

THE EFFECT OF DIETARY CALORIC RESTRICTION DURING PREGNANCY
ON MATERNAL AND FETAL BODY COMPOSITION IN THE
OBESSE SPRAGUE DAWLEY RAT

by

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INTRODUCTION

Obesity, a condition that is becoming increasingly more prevalent in the United States (Schneider et al, 1977), can be defined as a disorder in which excessive amounts of fat are stored in adipose tissue cells of the body. Many researchers refer to obesity as the condition in which overnutrition exists. Current terminology indicates that overnutrition may be a poor choice of terms and that the term "caloric excess" may be better. Obesity has been shown to be highly associated with coronary heart disease, hypertension, and maturity-onset diabetes (Schneider et al, 1977; Mayer, 1968; Krause et al, 1979). The obese woman entering pregnancy is at greater risk than is the normal weight woman entering pregnancy. These risks include the development of hypertensive disorders, diabetes mellitus, toxemia, and complications with wound healing (Tracy et al, 1969; Pritchard et al, 1976; Maeder et al, 1976; Schneider et al, 1977).

A positive correlation between maternal weight gain during pregnancy and birthweight of the infant has been thoroughly documented with weight gain ranking second only to duration of pregnancy as a determinant of infant birthweight (Schneider et al, 1977; Brown et al, 1979; Simpson et al, 1975). When dealing with the obese patient some researchers and physicians advocate restriction of weight gain during pregnancy so that the patient will end pregnancy with a net loss in weight (Schneider et al, 1977). The reasoning behind this dietary prescription is that maternal stores are already over-supplied and can theoretically be broken

down for energy and fetal needs during pregnancy. Several problems may result from the use of such a regimen. Caloric restriction during pregnancy limits the intake of nutrients that are essential for growth and development of the fetus and for maintenance of maternal stores and health (Krause et al, 1979; Lechtig et al, 1975).

Arroyo and co-workers in 1978 showed that the accumulation of fat during pregnancy depends on food intake, as it does in the non-pregnant woman. They observed a decrease in subcutaneous fat with food restriction. The decrease in subcutaneous fat, during the period in which fat accumulation generally occurs, probably indicates that low-weight women with the greatest food restriction use basal reserves to meet their energy/nutrient requirements during gestation. It was the conclusion of these researchers that the same should be true for overweight women.

Edwards et al in 1978 studied pregnancy in the massively obese woman. She and her co-workers found that obese women tended to gain less weight during pregnancy than did normal weight women but had larger babies. This finding is contrary to observations of others (Krause et al, 1979; Arroyo et al, 1978; Emerson, 1962; Lechtig et al, 1975). Inadequate maternal gain has generally been reported to result in a low birthweight infant. Judging from the data of Edwards et al (1978) one might conclude that minimal weight gain or actual weight loss during pregnancy is of less concern in obese women.

Naeye (1979) performed a further study concerning obesity and pregnancy. He examined data from the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke which had prospectively followed 53,518 pregnancies between 1959 and 1966.

Naeye used singleton infants and examined maternal weight gain during these pregnancies. His purpose in this research was to determine if, in overweight women entering pregnancy, a weight gain of 24 to 27 pounds is optimal, as is prescribed for normal weight women entering pregnancy. Naeye concluded that the obese woman needs to gain only one-half of the normal recommended weight gain of 24 to 27 pounds during pregnancy. Naeye postulated that the reason for this is that the obese woman has maternal stores to draw on for nutritional support. Naeye goes on to say that along with greater fat reserves "nonadipose nutritional reserves may also be greater in overweight mothers because cells in many organs, aside from adipose tissue, are larger in the obese than in the nonobese individual" (Naeye, 1979, page 7).

Lederman and Rosso (1980, 1981a, 1981b) have shown that food restriction in obese rats during pregnancy affects fetal growth. They performed a series of studies using 50 and 60% restrictions of food intake, restricting total nutrient as well as caloric intake. In one such study (1980) obese and control rats were mated and assigned to one of four groups: 1) Obese-ad lib fed; 2) Control-ad lib fed; 3) Obese-50% food restricted; and 4) Control-50% food restricted. Both obese and control rats fed ad lib produced normal fetuses of the same weight. Both obese and control rats, fed restricted diets, produced fetuses that showed evidence of growth retardation. Obese food restricted rats, however, maintained their obese state at term. Lederman and Rosso have postulated that all maternal stores cannot be mobilized to support fetal growth.

Most studies to date, have placed major emphasis on the effect of maternal diet on the fetal compartment rather than examining the effect of food restriction on both the fetal and maternal tissues. It is not clear whether food restriction and/or weight reduction have a positive or negative effect on maternal and fetal body composition. Changes in the maternal system may be significant and cannot be ignored. The present study was designed to determine the effect of various levels of dietary restriction in the mother rat during pregnancy. The effect of dietary restriction on both maternal and fetal compartments was considered. Obese Sprague Dawley rats were assigned to one of three dietary treatments: ad lib fed, 15% calorie restricted, and 30% calorie restricted. The diets were supplemented with protein, vitamins, and minerals. Restriction in intake thus constituted a carbohydrate restriction. The following components were measured at the termination of pregnancy: maternal weight gain; fetal weight and litter size; maternal total body and liver moisture, nitrogen (protein), and fat; fetal moisture, nitrogen (protein), and fat; maternal kidney size, moisture, and fat; maternal uterine size and moisture; placental size and moisture; and maternal serum protein concentrations.

REVIEW OF LITERATURE

Obesity As A Major Nutrition Related Health Problem

Obesity, a disorder in which excessive amounts of fat are stored in the adipose tissue cells of the body (Schneider et al, 1977), is one of the most prevalent nutrition related health problems in the United States. It is estimated that between 25 and 45% of the adult population of the United States is afflicted with the problem of obesity (Udall et al, 1978). The prevalence of obesity in the United States rises rapidly at age 25 and by the 50-59 age decade, virtually one-third of men and one-half of women exceed desirable weight by at least 20% (U.S. Public Health Service, 1965). Health professionals consider obesity to be a major nutrition related health problem and it is estimated that approximately 50 million Americans spend in the neighborhood of \$100 million each year trying to find an easy, quick way to lose weight (Schneider et al, 1977). Many researchers refer to obesity as a condition of overnutrition. As opposed to malnutrition this is not the best term to use. Instead, it may be better to refer to obesity as "caloric excess" (Schneider et al, 1977). Obesity has been shown to have a high association with coronary artery heart disease, hypertension, and maturity-onset diabetes (Schneider et al, 1977; Mayer, 1968; Krause et al, 1979). It is estimated that 70-80% of maturity-onset diabetics are obese (Mayer, 1968). Additional adverse health effects for the obese include complications during acute illness, complications following accidents, and/or complications following general anesthesia and surgery (Schneider et al, 1977). Obese women entering pregnancy

tend to have a higher incidence of obstetric complications (Schneider et al, 1977; Udall et al, 1978; Tracy et al, 1969; Kerr, 1962; Emerson, 1962; Pritchard et al, 1976; Maeder, 1975) including a greater incidence of toxemia, diabetes, hypertension, thromboembolism, and problems with wound healing. Other problems include prolonged labor, failure at breast-feeding, increased fetal loss, antepartum hemorrhage, and breech presentations that are less frequently diagnosed (Emerson, 1962). Obese patients generally deliver larger babies than do nonobese women (Udall et al, 1978; Emerson, 1962). Infants born to obese mothers are frequently more sluggish and not as alert at birth as are babies born to normal weight mothers as judged by the APGAR scale (Edwards et al, 1978). Subcutaneous fat accumulation in maternal tissues is related to increased subcutaneous fat accumulation in the infant (Udall et al, 1978). Maternal obesity is associated with increased subcutaneous fat in the newborn which could contribute to suboptimal infant outcome at the termination of pregnancy (Udall et al, 1978). Udall et al (1978) give several suggestions to explain why maternal obesity results in increased subcutaneous fat in the newborn:

- 1) Increased placental transfer of free fatty acids
- 2) Inheritance of a lower metabolic rate
- and 3) Decreased physical activity in utero.

Weight Gain During Pregnancy

Weight gain during pregnancy reflects the normal physiological effects of pregnancy. Twenty-four pounds or 12.5 kg is the recommended gain during pregnancy (National Research Council, 1970). However, the

normal range of weight gain varies greatly. Young women tend to gain more weight than do older women, primigravidas gain more than multi-gravidas, and thin women gain more weight than do fat women (Krause et al, 1979; Edwards et al, 1978; Naeye, 1979). It is generally agreed that weight gain follows a sigmoidal curve (Figure 1), when weight gain of the pregnant woman is plotted against the stage of pregnancy (Krause et al, 1979; Hytten et al, 1971). During the first trimester there is a small gain. A more rapid gain is observed during the second trimester; and a slower gain occurs during the third trimester (Schneider et al, 1977). Hytten and Leitch (1971) define the pattern of weight gain even further by saying that up to the 16th-18th week of pregnancy, an optimal rate of gain is 0.8 lb./week; from the 18th-28th week of pregnancy an optimal rate of gain is 1.0 lb./week; and from the 28th week of pregnancy until term an optimal rate of gain is 0.8 or 0.9 lb./week. A typical distribution of weight gained during pregnancy is shown in Table 1 and Figure 2. The components of weight gained and weight changes in each throughout gestation are shown in Table 2.

At term the maternal tissues account for slightly more than half and the fetus slightly less than half of the total weight gain (Schneider et al, 1977). The pattern of weight gain is as important as, or indeed more important than, is the total weight gain (Schneider et al, 1977; Krause et al, 1979). The total weight gain, however, has been shown to be positively correlated with birthweight of the infant (Schneider et al, 1977; Brown et al, 1979; Simpson et al, 1975) with weight gain ranking second only to duration of pregnancy as a determinant of infant birthweight. The maternal pre-pregnant weight is also related to

