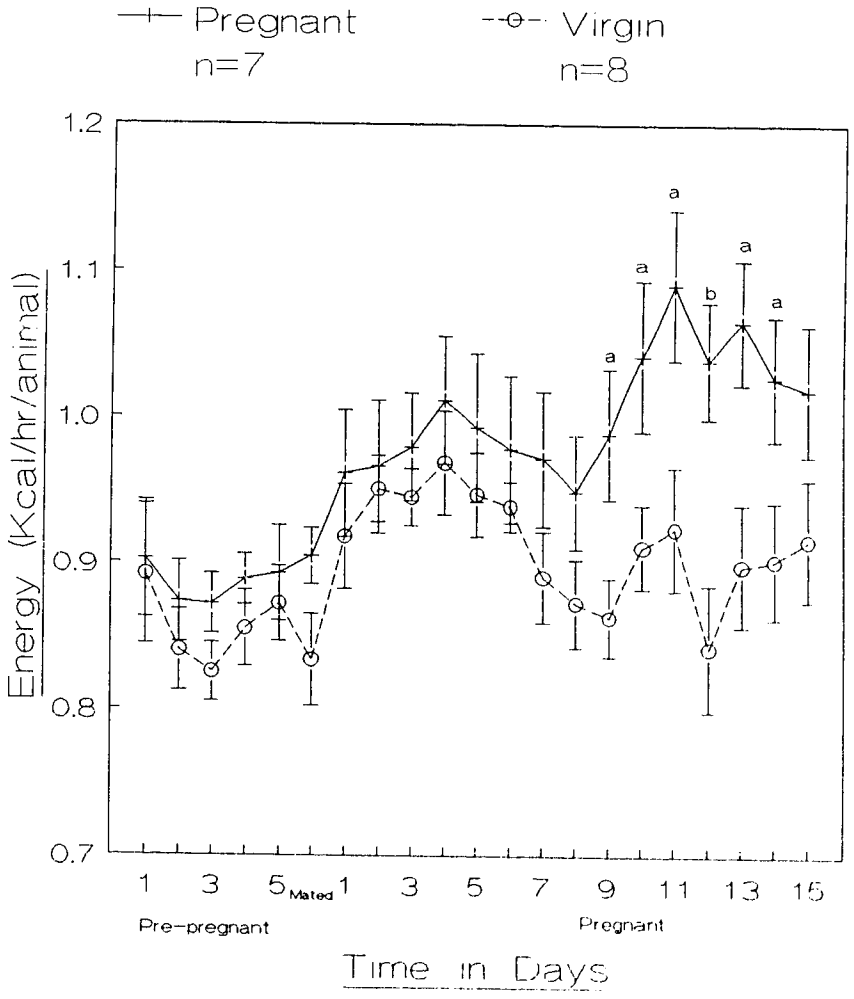


Values are means ± SE (bars)
n = number of animals
a : $p < 0.05$ vs. virgin controls

Fleming and Miceli 1983). There appears, however, to be a slight decrease in food intake during the last few days of pregnancy. Although this is not statistically significant, it appears to correlate with the previous study done by Wade et al (1986) which also found a slight decrease in food consumption before parturition.

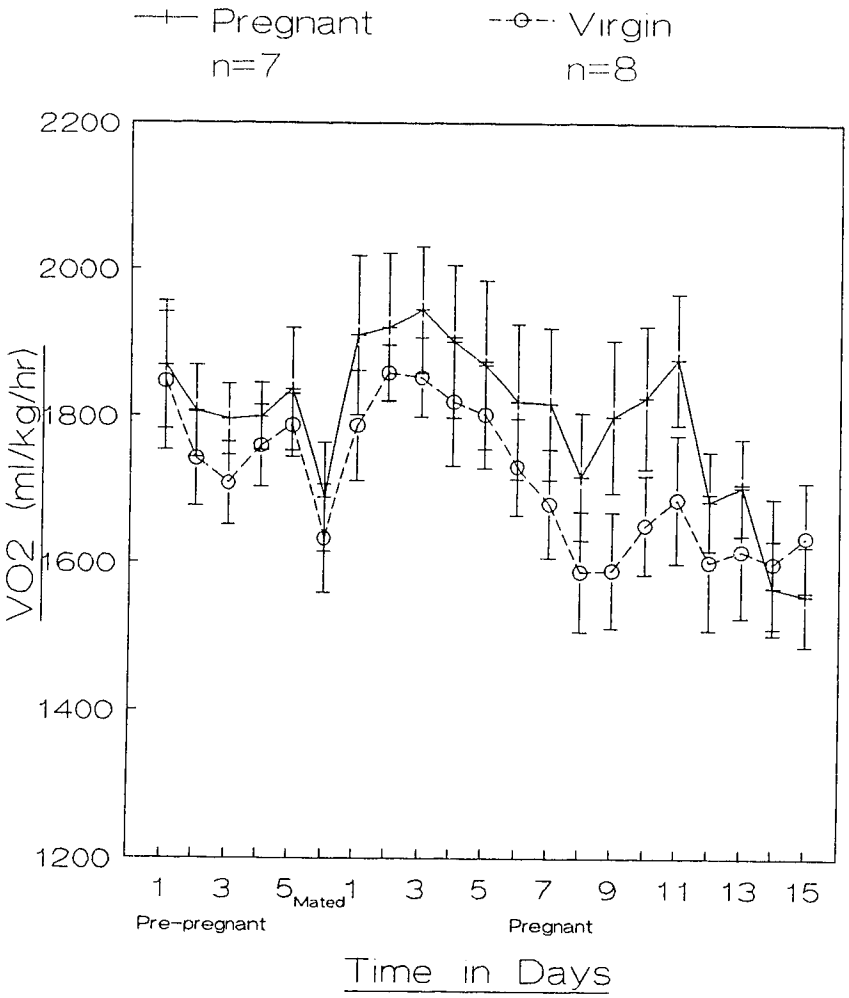
The average daily energy expenditure per animal is shown in Figure 7. There is no significant difference in energy expenditure between pregnant and virgin animals up to day 9 of pregnancy. However, on the 9th day of pregnancy there was a significant increase in energy expenditure ($P < 0.05$) in the pregnant group. The increase in expenditure continued until Day 14, but ceased to be statistically significant on Day 15, the day before parturition. The data in Figure 7 is expressed on a whole animal basis. When the energy expenditure is expressed on the basis of "per kilogram body weight", as in Figure 8, there does not appear to be an increase in oxygen consumed, and thus energy expenditure. This finding seems to correlate with the study done by Illingworth et al, 1987, which indicates that although the resting metabolic rate is increased during pregnancy in women, when expressed per unit of body weight no change was found. In practice, the trend in Figure 8 seems to suggest a decrease in oxygen utilization, especially during the last two days of

Fig 7: Daily Energy Expenditure



Values are means ± SE (bars)
n = number of animals
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

Fig 8: Daily Oxygen Consumption

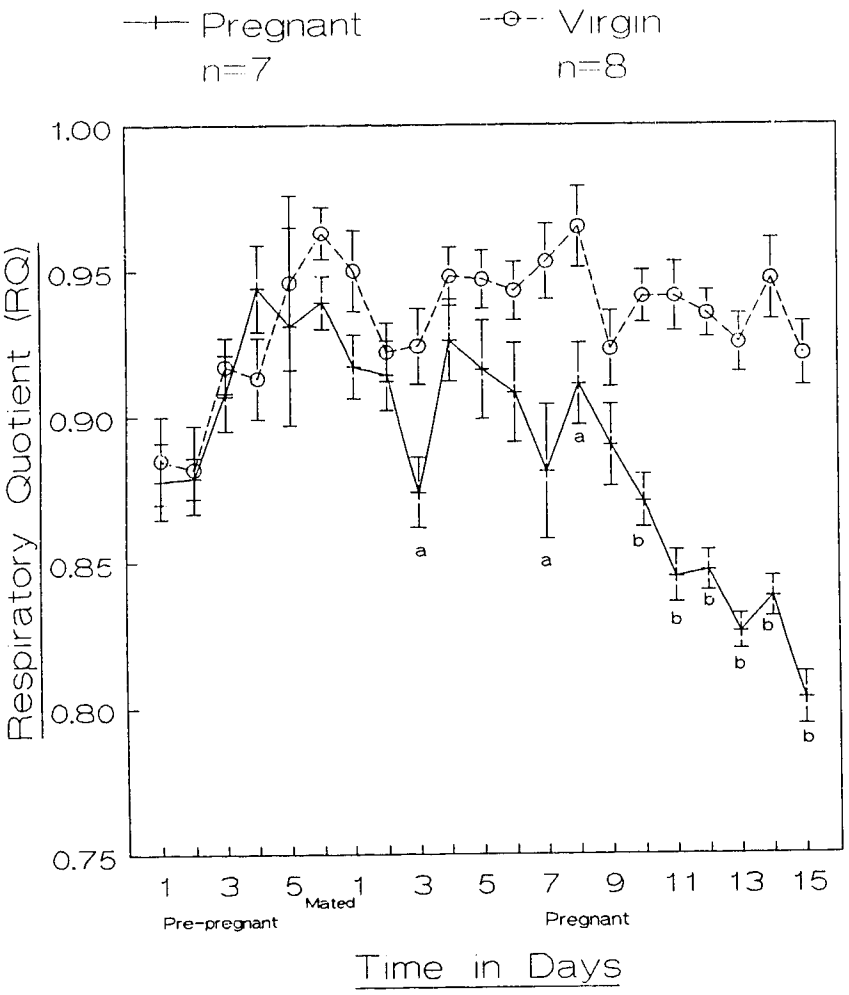


Values are means ± bars)
n = number of animals
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

pregnancy when oxygen consumption in the pregnant group is slightly lower than the control. This may be due to the fact that the weight gained during late pregnancy is probably mainly fluid thus in terms of per gram of body weight, oxygen consumption, and thus energy expenditure, appears to have decreased. However, no conclusions can be drawn from these observations as the findings are not statistically significant from that of the virgin controls.

It is interesting to note that with the significant increase in total energy expenditure (Figure 7) on Day 9, there is a subsequent sharp decrease ($P < 0.001$) in the respiratory quotient (RQ) on Day 10, as illustrated in Figure 9. This decrease in the RQ continued throughout the rest of pregnancy, which indicates that maternal fat is being utilized. Considering that RQ measurements are less accurate in relation to energy expenditure, Figure 9 shows remarkably small fluctuations within the virgin control group, with diminishing standard errors as the experiment progressed. It is also interesting to note that there appears to be a propensity for the RQ to decrease even before there is a significant increase in energy expenditure, as indicated by the occasional significant decreases ($P < 0.05$) in RQ on Days 3, 7 and

Fig 9: Average Daily RQ



Values are means ± SE (bars)
n = number of animals
a : p < 0.05 vs. virgin controls
b : p < 0.01 vs. virgin controls

8 of pregnancy. This may suggest that maternal body fat mobilization could be influenced by other factors, such as hormonal changes during pregnancy, besides the obvious nutritional influence, especially since it has been shown that brown adipose thermogenesis is decreased as early as Day 4 of pregnancy (Wade et al 1986). No significant increase in daily energy expenditure was noted however, nor was there a significant decrease in food intake during that period. In view of these last two observations, it would appear that the utilization of maternal fat could be accounted for by the observation that, although not statistically significant, food intake was marginally lower while energy expenditure tended to be slightly higher in the pregnant as compared to the control group. On the other hand, this could reflect the possibility of a decrease in energetic efficiency.

The results of Figure 7 show that energy expenditure was increased during the latter half of pregnancy. The slight decrease in expenditure during Day 15 may reflect the sudden curtailment of voluntary running activity during the last few days of pregnancy (Borer 1985). The decrease in voluntary activity may be a behavioral response to the increase in energy demands, analogous to the increased incidence of food hoarding (Fleming 1978) observed during pregnancy and lactation.

Total energy consumption and expenditure during pregnancy, Days 1 to 15, are shown in Table 2.2. The energy expenditure shown is the total energy utilized from Day 1, approximately 000 hour to Day 15, 2400 hour. The food consumed is the total for the 15 days. Energy content of the food was determined by bomb calorimetry (as described in Chapter 4). As illustrated, there is a significant ($P < 0.001$) total increase in energy expenditure of approximately 193 KJ compared to the virgin controls. Food intake, however, was not statistically different from that of the virgin control group. In view of the increased expenditure and maternal body fat mobilization, it would appear that energetic efficiency was not increased during pregnancy.

In order to evaluate energy balance, the total amount of food consumed was converted to digestible intake, since the digestibility of the diet is known (as described in Chapter 4), and further converted to metabolizable intake by multiplying by 0.96 (Mercer and Trayhurn 1987; Barr and McCracken 1983). The amount of energy utilized, as measured by indirect calorimetry, was then subtracted from the amount of metabolized energy. The remaining amount is presumably deposited, as part of the products of conception in the pregnant group, and mainly as body fat reserves and as part of

TABLE 2.2 TOTAL ENERGY CONSUMPTION AND ENERGY EXPENDITURE DURING PREGNANCY.

	Pregnant	Virgin
Total Metabolizable Intake (KJ): Day 1 to 15	1691.59±58.50	1778.33±43.42
Total Energy Expenditure (KJ): Day 1 to 15	1566.08 8 **	1373.08±17.87
Approximate Difference (KJ): 193		
Energy Balance: Amount of Energy Deposited (KJ)[a]	130.86±64.82 *	408.78±54.75

[a]Energy Deposited=Metabolizable Energy-Energy Expenditure

Values are means ± SE
* $p < 0.05$ vs. virgin controls
** $p < 0.001$ vs. virgin controls

growth, in the virgin control group (Table 2.2). The findings indicate that due to the increased demands of pregnancy, a significantly ($P < 0.05$) decreased amount of energy is "available" during pregnancy for deposition. In considering that body fat reserves were mobilized (Figure 9), the amount "available" appears to be insufficient.

The above data will be examined in further detail in the next chapter, along with carcass analysis studies, in order to consider the effects of energy expenditure on carcass fat and energy content at different stages of pregnancy and also in terms of carcass energy balance.

2.4 Discussion and Conclusions

The data obtained in this study reveal that, (i), food intake was not increased during pregnancy, which is in agreement with previous studies (Fleming and Miceli 1983; Wade et al 1986), (ii), body weight was significantly increased by Day 11 of pregnancy, (iii), daily energy expenditure was increased significantly by Day 9 of pregnancy, (iv), while correspondingly, RQ measurements were decreased substantially on Day 10 of pregnancy, indicating that body fat reserves were

utilized, (v), total energy expenditure was increased by approximately 193 KJ during pregnancy resulting in a substantially decreased amount available for deposition.

Thus, evidently maternal body fat stores were utilized to fund the cost of an increase in energy expenditure in the absence of an increase in food intake during pregnancy. The important finding in this study is the fact that there was a significant increase in energy expenditure on Day 9 which was paralleled by a corresponding drastic decrease in the respiratory quotient on Day 10, demonstrating that, without an increase in food intake, the fuel for the increase in expenditure came from maternal fat stores.

Furthermore, fat mobilization may be initiated, fairly early during pregnancy, without any apparent significant increase in daily energy expenditure. This could reflect the possibilities that other factors, such as hormonal influences, may play a role in the regulation of energy balance besides the effect of the physiologically imposed nutritional stress. This is especially so in view of the fact that brown adipose tissue thermogenesis is suppressed as early as Day 4 of pregnancy (Wade et al 1986) when the increased demand of fetal growth is not apparent. This could also suggest that energetic efficiency is actually decreased since the utilization of maternal body fat is

not accounted for by a significant increase in expenditure during the early stages of pregnancy. However, since RQ values are not quantitative but only indicative, the precise amount of body fat utilized is not known. Thus no definite conclusions can be drawn. Nevertheless, energy balance findings indicated that the demands of pregnancy resulted in a significant decrease in energy "available" for deposition, relative to that of the virgin controls.

It is interesting to note that some of the changes in the levels of circulating maternal hormones (Greenwald 1985) coincide with the decrease in RQ values, and hence, utilization of maternal body fat. This will be discussed in further detail in the last chapter.

In conclusion, the data obtained in these experiments indicate that in the absence of an increase in food consumption, maternal fat stores are utilized to support the increase in energy requirements during pregnancy. And in view of the utilization of maternal body fat, even during early pregnancy, it would appear that no increase in energetic efficiency occurs during pregnancy.

3. CARCASS ANALYSIS AT DIFFERENT STAGES OF PREGNANCY

3.1 Introduction

Pregnancy is usually associated with an increase in lipid deposition in humans (Hytten and Chamberlain 1980) and certain other mammalian species (Richard and Trayhurn 1985; Steingrimsdottir et al 1980). Exceptions to this include the Syrian (Wade et al 1986) and Djungarian hamsters (Schneider and Wade 1987). Carcass analyses have revealed that the Syrian hamster loses close to 40% of its body lipid by the end of pregnancy (Wade et al 1986). In the same study, it was also demonstrated that suppression of brown adipose tissue thermogenesis occurs as early as Day 4 of pregnancy raising the possibility that other influences, such as endocrine changes during early pregnancy may be involved.

Indirect calorimetry data presented in the previous study, indicated that fat was utilized especially during the last third of pregnancy and that daily energy expenditure was not significantly increased until Day 9 of pregnancy. The purpose of the work in this chapter is to, firstly, complement the indirect

calorimetry study with direct carcass analysis measurements at different points during the course of pregnancy, thus directly measuring maternal fat and energy content, plus fetal content. Carcass analysis should reveal whether a major loss of maternal body fat does indeed occur specifically between Days 10 and 15.

