THE EFFECT OF A MATERNAL DIETARY LYSINE DEFICIENCY ON TISSUE CARNITINE LEVELS IN THE RAT

by

MARY JANE MUISE TAYLOR

BSc.HEc. Mount Saint Vincent University, 1977

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Division of Human Nutrition

School of Home Economics

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1980

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

School
Department of Home Economics

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date April 14, 1980

ABSTRACT

The effect of a maternal dietary lysine deficiency on milk carnitine levels and on plasma and liver carnitine levels in dams, fetuses and neonates was studied. Experimental animals were fed either a low-lysine diet (0.27% lysine), a high-lysine diet (1.07% lysine) ad libitum, or the high-lysine diet pair-fed to the low-lysine group. All diets contained 20% wheat gluten, 20% corn oil and negligible carnitine.

Dams fed a diet, either low in lysine or restricted in total food intake, consumed significantly less food during pregnancy and lactation than high-lysine dams. When compared to high-lysine dams the low-lysine dams and their pair-fed controls gained significantly less weight during pregnancy and lost weight during lactation whereas the high-lysine dams gained weight during lactation.

Litter size was not affected by either a dietary lysine deficiency or by the small reduction in total food intake during gestation. However, birth weight of offspring in the low-lysine and high-lysine restricted groups was significantly lower than that of the high-lysine controls.

On day 15 of lactation the high-lysine pups weighed significantly more than the high-lysine restricted pups, which in turn weighed significantly more than the low-lysine pups, suggesting a superior lactation performance for those dams fed the high-lysine control diet and the poorest lactation performance for those dams consuming the low-lysine diet.

Liver and heart tissue samples were obtained from dams and their offspring on day 21 of pregnancy and day 15 of lactation.

When liver weight or heart weight were expressed as a percentage of total body weight for dams or pups, no significant difference between dietary groups was detected. These results indicate that liver and heart weights were proportional to body weight.

The low-lysine diet had no significant effect, on day 21 of gestation, on maternal plasma or liver carnitine levels or on fetal liver carnitine levels, whereas fetal plasma carnitine showed a small but significant increase compared to the high-lysine group. On day 15 of lactation plasma and liver carnitine levels were significantly higher in both dams and offspring fed the low-lysine diet, than in their respective controls. This increase in plasma and liver carnitine levels was probably due to a lowered food intake since animals fed the high-lysine diet pair-fed to the low-lysine group showed the same tissue carnitine response as did animals fed the low-lysine diet.

Milk carnitine levels on day 2 of lactation were highest in the high-lysine group and lowest in the high-lysine restricted group. On days 8 and 15 of lactation milk carnitine levels were significantly higher in dams fed the low-lysine diet than in those fed the high-lysine or the high-lysine restricted diet.

The results of this research indicate that plasma and liver carnitine levels in both dams and offspring and milk carnitine levels in dams, are not limited by the lysine content of the maternal diet under the experimental conditions of this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENT

I would like to thank my advisor Dr. P. Stapleton, Division of Human Nutrition, University of British Columbia, for his invaluable guidance, advise, and support. The helpful suggestions of my committee members, Dr. P. Hahn, Department of Pediatrics, University of British Columbia and Dr. P. V. Gallo, Division of Human Nutrition, University of British Columbia, are gratefully appreciated.

I would like to acknowledge Lewis James, Arts Computing, University of British Columbia, for his skills and assistance with the computer programs used to complete this thesis. Also acknowledged are the members of Dr. Hahn's lab, particularly Freda-Ann Smale and Nadia Hahn, for their instructions concerning the carnitine assay.

The financial support of the Natural Sciences and Engineering Research Council of Canada (1978-1979), the Summer Graduate Student Bursary (1979), and the University Teaching Assistantship (1977-1978) are gratefully acknowledged.

Special thanks are extended to my sister, Audrey Muise, for providing a "helping hand" when it was needed most.

Finally, to whom I am especially grateful, I would like to thank my husband, Paul Taylor, for his help, encouragement, and love throughout the course of this study.

To my parents

Gerald and Mary Muise

for a lifetime of support and encouragement

CHAPTER I

INTRODUCTION

Carnitine (β-hydroxy-γtrimethylaminobutyrate) plays a vital role in the intramitochondrial transport and subsequent oxidation of long-chain fatty acids (Fritz and Yue, 1963). It is synthesized from lysine and methionine, both of which are essential dietary amino acids (Broquist et al., 1975). Carnitine, although having the attributes of a vitamin is not considered one. It has been suggested that the relationship of carnitine to lysine is similiar to that which exists between niacin and tryptophan. Therefore if sufficient lysine is present in the diet, carnitine can be synthesized in adequate amounts in the body (Tanphaichitr and Broquist, 1976). It is not known how much lysine is required for carnitine synthesis although Horne et al. (1971) have estimated that 0.2% of the lysine requirement of Neurospora crassa is used for carnitine synthesis.

Because of its role in fatty acid oxidation, a carnitine deficiency may cause an impairment in fat utilization. Fat accumulation in tissues of the rat (Tanphaichitr et al., 1976), guinea pig (Wittles and Bressler, 1964), and human (Isaacs et al., 1976) has been associated with carnitine deficiencies.