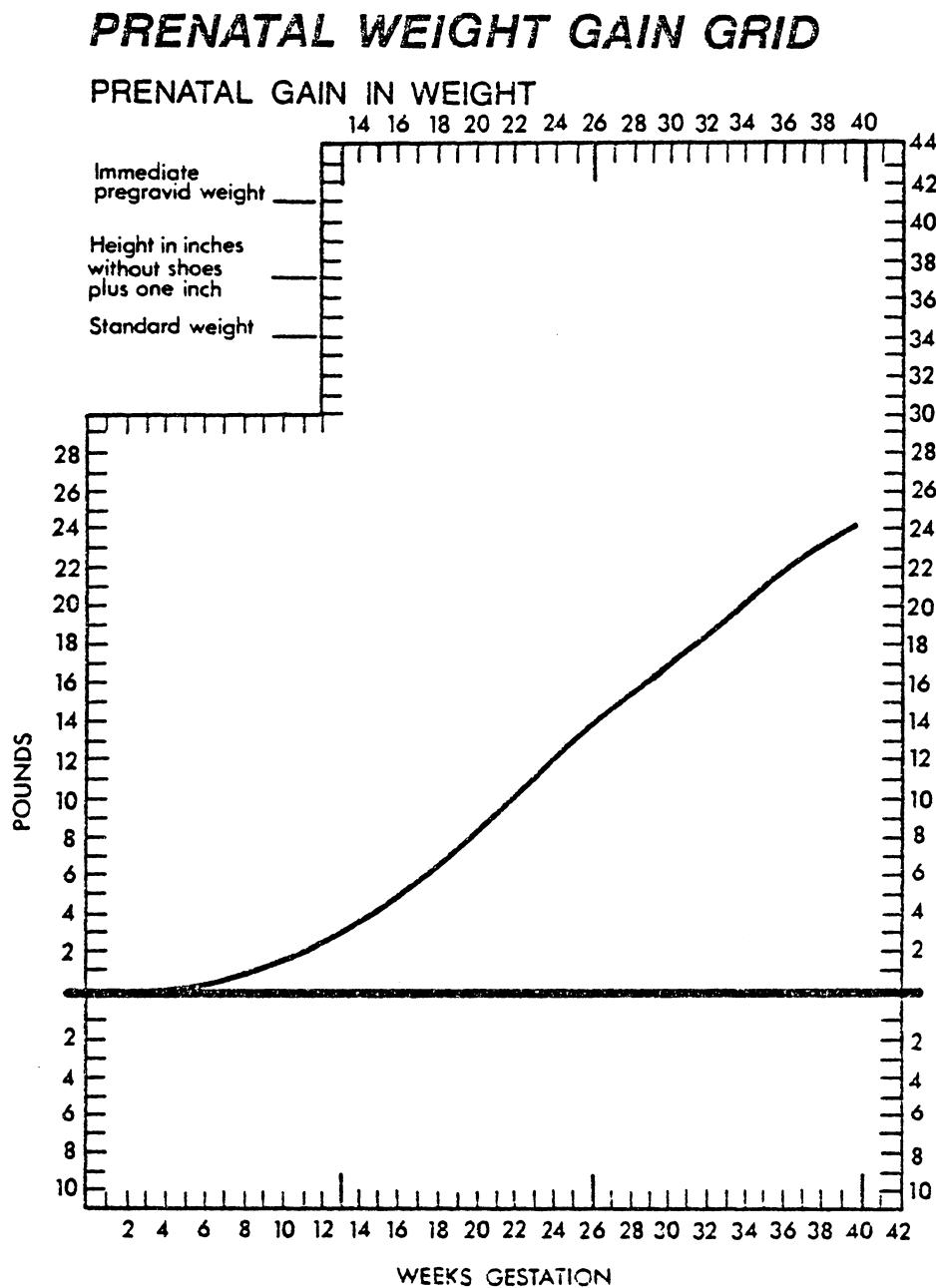


Figure 1. Prenatal Weight Gain Guide

From: Worthington, B. S., Vermeersch, J., Williams, S. R.
 Nutrition in pregnancy and lactation. St. Louis, The
 C. V. Mosby Company, 1981.

Table 1. Typical Distribution of Weight
Gained During Pregnancy

<u>Tissue</u>	<u>Weight (lbs.)</u>
Fetus	7.5
Uterus	2.0
Placenta	1.5
Amniotic Fluid	2.0
Blood Volume	3.0
Extracellular Fluid Increase	2.0
Breast Tissue	1.0
Fat	<u>4.0-9.0</u> 28.0

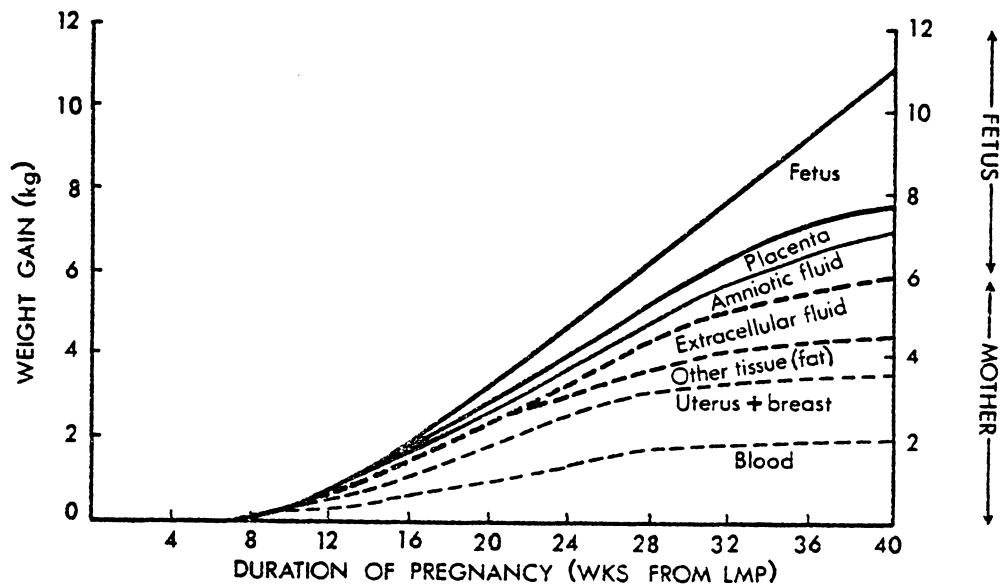


Figure 2. Average maternal weight gain during pregnancy

From: Committee on Maternal Nutrition, Food and Nutrition, Food and Nutrition Board, National Research Council, National Academy of Sciences: Maternal Nutrition and the Course of Pregnancy, Washington, D.C., 1970, Government Printing Office.

Table 2. Components of the Average Weight
Gained in Normal Pregnancy

Component	<u>Amount (gm) gained at</u>			
	10 weeks	20 weeks	30 weeks	40 weeks
A. Total gain of body weight	650	4000	8500	12,500
Fetus	5	300	1500	3300
Placenta	20	170	430	650
Liquor amnii	30	250	600	800
Increase of:				
Uterus	135	585	810	900
Mammary gland	34	180	360	405
Maternal blood	100	600	1300	1250
B. Total (rounded)	320	2100	5000	7300
C. Weight not accounted for (A-B)	330	1900	3500	5200

From Committee on Maternal Nutrition, Food and Nutrition, Food and Nutrition Board, National Research Council, National Academy of Sciences: Maternal Nutrition and the Course of Pregnancy, Washington, D.C., 1970, Government Printing Office.

birthweight but is not as strongly correlated as is the total amount of weight gained during pregnancy (Schneider et al, 1977; Brown et al, 1979).

Maternal Nutrition and Fetal Outcome

Energy needs during pregnancy in the adult woman are increased by 200 kcal/day due to the growth of the fetus and placenta as well as by increases in maternal tissues (National Research Council, 1970). Energy needs are also increased to support increases in maternal metabolism (Schneider et al, 1977). Caloric expenditure during pregnancy is not evenly distributed and does not parallel fetal growth (Schneider et al, 1977; Krause et al, 1979; Hytten et al, 1971; National Research Council, 1970). During the late first trimester and the second trimester there is a rapid increase in energy requirement. This added caloric cost is due to maternal changes such as increased blood volume, growth of uterus and breasts, and accumulation of storage fat. Energy requirements level off during the third trimester and remain fairly constant until term. The caloric cost at this time is associated with the growth of the fetus and placenta during the third trimester.

There is a marked increase in protein requirement during pregnancy due to increased needs of both the mother and fetus (National Research Council, 1970). Amino acids are needed for protein synthesis in maternal uterine and breast tissue, for increased maternal plasma amino acid levels, for fetal growth, and for fetal synthesis of proteins (Schneider et al, 1977; Dancis et al, 1958; Hurley, 1980). The fetus synthesizes

most of its protein from amino acids received from the maternal circulation (Hurley, 1980).

Infant birthweight has been shown to be related to maternal calorie and protein intake during gestation (Schneider et al, 1977; Hurley, 1980; Burke et al, 1943). Burke and co-workers (1943) examined dietary records and the outcome of pregnancy in a group of women attending a prenatal clinic. Infants were examined at birth by pediatricians who were unaware of the nutritional intake of the mothers. For mothers whose diets were classified as good or excellent, only 3% of the infants were classified as being in fair condition. The remaining 97% were classified as good to superior. When protein as a single nutrient was considered in these women's diets it was seen that birthweight and birth length of the infant correlated with the amount of protein in the maternal diet. When the amount of protein was under 45 gms/day the average birthweight of the infant was 5.8 lbs. When the protein intake was equal to 65.74 gms/day, the average birthweight was 8.0 lbs. If average daily protein intake exceeded 85 gms the average birthweight was 9.2 lbs. Infant birthweight, a major determinant of infant survival, is thus closely related to adequate maternal protein intake during pregnancy. Jeans et al (1955) and Primrose and Higgins (1971) found similar results relating maternal nutrient intake to outcome of pregnancy.

Animal studies have shown that the restriction of maternal dietary protein during pregnancy results in observable effects on the offspring (Schneider et al, 1977; Koshy et al, 1975). Changes in the structure and function of the central nervous system have been reported. Koshy

et al (1975) examined the effect of protein restriction on fetal growth. Pregnant rats were fed either 23% or 5% protein diets for either days 1-21 of gestation or days 7-21 of gestation. Fetal body development and fetal brain development were examined. A 5% protein diet inhibited fetal growth during both time periods. Fetal growth retardation was more severe in the group maintained on the 5% protein diet for the entire gestational period (days 1-21). Brain weight was reduced in the litters born to dams on the 5% protein diet for both time periods. Koshy et al (1975) report fewer brain cells in the pups of dams on a 5% protein diet throughout pregnancy as compared to the pups born to dams on the adequate 23% protein diet. Koshy et al (1975) conclude that fetal growth was retarded regardless of whether maternal protein restriction was imposed throughout the entire gestational period or only during the last two thirds of pregnancy, although the severity of growth retardation increased with the length of protein restriction.

Animal studies have consistently shown protein to be a critical nutrient in relation to normal fetal growth and development (Schneider et al, 1977; Koshy et al, 1975). However, even though human studies indicate the importance of adequate protein consumption during pregnancy there is evidence to suggest that energy may be the more important nutrient. Habicht et al (1974) have shown an increase in birthweight of infants born to nutritionally deficient women who received energy supplements in addition to the normal diet. Supplementary feedings of protein and/or energy were provided to pregnant women in 4 Guatemalan villages. The pregnant women attended clinics where they were able to

obtain supplements designed to augment their normal dietary intake. Record of attendance was one measure of compliance with the supplementary diet. Mean birthweight of infants increased with maternal caloric ingestion during pregnancy. Maternal caloric ingestion from the supplement increased birthweight of the infant whether the calories were ingested early or late in pregnancy. Protein ingestion from the supplement had little, if any, additional effect on birthweight. These observations were contrary to what the researchers had expected to find.

Fat Deposition During Pregnancy

Maternal fat stores are believed to increase during pregnancy to serve as a cushion for the maternal compartment during the latter part of pregnancy when fetal growth is occurring at its most rapid level (National Research Council, 1970). This increase in maternal stores may also provide a safety factor during lactation when caloric expenditure from the maternal tissues is great (Arroyo et al, 1978; Taggart et al, 1967; National Research Council, 1970). It has been postulated that increased maternal body fat, thereby, acts as a reserve for the fetus and newborn infant to draw on during these periods of rapid growth and development.

During pregnancy, energy is stored as fat in the maternal tissues mostly in fat depots but with a small amount stored in mammary glands (Krause et al, 1979). The average healthy pregnant woman, without food intake restriction, deposits 3-4 kg of subcutaneous fat between weeks 10 and 30 of gestation. This deposition of fat slows after the 30th

week of pregnancy (Arroyo et al, 1978; Taggart et al, 1967; National Research Council, 1970). After the 30th week of gestation most active growth is seen in the fetal compartment rather than in the maternal compartment (National Research Council, 1970). This is shown diagrammatically in Figure 3. This pattern of fetal development may be a possible explanation for the slowing of fat deposition after week 30 of gestation in maternal tissues.