Secondly, measurements of brown adipose tissue thermogenesis would give an indication of the adaptive changes that may be occurring during pregnancy in relation to carcass fat, carcass energy content, energy expenditure measurements (indirect calorimetry), and the time at which these changes occur. The changes in thermogenesis may give an indication of the factors, nutritional or otherwise, which may be regulating energy balance.

3.2 Materials and Methods

Animals and Housing

As before female Syrian hamsters weighing between 80 to 90 grams were purchased from the Charles River Breeding Laboratory and housed under the same conditions as previously described (previous chapter).

Timing of Estrous Cycle and Mating

As previously described.

Experimental Procedure

The hamsters were allowed to adjust and gain weight in the new environment after receipt. After timing their cycle, 24 female hamsters weighing between 89.9 and 107.5 grams were divided into 4 groups according to their day of estrus. Within each of these groups they were then tentatively assigned to one of the following groups matched for base-line body weight: Day 0 (Virgin control), Day 5, Day 10 or Day 15 (Table 3.1). Body weight was measured to the nearest 0.1 gram, with the unmated control group comprising the representative weight range. The unmated controls or baseline virgins were weighed and killed by cervical dislocation at approximately 1600 hour on Day 4 of their cycle. The rest of the animals on the same cycle were weighed, assigned to a group and mated (from 1800 to 2400 hour) that same day. On the next day, Day 4 of the estrous cycle for another group of animals, the procedure was repeated. This was continued until all 4 groups were processed.

Hamsters in Groups 1, 2 and 3 were killed the same way, by cervical dislocation, and at approximately the same time, 1600 hour, on the 5th, 10th and 15th day of pregnancy, respectively. The evening of mating is counted as Day 0. Day 1 of pregnancy starts on the day

after mating. The time of sacrifice, 1600 hour, is approximately 15 hours before parturition for Group 15.

Brown Adipose Tissue Measurements:

Interscapular brown adipose tissue was immediately dissected from the animal after cervical dislocation. The tissue was then placed on a stainless steel cooling plate kept on ice and "cleaned" by removal of any white adipose tissue, connective tissue and muscle tissue that might have been present. It was then weighed to the nearest 0.1 mg and placed in ice-cold sucrose buffer solution which was kept in an ice bucket. Homogenization of the tissue in the sucrose buffer medium was done in a cold room (4-6° C) by means of a glass Potter-Elvejhem homogenizer (Citenco Ltd., Boreham Wood, Herts, U.K.). Aliquots of the homogenate were then taken for cytochrome-c oxidase measurements. One ml of each tissue homogenate was mixed with 0.1 ml of "Lubrol" solution prior to the assay. Lubrol is a non-ionic detergent which solubilizes the cytochrome oxidase from the mitochondria, thus stimulating its activity.

Enzyme activity was assessed by measuring the rate of oxidation of ferrocytochrome c to ferricytochrome c. This was done spectrophotometrically by following the decrease in absorbance at 550nm. A Pye Unicam spectrophotometer (Philips: model PU8800 UV/VIS) was used to measure the decrease in absorbance due to

oxidation. The sample temperature was maintained at 24° C by means of a cuvette "jacket" connected via a circulation water pump to a thermostatically controlled water bath. A 1.5 ml cuvette was then positioned in this "jacket". 1.4 ml of diluted ferrocytochrome c was added to the cuvette and allowed to stabilize for a few seconds. 15 ul of the sample was then added and rapidly mixed to initiate the reaction. The decrease in absorbance was monitored via a graphic recorder and digital readout. After 1-2 min. the reaction was stopped by the addition of one drop of potassium ferricyanide to completely oxidized the cytochrome c. This reading was noted. Fully oxidized ferrocytochrome c, without the addition of a sample, was also documented.

The calculation of the enzyme activity is based on first order kinetics, as the decrease in the absorbance at 550nm is exponential. The rate of the reaction (v) is directly proportional to the concentration of the substrate (s) as $v=k \times s$, where k is the rate constant. The log of the absorbance versus time is linear, thus the slope of this line is the rate constant. Substrate concentration was calculated using an extinction coefficient of 9.2 $mM^{-1}cm^{-1}$ for ferricytochrome c (Yonetani and Ray 1965). Activity is expressed as umoles cytochrome c oxidized per min per

tissue. Enzyme activity was calculated using the formula:

$$k \times \text{time(min)} \times \frac{\text{reagent blank}}{\text{extinc.coeffi.}} \times \frac{\text{reagent vol.}}{\text{sample vol.}} \times \text{dilution}$$

with k= ln (1st absorbance/2nd absorbance)/time

Reagents

Sucrose buffer: 250mM sucrose, 1mM HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid) and 0.2mM dipotassium EDTA (ethylenediaminetetraacetic acid). The buffer was adjusted to pH 7.2 with 0.5M KOH at room temperature and stored at 4°C for no longer than 2 weeks.

Potassium phosphate buffer: 0.01M, pH 7.0

Potassium ferricyanide: saturated aqueous solution.

1% ferrocytochrome c: Cytochrome c was dissolved in phosphate buffer and completely reduced by the addition of a few crystals of ascorbic acid (Na salt). Excess ascorbate was removed by dialysis in visking dialysis tubing, against phosphate buffer. The buffer was changed 3 times over a 24 hour period at intervals of 45 min., 2 hours and overnight (Wharton and Tzagoloff 1967). This stock solution was kept frozen at -85°C. It was then diluted 16 times, when needed, with phosphate buffer.

give a final concentration of approximately 50uM cytochrome c for use in the assay .

Carcass Analysis: Gut contents were removed from all groups by removing the entire gastrointestinal tract, cleaning out the contents and returning the gut back to the carcass. Uterine contents were dissected, weighed and processed separately. The carcasses and uterine contents were autoclaved at 10^4 kg/m^2 (14 psi) for 50 min. in order to soften the tissues. They were then homogenized in an Osterizer(TM) blender with an approximately equal amount of distilled water. The homogenates were then transferred into aluminum pans and freeze-dried in a shelf freeze-dryer. Approximately 1 gram portions of the freeze-dried samples were then pressed into pellets with a Parr pellet press and the energy content determined by bomb calorimetry (Parr adiabatic calorimeter: model 1241EA). The calorimeter was calibrated with dry benzoic acid standards and the energy equivalent factor calculated (expressed as calories per ^{o} Celsius).

Essentially, the sample is completely combusted in a well insulated oxygen-rich container or bomb. All the heat produced is absorbed by a precisely weighed amount of water in which the container is immersed. The change in temperature of this fluid surrounding the combustion chamber is measured.

Theoretically, no heat exchange takes place with the environment since the chamber is very well insulated and has a temperature controlled "jacket" which adjusts itself according to the temperature of the water bath in which the bomb is placed. Therefore, the heat necessary for the increase in the water bath temperature must be derived from the sample that is completely burned, thereby releasing all of its energy, in the chamber (Guthrie 1975). Procedures and calculations are as per manufacturer's manual. All samples were determined in duplicate. They were repeated if the results were more than 10% apart. Most of the samples were well within 5% of each other. The results were then averaged.

Crude carcass lipid was determined by extracting approximately 1 gram of the freeze-dried carcass with 25 ml of petroleum ether (boiling point 35° to 60° C) 3 times (Wade et al 1986; Leshner et al 1972). Samples were weighed (to the nearest 0.1 mg) into centrifuge tubes, petroleum ether was then added and the tubes swirled and agitated. These were then centrifuged at 17000 rpm for 15 min. and the supernatant collected in previously weighed containers. This procedure was repeated 3 times. The fat extract, or supernate, and the fat-free residue, or precipitate, were both let to air dry separately in a fume hood at room temperature for approximately 48 hours. After the ether had evaporated,

only the extracted fat remained. This was then weighed. In order to obtain more reliable results, the amount of fat originally in the fat-free residue or precipitate was also calculated by subtracting the fat-free residue weight from the original weight of the sample. Thus, results from both the supernate measurements and that of the fat-free residue should be equivalent. Since all samples were done in duplicate, with 2 measurements per sample, all 4 results were pooled and averaged. Total fat content was obtained by multiplying the amount of fat extracted per gram of freeze-dried carcass with total freeze-dried carcass weight.

3.3 Results

Body weight and carcass analysis results of the 4 groups of hamsters are shown in Table 3.1. Interscapular brown adipose tissue results are presented in Table 3.2. The body weight shown is that of the whole live animal. Carcass weight is carcass minus gut contents and uterine contents. Figure 10 shows both whole body and maternal carcass weight. Body weight increased rapidly and is significantly ($P < 0.05$) greater on Day 5 relative to the virgin control group at

TABLE 3.1 CHANGES IN BODY COMPOSITION AT DIFFERENT STAGES OF PREGNANCY.

	CONTROL	EXPERIMENTAL GROUPS		
	Virgin Day 0	Group 1 Day 5	Group 2 Day 10	Group 3 Day 15
n	6	6	6	5
Initial Body Wt.(g)	99.4 ± 2.9	99.0 ±1.3	98.2 ±1.9	99.1 ±1.2
Final Body Wt.(g)	99.4 ±2.9	109.2[a] ±1.7	127.1[b] ±3.6	157.9[b] ±4.7
Carcass Wt. (g)	91.1 ±2.6	98.8[a] ±1.5	109.5[b] ±2.8	105.1[a] ±1.7
Fat Content (g)	12.04 ±0.77	10.34 ±0.88	9.87 ±0.7	6.95[b] ±0.45
Energy Content(KJ)	935.04 ±31.95	885.48 ±36.08	911.78 ±40.33	796.52[a] ±22.09
Fetuses:				
No. of fetuses	---	---	11.8 ±1.7	11.0 ±0.8
Wt.(g)	---	---	8.3 ±2.3	44.3 ±2.8
Total Energy Content (KJ)	---	---	---	107.9 ± 8.8

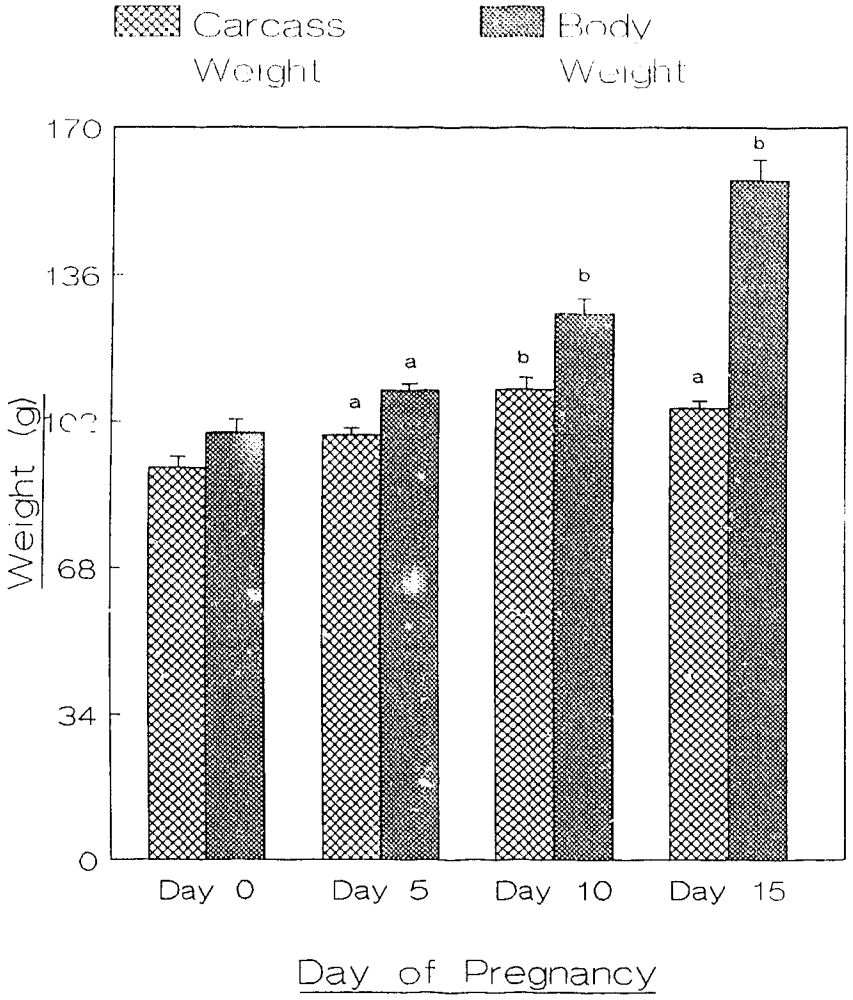
Values are means ± SE.
n= no. of animals
a: $p < 0.05$ vs virgin control (Day 0)
b: $p < 0.001$ vs virgin control (Day 0)

TABLE 3.2 CHANGES IN BROWN ADIPOSE TISSUE AT DIFFERENT STAGES OF PREGNANCY.

	CONTROL	EXPERIMENTAL GROUPS		
	Virgin Day 0	Group 1 Day 5	Group 2 Day 10	Group 3 Day 15
n	6	6	6	5
Interscapular brown adipose tissue:				
Wet Wt.(mg)	229.2 ±11	192.6[a] ±8.9	201.5 ±7.4	133.6[b] ±9.5
Cytochrome oxidase, (uMol/min/ tissue)	93.01 ±4.08	84.93 ±2.97	77.86[a] ±5.21	48.24[b] ±7.0

Values are means ± SE.
n = no. of animals
a: $p < 0.05$ vs virgin control (Day 0)
b: $p < 0.001$ vs virgin control (Day 0)

Fig 10: Body and Carcass Weight



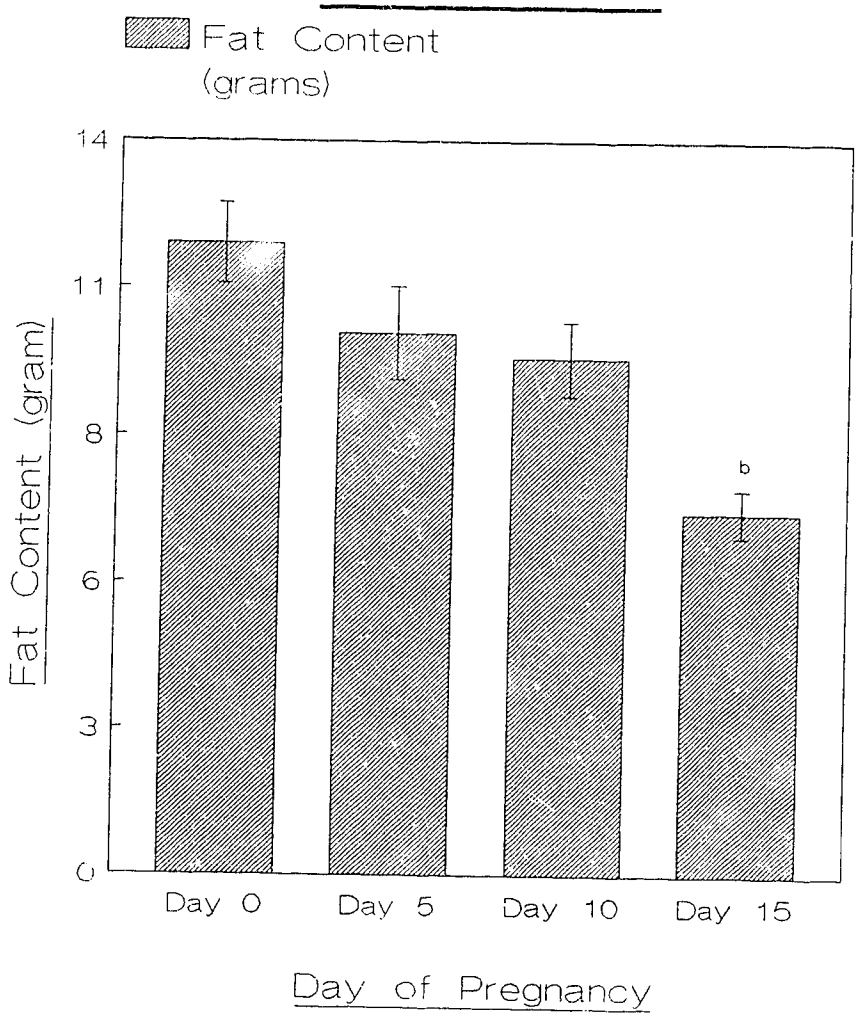
Values are means ± SE (bars)
All groups have 6 animals except Day 15 with 5 animals.
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

Day 0. It should be noted that the control group being compared here is at Day 0, unlike the virgin control group in the previous study, where the animals were the same age as the pregnant group and, hence, showed some growth since Day 0.

Maternal carcass weight on Day 5 was significantly different ($P < 0.05$) from that of the Day 0 virgin group. The weight gain is also consistent and steady from Day 0 to Day 10; by Day 15, however, there is a slight decrease. The initial weight gain may be suggestive of an increase of body fluid in the maternal tissues (Table 3.1). The slight decrease in weight seen on Day 15 may be indicative of the significant reduction in maternal body fat reserves during the latter part of pregnancy.