Dietary carnitine is generally obtained from animal foodstuffs, whereas plant foods are usually poor sources of carnitine (Fraenkel, 1954; Panter and Mudd, 1969). Plant foods are also likely to be deficient in lysine and/or methionine, the precursors of carnitine. Diets comprised mainly of plant foods may result in significantly lowered plasma carnitine levels in

humans (Mikhail and Mansour, 1976; Latifa and Bamji, 1977).

Availability of carnitine is of prime importance in physiological conditions that are associated with changes in fat metabolism. Pregnancy and lactation are examples of such physiological conditions. Since the neonatal rat derives most of its energy from milk fat, a deficiency of carnitine could impair utilization of dietary fat and thus adversely affect development of the neonate. The dam's milk provides the major source of carnitine for the suckling rat because neonatal carnitine synthesis appears insignificant, at least during the early stages of lactation (Ferre et al., 1978). Fetal tissues contain lower levels of carnitine, which appears to be derived mainly from maternal sources and is limited by placental transfer of carnitine (Hahn and Skala, 1975). Therefore a maternal carnitine deficiency could result in a carnitine deficiency in the offspring.

The relationship between dietary lysine and tissue carnitine has not been studied in the pregnant or lactating rat. In the post-weanling male rat, a dietary lysine deficiency reduced heart and skeletal muscle carnitine by 25% compared to control values. Female rats of the same age, responded differently to a lysine deficiency. Heart muscle carnitine of the female was not affected by dietary lysine levels (Borum and Broquist, 1977). Compared to the respective control values, plasma carnitine levels were higher in the plasma of female rats, but lower in the plasma of male rats, fed a low-lysine diet.

Stapleton and Hill (1972, 1980) previously reported a

significant drop in plasma lysine levels during the last week of gestation in dams fed a diet deficient in lysine and containing negligable carnitine or the same basal diet supplemented with lysine. However, the decrease in plasma lysine levels in the lysine deficient animals was significantly greater than that in the lysine supplemented animals. The quantity and quality of milk produced by the dams was also adversely effected by the dietary lysine restriction. Milk production was reduced, as was the quantity of protein in their milk. The effects of feeding a maternal diet deficient in lysine and containing negligible carnitine, during gestation and lactation, on the carnitine status of both the mother and her offspring is not known. However, the possibility that such a dietary condition does adversely affect the neonate is proposed since evidence suggests a dependency of the neonate on the mother rat for its major supply of carnitine to meet physiological requirements.

Therefore the major objectives of this thesis were to determine the effects of a maternal lysine deficiency on:

a) tissue carnitine levels of dams and their fetuses on day 21 of gestation.

b) tissue carnitine levels of dams and their pups on day 15 of lactation.

c) milk carnitine levels of dams on days 2, 8, and 15 of lactation.

CHAPTER II

REVIEW OF LITERATURE

FUNCTION OF CARNITINE

The existence of carnitine, β-hydroxy-trimethylaminobutyrate was known in the early 1900's but it was many years before its structure was finally established (Fraenkel, 1957). Carnitine, an essential growth factor for the mealworm Tenebrio molitar, was isolated in 1946 (Fraenkel and Blewett, 1946) and tentatively named Vitamin BT (T standing for Tenebrio). Several years later vitamin BT was recognized as carnitine (Carter et al., 1952). One of the first observations regarding the function of carnitine was by Fritz in 1955 who reported that the addition of carnitine to rat liver homogenates increased the rate of fatty acid oxidation. This led to an extensive study of the function of carnitine in fat metabolism, specifically long-chain fatty acids. Short chain fatty acids do not require carnitine for their oxidation (Fritz et al., 1962; Wittles and Bressler, 1964; Bressler and Wittles, 1965) and are activated by intra-mitochondrial enzymes (Rossi and Gibson, 1964; Bremer, 1968).

Long-chain fatty acids, the principal fuel for oxidative metabolism in tissues, are transported in the plasma as free fatty acids bound to albumin (Dole, 1956; Gordon, 1957; Gordon et al., 1958; Fredrickson et al., 1958). Carnitine plays an important role in the transport of the activated long-chain

fatty acyl groups from the site of activation to the mitochondrial matrix (Fritz and Yue, 1963; Pande, 1975), where the enzymes of B-oxidation are located (Beattie, 1968). Activation occurs when the long-chain fatty acid is acylated to coenzyme A (Figure 1). This process takes place in both extra-mitochondrial sites and the mitochondria (Rossi and Gibson, 1964; Norum et al., 1966; Bremer, 1968). The long-chain fatty acid activating system in the liver is localized mainly (70%) in the endoplasmic reticulum with the remaining activity in the mitochondria (Norum et al., 1966; Farstad et al., 1967). Most of the mitochondrial activity is found in the outer membrane fraction (Norum et al., 1966).