Arroyo and co-workers (1978) report that the accumulation of fat during pregnancy depends on food intake, as it does in non-pregnant women. They measured total skinfold thickness which they defined as the sum of seven skinfold sites in 57 pregnant women at weeks 24, 30, and 35 of gestation. All of these women had low energy and protein intake throughout pregnancy. This was determined by a trained nutritionist staying in the home of these women for a 24 hour period and carefully weighing all food items consumed by the pregnant woman. Food intake was collected between weeks 24 and 39 of gestation. The 57 women were subdivided into an overweight and a low weight group according to their weight-for-height at week 24 of gestation. Half of the pregnant women did not increase subcutaneous fat during pregnancy. In some of these cases decreased fat reserves were observed at the termination of pregnancy. The overweight women exhibited a greater loss in total skinfold thickness than the low weight women. Arroyo et al (1978) postulate that this decrease in total skinfold thickness is a reflection of restricted energy intake during pregnancy at a time when requirements are probably the greatest. Arroyo et al

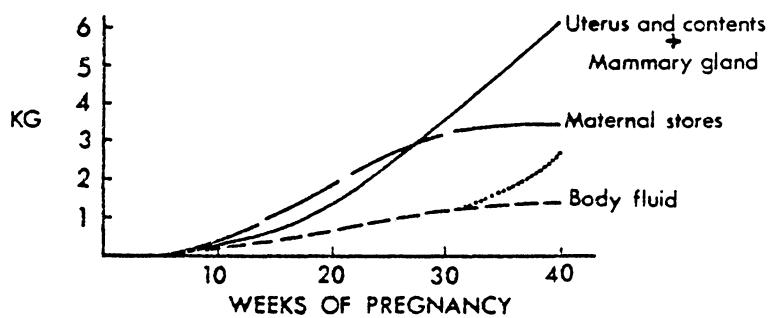


Figure 3. Components of Maternal Weight Gain During Pregnancy

From Committee on Maternal Nutrition, Food and Nutrition, Food and Nutrition Board, National Research Council, National Academy of Sciences: Maternal Nutrition and the Course of Pregnancy, Washington, D.C., 1970, Government Printing Office.

(1978) suggest that the decrease in subcutaneous fat during the period in which faster accumulation should be occurring probably indicates that energy supply is inadequate to meet maternal and fetal needs and that maternal tissue is being broken down to supply the basal needs of pregnancy.

Other researchers have examined skinfold measurements in an effort to study fat deposition during pregnancy (Gampel, 1965; Whitelaw, 1976; Tanner et al, 1975; Arroyo et al, 1978; Taggert et al, 1967). Taggert et al (1967) measured skinfold thickness in 84 women and total body water in 48 of these women throughout the course of pregnancy. Taggert and co-workers were attempting to follow the accumulation of stored subcutaneous fat throughout gestation. These researchers measured weight for height and skinfold thicknesses at seven body sites at 10, 20, 30, and 38 weeks of pregnancy as well as at the time of discharge from the hospital. This was followed up by obtaining the same measurements at 1 and 6-8 weeks post-partum. The body sites observed were triceps, biceps, scapula, costal, mid-thigh, knee-cap, and suprailiac. Absolute increases in skinfold thickness were seen at 6 of the 7 body sites between weeks 10 and 30 of gestation. The knee and triceps showed the least change in skinfold thickness and the greatest increase was seen at the suprailiac site. The authors concluded that the absolute and proportional changes in skinfold thicknesses were greatest at the central body sites and least at the peripheral body sites and were not proportional to the initial skinfold thickness. After the 30th week of gestation skinfold thickness increased only at the midthigh site.

Other body sites decreased or remained the same. From the 38th week of pregnancy through the first week post-partum, a sharp drop in measurements recorded for all sites was seen. Absolute changes in skinfold thicknesses during and after pregnancy are shown in Figure 4. The reduction in skinfold thickness was unrelated to the degree of edema and appeared to occur rapidly about the time of parturition. By 6-8 weeks post-partem total skinfold thickness had essentially returned to the initial value obtained at 10 weeks of gestation. Taggert et al (1967) point out that skinfold thickness increased more during pregnancy in underweight women than in overweight women.

Seitchik, Alper, and Szutka (1963) measured total body water and body density throughout pregnancy in 21 healthy, normal pregnant women. The intended purpose of this research was to determine if weight gain in pregnancy represents a fairly fixed amount of gain in water and fat-free solids and if a weight gain in excess of this "fixed" quantity is a gain in fat or edema fluid or both. One major methodological problem these researchers encountered was in the measurement of total body water. They employed a method whereby the subjects received deuterium oxide orally and collected serial urine specimens after ingestion. The researchers question even body distribution of the deuterium oxide. These researchers felt that they underestimated the values for total body water. The results of this research indicate that the majority of maternal weight gain during pregnancy is due to storage of fat tissue rather than to increases in lean tissue. Flanagan (1964) found results which support the work of Seitchik and his co-workers (1963).

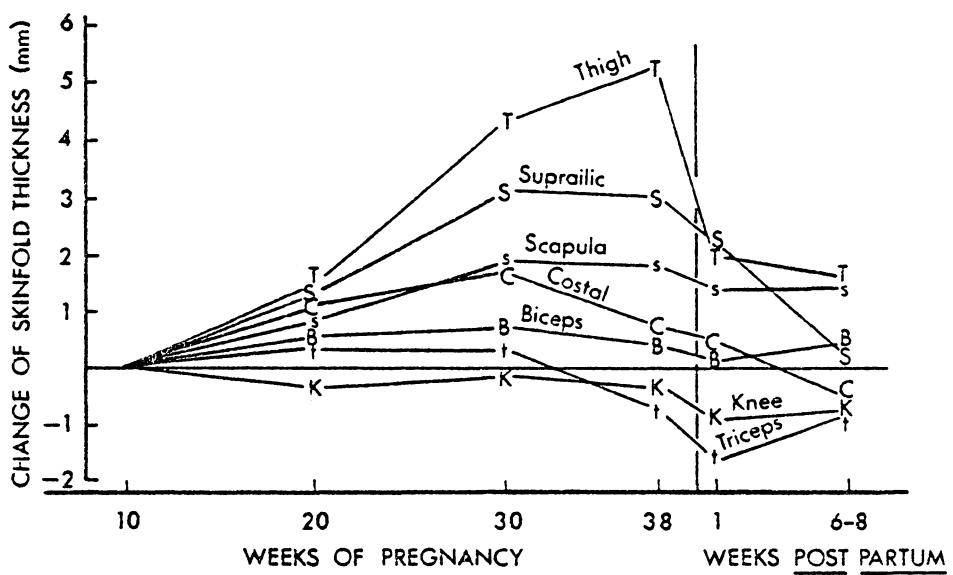


Figure 4. Absolute changes in skinfold thicknesses during and after pregnancy

From: Taggert et al, 1967

Weight Restriction During Pregnancy

In the early 1900's it was a common practice for physicians to restrict weight gain during pregnancy (Schneider et al, 1977; Hytten et al, 1971). The rationale for doing this was based on one or all of several reasons (Hytten et al, 1971): 1) For women with small pelvises it would be much easier to give birth to a small infant; 2) "a slim figure" was the ideal; it is easier to return to prepregnancy weight if only a small amount of weight is gained during pregnancy; and 3) pre-eclampsia occurs more often in women who gain weight in excessive amounts during pregnancy.

Some researchers and physicians advocate restriction of weight gain during pregnancy so that the patient will end pregnancy with a net weight loss (Schneider et al, 1977). This may be poor advice because:

1. Severe dietary restriction to limit calories may also result in the displacement of other essential nutrients from the diet (Schneider et al, 1977; Krause and Mahan, 1979).
2. Optimal protein utilization in pregnancy requires an intake of a minimum of 30 cal/kg body weight/day. If there is a deficit of energy intake below this minimum level ketosis may develop more quickly as protein is broken down to supply energy (Schneider et al, 1977).
3. Severe dietary restriction results in catabolism of fat stores which in turn produces ketonemia (Schneider et al, 1977).

Several studies were done in the 1960's looking at weight reduction, obesity, and pregnancy as they are inter-related. In 1962, Jacobson and co-workers gave intensive dietary instruction to 89 obese women who were between the 16th and 30th week of pregnancy. The obese women averaged 47 pounds above their desirable weight at the onset of

pregnancy. All obese women were placed on a 1500 calorie/day diet that contained 95 grams of protein. All women visited a specially established clinic every 2 weeks and talked to a nutritionist once a month throughout pregnancy. The women who stayed on the prescribed diet lost an average of 11.3 pounds. Weight loss occurred soon after the women began dieting. A plateau was reached or a slight gain occurred in the latter part of pregnancy. Complications of pregnancy occurred less often in the obese women who had successfully achieved a weight loss during pregnancy. The weights and heights of the infants born to obese mothers in this study did not differ significantly from the weights and heights of normal infants chosen as a control group. Female babies born to obese mothers who had not achieved weight loss during pregnancy were heavier than infants born to mothers who had lost weight.

In 1964 Sands performed a study on 132 pregnant women weighing 180-322 pounds when they entered pregnancy. Food intake was reduced to 900 kcal/day six days of the week. On the seventh day the patients were permitted to eat whatever they chose. Of the patients who started the program, 94.7% continued to term; 91.8% were successful in that they achieved negative weight benefit upon delivery. The authors do not make any comment on the outcome of pregnancy.

Edwards et al (1978) examined pregnancy in the massively obese woman. They studied 208 massively obese pregnant women and matched them to non-obese controls. Outcome of pregnancy, obstetrical performance, and incidence of infant obesity were measured. In the obese pregnant women the previous incidence of toxemia was greater than in the non-

obese control group. Likewise, a family history of diabetes was seen significantly more frequently in the obese group. The incidence of obstetrical complications was significantly greater in the massively obese patient. These complications included increased incidence of mild pre-eclampsia, hypertensive disorders of pregnancy, inadequate weight gain (<12 pounds), gestational diabetes, and wound infections. The infants born to the massively obese women were less responsive at birth twice as often as the infants born to the control group as judged by the APGAR score. Excessive-sized infants were born to 20.6% of the obese mothers as opposed to 5.3% of the control subjects. Low birth weight infants occurred twice as often among the nonobese controls. Infants of the massively obese women were an average of 209 grams heavier than those of the control subjects. Thirty-one percent of the obese women failed to gain an adequate amount of weight during pregnancy as opposed to 4.3% of the nonobese controls. Infants of obese women who gained less than 12 pounds during pregnancy had an average birth weight of 3,302 grams. This is compared to an average birth weight of 2,875 grams for infants born to nonobese women with inadequate weight gain during pregnancy. Judging from these data, one might conclude that minimal weight gain or actual weight loss during pregnancy is of less concern in the obese pregnant patient.

Naeye (1979) has shown that the optimum weight gain for an obese woman should approximate one-half the average weight gain of a normal pregnant woman since obese women have excess fat stores which can be catabolized to meet fetal needs. Naeye examined data from the Col-

laborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke which had prospectively followed 53,518 pregnancies between 1959 and 1966. Naeye examined the relationship of maternal weight gain and fetal outcome. The data of Naeye comparing perinatal mortality rates to pregnancy weight gain in pounds can be seen in Figure 5. Overweight mothers had the fewest fetal and neonatal deaths with a 15 or 16 pound weight gain in pregnancies that went to term. The optimal weight gain for normally proportional mothers (as judged by the Metropolitan Life Insurance Company desirable weights for height tables) was 20 pounds. For underweight mothers optimum fetal outcome was seen with a maternal weight gain of 30 pounds. Naeye concluded that a weight gain of the normal 24-27 pounds is not necessary for the obese woman during pregnancy.