Figure 11 and Table 3.1 show the depletion of maternal body fat at the different stages of pregnancy. Although the trend is fairly apparent, the decrease in total fat content is not statistically significant until Day 15 when the difference is substantial ($P < 0.001$). The findings here are consistent with the data from the previous chapter (Figure 9), which suggested that a significant decrease does not occur until after Day 10 of pregnancy, when the utilization of maternal body fat appeared to be considerable. The decrease between Day 0

Fig 1.1: Total Body Fat Content



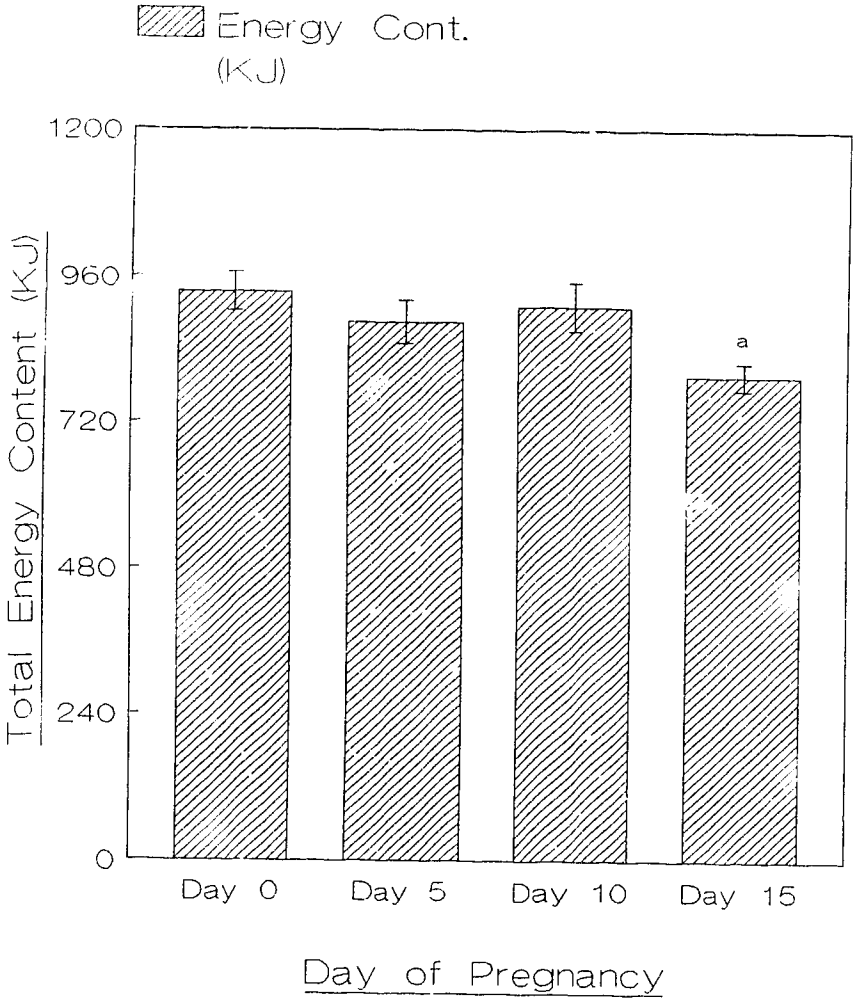
Values are means ± SE (bars)
All groups have 6 animals except Day 15 with 5 animals.
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

and Day 5 is almost 2 grams. However, the decrease between Day 5 and 10 is slight. The pregnant hamster lost close to 5.1 grams of fat during the course of pregnancy, which is approximately 42% of its initial total body fat. This value is very similar to that obtained in the study done by Wade et al (1986).

The energy content of the maternal carcass is shown in Figure 12 and Table 3.1. Similar to the body fat content, there was a significant decrease in total energy content by the end of pregnancy, Day 15 ($P < 0.05$). The maternal carcass lost close to 140 KJ of energy compared to the initial virgin control. When the energy content of the fetuses are added, to the Day 15 group, the total energy content of the carcass is approximately 904.42 KJ, which is almost equivalent (97%) to the baseline controls or estimated initial energy content. However, in view of the growth and thus increase in energy content which would normally occur if the animals were not mated, the depletion of energy content, and body fat, would be greater than that seen here. In other words, food intake during this period, and thus energy deposited, is not accounted for in this comparison.

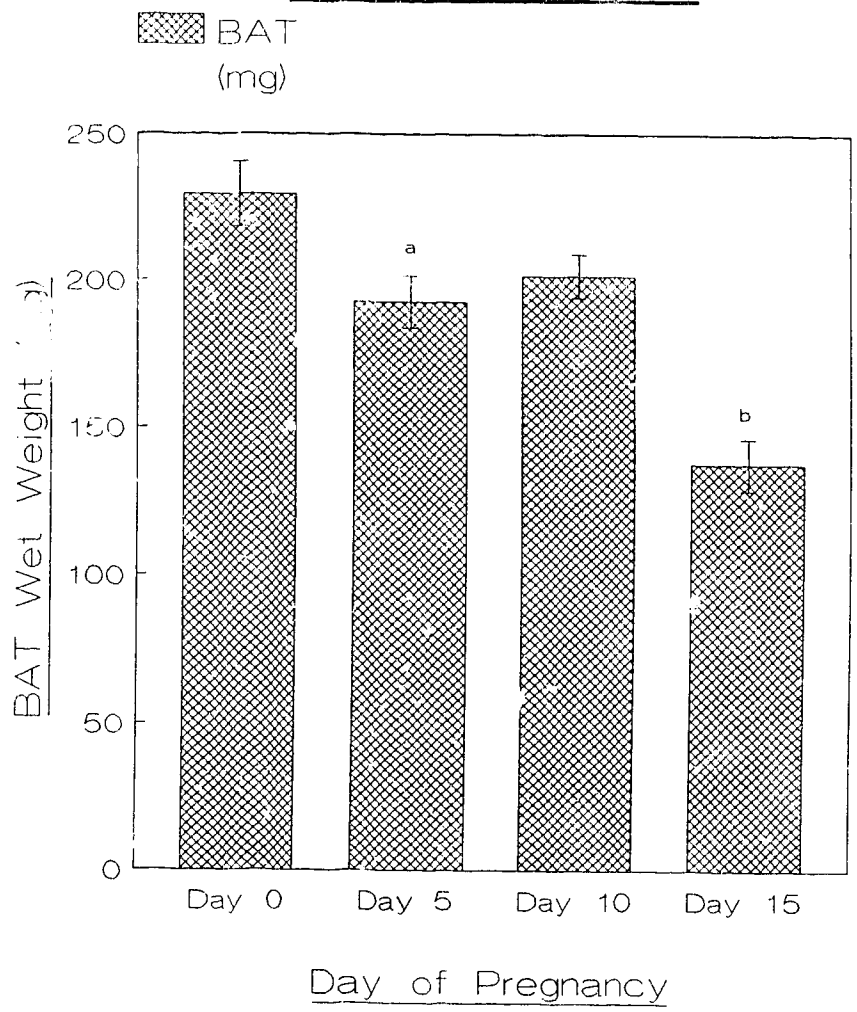
The interscapular brown adipose tissue (IBAT) weight is shown in Figure 13. There appears to be a

Fig 12: Energy Content



Values are means ± SE (bars)
All groups have 6 animals except Day 15 with 5 animals.
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

Fig 13: Brown Adipose Tissue Weight

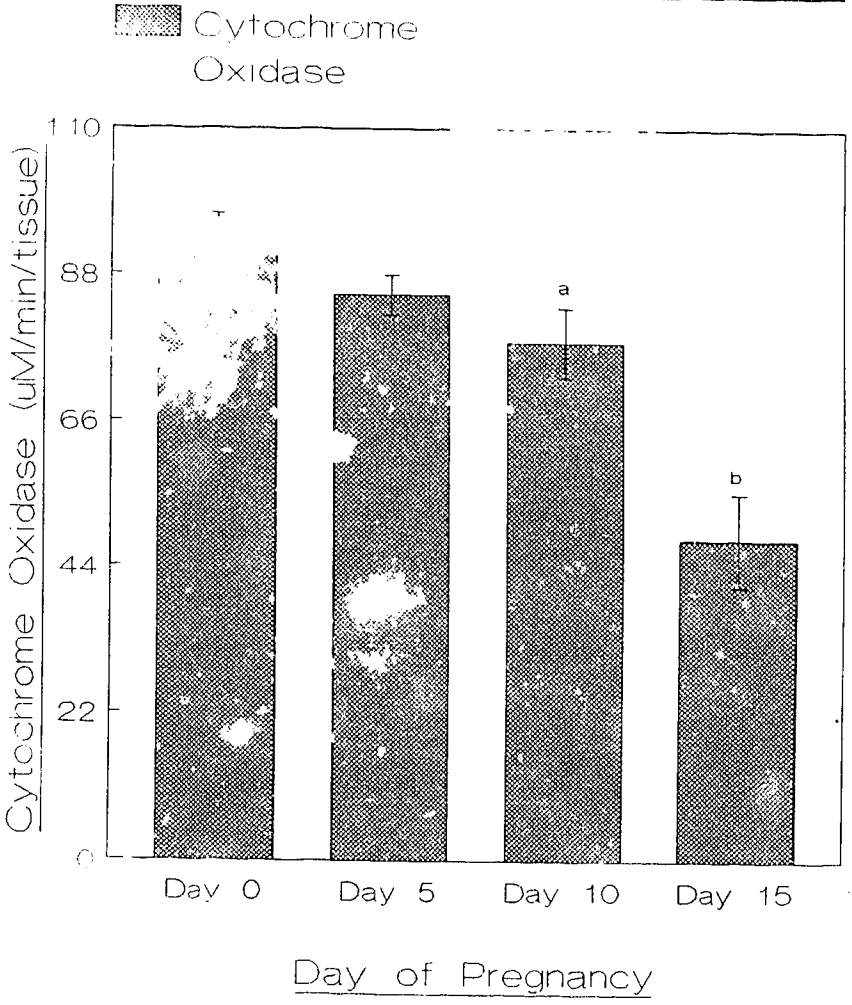


Values are means ± SE (bars)
All groups have 6 animals except Day 15 with 5 animals.
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

significant ($P < 0.05$) decrease in brown adipose tissue weight on Day 5 of pregnancy but not on Day 10, as compared to the initial control group. The difference between Days 5 and 10 is, however, very slight. A substantial decrease in BAT weight occurred between Days 10 and 15. By Day 15, BAT weight was considerably lower ($P < 0.001$) than in the baseline controls. This perhaps may be a reflection of the decrease in total body fat.

Figure 14 shows the cytochrome oxidase activity of the brown adipose tissue (expressed as uMol per min. per tissue). Although activity appears to be decreasing throughout the course of pregnancy, the decrease on Day 5 is not statistically significant. By Day 10 of pregnancy the difference is significant ($P < 0.05$) and by Day 15 the difference is highly significant ($P < 0.001$), as compared to the baseline control group. On the basis of this reduction in activity, which is an indication of a decrease in mitochondria content, whole tissue thermogenesis would appear to be decreased significantly. This progressive decrease seems to parallel the decrease observed in total body fat (Figure 11). This perhaps may suggest that body fat depletion may be associated with the suppression of BAT thermogenesis, considering that food deprivation apparently decreases BAT activity in hamsters.

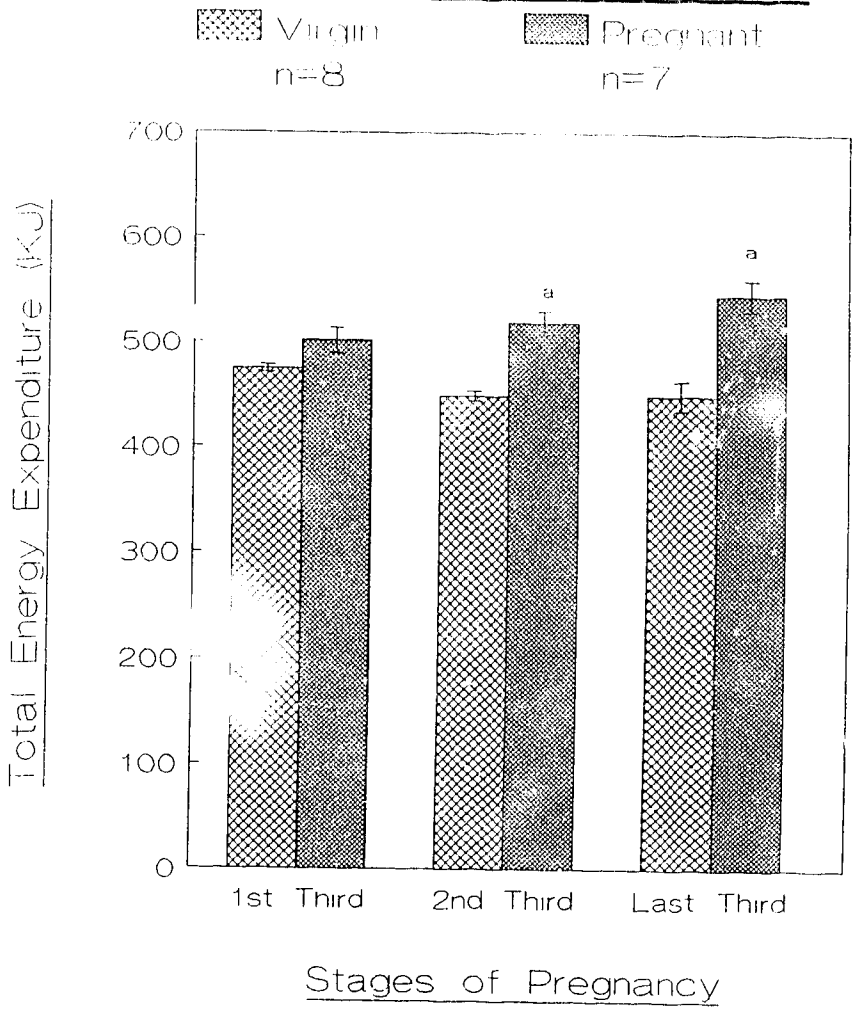
Fig 14: Cytochrome Oxidase Activity in BAT



Values are means ± SE (bars)
All groups have 6 animals except Day 15 with 5 animals.
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

Figure 15 shows energy expenditure data from the indirect calorimetry study. Energy expenditure during pregnancy has been divided into 5-day blocks, deleting data on the adjustment period. Daily energy expenditure is tabulated as follows: first 5 days of pregnancy, middle 5 days of pregnancy and the last 5 days of pregnancy. The sum of these 5-day blocks is then converted from kilocalories to kilojoules (1 kcal= 4.18 KJ). As shown in Figure 15, energy expenditure during the first third of pregnancy, although higher than the virgin controls, is not significantly different from the controls. The middle third of pregnancy, which includes Days 9 and 10, is significantly greater ($P < 0.05$) than that of the virgin controls. Carcass analysis data on fat and energy content, however, does not show a statistically significant decrease in the Day 10 group. Perhaps at this stage, the increase in expenditure was being supported mainly by food intake, but as daily expenditure increased, as seen in Figure 7, food consumption alone, which did not increase, was inadequate. Thus, body fat reserves were utilized (Figure 8) and carcass fat content was significantly ($P < 0.001$) decreased, as was carcass energy content ($P < 0.05$), in the Day 15 group (Figure 11 and 12 respectively).

Fig 15: Expenditure at Different Stages



Values are means ± SE (bars)
n = number of animals
a : $p < 0.05$ vs. virgin controls
b : $p < 0.001$ vs. virgin controls

Data on the fetuses are shown in Table 3.1. Fetal weight gain between Days 10 and 15 was dramatic. Within a 5 day period, the total conceptus weight increased from about 8 grams to 44 grams. Perhaps this may largely explain the significant increase in energy expenditure seen during the last third of pregnancy and in turn, the significant decrease in RQ (Figure 9), carcass fat and energy content. Fetal energy content was approximately 108 KJ (Table 3.1). In reviewing the calculations from Table 2.1, approximately 131 KJ of consumed energy was "available" for deposition. This appears marginally sufficient. It should be noted that the variation in the energy "available" for deposition is relatively large (SE $\pm$ 64.82 KJ). Maternal tissue deposition, i.e. uterus and mammary gland enlargements are still unaccounted for, although the amount of maternal protein gained during pregnancy is evidently insignificant (Wade et al 1986). In view of the significant lost in body fat, approximately 42% of initial carcass fat, it appears that energetic efficiency may actually be decreased during pregnancy.

One hamster was deleted from the Day 15 group. This was because of the "unusual" data obtained. The carcass weight was almost 10 grams above the average. Carcass fat content was 13.2 grams, which is almost twice the average amount. Similarly, the energy content

was over 200 KJ more than the average. Interestingly, the number of fetuses found in this hamster was only 4, less than half the average number. Brown adipose tissue weight was above average at 157.5 mg, as was the cytochrome oxidase activity, 55.06 uM/min/tissue. The inclusion of this animal may substantially bias the results since the very small number of fetuses clearly had a major effect on the energetics of pregnancy; this in itself is an interesting point.

3.4 Discussion and Conclusions

The results obtained in this study seem to confirm the findings of the previous calorimetry study in several respects. The indirect calorimetry study showed that daily energy expenditure was increased on Day 9, while on Day 10 RQ measurements were significantly decreased. The findings on total body fat (Figure 11) showed a significant ($P < 0.001$) decrease in the Day 15 group, with a loss of close to 3 grams of fat within a 5 day period. Carcass energy content also revealed a significant ($P < 0.05$) decrease in energy content by Day 15. Thus, the overall carcass analysis results appear to be in agreement with the indirect calorimetry data.

Although not statistically significant, carcass analysis revealed that body fat loss occurred early during pregnancy, as much as 2 grams being lost in the first 5 days (Table 3.1). This seems to be in keeping with the occasionally significant decrease in RQ values noted in Figure 9. This perhaps may suggest that other mechanisms, besides the obvious nutritional demands of the fetuses in later pregnancy, are affecting the regulation of energy balance.

The second objective of the present study was to evaluate BAT thermogenesis in relation to stage of pregnancy and carcass energy & fat content. The results obtained indicate that although a trend can be seen, the decrease in BAT thermogenesis was not statistically significant until Day 10. At this point in time (Day 10), maternal fat and energy content were still not significantly reduced (Table 3.1). On the other hand, energy expenditure was significantly increased (Figure 8 and 15) by this point in pregnancy. Thus, this finding may suggest that perhaps during increased expenditure due to tissue synthesis, i.e. fetal growth, BAT thermogenesis is suppressed as an adaptive response.