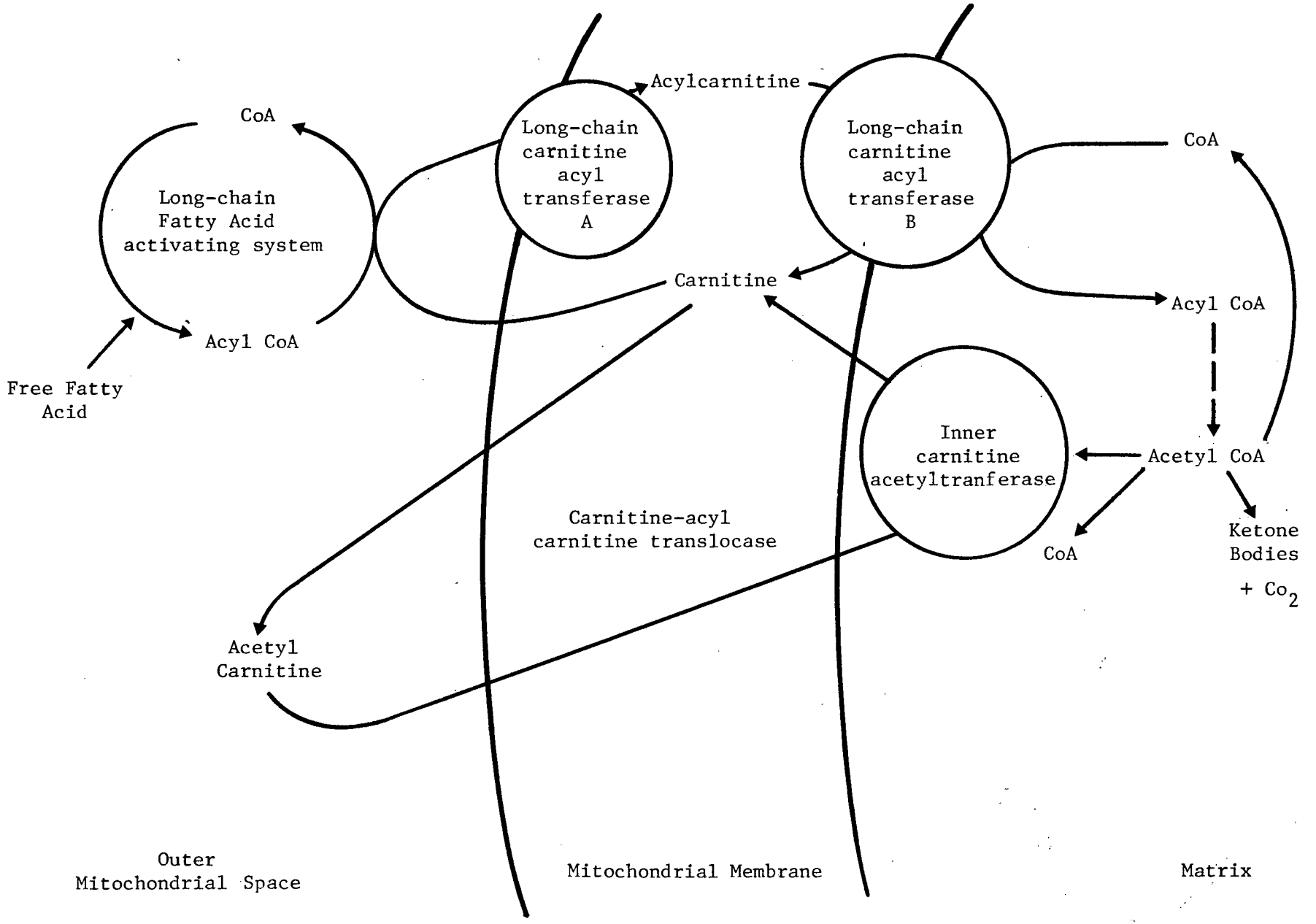
The transport of acyl-coenzyme A into the mitochondria is mediated by the formation of acyl carnitine. The mitochondrial inner membrane is permeable to carnitine esters (Yates and Garland, 1966) but it is impermeable to acyl-coenzyme A and free coenzyme A (Yates and Garland, 1966; Klingenberg and Pfaff, 1966). Transacylation of the coenyme A and carnitine esters is mediated by two distinctly separate enzymes (Figure 1).

One enzyme, carnitine palmityltransferase is specific for long-chain fatty acyl-coenzyme A (Fritz and Yue, 1963; Klingenberg and Pfaff 1966).

palmitlytransferase
palmityl-coenzyme A + (-)-carnitine ----------> (-)-palmitylcarnitine + coenzyme ASH

The effect of carnitine on fatty acid oxidation is mediated by

Figure 1 The function of carnitine in long-chain fatty acids oxidation


CoA
Long-chain
Fatty Acid
activating system
Acyl CoA
Free Fatty
Acid
Long-chain
carnitine
acyl
transferase
A
Acylcarnitine
Carnitine
Acetyl
Carnitine
Carnitine-acyl
carnitine translocase
Long-chain
carnitine
acyl
transferase
B
CoA
Acyl CoA
Inner
carnitine
acetyltranferase
Acetyl CoA
CoA
Ketone
Bodies
+ Co_2
Outer
Mitochondrial Space
Mitochondrial Membrane
Matrix

this enzyme which transfers the acyl group from coenzyme A to carnitine. The enzyme, carnitine palmityltransferase A, is localized on the outer surface of the inner membrane (Hoppel and Tomac, 1972). The acyl-carnitine crosses the inner membrane and is converted back to acyl-coenzyme A by carnitine palmityltransferase B, localized on the inner surface of the inner mitochondrial membrane (Hoppel and Tomac, 1972).