Luke and Petrie (1980) examined the influence of maternal nutritional status and nutrition during gestation on fetal growth and birth weight. Two hundred ninety-four uncomplicated term pregnancies were studied. Each woman was characterized by height and post-partum weight as underweight (70.0 to 119.9% of pre-pregnant weight), normal weight (120.0 to 159.9% of pre-pregnant weight), or overweight (160.0 to 209.9% of pre-pregnant weight). In the underweight group, infant birth weights increased an average of 214 grams with each 10% increase in maternal post-partem weight. A similar positive increase was seen for the infants born to normal weight women. An increase of 41.9 grams in infant birth weight was seen for every 10% increase in maternal gain. However, a negative relationship was seen between increasing maternal post-

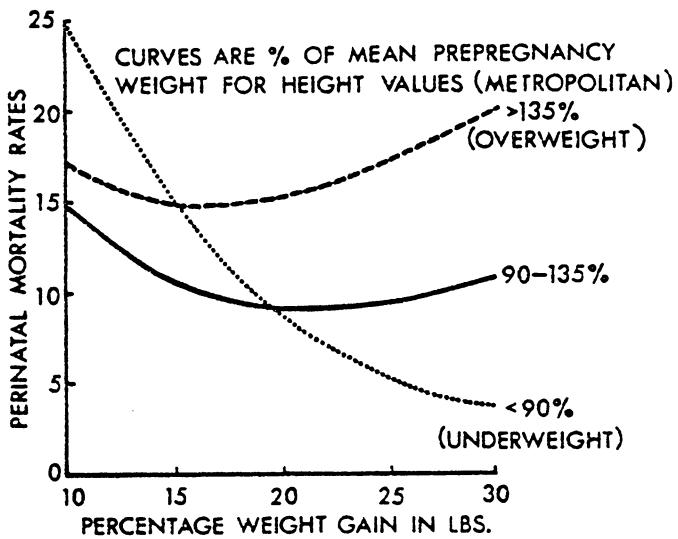


Figure 5. Perinatal Mortality Rates as Related to Pregnancy Weight Gain in Pounds in Overweight, Normal, and Underweight Women

From: Naeye, 1979

partem weight and infant birth weight for the overweight group of women. For the infants born to overweight women a loss of 29.8 grams was seen for every 10% increase in maternal post-partem weight. No severe growth retardation was observed in the infants born to women in the overweight group but the incidences of moderate and minimal growth retardation increased over that of the other two groups. The conclusions of Luke and Petrie (1980) are that the maternal compartment takes precedence over the fetal compartment in the obese woman resulting in observable intrauterine growth retardation. Luke and Petrie recommend the correction of underweight and overweight states before pregnancy is superimposed on an already existent state of malnutrition in the woman. In both underweight and overweight women, the normal pattern and amount of weight gain during pregnancy does not occur and, therefore, optimum fetal growth does not result. Luke and Petrie suggest that optimum intrauterine growth is observed only in normal weight women because the maternal compartment is favored in the other two observed maternal states.

Several animal studies have examined the effect of dietary restriction on fetal growth during gestation. Chow and Lee (1964) restricted the dietary intake of rats during gestation. Restriction of as little as 25% of total intake resulted in growth stunting and anemia as well as reduced resistance to the stress of pregnancy. Body weight gain of the offspring was low and was found to be correctible by early administration of pituitary growth hormone.

Berg (1965) fed 25, 50, and 75% restrictions of a stock diet to non-obese pregnant rats. Severe restrictions resulted in decreased maternal and fetal weight, ability to litter, and in poor fetal skeletal development.

Lederman and Rosso (1980, 1981a) have shown that food restrictions imposed on obese rats during pregnancy affect fetal growth. For purposes of their research, Lederman and Rosso have defined an obese rat as one that weighs 50 grams more than its chow-fed control. The question that Lederman and Rosso sought to answer was whether an obese animal with a surplus of pre-pregnancy stores could make these stores available to the fetus to support its normal growth if food consumption of the mother is restricted during gestation. In one study done by Lederman and Rosso (1980) obese and control rats were mated and divided into 4 groups: 1) obese-ad lib fed; 2) control-ad lib fed; 3) obese-50% food restricted; 4) control-50% food restricted. Both obese and control rats fed ad lib produced the same weight fetuses. Both obese and control rats, that were food restricted, produced litters that evidenced growth retarded fetuses. Obese food restricted rats maintained their obese state at term. Lederman and Rosso postulated from this data that maternal stores cannot be mobilized to support fetal growth.

Lederman and Rosso have performed two follow-up studies (1981a, 1981b). In the first, nonobese pregnant rats were pair-fed to a control group and fed a 50% food restriction based on average daily intake of the control group. The diet was fed in this pair-fed manner through

the fifteenth day of gestation. On this 50% restriction of food intake, rats maintained body weight close to prepregnancy weight. Beginning on day 16 of gestation and continuing to term the diets of the food restricted rats were supplemented with either carbohydrate or protein. Supplementation at this period in gestation should be beneficial to the fetus since this is the greatest period of fetal growth. Supplementation resulted in significant amounts of weight gain in food restricted dams on both carbohydrate and protein supplementation. However, the fetuses of these dams did not reach normal size by term. The conclusions of Lederman and Rosso were that the fetus cannot parasitize maternal stores nor have first access to nutrient supplements when they are available.

In the most recent study performed by Lederman and Rosso (1981b) food restriction of 60% was imposed after day 5 of gestation. Restricted rats were matched to two controls: 1) a pregnant ad lib chow fed rat; and 2) a nonpregnant ad lib chow fed rat. Both obese and non-obese rats were used. Obesity in these rats was achieved by feeding a diet of retail food items ad lib for a period of 2-7 weeks. The results of this study show that with a 60% food restriction obese rats lost weight during pregnancy. This loss was seen to be the result of loss in fat content of the carcass. Non-obese rats that were restricted 60% in food intake during pregnancy also lost weight but tended to lose more weight than their obese counterparts. The loss in the non-obese rats was observed in both fat and lean tissue composition. Fetal and placental weights were reduced by 25% in both the obese and non-obese

food restricted animals. Lederman and Rosso conclude that the fetal compartment cannot draw on excessive maternal nutrient stores to prevent growth retardation. Therefore, they conclude that the maternal compartment is favored over the fetal compartment at a level of 60% food restriction.

Only limited attempts have been made to define the impact of pre-existing obesity and the changes in maternal fat depots on growth and development of the fetus. An integrated analysis of the effects of nutrient restriction on both the mother and fetus would be a more desirable approach to establishing criteria for feeding regimens during pregnancy in the overweight/obese woman. The purpose of the present research was to determine if, in a caloric restriction situation the obese rat will break down maternal stores to support fetal growth. Changes in maternal and fetal composition induced in obese rats through manipulation of caloric intake during pregnancy were examined. Obese Sprague Dawley rats were placed on one of three dietary treatments: 1) ad lib; 2) 15% calorie-restricted which was expected to result in maintenance of body weight during pregnancy; and 3) 30% calorie-restricted which was expected to result in a maternal weight loss during pregnancy.

The diets were supplemented with protein, vitamins, and minerals. Thereby, food restriction constituted a carbohydrate restriction. It is believed that this dietary regimen approximates the human situation during pregnancy more closely than would a food or total nutrient restriction. The following components were measured at the termination

of pregnancy: maternal weight gain; fetal weight and litter size; maternal total body and liver moisture, nitrogen (protein), and fat; fetal moisture, nitrogen (protein), and fat; maternal kidney size, moisture, and fat; maternal uterine size and moisture; placental size and moisture; and maternal serum protein concentrations.

MATERIALS AND METHODS

Design of the Experiment

The animals used in this experiment were 44 female rats of the Sprague Dawley strain, obtained from Charles River Breeding Laboratories in Wilmington, Massachusetts. These female rats were purchased when they were two months old, housed in single suspended wire bottom cages and fed Purina Laboratory Chow #5001 ad libitum until they reached an age of six months. This period of aging was desired for several reasons: 1) It allowed the animals to reach an adult size and thus allowed for a slowing of active growth and thus less interference with both pregnancy and any dietary effects that might be observed; 2) It allowed the rats to reach an obese state so that excess fat stores would be available for mobilization to supply energy if needed. The animals were defined as obese since body weight exceeded 315 grams and average % carcass fat exceeded 29% of total carcass composition.

For breeding purposes Sprague-Dawley male rats of the same age were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. Throughout the breeding phase of the study the rats were housed in double suspended wire-bottom cages and fed Purina Laboratory Chow #5001 and water ad libitum. Rats were housed one male to one female to ensure mating. Breeding was staggered to facilitate sacrificing at the end of the study.

Prior to mating, five female rats were randomly chosen and carcass composition, including body moisture, fat, and nitrogen, was determined. Data collected on these rats represented baseline data and

was used to descriptively discuss changes that occurred due to dietary treatment. The remaining 39 rats were randomly assigned on a weight basis to one of three dietary treatments: ad libitum, 15% caloric restriction, and 30% caloric restriction (Figure 6). Average weight of animals in each treatment group was the same initially. Female rats were then placed with male rats. Mating was confirmed by the presence of a copulation plug. In some instances vaginal smears were also done to verify mating. When mating was confirmed, the female was removed from the male's cage and placed in a single suspended wire-bottom cage. This day was taken as Day 0 of the gestational period and experimental dietary treatment was begun. The animals were fed daily on a pair-fed schedule whereby the rats on the restricted diets were fed 15 or 30% less calories than what their respective controls ate on the same day of the gestational period.

All rats were housed in a room with controlled lighting (12 hours light, 12 hours dark) and constant temperature (22°C). The animals were weighed daily. Tap water was allowed ad libitum throughout the study.

Experimental Diets

Beginning on Day 0 of gestation each of the treatment groups was fed one of three diets: control (ad libitum), 15% caloric restriction, or 30% caloric restriction. The composition of the experimental diets is shown in Table 3. The 15 and 30% calorie-restricted diets were supplemented with protein, vitamins, and minerals in amounts of 15 and 30% respectively in order to ensure adequate (and equal) intake of these nutrients in all diets. This, in effect, means that these diets

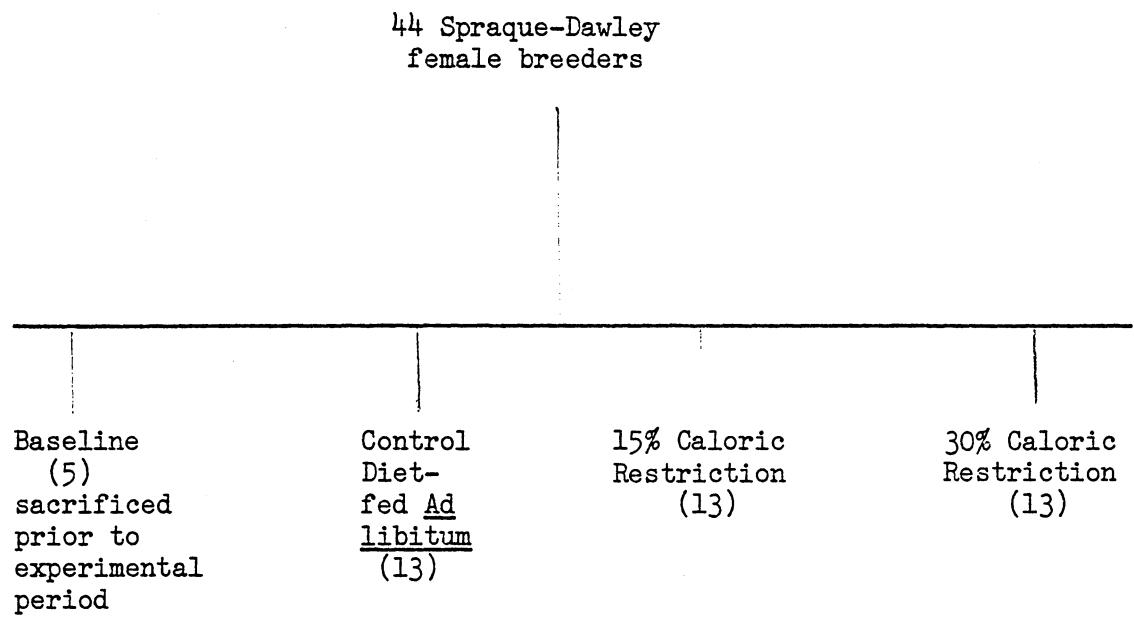


Figure 6. Experimental Design

Table 3. Composition of Experimental Diets

<u>Dietary Component</u>	<u>Control</u> <u>(gms/100 gms)</u>	<u>15% restricted</u> <u>(gms/100 gms)</u>	<u>30% restricted</u> <u>(gms/100 gms)</u>
Sucrose	35.00	33.50	32.00
Cornstarch	35.00	33.50	32.00
Fat ²	7.00	7.00	7.00
Casein ¹	14.90	17.15	19.40
Methionine ⁶	0.10	0.10	0.10
Minerals ⁴	4.00	4.60	5.20
Vitamins ⁵	1.00	1.15	1.30
Fiber ³	3.00	3.00	3.00

¹Casein. Vitamin Free. Nutritional Biochemicals Corp., Cleveland, Ohio.