Another possibility may be that the mechanisms regulating body fat mobilization may be involved since the net utilization of maternal body fat appears to be

initiated at this stage in pregnancy. Apparently the mitochondrial content of BAT in the Syrian hamster is not regulated by noradrenaline during pregnancy and lactation (Trayhurn and Wusteman 1987), which leaves the possibility that other factors may be suppressing BAT thermogenesis. Hormonal changes during pregnancy, such as prolactin, have been suggested (Wade et al 1986).

However, the data obtained in the present study is not in agreement with the findings of Wade et al (1986), in which BAT thermogenesis was found to be significantly decreased as early as Day 4 of gestation. One of the possible reason for this disagreement may be strain differences. Another likely possibility may be the differences in the initial body fat of the hamsters in these two studies. Although carcass fat was done in the first experiment of the study by Wade et al (1986), total body fat was not measured in the second experiment which was involved in the measurement of BAT thermogenesis throughout pregnancy and lactation. Body fat was estimated in the second experiment by means of parametrial white adipose tissue weight, which makes comparison between that study and the study done here difficult. Nevertheless, total body fat appears high in the present study compared to the carcass fat of the first experiment in the study by Wade et al, (1986). Perhaps it is possible that if maternal fat stores are

"adequate" or in relatively la[illegible]er quantities, [illegible] mechanism triggering the atrophy of BAT ther[illegible]sis during pregnancy may be subdued or delayed. [illegible] conceivable that the mechanisms involved i[illegible]ating energy balance are also in turn influenced b[illegible]eed back mechanisms associated with body reserves

From the data on energy intake and expenditure obtained in the indirect calorimetry study, along with the fat and energy content data from carcass analysis, the findings indicate that energetic efficiency was not increased during pregnancy.

4. THE EFFECTS OF DIET MANIPULATIONS ON PREGNANCY IN THE SYRIAN HAMSTER

4.1 Introduction

Dietary fat content (Wade 1982; Wade and Bartness 1983a), photoperiod (Wade 1983; Wade and Bartness 1983a) and exercise (Sandretto and Tsai 1988; Tsai et al 1981) have all been shown to effect body fat content in the Syrian hamster. High fat diets have been shown to increase body fat without any increase in food consumption. This increase in body lipid is mainly attributed to a decrease in energy expenditure (Wade 1982; Wade and Bartness 1983a). At the same time, the thermogenic capacity of brown adipose tissue has paradoxically been shown to increase in animals fed a high fat diet (Wade 1982).

During pregnancy, however, the Syrian hamster loses a significant amount of body fat despite a reduction in brown adipose tissue thermogenesis (Wade et al 1986). Thus if the animals are given a high fat diet during pregnancy, the loss of maternal body fat might be predicted to be minimized. Since a high fat diet is expected to increase metabolic efficiency, it would appear that metabolic efficiency during pregnancy should

also increase, perhaps by means of a decrease in energy expenditure. By the same token, because a high fat diet has been shown to increase brown adipose tissue thermogenesis, while pregnancy suppresses thermogenesis, a high fat diet could minimize the suppressive effects of pregnancy on brown adipose tissue.

The purpose of the experiments in this chapter was to evaluate the effects of a high fat diet on pregnancy in the Syrian hamster. Two formulated diets were used; one with a high fat content, 25% by weight or approximately 47.6% of energy content, and a low fat diet, 4% by weight or approximately 9.37% of energy content. Since the Syrian hamster has demonstrated the ability to maintain constant caloric intake when presented with food of different caloric density (Silverman and Zucker 1976; Wade 1982), the diets were formulated so as to give a constant nutrient density based on energy content. In other words, both high and low fat diets have the same nutrient content, in terms of vitamins and minerals, per calorie consumed.

An adjustment period is not possible due to the obvious biases it would introduce; since the High Fat group is expected to gain a significantly greater amount of body fat content relative to that of the Low Fat group, an adjustment period would result in the 2 groups having different weight and fat content even

before the start of the experiment. A third diet, the regular stock diet was also evaluated, to give an indication of the effects of adjustments and palatability of the experimental diets on pregnancy.

4.2 Materials and Methods

Animals and Housing

As previously stated.

Experimental Procedures

Two studies were performed. One study was on the effects of a high fat diet (25% fat w/w) versus a low fat diet (4% fat w/w). The other study was on the effects of the high fat diet (25% fat w/w) versus the regular laboratory chow diet ("Rodent Blox" Min. crude fat 4% w/w; refer to Table 2.1).

Study #1: Female hamsters purchased from Charles River (Quebec, Canada) were housed singly in acrylic cages under the same conditions as previously described. Estrous cycle was timed as before. The hamsters were then divided into 5 groups, with 8 animals in each group. One group was used as the initial baseline control. Two groups were assigned to a formulated low fat diet while the remaining two groups were assigned to a formulated high fat diet. These were

then subdivided into either a pregnant or virgin control group within each of the two diet groups. As in the previous chapter, assignments of these animals were based on matched base-line body weight and randomly distributed throughout the 4-day cycle. On Day 4 of their cycle, the animals assigned to the baseline virgin control group were killed by cervical dislocation, the other animals on the same cycle were either mated (approximately 1800 to 2400 hour) or left undisturbed, depending on the group to which they were assigned. At approximately 2400 hour all the animals on the same cycle, both mated and virgins, were then housed in acrylic cages with wire-mesh "false bottoms" lined with absorbent paper and given their respective assigned low or high fat diet. This procedure was repeated for the next consecutive 3 days. Food intake and body weight were taken every 2 days. Feces were collected throughout the study. The experimental pregnant animals were killed by cervical dislocation on Day 15 of pregnancy, along with the accompanying virgin controls (on the same cycle) at approximately 1600 hour, which is approximately 15 hours before parturition. A substantially reduced intake in the Low Fat group resulted during the first few days of pregnancy and this probably led to abortion in 3 of the pregnant hamsters.

They were promptly replaced but of these one also aborted, leaving a sample size of 7 for this group.

Study #2: All procedures and numbers of animals used were as in Study #1. The animals used in this study were matched for weight and age to those of the first study. The same formulated high fat diet was used. The other group, however, was given the usual laboratory chow pellets, i.e. there was no change in their diet. One of the animals in the High Fat pregnant group apparently aborted, leaving a sample size of 7 for this group. Food intake and body weight measurements were made daily.

Carcass Analysis and Brown Adipose Tissue Measurements

As previously described in Chapter 3.

Formulated High and Low Fat Diet

Components of both diets are listed in Table 4.1. The protein source used was casein. The gross energy content of the diet was measured by bomb calorimetry. (For Stock Diet refer to Table 2.1)

Digestible and Metabolizable Energy

Fecal matter was collected throughout the whole 15 days for all groups (Figure 31). Gross energy content was assessed by bomb calorimetry. Digestible energy intake was calculated by subtracting the total energy content of feces from the total energy content of

TABLE 4.1 COMPOSITION OF THE FORMULATED HIGH FAT AND LOW FAT DIET

Nutrient	Grams per 1000 g	Density g per 1000 kcal
Low Fat Diet (4% fat w/w):		
Fat[a]	40	10.59
Protein(Casein)	230.1	61
Glucose	177.3	47
Vitamin mix*	8.6	2.3
Mineral mix*	43.5	11.55
Choline	3.3	.9
Inositol	5.2	1.4
L-Methionine	2.2	.6
Cornstarch	447.6	118.6
Non-nutri.Cellu.	42.19	11.17
High Fat Diet (25% fat w/w):		
Fat[b]	250	52.95
Protein(Casein)	287.9	61
Glucose	221.8	47
Vitamin mix*	10.8	2.3
Mineral mix*	54.5	11.55
Choline	4.2	.9
Inositol	6.6	1.4
L-Methionine	2.8	.6
Cornstarch	108.7	23.02
Non-nutri.Cellu.	52.7	11.16

[a]Fat: 10% linseed oil (4 g)
90% corn oil (36 g)
% Fat as energy -9.4%

[b]Fat: 1.6% linseed oil (4 g)
14.4% corn oil (36 g)
84% beef tallow (210 g)
% Fat as energy=47.6%

*Vitamin and mineral mixture; Teklad Diets, Harlan Sprague Dawley Co. Inc., Madison, WI. USA

food consumed per animal. Metabolizable energy was calculated by multiplying digestible energy by 0.96 (Mercer and Trayhurn 1987; Barr and McCracken 1984).

Statistical Analysis

The statistical significance of differences between groups was assessed using Student's t-test and where indicated, using 2-way analysis of variance (ANOVA) based on a complete and balanced factorial design. In order to balance some of the data (i.e. Low fat pregnant group: n=7), the mean of that group was used as an added data point (Statistical Consultant, Computing Services, University of Alberta).

4.3 Results and Discussions

The results for Study 1 are summarized in Table 4.2. There were no differences in food consumption between the pregnant and virgin control groups in the Low Fat diet (Figure 16). The High Fat pregnant group appeared to have a slightly lower food intake than the control group but this difference was not statistically significant. The differences in energy consumed between the two diets were, however, statistically significant. It thus appears that, considering that there were no differences in food consumption between virgin and

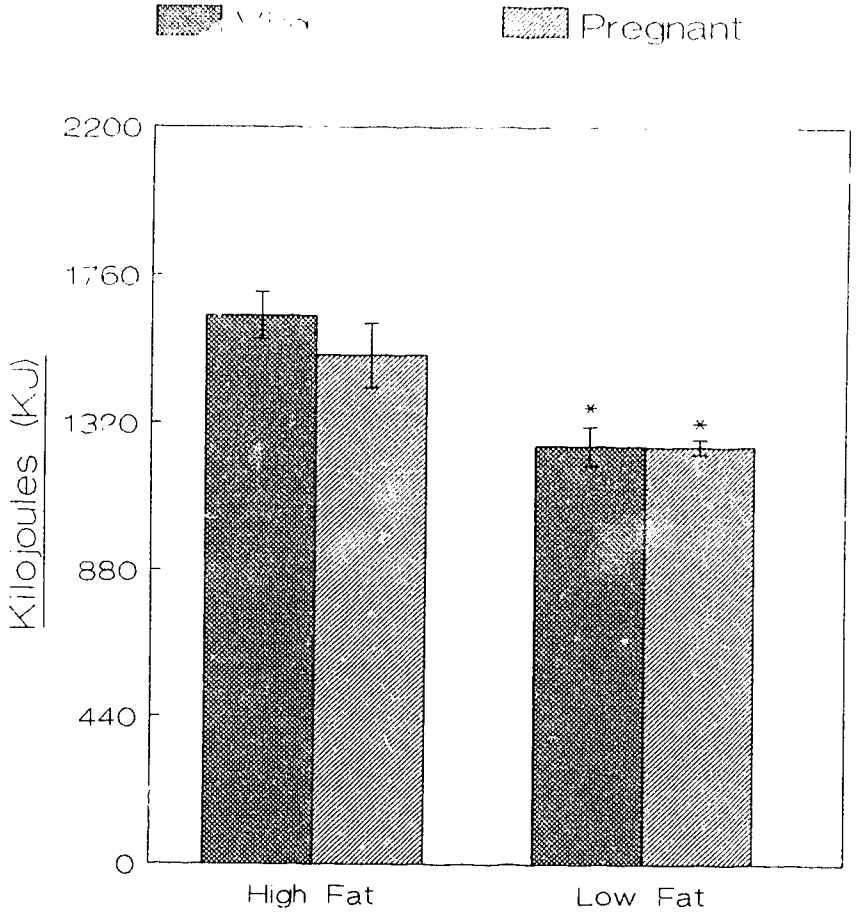
TABLE 4.2 HIGH VS LOW FAT DIET: EFFECTS ON BODY COMPOSITION AND BROWN ADIPOSE TISSUE

STUDY #1

	High Fat		Low Fat	
	Virgin n=8	Pregnant n=8	Virgin n=8	Pregnant n=7
ME Intake (KJ)	1640.56 ± 69.74	1522.42 ±95.22	1253.23* ± 58.17	1251.87* ±22.66
Carcass Energy (KJ)	814.22 ±42.04	573.73 [a] ± 39	586.69* ±39.61	428.38[a]** ±27.8
Body Fat (g)	8.82 ±0.91	3.39 [a] ±0.86	3.02** ±0.66	0.87[a]* ±0.34
Interscapular BAT:				
Wt.(mg)	196.6 ±8.7	126.5 [a] ±8.8	165.4 ±13	75.2[b]** ±6.4
Cytochrome Oxidase Activity (uM/min)	61.01 ±4.7	24.75 [b] ±2.34	50.51 ±2.81	15.91[b]* ±2.27
Fetuses:				
No.	--	11.4 ±0.5	--	13.0 ±0.4
Wt.(g)	--	34.0 ±1.4	--	33.1 ±2.4
Total Energy Content(KJ)	--	78.3 ±3.3	--	73.4 ±4.6
Total Fat Content(g)	--	0.17 ±0.02	--	0.16 ±0.03

Values are means ± SE ; ME - Metabolizable Energy
a : P < 0.05 vs virgins ; b : P < 0.001 vs virgins
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 16: Metabolizabl[illegible] Intake-High vs Low Fat



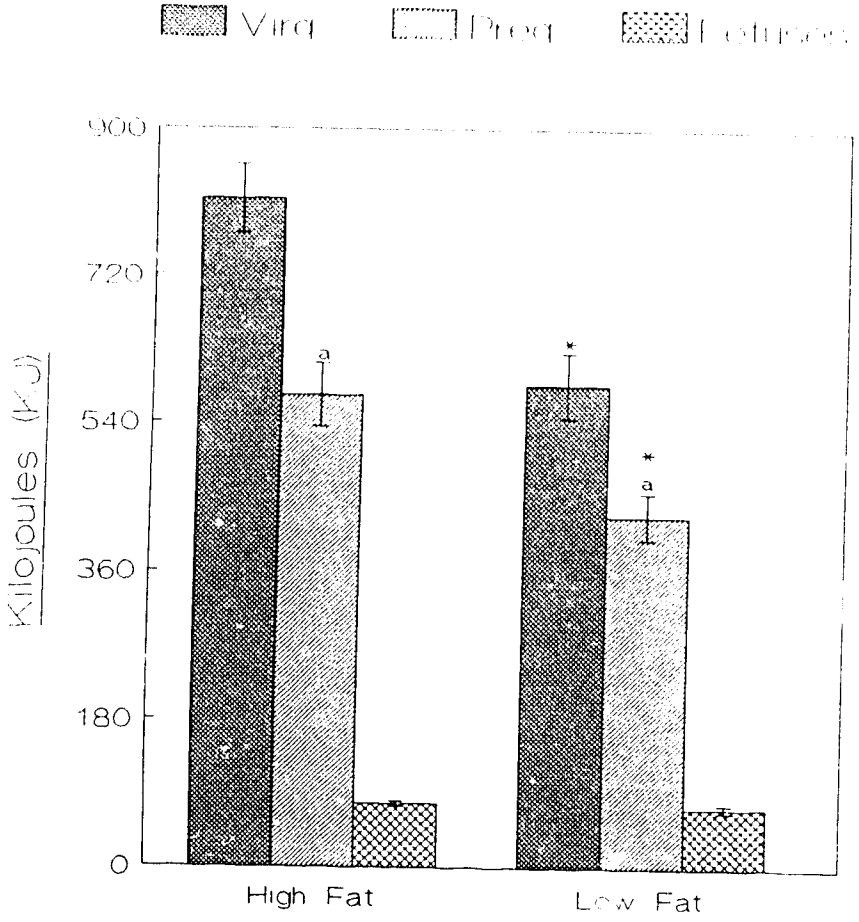
Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

pregnant groups, palatability of the two diets may have an influence on the difference in intake especially during the initial period.

In both the High Fat and Low Fat groups, pregnancy significantly decreased total energy content (Figure 17), total fat content (Figure 18), brown adipose tissue weight (Figure 19) and cytochrome oxidase activity (Figure 20), compared to the virgin control groups.