The acyl coenzyme A within the mitochondria may then be oxidized by B-oxidation to two carbon fragments of acetyl coenzyme A. These two carbon fragments can either:

a) enter the Krebs Cycle

b) be used in the production of Ketone bodies

c) or be transported out of the mitochondria.

The second enzyme, carnitine acetyltransferase, is specific for acetyl coenzyme A and short chain acyl coenzyme A (Bremer, 1962; Fritz, 1963; Fritz and Yue, 1963; Norum and Bremer, 1963).

carnitine acetyltransferase
---------->

(-)acetyl carnitine + coenzyme A

(-)-carnitine + acetyl-coenzyme A

The function of carnitine acetyltransferase has been difficult to understand. Several researchers have proposed that carnitine acetyltransferase functions as a transporter of acetyl groups between their site of formation by B-oxidation, to the outer mitochondrial space (Bressler, 1970). However, no such carnitine-dependent transfer has been substantiated, and it is also interesting that very little or no outer acetyltransferase

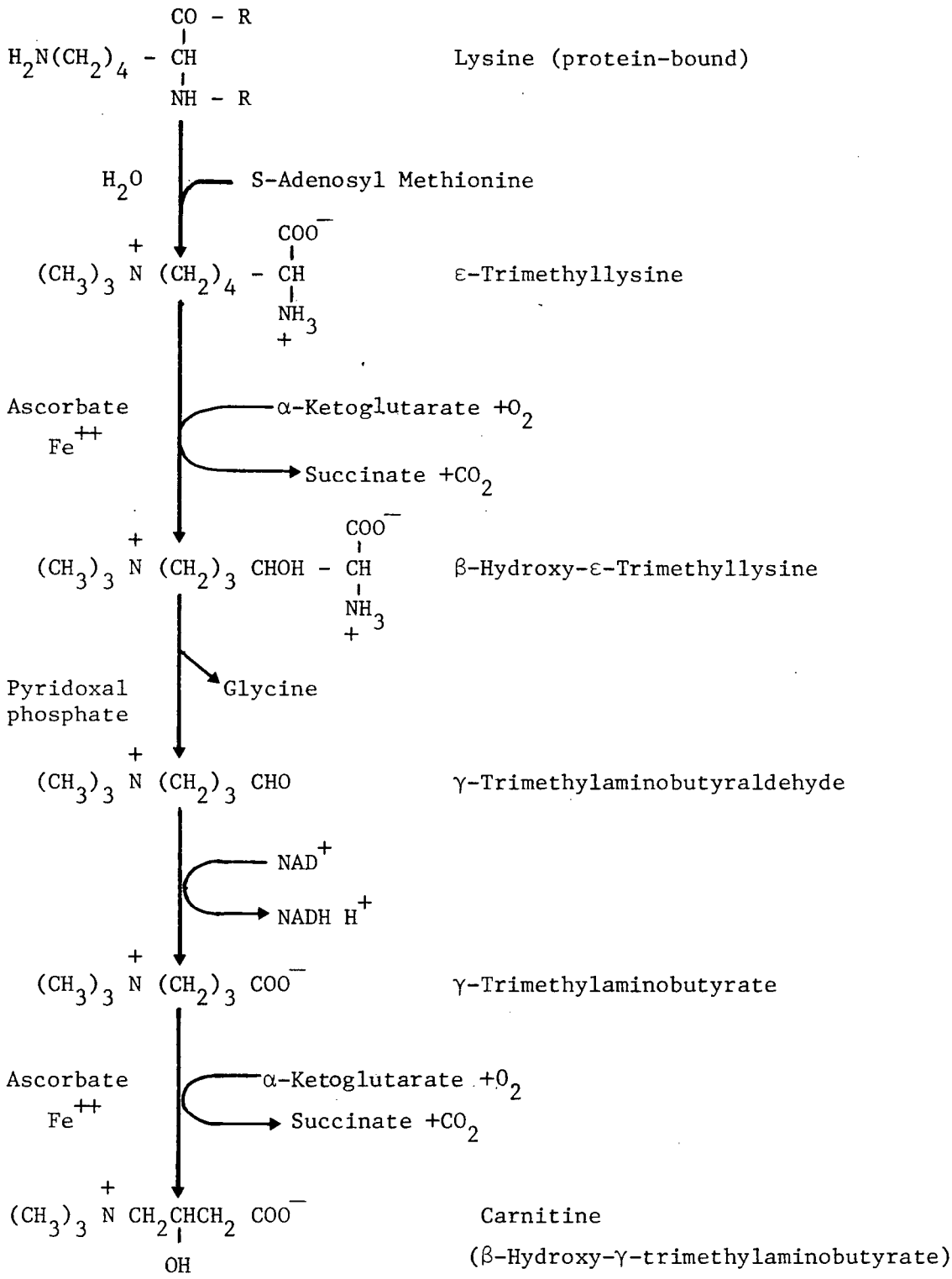
is found in the mitochondria (Solberg, 1970). Acetylcarnitine may instead represent a buffering system or depot for mitochondrial acetyl residues (Kopec and Fritz, 1973; Brosman et al., 1973; Brener, 1977) leaving coenzyme A available for fatty acid oxidation, particularly during periods of increased ketogenesis. Sufficient evidence at present is not available to support this hypothesis.