²Crisco Hydrogenated Vegetable Shortening. Proctor and Gamble, Cincinnati, Ohio.

³Alphacel Non-nutritive Bulk. Nutritional Biochemicals Corp., Cleveland, Ohio.

⁴AIN Mineral Mixture 76. Nutritional Biochemicals Corp., Cleveland, Ohio.

⁵AIN Vitamin Mixture 76. Nutritional Biochemicals Corp., Cleveland, Ohio.

⁶dl Methionine. Nutritional Biochemicals Corp., Cleveland, Ohio.

provided for a carbohydrate restriction in order to achieve a reduction in calories. Dietary casein was supplemented with methionine in the proportion recommended by the National Research Council (1966) in order to assure adequate intake of the sulphur-containing amino acids. The experimental diets were fed from Day 0 of gestation until Day 20 of gestation. Animals were sacrificed on Day 20 of gestation.

Sacrifice of Animals

On the morning of Day 20 of gestation each rat was anesthetized with carbon dioxide and blood was drawn by heart puncture. Ten ml Venoject vacutainers (Kimble-Terumo, Inc., Elkton, Maryland) treated with EDTA and equipped with 21 guage $1\frac{1}{2}$ " needles were used. After collection each tube was inverted gently several times to mix the anti-coagulant with the blood. Blood was centrifuged in the vacutainer tubes in which it had been collected in an IEC Refrigerated Centrifuge Model DPR-6000 (International Equipment Corp., Needham Heights, Mass.). Blood was spun at 4°C for 30 minutes at 3000 rpm. Plasma was removed from the red cells using Pasteur pipets and stored frozen in polycarbonate vials until analysis.

If, after blood was drawn, the animal was still alive, carbon dioxide was administered until death occurred. Immediately after sacrifice the entire liver was removed, rinsed, blotted, weighed, and quickly frozen in a polycarbonate specimen cup with a snap-on lid. The kidneys were removed and treated in the same manner. Fetuses were removed by Caesarean section, weighed, and frozen until further analysis. Placentas (both maternal and fetal portions) were removed,

weighed, and frozen in polycarbonate specimen cups. The remaining uterine tissue was removed, weighed, and frozen. The maternal animal was weighed and frozen until further treatment and analysis.

Carcass Analysis

Total Carcass Moisture Content

Carcass in this study refers to the total animal body minus the liver, kidneys, uterus, fetuses, placentas, and blood drawn at sacrifice. Each frozen carcass was chopped into pieces one inch square with a meat cleaver and rubber mallet. The pieces were placed in an aluminum tin, covered with cheesecloth, and freeze-dried for 72 hours to ensure a completely moisture-free carcass. Upon removal from the freeze-drier each carcass was placed in a 55⁰C oven overnight to expel any moisture that may have accumulated in handling after freeze-drying. After drying, each carcass was reweighed and stored under desiccant until further processing could take place. Carcass water content was calculated as the weight lost during freeze-drying.

Total Carcass Fat and Nitrogen Content

The freeze-dried maternal carcass was ground with a 4:1 ratio of sodium sulfate to rat weight using a Hobart grinder. The sodium sulfate was used to promote mixing and thorough grinding of the carcass. During the grinding procedure care was taken to retain all carcass pieces. Grinding took approximately 25 minutes per animal. When the grinding procedure was completed, the carcass plus sodium sulfate was stored in Mason canning jars for further analysis.

Fat and Kjeldahl Nitrogen content of the carcass were determined. A ten gram sample was weighed in duplicate for the Soxhlet fat extraction (Appendix B). Nitrogen was determined using the Kjeldahl method (Appendix C, AOAC, 1970) with zinc and copper as catalysts. A duplicate 1.5 gm sample was used for the Kjeldahl determination.

Liver Analysis

Liver tissue was freeze-dried for 48 hours. It was then weighed and moisture content was determined as the weight lost during freeze-drying (Appendix A). The liver tissue was then ground using a mortar and pestle. Between 0.500 and 0.800 grams was then weighed out and used for the determination of fat content. The Soxhlet fat extraction method was used to determine fat content of the livers (Appendix B). A sample of fat-free liver was then analyzed for nitrogen using the Kjeldahl procedure (Appendix C, AOAC, 1970). A sample of 0.200 to 0.250 gms was used for Kjeldahl nitrogen determination.

Kidney Analysis

Kidney tissue was freeze-dried for 48 hours. It was then weighed and moisture content was determined as the weight lost during freeze-drying (Appendix A). The kidney tissue was then ground using a mortar and pestle. A 0.200 gm sample was weighed and used for the analysis of fat content. The Soxhlet fat extraction method was used (Appendix B).

Placenta and Uterine Analysis

Placentas and uteri were freeze-dried for 48 hours. They were

then weighed and moisture content was determined as the weight lost during freeze-drying (Appendix A).

Fetal Analysis

The fetal tissue was freeze-dried for 48 hours. It was then weighed and moisture content was calculated as the moisture lost in freeze-drying (Appendix A). The fetuses were then ground with mortar and pestle and one combined sample was prepared for each litter of pups. Two pup samples from each dietary treatment were assayed for fat content using the Soxhlet method (Appendix B). As no differences in fat content were found, it was decided that the remaining fetal samples not be analyzed for fat content. Fetal nitrogen was determined using the Kjeldahl method (Appendix C, AOAC, 1979). Duplicate samples of 0.200-0.300 gms were used for nitrogen determination.

Blood Analysis

Plasma samples were thawed at room temperature. Serum protein was determined using the Technicon¹ AAII method. This is a modified biuret procedure (AAII method #14).

Statistical Analysis

A multivariate analysis of variance procedure was used to compare carcass and liver moisture, fat, and protein as well as kidney moisture and fat. Weight change from Day 0 to Day 20 across treatments was also

¹Technicon Instruments, Inc., Tarrytown, NY.

examined by this statistical procedure. Pregnancy and dietary effects were examined between the three dietary treatments, as well as the existence of an interaction between pregnancy and diet. Multivariate one-way analysis of variance was used to compare fetal weight, fetal nitrogen, and placental weight between dietary groups. Univariate one-way analysis of variance was used to compare pup weight as a percentage of maternal gain across treatments. One-way analysis of variance was used to compare serum protein data. When a significant F value was observed, Duncan's Multiple Range Test was then used to determine which groups were significantly different when results were combined across pregnancy and dietary effects. Least Squares Means Multiple Comparison tests were used to locate significant differences between the six dietary treatment groups. The Least Squares Means procedure was used because environmental effects are eliminated from the estimates of treatment effects and because missing data can be handled by applying the least squares procedure to all observations that are not missing.

RESULTS AND DISCUSSION

Breeding of Animals

All female rats were housed on a one-to-one basis with a male rat. Therefore, all rats had an equal chance of becoming pregnant. Although copulation plugs were observed for all rats, pregnancy did not occur in 19 of the animals. Nicholas (1949) and Long and Evans (1922) have shown that up to one-third of all mating attempts do not result in successful pregnancies.

When the copulation plug was observed, the female rat was removed from the male's cage and housed individually. It was at this time that dietary treatment began. Because not all animals became pregnant, they could be assigned to a pregnant or a nonpregnant group for all dietary treatments and subsequent analysis.

Food Intake and Change in Body Weight

Average daily food intakes of the animals are shown in Table 4. It had been assumed initially when copulation plugs were observed that all rats were pregnant. Pair-feeding, or food restriction, was based on the assumption that each control, ad lib fed rat was pregnant. Pregnant animals have been shown to consume more food than do non-pregnant animals due to increased needs to support fetal growth (National Research Council, 1966). Pregnancy in this study occurred randomly. As a result not all control rats were pregnant. Consequently a pregnant calorie-restricted rat may have been paired to a nonpregnant control. This accounts for discrepancies between actual dietary

Table 4. Average Daily Food Intake
of Adult Female Rats

<u>Treatment Group</u>	<u>Food Intake (gms)</u>	<u>Expected Restriction</u>	<u>Actual Restriction</u>
		<u>Level</u>	<u>Level</u>
Ad Lib-Pregnant	20.49	---	---
Ad Lib-Nonpregnant	21.89	---	---
15% Calorie Restricted-Pregnant	17.71	15%	13.57%
15% Calorie Restricted- Nonpregnant	18.53	15%	15.35%
30% Calorie Restricted-Pregnant	13.52	30%	34.02%
30% Calorie Restricted- Nonpregnant	15.36	30%	29.83%

L7

restriction levels and calculated or expected restriction levels. Pregnant ad lib fed rats had a lower daily food intake than the nonpregnant ad lib fed rats. This is contradictory to other research (National Research Council, 1966). This may be explained by the fact that during the first five days of the study ad lib fed rats were meal-fed in an attempt to match food consumption patterns of the restricted animals. Several of the animals were subjected to this regimen before meal-feeding was stopped. Food intake data collected during the 5-day meal feeding period served to lower the average daily food intake. Meal-feeding was stopped because ad lib fed rats on this regimen continued to lose weight. Once ad lib feeding was resumed, weight gain began to follow normal expected patterns.

Average body weight changes for each dietary treatment group are shown in Table 5. Initially all animals were matched for weight when placed into treatment groups. All pregnant animals gained weight regardless of dietary restriction. Nonpregnant animals gained less weight than did pregnant animals on all dietary treatments and actually showed a loss in weight on the 29.83% restricted intake. The nonpregnant animals performed as had been originally expected based on dietary treatment with the ad lib fed group gaining weight, the 15.35% calorie restricted group approaching maintenance of body weight, and the 29.83% calorie restricted group losing body weight. It is evident that the pregnant animal is more metabolically efficient than is the nonpregnant animal. Body weights of pregnant animals combined over dietary treatment groups were significantly greater than those of the nonpregnant

Table 5. Least Squares Means for Body Weight Changes of Adult Female Rats

	Mean Beginning Wt. Day 0 (gms)	Total	Maternal Compartment Weight	Fetal Compartment Weight	Wt. Change (gms)	
Ad Lib-Pregnant	353	438	396.460 \pm 21.614	41.707 \pm 12.253	+85 \pm 8	
15% Restricted-Pregnant	367	448	389.316 \pm 20.720	59.084 \pm 10.466	+81 \pm 9	
30% Restricted-Pregnant	318	374	318.181 \pm 6.294	55.486 \pm 7.385	+56 \pm 11	
Ad Lib-NonPregnant	365	418			+53 \pm 7	
15% Restricted-NonPregnant	357	375			+18 \pm 8	
30% Restricted-NonPregnant	362	355			-7 \pm 8	
$P > t $ $H_0: \text{LSM } (i) = \text{LSM } (j)$						
i/j	1	2	3	4	5	6
1	---	0.7869	0.0467	0.0063*	0.0001**	0.0001**
2	---	---	0.0843	0.0174	0.0001**	0.0001**
3	---	---	---	0.8014	0.0100*	0.0001**
4	---	---	---	---	0.0034*	0.0001**
5	---	---	---	---	---	0.0355
6	---	---	---	---	---	---

* $p < 0.01$
** $p < 0.001$

animals ($p \leq 0.05$). Pregnant animals gained an average of 74.1 grams over the course of pregnancy. Nonpregnant animals gained an average of 21.0 grams over the 20 days of dietary treatment.

Dietary treatment significantly affected changes in body weight ($p \leq 0.05$) when pregnant and nonpregnant animals were grouped. Ad lib fed control rats gained 68.7 grams, 15% calorie restricted gained 49.6 grams and 30% calorie restricted rats gained an average of 24.3 grams over the 20 day period of dietary treatment.