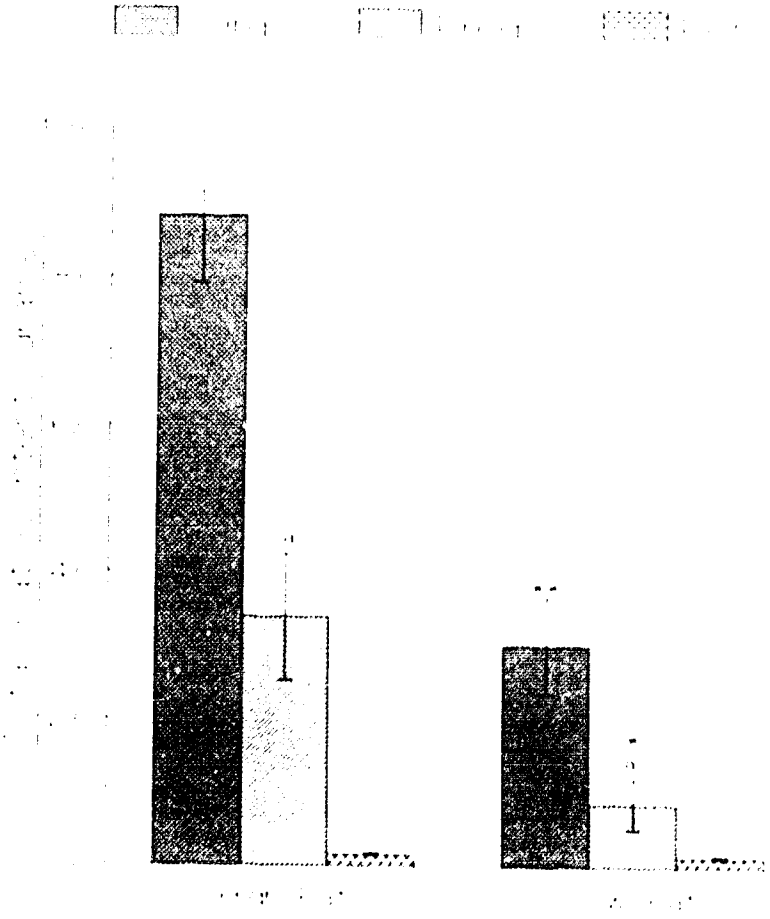
The High Fat diet, however, appeared to suppress the effects of pregnancy on BAT weight and cytochrome oxidase activity, as the BAT weight ($P < 0.001$) and cytochrome oxidase activity ($P < 0.05$) were significantly lower in the Low Fat pregnant group than in the High Fat pregnant group (Table 4.2). Nevertheless, it could be argued that the increased food consumption in the High Fat pregnant group may contribute to the attenuated suppression of BAT weight and cytochrome oxidase activity. On the other hand, the difference in diets did not affect the BAT and cytochrome oxidase activity of the control groups significantly although the food consumed is significantly different ($P < 0.05$). Thus, this may suggest that perhaps it takes a longer time to see the

Fig 17: Energy Content
High vs Low Fat



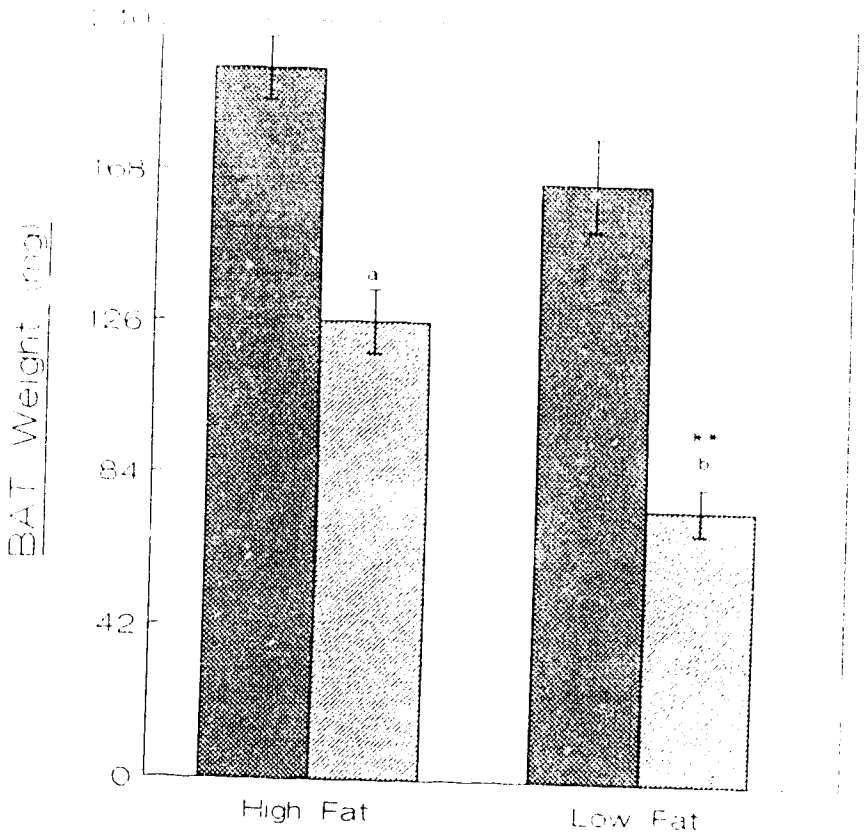
Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 18: Carcass Fat
High vs Low Fat



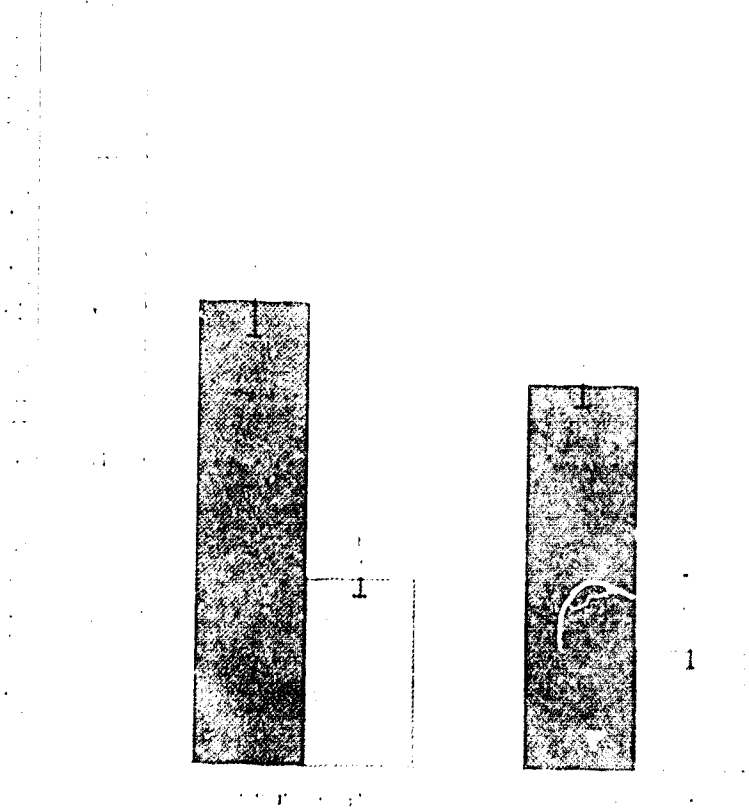
Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 19: BAT Weight
High vs Low Fat Diet



Value are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 20:Cytochrome Oxidase
High vs Low Fat Diet



Values are means ± SE (bars). Group size=
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

effects of the diet on virgin controls but with pregnancy the effects of a High Fat diet may be more pronounced.

Despite the low intake and virtually complete depletion of maternal body fat in the Low Fat pregnant group, it is interesting to note that none of the fetal parameters were affected. The number of fetuses and their weight, total energy content and total fat content were virtually identical to those of the High Fat group. Thus, it would seem to indicate that regardless of the low level of voluntary food intake, and substantial depletion of maternal body reserves, the fetuses are virtually unaffected (Table 4.2).

The results of Study 2 are shown in Table 4.3. The groups fed the regular laboratory stock pellet diet had a higher energy consumption than those fed the formulated High Fat diet (Figure 21). This is probably due to the fact that the High Fat group had to adjust to the new diet, resulting in a decrease in consumption during the initial period whereas the Stock diet group had relatively no adjustment to make (Figure 22). There were no differences between the pregnant and virgin groups consuming the Stock diet. Similar to the High Fat pregnant group in the previous study, the pregnant group in the present study had a lower intake compared to the

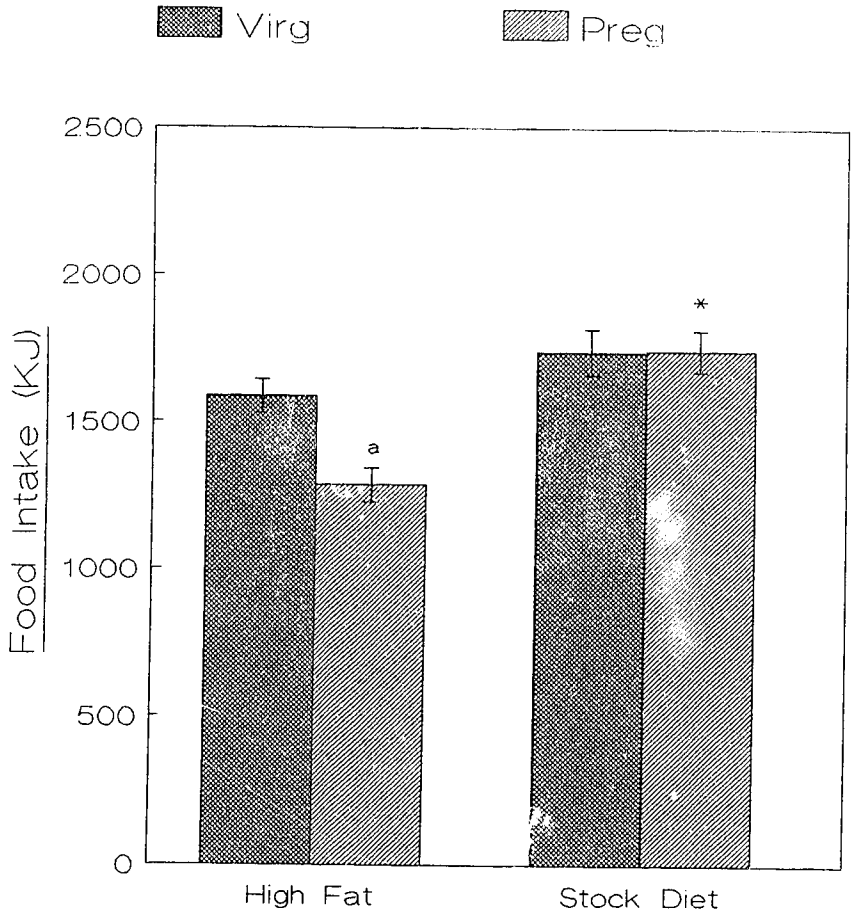
TABLE 4.3 HIGH FAT VS STOCK DIET: EFFECTS ON BODY COMPOSITION AND BROWN ADIPOSE TISSUE

STUDY #2

	High Fat		Stock Diet	
	Virgin n=8	Pregnant n=7	Virgin n=8	Pregnant n=8
ME Intake (KJ)	1586.50 ± 57.12	1284.38 [a] ±58.39	1740.03 ± 76.71	1748.23* ±69.96
Carcass Energy (KJ)	904.82 ±43.13	592.41 [b] ±40.92	876.41 ±84.07	701.64 ±41.94
Body Fat (g)	10.33 ±0.86	4.18 [a] ±0.89	8.94 ±1.81	4.98 ±0.79
Interscapular BAT:				
Wt.(mg)	232.0 ±15.8	127.2 [b] ±12.8	228.3 ±20.9	126.1 [a] ±9.7
Cytochrome Oxidase Activity (uM/min)	72.35 ±5.0	22.96 [b] ±3.24	80.74 ±11.9	27.2 [a] ±1.74
Fetuses:				
No.	--	9.4 ±1.1	--	12.0 ±0.6
Wt.(g)	--	26.9 ±1.7	--	41.0 ** ±2.2
Total Energy Content(KJ)	--	59.3 ±3.7	--	91.1 ** ±4.2
Total Fat Content(g)	--	0.17 ±0.02	--	0.19 ±0.02

Values are means ± SE ; ME = Metabolizable Energy
a: $P < 0.05$ vs virgin ; b : $P < 0.001$ vs virgin control
*: $P < 0.05$ vs corresponding High Fat Group
**: $P < 0.001$ vs corresponding High Fat Group

Fig 21: Metabolizable Intake-High Fat vs Stock



Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

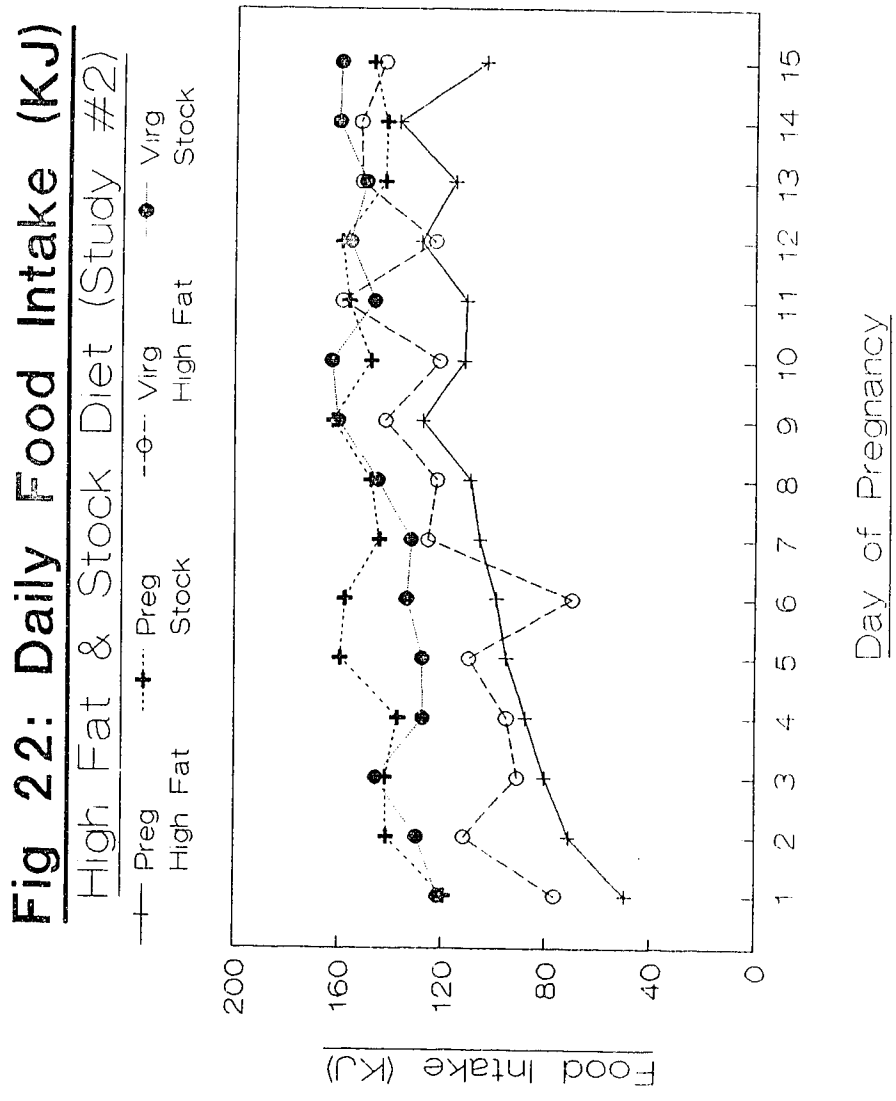
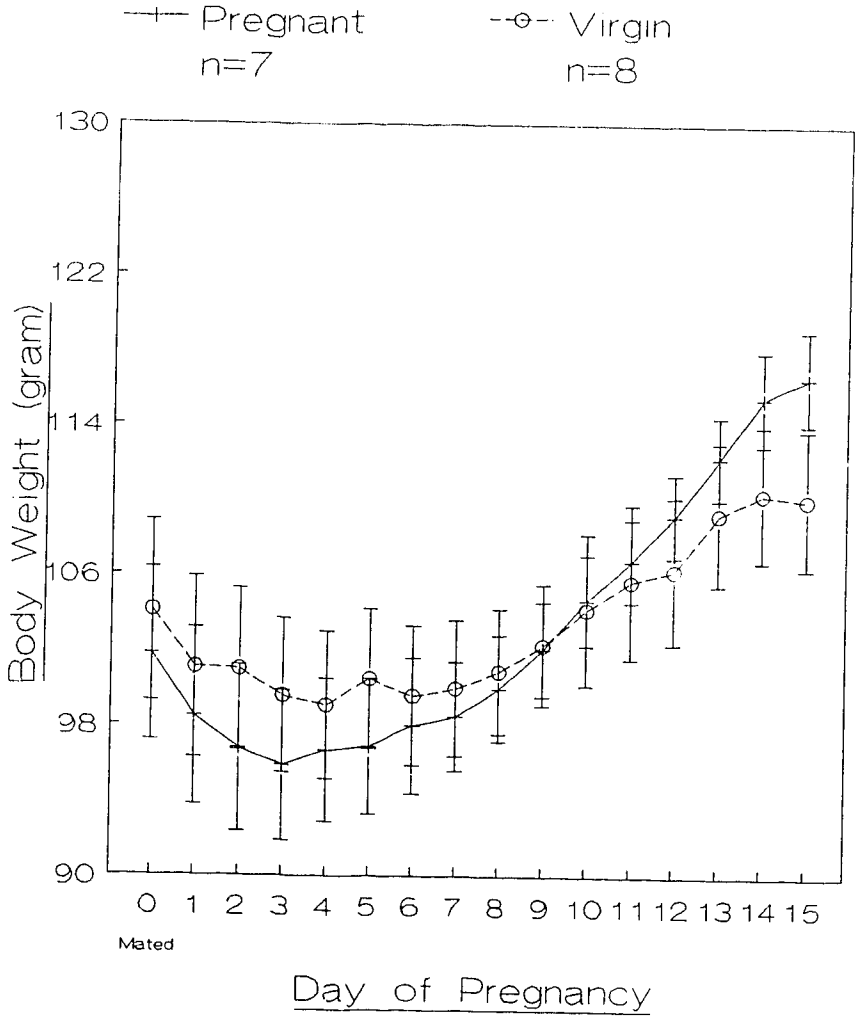


Fig 22: Daily Food Intake (KJ)
High Fat & Stock Diet (Study #2)
Preg High Fat
Preg Stock
Virg High Fat
Virg Stock
Food Intake (KJ)
200
160
120
80
40
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Day of Pregnancy