CARNITINE METABOLISM

Carnitine is widely distributed in nature and its highest concentrations are found in animal products (Fraenkel, 1954). Plant products generally contain low levels of carnitine (Panter and Mudd, 1969). In addition to dietary carnitine, the rat is able to synthesize carnitine from its amino acid precursors lysine and methionine (Tsai et al, 1974; Cederblad and Lindstedt, 1976).

The pathway for carnitine biosynthesis is represented in Figure 2. The carbon chain and quartenary nitrogen atom of the carnitine are derived from lysine (Cox and Hoppel, 1973a; Horne and Broquist, 1973). Similarly, ε-trimethyllysine is synthesized via the methylation of protein bound lysine by S-adenosyl methionine (LaBadie et al., 1976). Thus ε-trimethyllysine is an established precursor of γ-trimethylaminobutyrate which in turn is hydroxylated to yield L-carnitine (Lindstedt, 1967; Lindstedt and Lindstedt, 1965; Cox and Hoppel, 1973b). Holchalter and Henderson (1976) reported that the two carbon fragment lost from the trimethyllysine was

Figure 2 Biosynthesis of carnitine from lysine and methionine



adapted from Hulse and Ellis (1978)

glycine and proposed the pathway for the conversion of trimethyllysine tc carnitine. Hulse et al., (1978) recently succeeded in establishing the whole pathway from trimethyllysine to carnitine in the rat. Thus carnitine synthesis requires two amino acids in addition to three vitamins (ascorbic acid, pyridoxine, and niacin).

The liver is the primary site of carnitine biosynthesis in the rat (Tanphaichitr and Broquist, 1974), although the testes have a limited capacity for such synthesis (Casillas and Erickson, 1975). Reports of studies *in vivo* (Tanphaichitr and Broquist, 1974) and *in vitro* (Haigler and Broquist, 1974; Tanphaichitr and Broquist, 1974) indicate that several tissues, including heart, liver, kidney, muscle, and testes can convert ε-trimethyllysine to γ-butyrobetaine. Transport of γ-butyrobetaine via the plasma to the liver is required for the final conversion to carnitine (Lindstedt, 1967; Haigler and Broquist, 1974; Tanphaichitr and Broquist, 1974).

Exactly how much of the lysine ingested by the rat is used to synthesize carnitine is not known. Horne et al. (1971) have estimated that 0.2% of the lysine requirement of *Neurospora crassa* is used for carnitine synthesis. The estimated total body concentration of carnitine in rats is 0.33 u moles per 100 g of body weight (Tsai et al., 1974; Cederblad and Lindstedt, 1976). The daily synthesis of carnitine as computed by Cederblad and Lindstedt (1976) would approximate 2 u moles per 100 g body weight per day. Thus the dietary requirement as calculated by Tsai et al. (1974) of 1.5 u moles of carnitine per 100 g body weight per day can be met by daily biosynthesis

of carnitine from its amino acid precursors lysine and methionine.

MATERNAL-NEONATAL CARNITINE RELATIONSHIP

The carnitine relationship that exists between the mother rat and her offspring, both pre- and postnatally, has recently aroused considerable attention. Evidence suggests a dependence of the neonate on the mother rat as its major supplier of carnitine to meet its physiological requirements (Hahn and Skala, 1975; Robles-Valdes et al., 1976; Ferre et al., 1978). Human studies have also emphasized the important role the mother plays in supplying carnitine to her breast-fed infant (Novak et al., 1979).

Prenatal

The mammalian fetus gains most of its energy from maternally derived carbohydrates (Hahn and Koldovsky, 1966). It is well established that a high carbohydrate diet supports fatty acid synthesis rather than fatty acid oxidation. The biochemical findings regarding fat utilization by the rat fetus are in keeping with this concept.

In addition, the capacity for long-chain fatty acid oxidation in rat fetal liver is low. The concentration of carnitine palmityltransferase required for long chain fatty acid oxidation (Augenfeld and Fritz, 1970; Lockwood and Bailey, 1970) and the concentration of carnitine acetyltransferase are also

low during fetal development (Lee and Fritz, 1971). Correspondingly, fetal plasma (Hahn and Skala, 1975; Robles-Valdes et al., 1976; Seccombe et al., 1978) and liver (Robles-Valdes et al., 1976) carnitine concentration in the rat are low compared to postnatal values.

At present, there is no evidence to indicate that fetal synthesis of carnitine occurs in mammals. Fetal demands for carnitine may instead be met by supplies derived from maternal sources (Hahn and Skala, 1975; Hahn et al., 1977), even though placental transfer of carnitine is limited (Hahn and Skala, 1975). In both the sheep (Hahn et al., 1977) and rat (Hahn and Skala, 1975) blood levels of carnitine in the fetus are low as compared with the maternal blood levels. Apparently this restriction is due in part to retention of carnitine by the placenta (Hahn and Skala, 1975). In both species, there appears to be a slow exchange of carnitine between mother and fetus. The rapid intravenous injection of L-carnitine into the pregnant ewe led to a transient rise in the maternal blood but had no effect on the fetal blood levels (Hahn et al., 1977). Twenty-four hours after the injections of labelled carnitine into mother rats (Hahn and Skala 1975), radioactive carnitine was found in fetal liver and brown adipose tissue, although only in small amounts. Since the source of fetal carnitine appears to be mainly maternal, a carnitine deficiency in the mother may result in a carnitine deficiency in the offspring.