In order to define the exact location of significant differences in maternal weight changes between the six treatment groups the Least Squares Means Multiple Comparison Procedure was used. The results are shown in Table 5. Division of weight gain between maternal and fetal compartments is also shown in Table 5. This provides a more detailed picture of the overall effect of diet and pregnancy on weight change in the maternal animal. These results indicate that there was essentially no difference in total weight change in any of the pregnant animals regardless of dietary treatment. Pregnant animals fed ad lib gained significantly more weight than did any of the nonpregnant animals. Pregnant animals, restricted 15%, did not differ significantly in weight gain from nonpregnant ad lib fed animals, although they did gain significantly more weight than did nonpregnant animals fed 15 and 30% caloric restricted diets. Pregnant animals, restricted 30%, did not gain more weight than ad lib fed pregnant animals but did gain significantly more weight than did nonpregnant animals than were 15 and 30% restricted. Ad lib fed nonpregnant animals gained significantly more weight than did

either of the nonpregnant restricted groups. Nonpregnant 15% calorie restricted animals did not differ significantly in weight gain from nonpregnant 30% calorie restricted animals.

Total pup weight of each litter was calculated as % of maternal weight gain during pregnancy. The mean total pup weight as a percentage of maternal weight gain for each dietary treatment is shown in Table 6. Analysis of Variance showed significant differences in litter weight as percent of maternal gain due to dietary treatment. Duncan's Multiple Range procedures were used to locate differences ($p \leq 0.05$). The results of the Duncan's Multiple Range Test is shown in Table 6. In summary, no differences existed in maternal weight gain of pregnant animals but differences did occur in litter weight expressed as a percentage of maternal weight gain.

Total Serum Proteins

Analysis of variance procedures indicated that serum protein levels were significantly affected by pregnancy state ($p \leq 0.05$) but not by dietary treatment ($p \leq 0.05$). A significant interaction was seen between pregnancy and diet ($p \leq 0.05$). The Least Squares Means Multiple Comparison Procedure was used to determine the location of significant differences between serum protein levels for the six treatment groups. The results are shown in Table 7.

Serum protein was lower in all pregnant groups regardless of dietary treatment. When all pregnant animals were grouped as one group with all nonpregnant animals composing a second group, significant differences

Table 6. Pup Weight As % of Maternal Gain

Treatment Group	Average Number of pup/litter	Total Average Litter Weight (gms) ¹	Total Maternal Weight Gain (gms)	Litter Weight As % of Maternal Gain ¹
Ad Lib	8.000 ± 2.683	25.37 ± 8.946	+85 ± 8	29.85 ± 8.249 ^a
15% Calorie-Restricted	12.200 ± 2.608	38.95 ± 6.909	+81 ± 9	48.09 ± 4.286 ^{a,b}
30% Calorie-Restricted	11.000 ± 1.155	39.91 ± 4.676	+56 ± 11	71.27 ± 6.332 ^b

¹All values are expressed as group means ± SEM

^aDifferent superscripts indicate values which are significantly different ($p \leq 0.05$)

Table 7. Least Squares Means for Total Serum Protein Values of Pregnant and NonPregnant Rats Fed Ad Lib, 15% and 30% Calorie Restricted Diets

<u>Treatment Group</u>	<u>Serum Protein (gms/100 mls)</u>	$p > t $						
		<u>i/j</u>	<u>1</u>	<u>2</u>	<u>$H_0 = LSM (i) = LSK (j)$</u>	<u>3</u>	<u>4</u>	
Ad Lib-Pregnant	<u>6.40±0.25</u>	1	---	0.4872	0.0334	0.0384	0.0073*	0.0004**
15% Restricted-Pregnant	<u>6.15±0.25</u>	2	---	---	0.1127	0.0074*	0.0013*	0.0001**
30% Restricted-Pregnant	<u>6.48±0.32</u>	3	---	---	---	0.0003*	0.0001**	0.0001**
Ad Lib-NonPregnant	<u>7.14±0.23</u>	4	---	---	---	---	0.3935	0.5272
15% Restricted-NonPregnant	<u>7.43±0.25</u>	5	---	---	---	---	---	0.3107
30% Restricted-NonPregnant	<u>7.78±0.23</u>	6	---	---	---	---	---	---

* $p < 0.01$

** $p < 0.001$

were seen in serum protein levels between pregnant and nonpregnant animals ($p \leq 0.05$). Pregnant animals had a mean serum protein level of 6.01 ± 0.16 gms % while nonpregnant animals had a mean serum protein level of 7.45 ± 0.14 gms %. Serum protein levels tended to decrease in pregnant rats as the level of dietary restriction increased although this was not significant at the $p \leq 0.01$ level. Ad lib fed pregnant rats had significantly lower serum protein concentrations than non-pregnant 15 and 30% calorie restricted. Pregnant rats fed 15 and 30% caloric restrictions had significantly lower serum protein concentrations than any of the nonpregnant rats regardless of dietary treatment. In nonpregnant rats serum protein levels tended to increase as dietary restriction increased although this was not significant at the $p \leq 0.01$ level.

Serum protein concentration falls during pregnancy (Committee on Maternal Nutrition, 1970). In a normal human pregnancy there is a sharp decrease in blood protein levels from a concentration of over 7 gms/100 mls of plasma to a concentration of 5.5-6.0 gms of protein/100 mls of plasma during the first trimester. This decreased concentration remains constant for the duration of pregnancy (Committee on Maternal Nutrition, 1970). The decrease in serum protein concentration is thought to be due to one or all of the following mechanisms:

- (1) A decrease in serum albumin content during the first two trimesters of pregnancy. Albumin falls from 4 grams per 100 mls to 2.5-3.0 grams per 100 mls in early pregnancy (Committee on Maternal Nutrition, 1970);
- (2) The normal blood volume increase and subsequent dilution of blood

components (hemoglobin, hematocrit, serum proteins) (Committee on Maternal Nutrition, 1970); (3) Transplacental transfer of proteins as well as amino acids (Dancis and Shafran, 1958). Several investigators (Eurley, 1980; Dancis and Shefran, 1958) have shown that there is evidence for placental transfer of proteins as well as amino acids from maternal circulation to the fetal circulatory system. Amniotic fluid has also been shown to be a medium for the transfer of proteins from the maternal compartment to the fetal compartment. Placental and amniotic transfer of proteins, as well as a dilution effect due to an increase in blood volume (a normal physiologic adjustment associated with the pregnant state) probably account for lowered total serum protein levels in the pregnant animals.

Kidney Composition

Size, moisture, and fat content of the kidney for each group are shown in Table 8. No significant differences in tissue size, % moisture, or % fat were observed between any of the treatment groups ($p \leq 0.05$). Neither pregnancy nor dietary caloric restrictions of 15 or 30% caused any significant change in kidney composition. This suggests that the kidney organ system is a very stable system and is not an indicator of changes that may occur as a result of pregnancy or calorie restriction situations.

Liver Composition

Percent liver fat, protein (nitrogen \times 6.25) and moisture content

Table 8. Kidney Size, % Moisture and % Fat Composition of Adult Female Rats Fed Ad Lib, 15 and 30% Caloric Restrictions¹

<u>Treatment Group</u>	<u>Kidney weight (gms)</u>	<u>% Moisture</u>	<u>% Fat, wet</u>
Ad Lib-Pregnant	2.20 \pm 0.08 ^a	76.89 \pm 0.41 ^b	9.41 \pm 0.56 ^c
15% Restricted-Pregnant	2.22 \pm 0.09 ^a	77.06 \pm 0.45 ^b	8.61 \pm 0.62 ^c
30% Restricted-Pregnant	1.90 \pm 0.15 ^a	77.33 \pm 0.58 ^b	9.61 \pm 0.80 ^c
Ad Lib-NonPregnant	2.40 \pm 0.19 ^a	77.12 \pm 0.38 ^b	8.74 \pm 0.52 ^c
15% Restricted-NonPregnant	2.36 \pm 0.06 ^a	77.12 \pm 0.41 ^b	9.95 \pm 0.56 ^c
30% Restricted-NonPregnant	2.14 \pm 0.09 ^a	77.51 \pm 0.41 ^b	8.91 \pm 0.56 ^c

¹Kidney weights, % Moisture and % Fat are expressed as Least Squares Means \pm SEM

^aMeans with common superscripts are not significantly different

for each group are shown in Tables 9, 10, and 11 respectively. Pregnant animals combined across dietary treatments had a mean % liver fat of $12.48 \pm 1.16\%$. Nonpregnant animals combined across dietary treatments had a mean value of $7.46 \pm 0.95\%$ fat in the liver. Analysis of Variance procedures showed significant differences in % liver fat due to pregnancy. Least Squares Means Multiple Comparison procedures were used to locate where the significant differences occurred between the six treatment groups. Significant differences existed only between the pregnant 15% calorie restricted animals and the nonpregnant 15 and 30% calorie restricted groups for liver fat composition. This may be questioned in that the pregnant 15% restricted group had a mean % liver fat that was greater even than the ad lib pregnant animals. Larger numbers of observations within each group may have explained this phenomenon more clearly. As analyzed by Analysis of Variance procedures no significant differences were observed in liver fat due to dietary treatments and no interaction between pregnancy and diet was observed ($p \leq 0.05$).

No significant differences were observed in percent liver moisture content between any of the six treatment groups as analyzed by Analysis of Variance ($p \leq 0.05$) and the Least Squares Means Multiple Comparison Procedure the results of which are included in Table 10.

Analysis of Variance procedures showed that percent protein in the liver was affected both by dietary treatment and by the pregnant state

Table 9. Least Squares Means for % Liver Fat in Adult Female Rats
Fed Ad Lib, 15% and 30% Calorie Restricted Diets

Dietary Treatment	% Liver Fat, wet	i/j	1	p > t	$H_0:$	LSM (1) = LSM (j)		
				2	3	4	5	6
Ad Lib-Pregnant	11.18±1.69	1	---	0.0701	0.7794	0.4257	0.0730	0.0546
15% Restricted-Pregnant	15.92±1.86	2	---	---	0.0771	0.0113	0.0011*	0.0008**
30% Restricted-Pregnant	10.35±2.40	3	---	---	---	0.7200	0.2255	0.1857
Ad Lib-NonPregnant	9.31±1.57	4	---	---	---	---	0.2696	0.2127
15% Restricted-NonPregnant	6.71±1.69	5	---	---	---	---	---	0.8867
30% Restricted-NonPregnant	6.36±1.69	6	---	---	---	---	---	---

*p < 0.01

**p < 0.001

Table 10. Least Squares Means of Liver Moisture in Adult Female Rats
Fed Ad Lib, 15% and 30% Calorie Restricted Diets

<u>Treatment Groups</u>	<u>% Liver Moisture</u>	<u>i/j</u>	<u>1</u>	<u>p > t </u>	<u>H₀:</u>	<u>LSM (i) = LSM (j)</u>	<u>6</u>
				<u>2</u>	<u>3</u>	<u>4</u>	
Ad Lib Pregnant	66.10 \pm 1.12	1	---	0.3610	0.2845	0.9223	0.2323 0.0873
15% Restricted-Pregnant	64.55 \pm 1.23	2	---	---	0.0787	0.3934	0.0458 0.0142
30% Restricted-Pregnant	68.22 \pm 1.59	3	---	---	---	0.2413	0.9257 0.7242
Ad Lib-NonPregnant	65.95 \pm 1.04	4	---	---	---	---	0.1831 0.0630
15% Restricted-NonPregnant	68.03 \pm 1.12	5	---	---	---	---	---
30% Restricted-NonPregnant	68.91 \pm 1.12	6	---	---	---	---	---