Fig 23: Body Weight

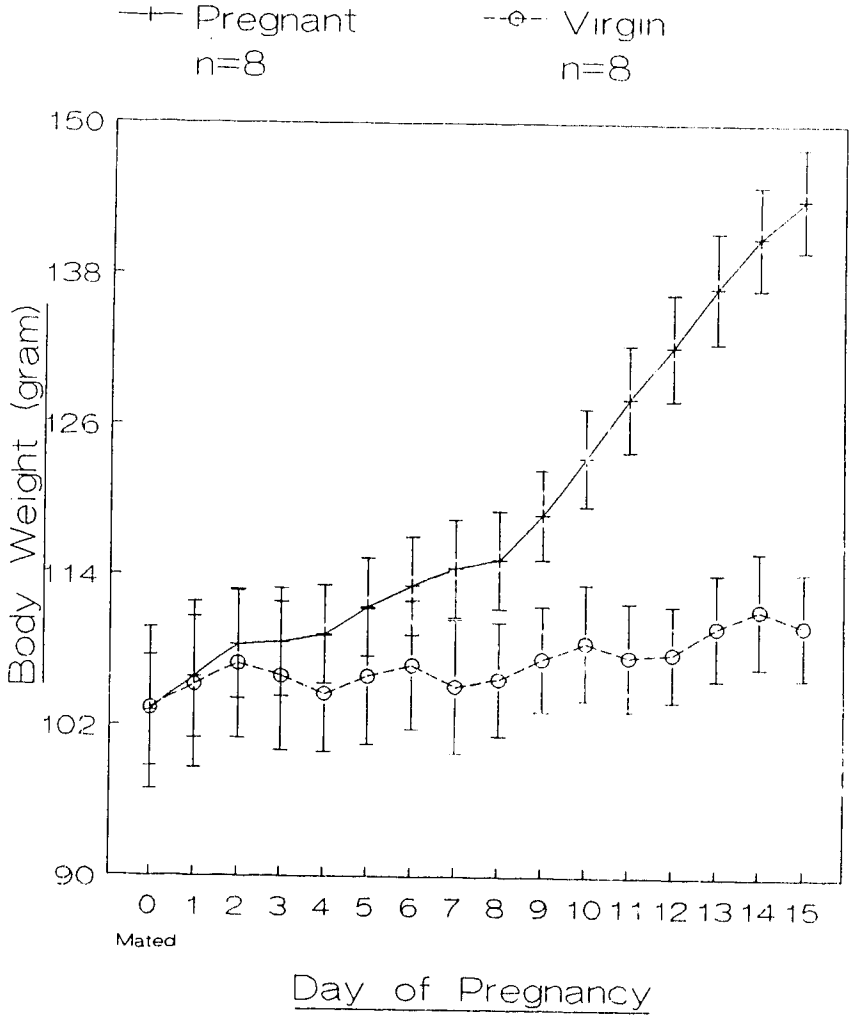
High Fat Diet (Study #2)



Values are means ± SE (bars)
n = no. of animals

Fig 24: Body Weight

Stock Diet (Study #2)

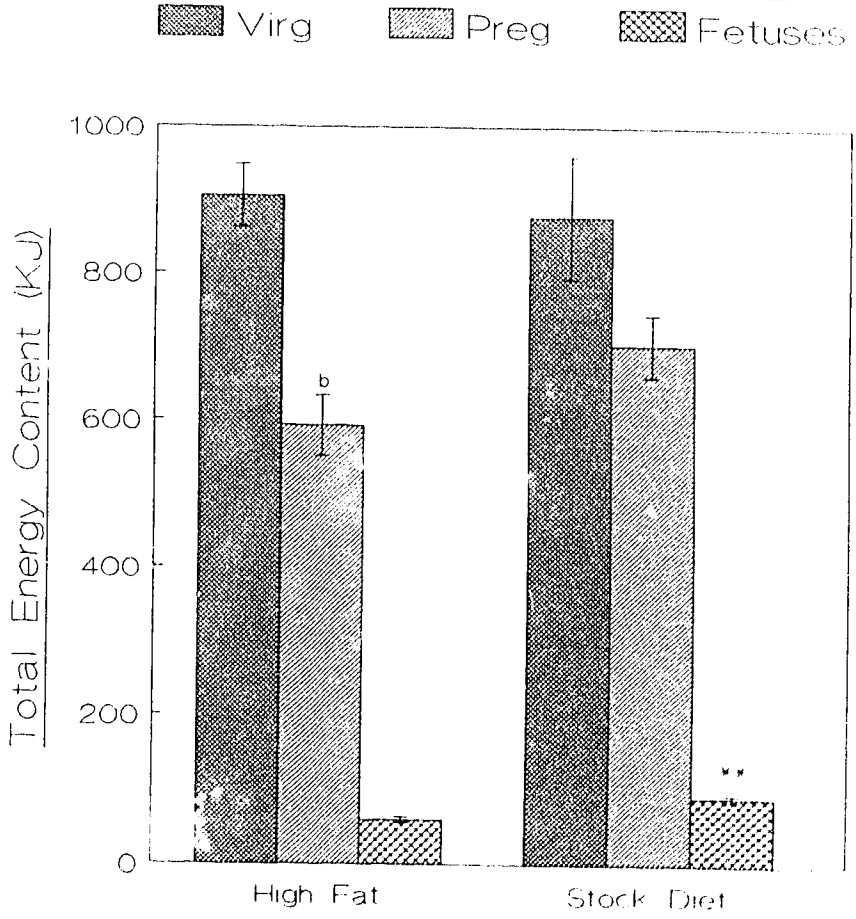


Values are means ± SE (bars)
n = no. of animals

virgin control group, however, the difference in this case was statistically significant ($P < 0.05$). Considering the short experimental time involved and the initial adjustment required, the difference in energy consumption probably reflects the initial decrease in food intake (Figure 22). The changes in body weight for both High Fat and Stock diet groups are shown in Figure 23 and 24, respectively. There was virtually no difference between the weight of the pregnant and control group fed the High Fat diet, Figure 23, evidently due to the decrease in food intake.

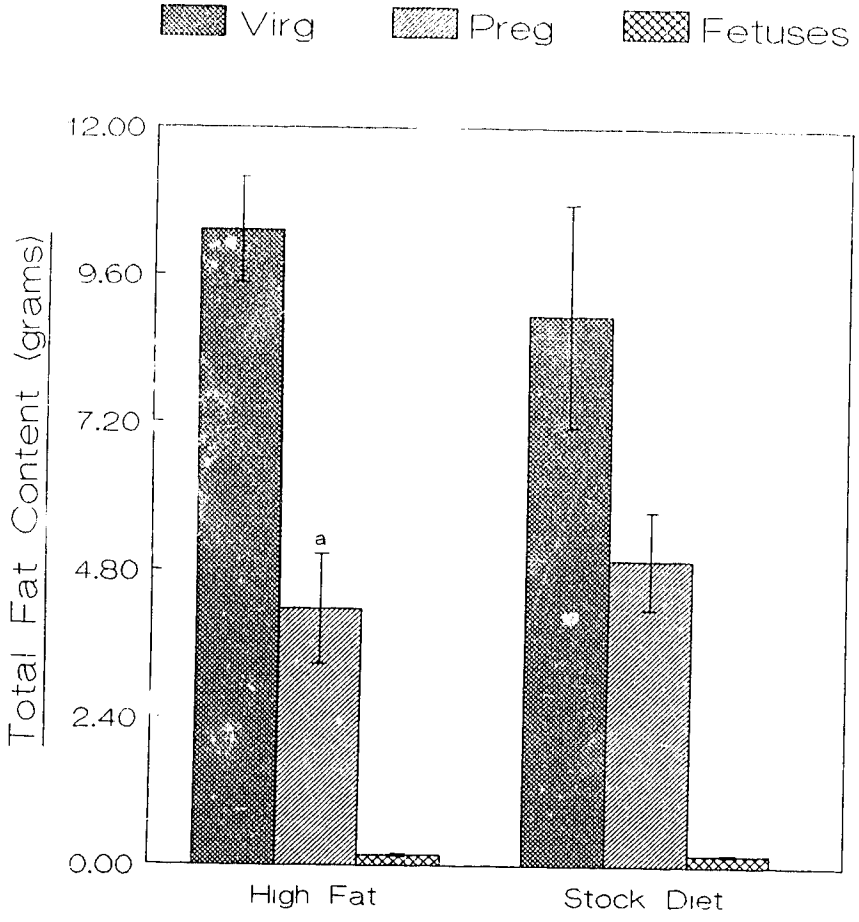
Total energy (Figure 25) and fat content (Figure 26) of the pregnant group was significantly lower than the control in the High Fat diet groups. However, the differences between the pregnant and virgin groups fed the Stock diet were not statistically significant although the decrease in the pregnant group is apparent. Nevertheless, BAT thermogenesis is significantly suppressed in the pregnant groups on both diets by virtually the same amount (Table 4.3), ie both BAT weight (Figure 27) and cytochrome oxidase activity (Figure 28) were virtually equivalent in both pregnant groups fed the two diets. This appears to support the suggestion that the suppression of BAT thermogenesis is regulated, or influenced perhaps more strongly, by

Fig 25: Carcass Energy High Fat vs Stock Diet



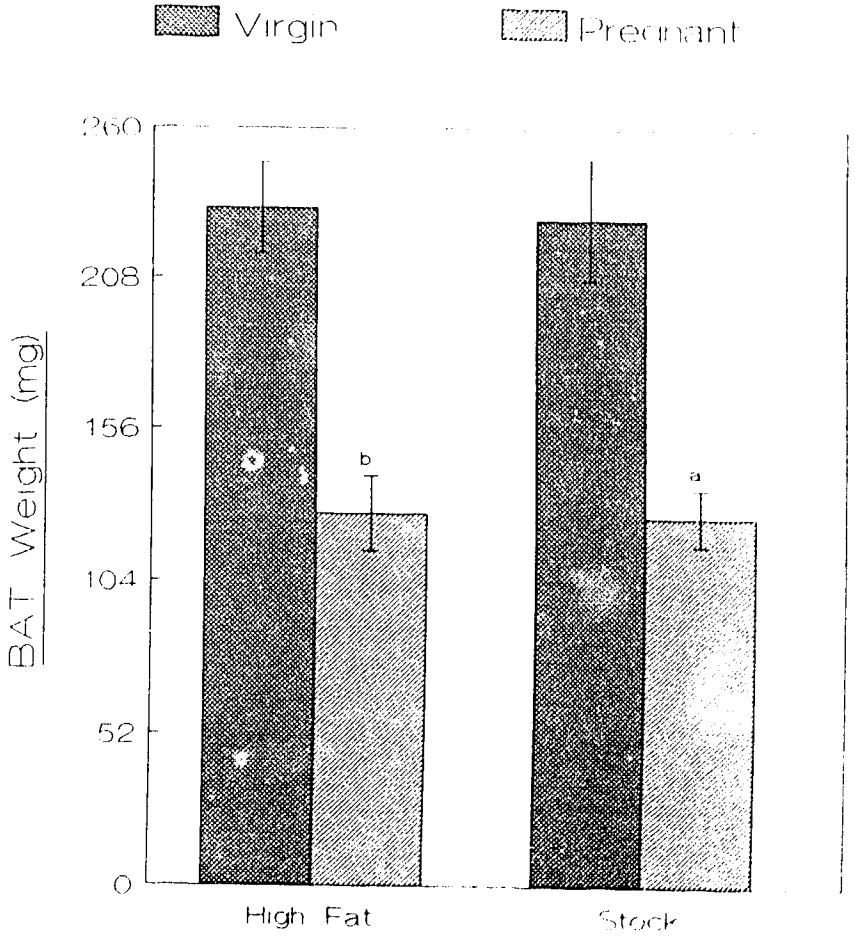
Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 26: Carcass Fat
High Fat vs Stock Diet



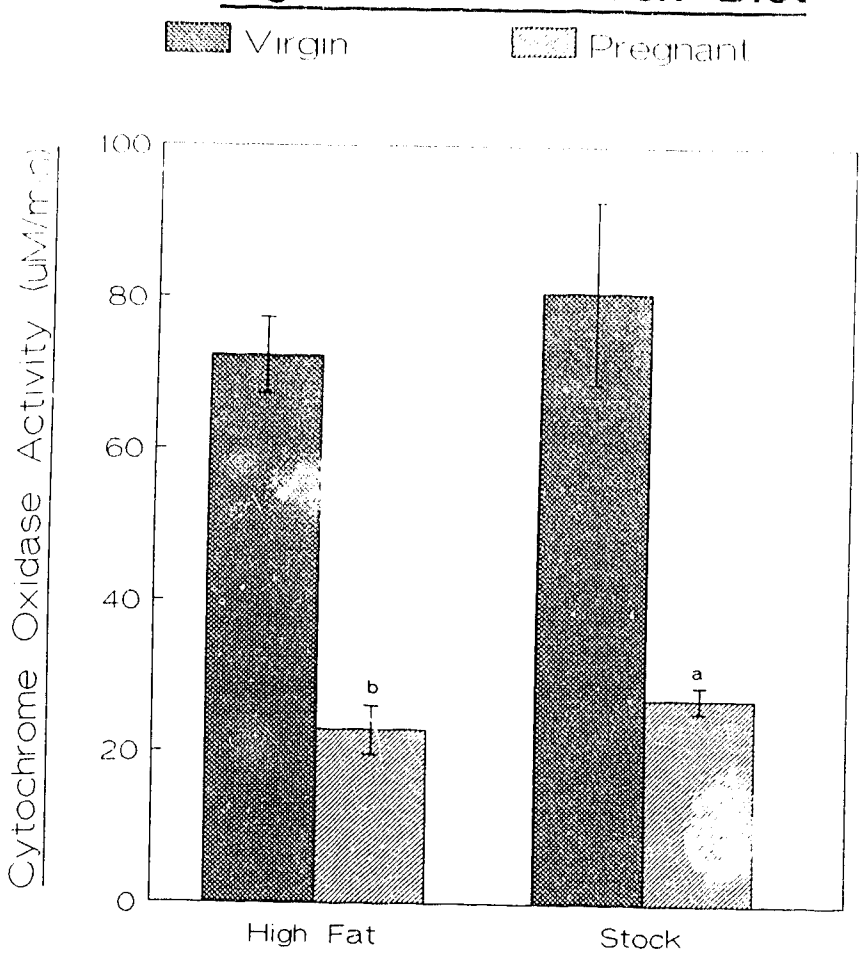
Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 27: BAT Weight
High Fat vs Stock Diet



Values are means ± SE (bars); Group size-7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

Fig 28:Cytochrome Oxidase
High Fat vs Stock Diet



Values are means ± SE (bars); Group size=7 to 8 animals.
a : P < 0.05 vs Virgin Controls
b : P < 0.001 vs Virgin Controls
* : P < 0.05 vs corresponding High Fat Group
** : P < 0.001 vs corresponding High Fat Group

factors other than the physiologically imposed nutritional stress, in view of the significant ($P < 0.05$) differences in energy consumption and fat gain between the two pregnant groups.

The energy consumed by the virgin High Fat diet group was slightly lower than that of the Stock diet group; however, carcass energy and fat content of the High Fat group were slightly higher. This seems to suggest that high fat diets increase metabolic efficiency in the virgin control groups, which would be in agreement with other published results (Wade 1982). The findings are, nevertheless, statistically insignificant. This is probably due to the relative short duration of the experiment compared to those of Wade (1982). Since there seems to be a trend, a longer exposure may result in obesity in the high fat group.

Nonetheless, the pregnant groups in Study 2 show a significant difference ($P < 0.05$) in energy intake, while carcass fat and energy content were not significantly different between the two pregnant groups on different diets. This may, thus, indicate that energetic efficiency is increased in the pregnant High Fat group.

Although there appears to be no significant differences in the maternal energy and fat content of both pregnant groups on these two diets, the fetuses of

the Stock diet group were significantly ($P < 0.001$) heavier and had a greater energy content compared to the fetuses of the High Fat group. This may be partially attributed to the significant differences in food consumed (Table 4.3). Perhaps food deprivation, voluntary or otherwise, during embryogenesis in early gestation may trigger some negative influences on the fetuses. On the other hand, this may be a reflection of the total decrease in food consumed during pregnancy. Since fat content is not significantly different while the energy content appears higher in the Stock diet group fetuses, it would seem that the increase in energy content is due to an increase in protein deposition. Protein content was not, however, directly measured.

Total fetal fat content of both the High Fat and Stock diet groups was essentially equivalent. Interestingly, all 4 groups (Study #1 and #2) have virtually identical amounts of fetal fat regardless of maternal intake or body reserves. This may indicate that fetal fat deposition is tightly regulated by mechanisms, genetic, hormonal or otherwise, unrelated to maternal intake or reserves and thus "environmental" influences.