Postnatal

At birth, there is a sudden change in availability of energy yielding substrates. Maternal blood glucose is replaced by a high fat diet of milk (Cox and Mueller, 1937; Luckey et al., 1954) of which 69.5% of the ingested calories are fat (Cox and Mueller, 1937). The release of energy from dietary lipid, requires oxidation of fatty acids in the mitochondria. An adequate amount of carnitine is therefore necessary for the formation of acyl carnitines which are then able to cross the mitochondrial membrane (Fritz, 1963).

Increased rates of fatty acid utilization during the suckling period are indicated by raised plasma concentrations of ketone bodies (Drahota et al., 1964 ; Lockwood and Bailey, 1971; Page et al., 1971; Ferre et al., 1978). In vitro studies have shown more precisely that an increased capacity for fatty acid degradation coincides with the intakes of the high-fat diet of suckling rats. Oxidation of ^{14}C-palmitate to $^{14}CO_2$ by liver mitochondrial preparations and liver slices increases rapidly after birth so that the maximum rate of oxidation is achieved at 2 days of age. The high level is maintained until weaning (21 days of age), after which time the oxidation rate falls gradually to adult values (Taylor et al., 1967; Augenfeld and Fritz, 1970; Lockwood and Bailey, 1970).

After birth the rapid increase in palmitate oxidation is accompanied by a similiar rapid increase in the activity of carnitine palmityltransferase. The activity of this enzyme increases sharply and has its highest activity during the

suckling period (Augenfeld and Fritz, 1970; Lockwood and Bailey, 1970). Likewise, carnitine concentration in the plasma (Robles-Valdes et al., 1976; Borum, 1978; Seccombe et al., 1978) and liver (Robles-Valdes et al., 1976; Borum, 1978) increase rapidly following birth, while the pup is consuming the milk diet, and decrease when the pup is weaned to a high carbohydrate diet. Prior to weaning there are no sex differences in plasma or liver carnitine levels (Borum, 1978).

The primary source of carnitine in neonatal tissue, at least 24 hours post partum, is the milk from the mother rat (Ferre et al., 1978). Milk carnitine content is very high initially and falls as nursing continues (Robles-Valdes et al., 1976). Robles-Valdes et al., (1976) reported liver carnitine concentrations in mother rats during late gestation to be high but decreased to normal at approximately the seventh day of lactation accompanying the decrease in milk carnitine levels. Experiments in which the fate of ^{14}C-butyrobetaine, the immediate precursor of carnitine, was followed after injection into nursing rats indicated movement of carnitine from maternal liver to maternal plasma to maternal milk and finally into neonatal heart and liver within 24 hours after its injection.

Cycling of carnitine between mother and infant rat has been documented by Hahn and Skala (1975). Since infant rats are unable to urinate on their own, the mother animal empties their bladder by licking the perineal region and swallowing the urine (Capek and Jelinek, 1956). Thus substances injected into the suckling rat can be passed back to the mother and eventually be secreted in her milk. When infant rats were injected with ^{14}C-

carnitine, the label appeared in the blood and other organs of the mother rat and also in its milk thereby returning tc the pups of the litter (Hahn and Skala, 1975). Thus a maternally induced deficiency of carnitine may adversely affect the carnitine status of suckling offspring, since the primary source of carnitine for the mother-infant cycle, at least during the initial stages of lactation, is maternal.

RELATIONSHIP OF CARNITINE TO KETOSIS

Increased ketogenesis indicates enhanced fatty acid oxidation. Whenever the availability of carbohydrate is inadequate more lipid is oxidized and ketosis develops. A high rate of free fatty acid uptake and oxidation by the liver is the most important factor underlying ketogenesis. States such as starvation and diabetes are accompanied by an increased hepatic fatty acid oxidation and ketogenesis (Fritz, 1967; McGarry and Foster, 1971; McGarry et al., 1973; McGarry and Foster, 1974; McGarry et al., 1975), processes which require the carnitine acyltransferase system (Fritz and Yue, 1963).

McGarry et al. (1975) suggested, on theoretical grounds, that the B-oxidation of fatty acids might be governed, if only in part, by the concentration of carnitine. They based this notion on two considerations. First, carnitine is necessary for the initial step in the oxidation of long chain fatty acids, specifically the carnitine acyltransferase reaction (Hoppel and Tomac, 1972). Second, studies conducted by Fritz (1955) established that carnitine was capable of stimulating fatty acid

oxidation when introduced into rat liver homogenates.

Furthermore, McGarry et al. (1975) showed that ketotic states, such as fasting or alloxan diabetes, in the weaned rat were accompanied by an increased carnitine content in the liver. The ketogenic capacity of hepatic tissue correlated with carnitine concentrations. In addition, carnitine alone had the capacity to directly activate ketogenesis in isolated perfused liver from nonketotic (fed) rats.