*p < 0.01

of the animals ($p \leq 0.05$). Pregnant animals had a mean % liver protein of $25.00 \pm 0.70\%$ and nonpregnant animals had a mean % liver protein content of $22.47 \pm 0.58\%$. Ad lib fed animals combined across the pregnant and nonpregnant state had a mean % liver protein of $21.89 \pm 0.70\%$, 15% calorie restricted animals $25.23 \pm 0.76\%$, and 30% calorie restricted animals $24.07 \pm 0.89\%$. Analysis of Variance procedures indicated a significant effect on liver protein due to an interaction between pregnancy and diet ($p \leq 0.05$). The Least Square Means Multiple Comparison Procedure was used to locate where significant differences occurred between the six treatment groups. The results of the Least Square Means procedure are shown in Table 11. Ad lib fed pregnant rats had significantly lower % liver protein than the 15% calorie restricted pregnant animals but were not significantly different from any of the other treatment groups. Pregnant 15% restricted animals had significantly more % liver protein than any of the nonpregnant animals regardless of dietary treatment. Pregnant 30% calorie restricted animals did not differ significantly in % liver protein from any of the nonpregnant animals regardless of dietary treatment. No significant differences in % liver protein were observed between any of the nonpregnant animals. In summary, as dietary restriction increased, the percentage of protein in the liver also tended to increase. The higher value for the 15% calorie restricted group may be explained by one extremely high value

Table II. Least Squares Means for % Liver Protein in Adult Female Rats
Fed Ad Lib, 15% and 30% Calorie Restricted Diets

<u>Treatment Groups</u>	<u>% Liver protein, wet</u>	<u>t/j</u>	<u>1</u>	<u>p > t </u>	<u>II : LSM (1) = LSM (j)</u>			
				<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
Ad Lib-Pregnant	21.98 \pm 1.03	1	---	0.0004**	0.1241	0.8985	0.8414	0.3633
15% Restricted-Pregnant	28.19 \pm 1.13	2	---	---	0.0785	0.0002**	0.0006**	0.0037*
30% Restricted-Pregnant	24.82 \pm 1.46	3	---	---	---	0.0950	0.1663	0.4125
Ad Lib-NonPregnant	21.80 \pm 0.95	4	---	---	---	---	0.7377	0.2861
15% Restricted-NonPregnant	22.28 \pm 1.03	5	---	---	---	---	---	0.4761
30% Restricted-NonPregnant	23.33 \pm 1.03	6	---	---	---	---	---	---

*p < 0.01

**p < 0.001

in that treatment group. The increase in liver protein as dietary restriction increases shows that there is a higher percentage of lean tissue in the groups that are more restricted.

Research in the area of liver composition and changes during pregnancy and weight reduction is very limited. It appears from this data that this may be an area noteworthy of further investigation.

Total Carcass Composition

Carcass analysis data for all treatment groups is presented in Tables 12, 13, and 14. For purposes of analysis, carcass refers to the entire animal carcass minus the liver, kidneys, fetuses (if applicable), uterus, and blood drawn at sacrifice.

Pregnant animals had a mean percent carcass fat content of $27.39 \pm 1.35\%$; nonpregnant animals had a mean percent carcass fat of $32.76 \pm 1.11\%$ combined over dietary treatments. Carcass fat appeared to decrease as dietary restriction increased. Ad lib fed animals combined over the pregnant and nonpregnant states had a mean % carcass fat content of $33.88\% \pm 1.34\%$, 15% calorie restricted animals $30.58 \pm 1.46\%$, and 30% calorie restricted animals had a carcass fat percentage of $25.77 \pm 1.71\%$. Analysis of Variance procedures showed significant differences to be present in the carcass fat components as a result of pregnancy and dietary treatment ($p \leq 0.05$). The Least Square Means Multiple Comparison procedure was used to identify the exact location of significant differences between the six treatment groups. The

Table 12. Least Squares Means for Percent Carcass Fat In Pregnant and NonPregnant Female Rats
Fed Ad Lib, 15% and 30% Calorie Restricted Diets

Treatment Group	% Carcass Fat, wet			$p > t $	$H_0:$	LSM (i) = LSM (j)		
		1/j	1			4	5	6
Ad Lib-Pregnant	30.92±1.97	1	---	0.7994	0.0077*	0.0366	0.9811	0.8652
15% Restricted-Pregnant	30.17±2.16	2	---	---	0.0158	0.0260	0.7820	0.9264
30% Restricted-Pregnant	21.09±2.79	3	---	---	---	0.0001**	0.0073*	0.0108
Ad Lib-NonPregnant	36.84±1.83	4	---	---	---	---	0.0386	0.0248
15% Restricted-NonPregnant	30.99±1.97	5	---	---	---	---	---	0.8466
30% Restricted-NonPregnant	30.45±1.97	6	---	---	---	---	---	---

* $p < 0.01$

** $p < 0.001$

results of the Least Squares Means procedure are shown in Table 12. Ad lib fed pregnant rats had significantly greater carcass fat content than the 30% calorie restricted pregnant rats but did not differ significantly from the pregnant 15% calorie restricted group or any of the nonpregnant treatment groups. Pregnant 15% calorie restricted rats did not differ significantly in carcass fat content from any of the other five treatment groups. Significant differences were observed between the pregnant 30% restricted animals and the ad lib pregnant animals as well as the nonpregnant ad lib fed and 15% calorie restricted groups. Pregnant 30% calorie restricted animals had significantly lower carcass fat content than these three groups. The Analysis of Variance procedure did not show any significant effect of a possible interaction between pregnancy and diet on carcass fat content ($p \leq 0.05$).

Moisture content of the carcasses showed an inverse relationship with carcass fat as was expected. Ad lib fed nonpregnant animals had the lowest % carcass water and the highest % carcass fat. In the 30% calorie restricted pregnant animals the opposite was seen with this group having the highest % carcass water and the lowest % carcass fat. This inverse relationship between moisture and fat has been documented by many researchers (Farris and Griffith, 1949). Lederman and Rosso (1981b) observed that obese, food restricted rats do not expand their total body water content during pregnancy which is slightly contradictory to the data presented here. In this research carcass water content increased in pregnant animals as caloric restriction increased. Total carcass water content did not change greatly in nonpregnant rats

as caloric restriction increased although a slight increase was seen.

Results from the Least Squares Means Multiple Comparison procedure are shown in Table 13. Pregnant 30% calorie restricted rats had significantly greater carcass moisture content than pregnant ad lib fed animals and all nonpregnant animals regardless of dietary treatment. It may be speculated that this difference can be related to decreased fat content in the pregnant 30% calorie restricted group. Another speculative explanation may be that in this research not enough dietary stress was imposed to cause a decrease in normal blood volume expansion as may have occurred in the research of Lederman and Rosso (1981).

Total carcass nitrogen (Table 14) decreased as caloric restriction increased. Lederman and Rosso (1981) also observed decreases in total carcass nitrogen with food restriction during pregnancy. Pregnant animals restricted by 30% had the lowest total carcass nitrogen. This may have been a manifestation of weight differences rather than dietary treatment.

Percent carcass protein tended to increase as dietary caloric restriction increased with ad lib fed animals having a % carcass protein content of $16.92 \pm 0.35\%$; 15% calorie restricted animals $17.55 \pm 0.38\%$, and 30% calorie restricted animals having a mean % carcass protein of $18.95 \pm 0.44\%$ when pregnant and nonpregnant animals were grouped together to examine the effect of dietary treatment. Analysis of Variance procedures showed significant differences ($p \leq 0.05$) in percent carcass protein due to pregnancy and dietary treatment although no statistically significant interrelationship between the two was

Table 13. Least Squares Means for Percent Carcass Moisture in Pregnant and NonPregnant Female Rats
Fed Ad Lib, 15% and 30% Calorie Restricted Diets

Treatment Group	% Carcass Moisture	i/j	1	p > t	H ₀ :	LSM (1) = LSM (j)		
		1/1	1	2	3	4	5	6
Ad Lib-Pregnant	50.53±1.41	1	---	0.9241	0.0092*	0.0129	0.4454	0.7677
15% Restricted-Pregnant	50.73±1.55	2	---	---	0.0137	0.0139	0.4113	0.7065
30% Restricted-Pregnant	57.39±2.00	3	---	---	---	0.0001**	0.0019*	0.0051*
Ad Lib-NonPregnant	45.41±1.31	4	---	---	---	---	0.0740	0.0262
15% Restricted-NonPregnant	48.99±1.41	5	---	---	---	---	---	0.6379
30% Restricted-NonPregnant	49.94±1.41	6	---	---	---	---	---	---

*p < 0.01

**p < 0.001

Table 14. Total Carcass Nitrogen and Least Squares Means for Percent Carcass Protein
in Pregnant and NonPregnant Female Rats for Ad Lib,
15% and 30% Calorie Restricted Diets

Treatment Group	Total Carcass Nitrogen (gms)	% Carcass Protein	p > t H ₀ : LSM (i) = LSM (j)					
			i/j	1	2	3	4	5
Ad Lib-Pregnant	10.10	17.52 ⁺ 0.51	1	---	0.8933	0.0111	0.0971	0.8247
15% Restricted-Pregnant	9.87	17.42 ⁺ 0.56	2	---	---	0.0105	0.1470	0.7301
30% Restricted-Pregnant	9.26	19.93 ⁺ 0.72	3	---	---	---	0.0003** 0.0170	0.0359
Ad Lib-NonPregnant	10.09	16.33 ⁺ 0.47	4	---	---	---	---	0.0615
15% Restricted-NonPregnant	9.84	17.68 ⁺ 0.51	5	---	---	---	---	0.6845
30% Restricted-NonPregnant	9.41	17.98 ⁺ 0.51	6	---	---	---	---	---

**p < 0.01; Total Carcass Nitrogen expressed as group means

observed ($p \leq 0.05$). The Least Squares Means Multiple Comparison Procedure was used to locate the significant differences between the six treatment groups. Results of the Least Squares Procedure are shown in Table 14. As indicated by the results of this procedure, no significant differences in percent carcass protein were present between the six treatment groups except for the pregnant 30% calorie restricted animals and ad lib fed nonpregnant animals. Differences in percent carcass protein for several other groups were approaching significance and larger numbers of observations per group may have shown significant differences to be present.

Placental and Uterine Analysis

Placentas were dissected out of the uterine tissue and separated from the fetuses at sacrifice, placental weight/pup was calculated with the following values being obtained: Ad lib = 0.681 grams; 15% calorie-restricted = 0.706 grams; and 30% calorie-restricted = 0.663 grams. Dietary treatment did not exert a significant effect on placental size which is contrary to the findings of other researchers (Lederman and Rosso, 1980, 1981). Lederman and Rosso (1981b) observed a reduction of 25% in placental weight in food restricted obese and nonobese animals. Dietary restriction was not as severe in this research as that invoked by other researchers (Lederman and Rosso, 1980, 1981). Another point to be made again is that Lederman and Rosso imposed total food restrictions rather than just a caloric restriction as imposed in this study. This would indicate that a break off point may exist between the 30% calorie restriction imposed in

this research and the levels of restriction imposed in other studies in which placental growth retardation was observed (Lederman and Rosso, 1980, 1981).

Uterine tissue weights were greater in pregnant than nonpregnant animals as expected due to expansion of tissue during pregnancy. Dietary restriction did not have a significant effect on uterine size ($p \leq 0.05$).

Fetal Composition

Average fetal weight, % moisture, and nitrogen content are shown in Table 15. There were no significant differences between fetal size, % moisture or nitrogen content between dietary treatments ($p \leq 0.05$). This indicates that maternal stores were sacrificed at these levels of restriction to support growth and development of the fetal compartment. Total nitrogen content of the litter is shown in Table 15. As can be seen more nitrogen was laid down in the fetal compartment in the 15 and 30% restricted groups. This may be a manifestation of number of pups in each litter since this calculation is based on total litter weight. Lederman and Rosso (1980, 1981) have observed fetal growth retardation of approximately 25% in litters born to dams that were food restricted by as much as 60% of a matched pregnant control group during gestation. The restrictions that Lederman and Rosso imposed during pregnancy involved restrictions of all nutrients. Their dietary regimen differs from that used in the present experiment in that protein, vitamins, and minerals were supplemented in this research so that a protein depletion effect could be ruled out as possibly influencing any results that were obtained.