In Figures 29 and 30, the Baseline virgin group was used to estimate the initial carcass energy and fat content of the animals. Tables 4.4 and 4.5 show

Fig 29: Baseline Virgins

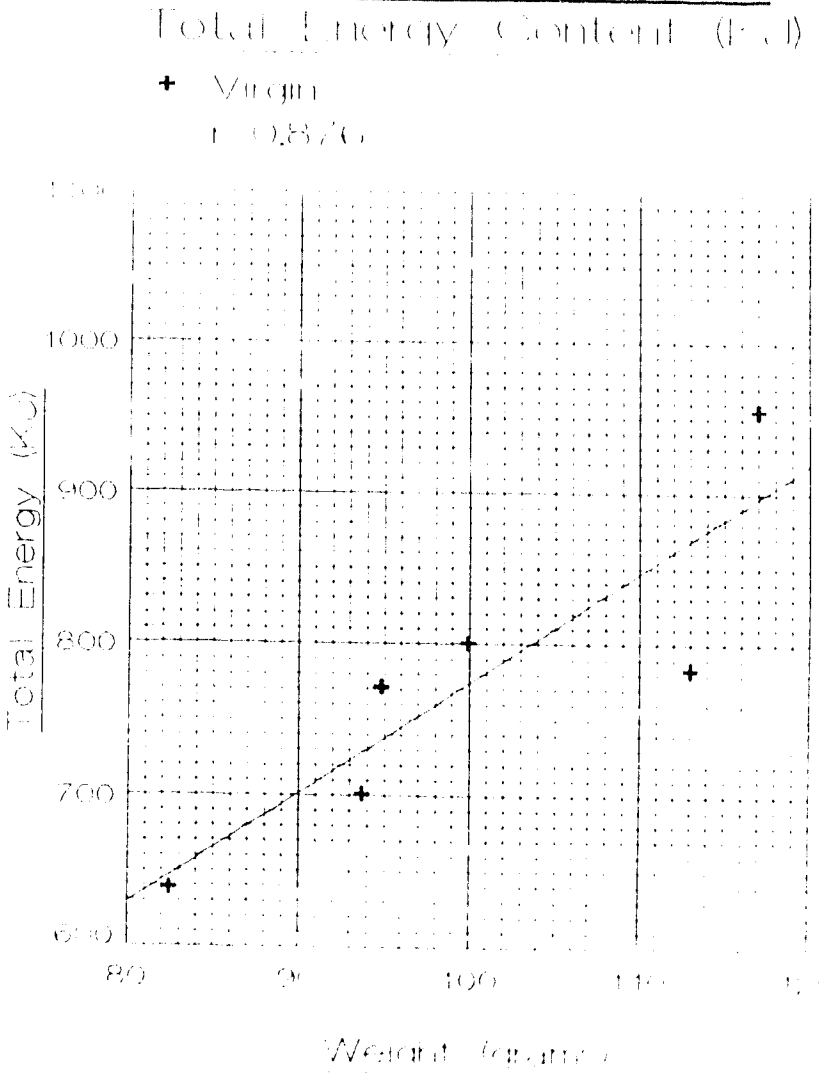


Fig 30: Baseline Virgin

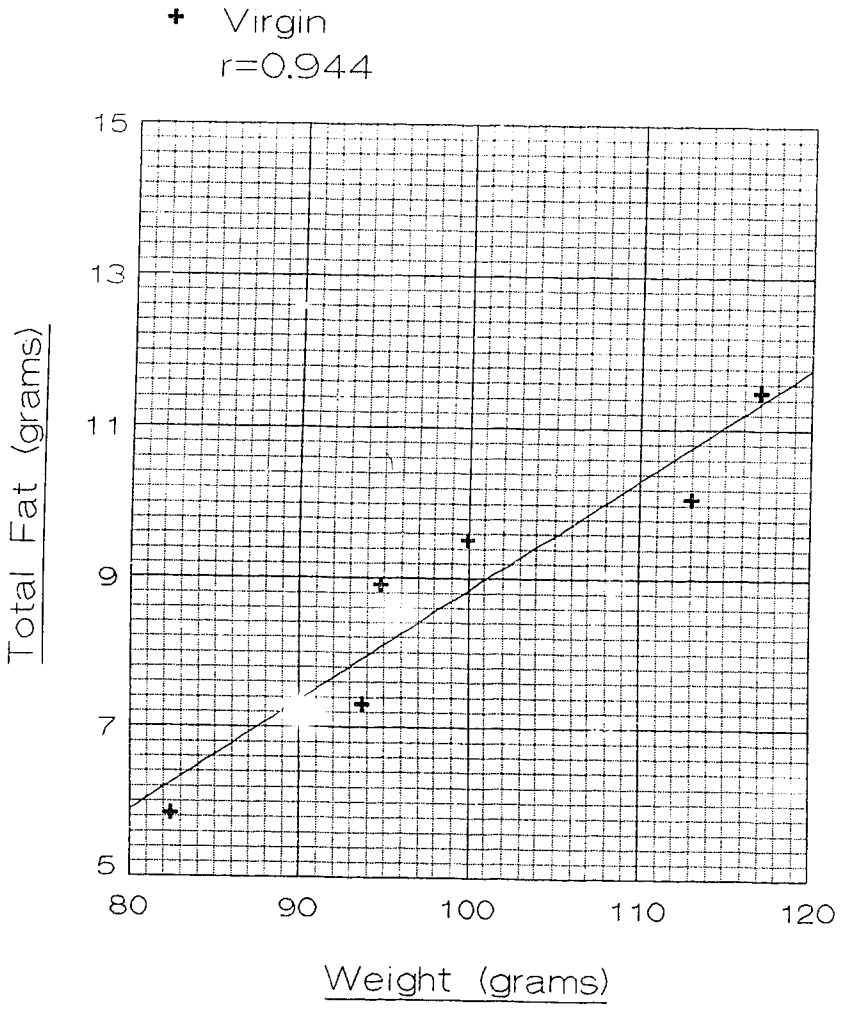


TABLE 4.4 EFFECTS OF PREGNANCY AND DIET ON ENERGY BALANCE AND BODY FAT IN SYRIAN HAMSTERS

Study #1:

	Pregnant		Virgin	
Low Fat Diet:				
	Energy (KJ)	Fat (g)	Energy (KJ)	Fat (g)
Init.	853.1±30.1	10.5±0.6	830.6±11.9	10±0.3
Final	428.4±27.8		586.7±38.6	3.0±0.7
Loss	-425.0±38.2	.7	-244.0±31.3	-7.0±0.5
Fetuses	73.3±4.6			
Net Loss:	-351.5 ±38.7		Difference: -107.5	-2.6
High Fat Diet:				
	Energy (KJ)	Fat (g)	Energy (KJ)	Fat (g)
Init.	834.7 ±21.8	10.1±0.5	840.0±18.2	10.3±0.4
Final	573.7 ±39	3.4 ±.9	814.2±42.0	8.8±0.9
Loss	-249.2±45.0	-6.4±1.0	-25.8±37.8	-1.4±.8
Fetuses	78.3±3.3			
Net Loss:	-170.2 ±48.0		Difference: -144.4	-5.0

Values are means ± SE.
Initial energy content and body fat are estimated from the Baseline Virgin group, Graphs 4K and 4L.

TABLE 4.5 EFFECTS OF PREGNANCY AND DIET ON ENERGY BALANCE AND BODY FAT IN SYRIAN HAMSTERS

STUDY #2

	Pregnant		Virgin	
Stock Diet:				
	Energy (KJ)	Fat (g)	Energy (KJ)	Fat (g)
Init.	832.7 ±34.7	10.4±1.0	834.8+50.8	10.2±1.1
Final	701.6 ±41.9	4.98±0.8	876.4 ±84.1	9±1.8
Loss	-148.6 ±30.8	-5.5±0.6	+41.6±65.6	1.2±1.3
Fetuses	91.2 ±4.2			
Net Loss:	-61.2 ±30.7		Difference: -102.8	-4.3
High Fat Diet:				
	Energy (KJ)	Fat (g)	Energy (KJ)	Fat (g)
Init.	837.9 ±35.1	10.1±0.7	844.9 ±40.0	10±0.8
Final	592.4 ±40.9	4.2 ±0.9	904.8 ±43.1	10.3±0.8
Loss	-231.0±59.6	-5.7±1.3	+91.3±28.0	0.1±0.8
Fetuses	59.3 ±3.7			
Net Loss:	-171.6±57.8			

Values are means ± SE
Initial energy content and body fat are estimated from the Baseline Virgin group, Graphs 4A and 4B.

the energy balance and body fat depletion of the animals. Assuming that the pregnant group loses the same amount of carcass energy and fat due to the effects of the diet as the virgin control group, especially since there is no significant difference in food intake, then the amount of carcass energy and fat content lost due to the effects of pregnancy can be roughly estimated by subtracting the losses of the pregnant group from those of the control group. The difference should be the estimated losses due to pregnancy on that particular diet.

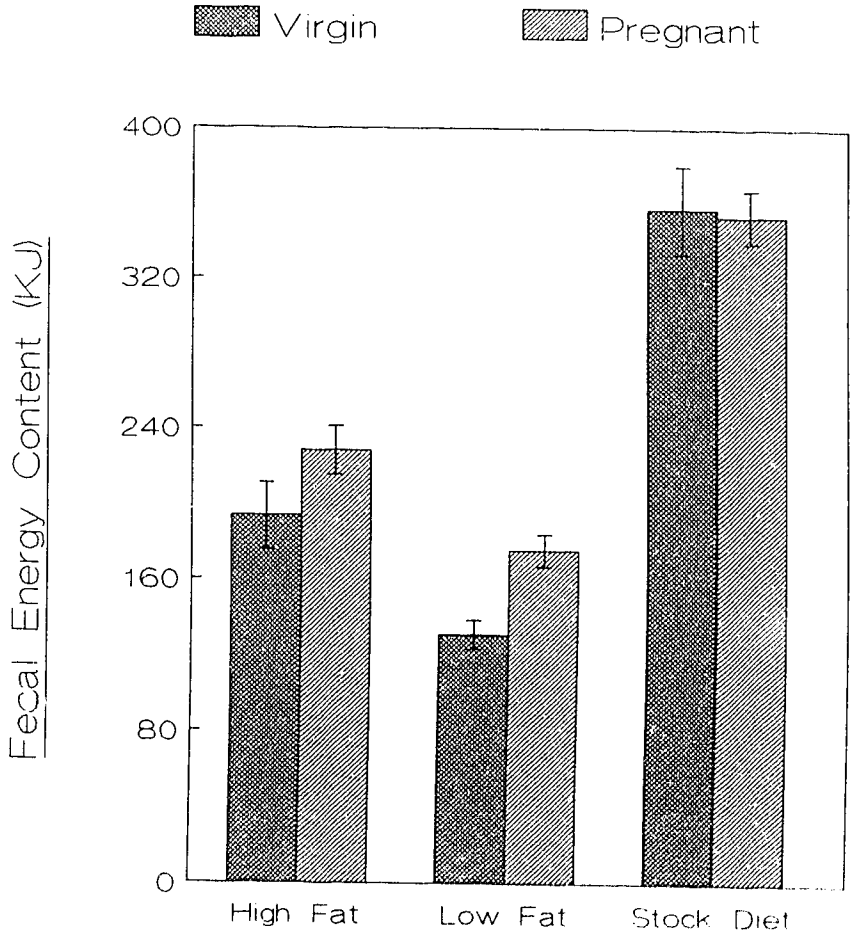
In the Low Fat fed animals (Table 4.4), the pregnant group, with fetal energy included, lost approximately 352 KJ of total carcass energy while the control group lost approximately 244 KJ, giving a difference of 108 KJ (Table 4.4). This "extra" amount lost by the pregnant group is probably the amount used to fund the cost of pregnancy. The difference in the High Fat group in Study #2 (Table 4.4) is not valid since the food intake was significantly lower in the pregnant group compared to the virgin control. Nevertheless, based on these estimates, it would seem that energy lost during pregnancy appeared to be slightly higher, 144 KJ, in the High Fat (Table 4.4, Study #1) group than those fed on a Low Fat or Stock diet, 108 KJ and 103 KJ respectively. It would also

appear that the energy utilized during pregnancy is very similar in both the Low Fat and Stock diet groups, even though the Stock diet group consumed significantly more and maternal reserves are not as depleted as in the Low Fat group. However, the amount of energy deposited in the fetuses is higher in the Stock diet group (fetal energy content 91 KJ). As discussed previously, this may be a reflection of the relative food deprivation. It may also indicate that the effects of a High Fat diet do not have any beneficial effects on fetal outcome, although the diet appears to increase the efficiency with which energy is stored in the maternal body.

Total fecal energy content from the different diets is shown in Figure 31. The digestible energy intake of the 3 diets are shown in Figure 32. The High Fat group shown is that of Study 1. The digestibility of the diets are given in Figure 33, which indicates that the formulated diets have a slightly greater digestibility than the Stock diet. However, the difference in food intake appears more substantial than the difference in digestibility.

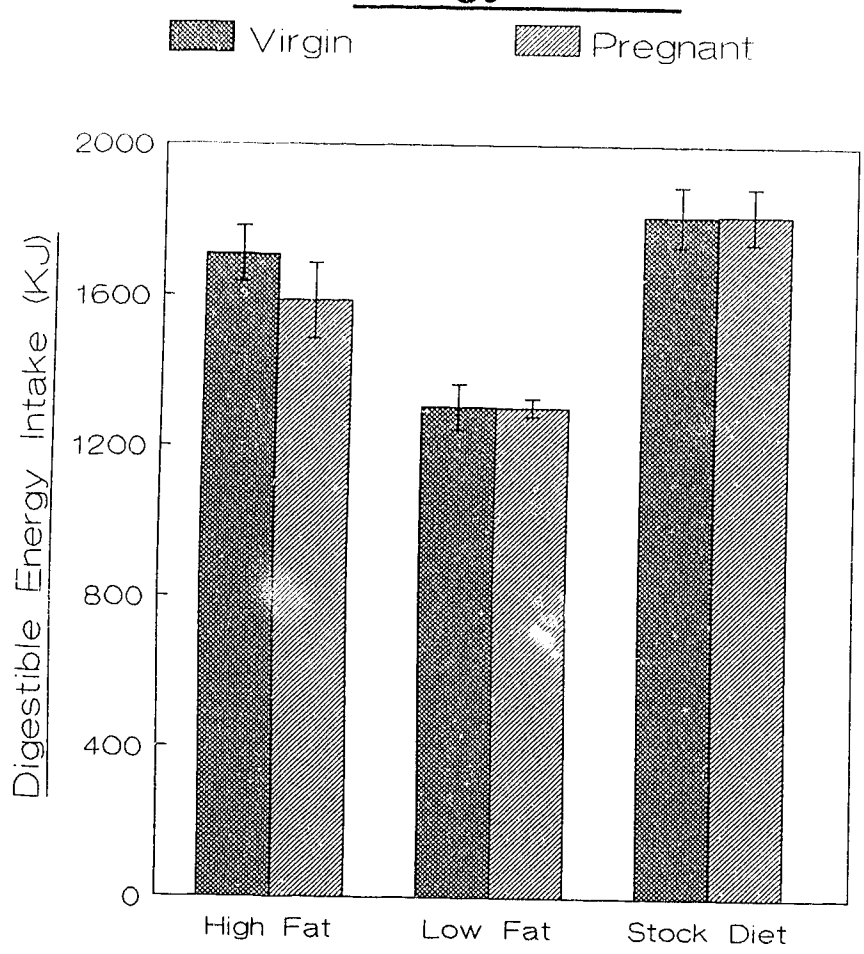
A 2-way analysis of variance was done to evaluate the effects of pregnancy, diet and the possible interactions between these two variables on food intake, carcass energy and fat content. Table 4.6 lists the F

Fig 31: Total Fecal Energy Content



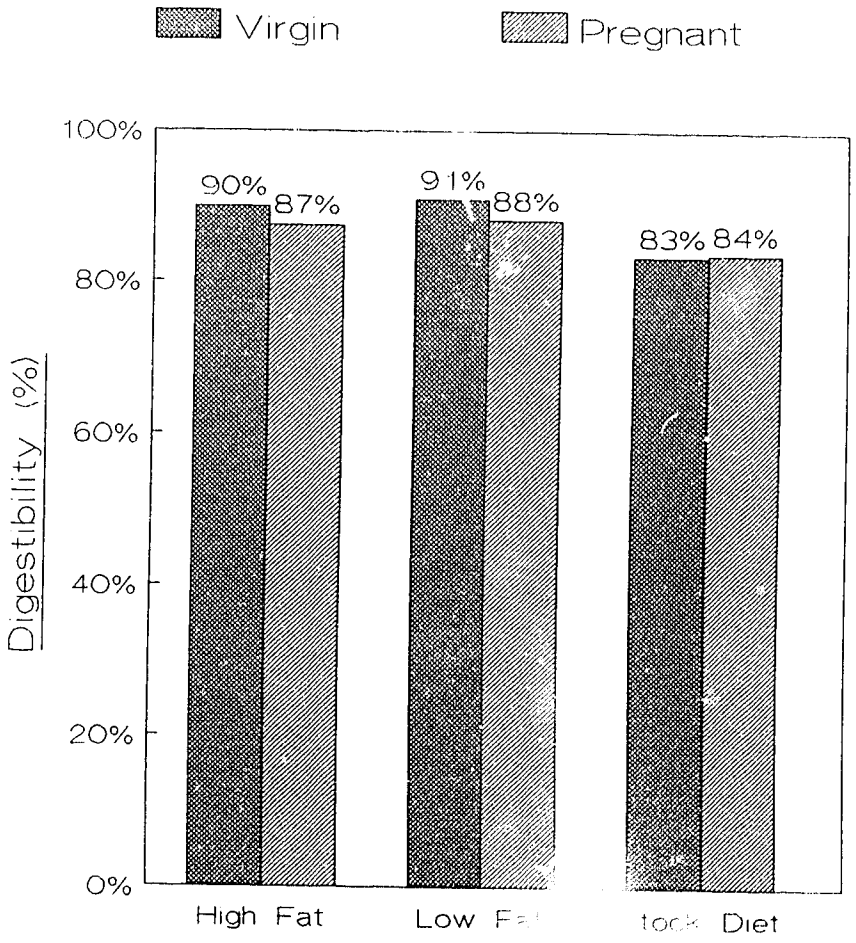
Values are means ± SE (bars)
Group size=7 to 8 animals.

Fig 32: Digestible Energy Intake



Values are means ± SE (bars)
Group size=7 to 8 animals.

Fig 33: Digestibility of Diets



Values are means ± SE (bars)
Group size=7 to 8 animals.