Carnitine tissue levels and production of ketone bodies during perinatal development have also been studied. Ketone body formation is very low in fetal tissues (Drahota et al., 1964; Augenfeld and Fritz, 1970; Robles-Valdes et al., 1976). Fetal liver (Robles-Valdes et al., 1976) and plasma (Robles-Valdes et al., 1976; Borum, 1978) carnitine concentrations are also low during the prenatal period. In contrast, during the suckling period the plasma levels of ketone bodies are high (Drahota et al., 1964; Lockwood and Bailey, 1971; Page et al., 1971) and the neonate may be described as being in a physiological state of ketosis as a direct result of high rates of utilization and oxidation of fatty acids. The concentration of ketone bodies in the blood increases rapidly after birth (Drahota et al., 1964; Lockwood and Bailey, 1971; Page, 1971; Robles-Valdes et al., 1976; Ferre et al., 1978), remains high during the suckling period and falls after weaning (Lockwood and Bailey, 1971; Page, 1971; Robles-Valdes et al., 1976). An almost identical profile has been observed for liver carnitine concentrations in the neonate (Robles-Valdes et al., 1976; Ferre et al., 1978; Borum, 1978).

These data indicate a relationship between hepatic levels of carnitine and the rate of ketogenesis. When fatty acid oxidation is substantially increased, tissue carnitine levels are higher than normal.

MATERNAL DIETARY DEFICIENCIES

The importance of a nutritionally sound diet during gestation and lactation for the maintenance of pregnancy and the health of the offspring is well documented (Barry, 1920; Chow and Lee, 1964; Niiyama et al., 1970, 1973). In this particular review, the effects of a maternal lysine deficiency and/or maternal dietary food restriction on dams and their offspring, are discussed.

Pregnancy

The maintenance of pregnancy in rats fed purified diets devoid of a single amino acid was examined by Niiyama et al. (1970, 1973). Rats fed a lysine-free diet showed food intake comparable to control animals and maintained pregnancy. Litter size (Niiyama et al., 1970, 1973) was not affected. However, fetal weight (Niiyama et al., 1973) was significantly lower than for control animals. Niiyama suggested that animals fed lysine deficient diets may have maintained pregnancy because of the lysine sparing mechanism shown to exist in non-pregnant rats (Yamashita and Ashida, 1969; Canfield and Chytil, 1978). The basis for the extensive preservation of lysine in rats subjected

to a lysine deficiency, appears to be the adaptive decline of the first enzyme in the pathway of lysine degradation, lysine-ketoglutarate reductase (Chu and Hegsted, 1976).

Stapleton and Hill (1972, 1980) reported that a diet containing 0.42% lysine, fed to rats during gestation, did not have a significant effect on food intake or litter size when compared to a 1.12% lysine diet. The average birth weight of pups in the low-lysine group was significantly lower than in the control group. Plasma amino acid analysis revealed a significantly lower plasma lysine concentration on day 7 and day 21 of pregnancy in dams fed the low-lysine diet than in those consuming the high-lysine diet. This resulted in a reduced amount of lysine available to the offspring during the last week of pregnancy, a factor possibly contributing to the lower birth weight of the lysine deficient group.

Restricting food intake during gestation has also been reported to have a significant effect on reproduction. Chow and Lee (1964) reduced the dietary intake of rats by 25% and observed a significant reduction in fetal weight. A decreased birth weight has also been reported in the offspring of rats consuming a diet restricted in total food intake during gestation (Barry, 1920; Niiyama et al., 1973). Thus both the quantity and quality of the maternal diet affected reproductive performance.

Lactation

Imposing a dietary deficiency during lactation affects not

only food intake of the dams but also weight gain of the offspring (Stapleton and Hill, 1972, 1980; Jansen and Chase, 1976; Chow and Lee, 1964). Stapleton and Hill (1972, 1980) fed a wheat gluten based diet, deficient in lysine, to rats during pregnancy and lactation. Food intake of lysine deficient dams during lactation was significantly reduced, as was the average weaning weight of their offspring. Milk production and its protein content were also significantly reduced in dams fed the lysine deficient diet. This effect on milk probably contributed to the lower weight gain of suckling rats in the lysine deficient group. Crnic and Chase (1978) reported that dams fed a low protein diet during lactation showed little engorgement of the mammary tissue and yielded milk less readily than dams fed either a caloric restricted or ad libitum diet.

Jansen and Chase (1976) reported a significant reduction in food intake during pregnancy and lactation in rats fed a basal bread diet when compared with dams consuming a lysine fortified bread diet. Lysine fortification of bread decreased maternal weight loss during lactation, and significantly increased weaning weight of the offspring. When Chow and Lee (1964) imposed a total food restriction on dams during both pregnancy and lactation, growth stunting resulted in the offspring.

Thus some of the major effects of a maternal lysine deficiency on the offspring are decreased birth weight and decreased weaning weights. A maternal lysine deficiency also decreases the food intake of dams, at least during lactation, and depresses milk flow.