Table 15. Fetal Composition of Litters Born to Ad Lib-Fed,
15% Calorie Restricted and 30% Calorie Restricted
Dams During Gestation¹

Treatment Group	Average Fetal Weight (gms)	% Moisture (gms)	Nitrogen mg N/gm wet tissue	Total Nitrogen Content of Litter (mg Nitrogen)
Ad Lib	3.398 \pm 0.109 gms	86.142 \pm 0.607	15.201 \pm 0.758	432.447 \pm 163.806
15% Calorie Restricted	3.362 \pm 0.096 gms	85.710 \pm 0.792	15.772 \pm 1.056	650.005 \pm 156.538
30% Calorie Restricted	3.635 \pm 0.057 gms	85.604 \pm 1.119	15.991 \pm 1.499	650.895 \pm 162.090

¹All means are expressed as group means \pm SEM

SUMMARY AND CONCLUSIONS

The present experiment was conducted to determine the effect of 15 and 30% caloric restrictions during pregnancy on body composition of obese female rats and on fetal composition. Thirty-nine six month old obese female Sprague-Dawley rats were assigned on a weight basis to one of three dietary treatments: ad lib, 15% caloric restriction, and 30% caloric restriction. All other nutrients were fed at levels to meet dietary requirements. Each treatment group was further sub-divided into pregnant and nonpregnant animals. Rats were sacrificed on Day 20 of gestation and fetuses were taken by Caesarean section. The effects of the dietary treatment on maternal body composition, liver composition, serum protein concentration, and on fetal composition were determined.

Serum protein levels tended to decrease in pregnant animals as caloric restriction increased. Serum protein levels were significantly lower in pregnant animals than in nonpregnant animals. Serum protein levels in nonpregnant animals tended to increase as dietary restriction increased. Placental transfer of proteins may be an interesting phenomenon to examine in relationship to the data presented with this research.

Weight change for the pregnant rats was +85 gms in the ad lib group, +82 gms in the 15% calorie restricted group, and +56 gms in the 30% calorie restricted group. Weight changes in the non-pregnant rats for 20 days of dietary treatment were +53 gms, +18 gms, and -7 gms respec-

tively. Weight changes observed suggest that the maternal compartment is more efficient at metabolizing available food during pregnancy.

Fetal body nitrogen and average pup weight did not differ between treatment groups. This indicates that the fetal compartment is not affected by maternal caloric restriction up to 30% provided that all other nutrients are adequate. Pup weight as % of maternal gain was significantly different between treatment groups. Pup weight comprised almost 75% of the weight gained by the 30% calorie restricted animals during pregnancy which indicates that the fetal compartment is favored rather than the building up of maternal stores at this level of dietary intake.

Total body nitrogen was not decreased during pregnancy in the ad lib and 15% calorie restricted animals but a decrease was seen in the carcass nitrogen content of the 30% calorie restricted pregnant animals. No differences were observed when nitrogen was expressed as a percentage of total body composition.

Pregnant ad lib fed and 15% calorie restricted animals showed similar carcass fat content at the termination of pregnancy. Pregnant animals on the 30% calorie restricted diet had a carcass fat content that was 10% lower than the ad lib-fed and 15% restricted groups. This would indicate that maternal fat stores were being mobilized to support the fetal compartment at this level of caloric restriction.

Based on Analysis of Variance procedures, liver composition appeared to be affected both by pregnancy and diet. Least Squares Means Multiple comparison procedures however showed that % liver fat was only

significantly different between the pregnant 15% calorie restricted animals and the nonpregnant 15 and 30% restricted groups. Percent liver protein was significantly different between dietary treatments for pregnant animals. Pregnant 15% restricted animals had a significantly higher percentage of liver protein than did pregnant ad lib fed animals but values were not significantly different from those seen in pregnant 30% calorie restricted animals. In summary as the level of dietary restriction increased the percentage of protein in the liver also tended to increase although all of the differences were not significant. This may be indicative of the fact that the caloric restriction was not severe enough to significantly affect tissue systems in the maternal animal. Literature on liver composition during pregnancy is limited and this data may be worth reviewing more seriously.

In comparing the data presented in this research to that of other researchers in the area, most notably Lederman and Rosso, two problems may exist with the methodology of Lederman and Rosso. Lederman and Rosso defined obesity in their experimental animals as being 50 grams heavier in body weight than chow-fed controls. By using this definition, they may have been identifying the fast-growers as obese rather than achieving a true obese state. Also, the dietary restriction imposed by Lederman and Rosso is a total restriction of all nutrients. This does not eliminate the possibility of a malnutrition effect (lack of dietary protein) rather than a weight loss effect.

In a future study it would be beneficial to look at 40 and 50% caloric restrictions during pregnancy while maintaining adequate protein intake. Differences in amount and kind of restriction between this study and those found in the literature make it impossible to draw any conclusions about a break-off point above which the fetal compartment is favored at the expense of the maternal compartment when calorie restriction is superimposed on the pregnant state. In the present study, a level of 30% caloric restriction while maintaining adequate protein, vitamin, and mineral intake did not adversely affect the fetal compartment. Contrary to this, Lederman and Rosso have shown that food restrictions of 50 and 60% of total nutrient intake did adversely affect fetal and placental growth. Further investigation with increases in caloric restriction and larger numbers of observations are needed to begin to answer the questions about obesity and weight reduction during pregnancy.

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Appendix A

Moisture Determination

1. Dry samples for 48-72 hours in freeze-dryer. Remove samples from freeze-dryer and place in 50°C oven overnight.
2. Cool in dessiccator and weigh. Report loss in weight as moisture.

Calculations:

$$\% \text{ Moisture} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Appendix B

Soxhlet Fat Extraction Method for Total Fat

1. Label filter paper. Weigh paper and 2 paper clips. Record this weight. Weigh and record sample weight (10 grams for carcass; 1 gram for liver; 0.100 grams for kidney). Fold sample in filter paper, fasten with paper clips, and place in large Whatman thimble.
2. Fill the Soxhlet flask over the 5000 ml mark with petroleum ether.
3. Assemble flask, thimble in thimble reservoir, and condenser.
4. Turn on cold water to circulate through condenser.
5. Turn on heat to approximately 90° C.
6. Operate 48 hours checking the ether level several times during this period.
7. Turn off heat. Allow petroleum ether to stop boiling and then turn off cold water. Remove thimble and pour ether in chamber back into the 5000 ml flask. Place samples in a pipette drying rack that has been lined with paper towels. Leave rack under fume hood for 1-2 hours until no trace of petroleum ether is left.
8. When the ether smell is gone, place rack containing samples in drying oven at 45-50° C and allow samples to dry overnight.
9. Following drying, place samples in dessicator to cool. Weight when cool.

Calculations:

$$\% \text{ Fat dry} = \frac{\text{sample weight (dry)} - \text{Extracted sample weight (dry)}}{\text{sample weight (dry)}} \times 100$$

$$\% \text{ Fat wet} = \% \text{ Fat dry} \times \frac{\text{wet weight of sample}}{\text{dry weight of sample}}$$

Appendix C

Modified Kjeldahl Procedure for Nitrogen and Protein

Principle:

The nitrogen is oxidized to $(\text{NH}_4)_2\text{SO}_4$ by digestion with concentrated H_2SO_4 . The digest is made alkaline with concentrated NaOH and the NH_3 is distilled into a saturated solution of boric acid. The amount of nitrogen obtained is multiplied by the factor 6.25 to arrive at the crude protein content of the sample.

Procedure:

Sample Size: 0.5 - 2.0 g (equivalent to 15 to 40 mg. of nitrogen).

Digestion: Place sample in the kjeldahl flask. Also, prepare two blanks. Add 10 gms. Na_2SO_4 - CuSO_4 mixture and 4 glassbeads to each flask. Pour 25 ml H_2SO_4 down the side of the flask to wash down any sample adhering to the neck of the flask. If sample is larger than 2 g add 10 ml H_2SO_4 for each additional g of sample.

Place flask on burners and turn heat to 2. Heat at this setting until samples stop frothing and begin to clear. Throughout this period, turn flasks frequently to rinse down carbonaceous material. Turn heat to 4 and heat until sample turns clear green in color. When all samples have turned clear green in color, turn heat up to 7 and heat for 30 minutes. After 30 minutes, turn heat off and allow samples to cool on the heating rack until vapors are no longer apparent.

When flasks can be handled by hand, move the flasks to the rack and stopper them tightly. Allow samples to cool completely.

When samples are completely cool, add 250 ml of distilled water. Add slowly at first, mixing during the addition. Re-stopper the flask.

Distillation: When samples are thoroughly cool, pour about 25 ml of 4% boric acid and 25 ml H_2O into 500 ml Erlenmeyer flasks. Add 4 drops of mixed indicator (methyl red-methylene blue). Place flask under the distillation rack and insert the delivery tube under the surface of the liquid. Turn on water to the condenser. Check to be sure cold water is flowing.

Turn on burners to 2 to let them warm up. Swirl each flask to mix contents. To each kjeldahl flask add approximately 1/16 teaspoon of granular zinc and then immediately add 70-80 ml of 50% NaOH -- add slowly down the side of the flask to layer the NaOH below the diluted sulfuric acid digest.

Connect the flask with the distillation rack. Swirl slowly and then vigorously to mix the contents of the flask. Turn the heat up to 5 immediately and place the label on the receiving flask. If the mixture does not turn blue, the acid was not neutralized and more NaOH should be added to the samples.

Distill until about 200 ml are in the collection flask. Lower the collection flask so the tube is out of the liquid. Distill to 225 ml total. Turn off heat.

Titration: Titrate in order of coming off the distillation. Titrate to a slight purple color. Titrate all samples until the color matches the end-point of the blank.

Calculations:

One equivalent of HCl reacts quantitatively with one equivalent of N as ammonium borate. Therefore:

Normality of acid x 14.000 = mg of N equivalent to 1 ml of acid

Total N = $\frac{(\text{ml HCl} - \text{ml blank})}{(\text{normality of acid} \times 14.000)}$ x equivalent of N

$$\% \text{ Protein} = \frac{\text{mg N}}{\text{g sample (wet)}} \times 6.25 \times 0.1$$

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THE EFFECT OF DIETARY CALORIC RESTRICTION DURING PREGNANCY
ON MATERNAL AND FETAL BODY COMPOSITION IN THE
OBESE SPRAGUE-DAWLEY RAT

by

Leslie Kirby Reynolds

(ABSTRACT)

Obese, female Sprague-Dawley rats were assigned, on a weight basis, to one of three dietary treatments: ad lib, 15% caloric restriction, and 30% caloric restriction. All other nutrients were fed at levels to meet dietary requirements. Each treatment groups was further sub-divided into pregnant and non-pregnant animals. Rats were sacrificed on Day 20 of gestation and fetuses were taken by Caesarean section. Maternal and fetal body composition, maternal serum protein concentrations were examined. Weight change for pregnant rats was +85 gms in the ad lib-fed group, +82 gms in the 15% calorie restricted group, and +56 gms in the 30% calorie restricted group. Weight changes for the non-pregnant rats for 20 days of dietary treatment were +53 gms, +18 gms, and -7 gms respectively. Fetal body nitrogen and average pup weight did not differ between treatment groups. Total maternal body nitrogen was not decreased during pregnancy in ad lib-fed and 15% calorically restricted animals. It did decrease in 30% calorically restricted animals. Ad lib-fed animals showed no changes in total body fat. Animals on the 15% calorie restriction diet showed no change in total body fat percentages. Animals on the 30% calorie restriction showed a 10% decrease in total body fat content as compared

to the ad lib and 15% restricted pregnant group. Serum protein levels decreased in pregnant animals as caloric restriction increased. Serum protein levels in nonpregnant animals increased as dietary restriction increased. The fetal compartment was not affected by maternal caloric restriction up to 30% provided that all other nutrients were adequate. Maternal stores were affected at a level of 30% caloric restriction.