TABLE 4.6 SUMMARY OF 2-WAY ANALYSIS OF VARIANCE

Critical F value (F Table): $F_{0.05(1)\ 1,28} = 4.20$

	Low Fat vs High Fat		Stock vs High Fat	
	F value		F value	
Metabolizable Intake (KJ)				
Preg/Virg	0.26	NS	5.02	Signif.
Diet	24.20	Signif.	20.97	Signif.
Interaction	0.24	NS	5.21	Signif.
Carcass Energy (KJ)				
Preg/Virg	29.30	Signif.	37.41	Signif.
Diet	25.64	Signif.	0.04	NS
Interaction	1.23	NS	5.03	Signif.
Carcass Fat Content (g)				
Preg/Virg	27.62	Signif.	19.62	Signif.
Diet	33.29	Signif.	0.08	NS
Interaction	5.18	Signif.	0.92	NS

Calculation was done in APL language using the statistical package "STATPACK 3:STATPACK 700 ANOVAGP" from Computing Services Library, University of Alberta.

values obtained ($P < 0.05$, 1-tail test, degrees of freedom 1 and 28 respectively). As noted before, pregnancy "status" did not affect food intake in Study #1. In Study #2, food intake was affected by pregnancy. The type of diet significantly affected the amount of food consumed in both Study #1 and #2. In Study #1 this is probably due to the effects of palatability, a high fat diet being more palatable than a low fat diet. Study #2 probably reflects the adjustment required when a new high fat diet is given. Interaction of pregnancy status and diet was not significant in Study # 1. In other words, pregnancy and diets were independent factors in terms of food intake. In Study # 2, interaction of pregnancy and diet was significant, which means that they are dependent on each other, hence interpretations are difficult. If the factors are largely independent, the analysis of variance summarize the data well. When the factors are not independent, the data require a detailed study. The difficulty lies in the complex nature of the situation and not in the factorial approach to it (Steel and Torrie 1980).

Pregnancy has a definite effect on carcass energy in both studies. Thus, regardless of diet, pregnancy decreases maternal carcass energy significantly. In Study #1, the difference in diets apparently affects carcass energy and fat content

significantly. But in view of the fact that food intake was significantly different for the two diets no solid conclusions can be drawn. In Study #2, there was no significant difference in carcass energy or fat content between the 2 diets, which perhaps may suggest that the initial decrease in consumption in the High Fat group relative to the Stock diet, is balanced out by the increase in metabolic efficiency of the High Fat diet.

In terms of carcass fat content Study #1 shows a significant interaction between pregnancy and diet. In other words, there appears to be an interdependent relationship between diet and pregnancy. Perhaps this may suggest that in terms of carcass fat content the effects of a high fat diet attenuate the effects of pregnancy or conversely the effects of a low fat diet are synergistic to the effects of pregnancy. However, due to the differences in food consumed, firm conclusions cannot be drawn. A possible reason for not observing a significant interaction in Study 2 may be the substantially greater ($P < 0.05$) food intake of the Stock diet group, which has the low fat content, thereby counter-balancing the effects of the High Fat diet.

The interaction of pregnancy and diet on carcass energy seen in Study 2 appears to be a difference in the magnitude of response (Steel and Torrie 1980). In other words, pregnancy appears to

decrease energy content in the High Fat groups more substantially ($P < 0.001$) than it does with the Stock diet groups (not significant), Table 4.3. Conversely, this effect is not seen in Study #1. The reason for this is unclear.

4.4 Conclusions:

It would appear that the effects of the diets are difficult to interpret due to the differences in energy intake. These differences are due partly to the adjustment required by the animals and also partly because of the differences in palatability of the diets. In the study done by Wade (1982), the energy intake of the animals started to level off only sometime after being on the diet for a week. The high fat diet in that study was not a formulated diet but consisted of the regular stock diet mixed with vegetable shortening, which means the nutrient density is diluted and since it is still a stock diet the adjustment required may not be as great compared to a new, powdered, formulated diet. The study was also 4 weeks in length, a comparatively longer period, with the possibility of more pronounced effects being achieved.

Nevertheless, the data presented shows several interesting findings. Despite the substantial differences in food intake, maternal carcass energy content, and body fat, the amount of fetal fat deposited appears remarkably unaffected. The findings also suggest that although a high fat diet appears to increase maternal fat reserves in terms of increased fat deposits, fetal outcome is apparently not affected.

5. GENERAL DISCUSSION AND CONCLUSIONS

In the past decade or so, epidemiological studies have raised the possibility that an increase in energetic efficiency may occur during pregnancy. This is based on observations that maternal food consumption does not appear to increase significantly, as would be expected according to recommendations made by the World Health Organization (WHO), Food and Agriculture Organization (FAO) and the United Nations University (UNU) (Durnin 1987a; 1987b; Whitehead et al 1986). This discrepancy between the apparent increase in energy requirement during pregnancy, and the lack of a significant increase in food consumed by pregnant women, however, is difficult to quantify in such studies.

The studies done on animals so far have not shown any increase in energetic efficiency during pregnancy (Richard and Trayhurn 1985; Wade et al 1986) as demonstrated by energy balance measurements. The naturally hyperphagic pregnant mouse, when pair-fed to virgin controls, does not show any increase in energetic efficiency (Richard and Trayhurn 1985). A carcass energy balance study on Syrian hamsters also suggested that energetic efficiency is not increased during pregnancy (Wade et al 1986). Since the Syrian hamster does not

increase its food consumption dur g pregnancy, it is an ideal animal model in which to study the energetic adaptations of pregnancy as metabolic efficiency can be investigated without dietary manipulation. The major findings of the studies undertaken here will be reviewed and discussed in the following sections.

The indirect calorimetry study showed some important results regarding energy expenditure and fat utilization in the Syrian hamster during pregnancy. Continuous measurements of energy expenditure during pregnancy have not been presented previously and the findings are interesting in several respects.

First, they illustrated the fact that fat stores are being utilized during pregnancy. This is in agreement with carcass analysis data done here and elsewhere (Wade et al 1986) which showed that by the time of parturition, maternal body fat is decreased by more than a third of its initial level.

Secondly, the data obtained demonstrated that the Syrian hamster does not increase its food intake during pregnancy, confirming results from previous studies (Fleming and Miceli 1983; Wade et al 1986).

Thirdly, the indirect calorimetry study showed that total energy expenditure is increased significantly ($P < 0.001$) during pregnancy in the Syrian hamster. This

increase is not apparent until Day 9, when a significant increase in daily energy expenditure is evident.

Fourthly, this increase in energy expenditure is followed on Day 10 by a significant decrease in RQ ($P < 0.001$). The data shows that from Day 10 onwards there is a sharp decrease in RQ, indicating that maternal body fat is being utilized. The time period in which utilization occurs parallels some of the maternal hormonal changes which occur during pregnancy.

Progesterone is one of those hormones that appears to coincide with the decrease in RQ values. Progesterone levels increase steadily during pregnancy with a small peak occurring on Day 8 followed by a sharp peak on Day 14 which then ends in an abrupt fall to barely detectable levels just before parturition. It is this abrupt decrease which induces parturition (Greenwald 1985). Administration of progesterone leads to hyperphagia and body weight gain in gonadectomized hamsters but adult and prepubertal female hamsters are apparently unresponsive to this treatment (Zucker et al 2; Borer 1985). In rats, progesterone does not appear to affect energy intake nor weight gain (Richard 1986). Although progesterone levels are increased during the latter stages of pregnancy, the correlation does not appear very strong. Thus, the influence of this hormone on maternal fat utilization does not appear persuasive.

Prolactin, however, appears to have a much stronger influence on body fat regulation. The level of circulating prolactin increases after mating, peaks at approximately Day 6 of pregnancy, and then decreases rapidly by Day 10 (Greenwald 1985), which coincides with the concomitant sharp decrease in RQ on Day 10. Prolactin remains at this "lower" level throughout pregnancy from Day 10 onwards which parallels the observations made on the decreased RQ, and thus lipolysis, during the latter part of pregnancy. This peptide hormone apparently stimulates lipogenesis and fattening in many vertebrate species including the Syrian hamster (Joseph and Meier 1974; Cincotta and Meier 1985a). In the Syrian hamster, prolactin has been shown to facilitate hepatic lipogenesis and the expression of circadian variations in insulin receptor profile (Cincotta and Meier 1985a and 1985b). In other words, prolactin enhances insulin stimulation of hepatic lipogenesis during certain times of the day.

Bromocriptine injections have been shown to reduce lipogenesis and block lipogenic responsiveness to insulin in isolated hamster hepatocytes (Cincotta and Meier 1985a) and reduce the number of insulin receptors in the liver (Cincotta and Meier 1985b). However, prolactin levels are elevated above those of unmated hamsters (Bast and Greenwald 1974; Greenwald 1985)

during pregnancy and lactation, which thus, seems to complicate the issue. Nevertheless, it is conceivable that the rapid fall in prolactin levels, from approximately 170 ng/ml serum on Day 6 to 35 ng/ml serum on Day 10 (Greenwald 1985), may have some influence on maternal fat mobilization, regardless of the pre-pregnant levels.

Another more direct study which evaluated the effects of prolactin reduction on body fat stores in the Syrian hamster showed a significant (25% to 49%) reduction in body fat when the animals were treated with bromocriptine (Cincotta and Meier 1987). Interestingly, food consumption was unaffected and the animals did not show any body weight loss despite the fat loss observed in the study. Animals in the study were maintained on a 14-hour daily photoperiod.

On a short-day nonstimulatory photoperiod, however, substantial increments in serum prolactin concentration are associated with reductions in body weight and fat while low serum prolactin levels are associated with higher body fat (Borer 1985). The effects of prolactin on lipogenesis and body fat stores are, thus, apparently dependent on circadian variations and on photoperiod (Joseph and Meier 1974; Borer 1985; Cinocotta and Meier 1985a, 1985b and 1986).

Photoperiod itself also has a significant influence on body fat in the female Syrian hamster (Wade 1983; Wade and Bartness 1983a). It is very possible that during pregnancy, prolactin levels may exert an influence on the utilization of maternal body fat via the critical permissive role this hormone plays in the lipogenic effects of insulin (Cincotta and Meier 1985a; Cincotta and Meier 1986), although the understanding of this relationship is still incomplete especially in view of the confounding factors involved.

It is also conceivable that estrogen levels may influence energy balance during pregnancy. The rise in estradiol level is gradual during the first half of pregnancy, but the rate increases rapidly from Day 10 onwards and peaks on Day 14 (Greenwald 1985). Estradiol apparently reduces body fat and weight (Borer 1985), although the weight suppressing effects of estradiol is less pronounced in hamsters than in rats, in which the sensitivity is 20 times greater (Morin and Fleming 1978; Borer 1985). This may be partially explained by the observation made by Richard (1986), that in estradiol-treated rats energy gain is suppressed mainly by alteration of food intake whereas in female hamsters ovarian hormones appear to have less of an influence on food consumption (Wade et al 1986; Zucker et al 1972). Another possibility may be that the affinity of anterior

pituitary and uterine tissues for estradiol are different in the two species (Feder et al 1974). These two possibilities seems to fit in well with the observation seen here where food intake was not altered.

A more recent study demonstrated the relationship between estradiol and Syrian hamster adipocytes (Pecquery et al 1987). The authors found that after in vivo treatment with estradiol hamster adipocytes tested in vitro had a reduced lipolytic response. The basis of the defective lipolytic response is evidently an impairment of the adenylate cyclase function. Furthermore, the lipolytic effects of estradiol are independent of the possible increase in prolactin which may occur with estradiol treatments (Pecquery et al 1987). Moreover, the plasma estrogen levels reached in the male hamsters used in the study are similar to those found in female hamsters at the end of pregnancy (Pecquery et al 1987). Thus it seems likely that the increase in estrogen levels seen during the latter part of pregnancy may have an influence on maternal fat mobilization, especially since the relatively rapid rise in estradiol occurs on Day 10 of pregnancy which corresponds to the concomitant decrease in RQ observed on Day 10 in the data obtained in the present study. The abrupt decrease in estradiol just before parturition, however, does not seem to affect

maternal fat utilization. Perhaps, this may be partially due to the, by then strong, nutritional stress imposed by the fetuses. Nevertheless, the in vivo data obtained here along with the in vitro findings of the lipolytic effects of estradiol indicate that estrogen may play a significant role in the lipolytic changes during late pregnancy and thus, energy balance regulation in this species.

It seems possible that the mobilization of maternal body fat observed is influenced by the synergistic effects of both estrogen and prolactin. If so, it would appear to support the hypothesis that the effects of sex steroid hormones on adipocyte metabolism may be responsible for the secondary sex characteristics of adipose tissue distribution in women (Rebuffe-Scrive et al 1986).

Carcass analysis performed at the different stages of pregnancy demonstrated several points with regards to energy balance and maternal fat utilization.

First, the data showed that maternal body fat and carcass energy content decreased significantly by the end of pregnancy; thus, body fat was used during pregnancy. This is in agreement with the indirect calorimetry findings and a previous study done by Wade et al, (1986).

Secondly, the findings obtained in this study showed that total body fat was not significantly decreased until after Day 10 of pregnancy. This is consistent with RQ measurements which indicated that significant net mobilization of maternal fat does not occur until after Day 10 of pregnancy.

Thirdly, brown adipose tissue thermogenesis measurements indicate that there is a reduction in thermogenesis by virtue of the decrease in cytochrome oxidase activity. Although the reduction in cytochrome oxidase activity was not significant until Day 10, which is not consistent with data from the previous study by Wade et al (1986) showing a significant decrease by Day 4, there is nonetheless a trend indicating a steady decrease in cytochrome oxidase activity throughout pregnancy. This decrease in thermogenesis has been suggested to be the result of hormonal influences since fetal substrate demands are insignificant during early pregnancy.

Fourthly, it has also been suggested that the decrease in BAT thermogenesis is part of a maternal the adaptive mechanism to "save" energy, and thereby increase energetic efficiency. Despite this energy "saved", however, there does not appear to be any overall increase in energetic efficiency. Energy balance data from both indirect calorimetry and carcass analysis

studies indicate that energetic efficiency is not increased during pregnancy. In view of the indirect calorimetry study which showed an increase in energy expenditure on Day 9 onwards, it would appear that the suppression of BAT thermogenesis occurs when total energy expenditure actually increases. Thus, it could be that the suppression of BAT thermogenesis is a response to the effects of the increased fetal requirement and perhaps may be regulated by some of the mechanisms which appear to be regulating maternal body fat utilization, i.e. maternal hormones. On the other hand, this decrease in BAT thermogenesis could also be a consequence of the increase in energy expenditure leading to the decrease in requirement for BAT heat production.

The studies on diet manipulation illustrated several factors in terms of energy regulation during pregnancy, despite the differences in energy consumed with the different diets.

First, the studies indicated that pregnancy per se does not increase the amount of energy consumed (Wade et al 1986; Fleming and Miceli 1983), unlike the situation in rats and mice where hyperphagia usually accompanies pregnancy (Steingrimsdottir et al 1980; Richard and Trayhurn 1985). If anything, pregnancy seems to decrease food consumption in the Syrian hamster, as

is the case with the High Fat diet where the pregnant group consumed significantly less than the virgin control group (Study #2). This finding, however, probably reflects more on the adjustment required by the pregnant hamsters to a new diet relative to the virgin control hamsters.

Secondly, when the daily food intake was plotted out comparing High Fat diet to that of Stock diet, it seems to indicate that the difference in energy intake was due to the initial adjustment since daily food intake of the High Fat group slowly increases and leveled off at approximately the same value as that of the Stock diet group. This appears to be in keeping with other studies indicating that the Syrian hamster tends to maintain constant caloric intake when presented with food of different caloric density (Silverman and Zucker 1976; Wade 1982). However, the data does show that palatability influences food consumption, at least initially.

Thirdly, even though it may be difficult to assess the effects of the High Fat diet due to the differences in energy consumed with the different diets, the general picture indicates that a High Fat diet appears to reduce the effects of pregnancy on BAT thermogenesis. In Study #1, BAT weight and cytochrome oxidase activity were significantly lower in the Low Fat

as opposed to the High Fat pregnant group even though the virgin control groups of both diets did not exhibit any significant difference in BAT thermogenesis. The difference seen cannot thus be totally accounted for by the difference in food consumed by the two diet groups. Furthermore, in Study #2, despite the significantly lower energy intake of the High Fat pregnant group, BAT weight and cytochrome oxidase activity were virtually similar to that of the Stock diet group. Previous studies (Wade 1983; Wade and Bartness 1983) indicate that a High Fat diet tends to increase BAT thermogenesis. In pregnancy, this effect seems to still prevail as observed in the findings obtained here where the effects of a High Fat diet appear to diminish the suppressive effects of pregnancy on BAT thermogenesis.

The fourth point is that a High Fat diet appears to increase body fat content. Even though energy consumption was lower in the High Fat pregnant group in comparison to the corresponding Stock diet group, body fat and energy content were similar. This thus appears to be in keeping with other studies which indicated that High Fat diets increase metabolic efficiency (Wade 1982; Wade and Bartness 1983a; Mercer and Trayhurn 1987).

Fetal fat content, however, does not appear to be influenced by either diet or maternal body reserves. This perhaps may suggest that the regulation of fetal

fat deposits are influenced more by genetic or other mechanisms, and less affected by maternal energy balance.

Interestingly, although not precise, energy balance estimates (Tables 4.5 and 4.6) appear to suggest that the amount of energy required for pregnancy is similar regardless of body reserves, i.e. body reserves do not affect the amount of energy being used for pregnancy as both Low Fat and Stock diet groups seem to expend the same amount of energy.

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