LYSINE DEFICIENCY AND TISSUE CARNITINE LEVELS

A large proportion of the world's population consumes cereal based diets which are likely to be low in carnitine and limiting in its amino acid precursors lysine and methionine. Consumption of diets consisting mainly of plant foods may result in a carnitine deficiency in humans (Latifa and Bamji, 1977). Because of carnitine's role in fatty acid oxidation an impairment in fat utilization may result from such a state of carnitine deficiency. Carnitine deficiencies have been associated with fat accumulation in the tissue of the rat (Tanphaichitr et al., 1976), guinea pig (Wittles and Bressler, 1964), and human (Isaacs et al., 1976).

Tanphaichitr and Broquist (1973) measured the effect of feeding a 20% wheat gluten diet, limiting in lysine and containing negligible carnitine, on tissue carnitine levels in male rats. When compared with control animals receiving a 0.8% lysine supplemented diet, carnitine levels in heart and skeletal muscle were generally 33% lower in the lysine deficient group. The carnitine level of the liver however, was significantly higher in the unsupplemented group than in the lysine supplemented group. The authors suggest that this latter finding may reflect the need for increased hepatic synthesis of carnitine under the stringent nutritional conditions employed, i.e., a lysine deficiency and significantly reduced food intake. A third group of animals in this study fed a laboratory chow diet, which contained a higher level of carnitine, were reported to have higher levels of tissue carnitine compared to animals

given the carnitine-free, lysine supplemented diet. The results suggest that tissue carnitine levels are higher when dietary carnitine is available.

The effect of cereal based diets, deficient in lysine, on tissue carnitine levels and fatty acid oxidation has also been observed (Tanphaichitr et al., 1976; Khan and Bamji, 1979). Tanphaichitr et al. (1976) fed male weanling rats a 72% rice diet containing no detectable carnitine and limiting in threonine and lysine. They observed liver lipid accumulation, a major part of which was triglycerides. Reduction in liver fat content was partly achieved by feeding carnitine. However, complete relief of the condition was not attained until the diet was supplemented with threonine and lysine. Therefore liver lipid accumulation in these rats may occur as a result of insufficient lipoprotein transporters for removing triglycerides from the liver, and of course, a carnitine deficiency resulting in impaired fatty acid oxidation.

Khan and Bamji (1979) reported marked elevation in triglyceride and total lipid content of liver, heart, and skeletal muscle in rats fed a carnitine free and lysine depleted diet (5% wheat protein). Oxidation of fatty acids by heart homogenate was impaired in animals fed the wheat gluten diet ad libitum, as well as in their pair-fed controls. Supplementation of wheat gluten with 0.2% carnitine produced a significant increase in plasma, muscle, and liver carnitine levels, restored fatty acid oxidation, and reduced the triglyceride level of tissue. Supplementation of the wheat diet with lysine and threonine at 0.2% level improved muscle carnitine levels and

palmitate oxidation and diminished the triglyceride levels of tissues. These data suggest the essentiality of carnitine as a dietary nutrient, either through preformed carnitine or its amino precursors lysine and methionine.

Sex differences in tissue carnitine levels have been observed in both rats (Borum and Broquist, 1977; Borum, 1978) and humans (Cederblad and Lindstedt, 1971; Maebashi et al., 1976). Female rats have only about half as much carnitine in the plasma as male rats, and carnitine content of liver and heart in males is slightly higher than in females (Borum and Broquist, 1977; Borum, 1979). Borum and Broquist (1977) investigated the effects of a carnitine deficiency in male and female rats fed a 20% wheat gluten diet. When compared to control females consuming a lysine supplemented diet, the lysine deficient female rats had higher tissue carnitine levels in all three tissues analyzed, i.e., plasma, liver, and heart. Only the plasma carnitine levels however, were significantly higher. Male rats subjected to the lysine deficient diet had moderately decreased carnitine levels in the plasma and heart, but elevated carnitine levels in liver. The mechanism underlying the sex differences in plasma carnitine levels of rats, and their opposite response to a dietary lysine deficiency, is not well understood.

CHAPTER III

METHODS AND MATERIALS

ANIMALS

White rats of Wistar strain (purchased from Woodlyn Breeding Laboratories, Guelph) were used in all experiments. Female rats, weighing approximately 175 g, were housed in plastic breeding cages (3 per cage) and fed Purina Rat Chow and water *ad libitum* until mated. Male breeding rats weighing no less than 250 g were fed a similar diet. Females averaging 225 g were mated by placing 1 male rat with 3 females in a breeding cage overnight. From day one of gestation, the day sperm was found in a vaginal smear, dams were placed on their respective diets.

Once mated the animals were individually housed in stainless steel cages with raised wire floors under conditions of controlled light (lighting 0600-1200 hours) and temperature (24°C). The cages were equipped with external water bottles and internal cup feeders allowing *ad libitum* intake in all experiments unless specified otherwise. Pregnant dams were transferred at 18 days of gestation to larger plastic breeding cages with wood shavings as bedding. Daily food intake and weekly weight changes during pregnancy and lactation were recorded.

DIETS

A diet deficient in lysine (the low-lysine diet) was based on wheat gluten as the source of protein. The composition of the diet is shown in Table I. The amino acid composition of the diet was calculated from amino acid composition of wheat gluten according to FAO Nutritional Studies (1970). Those amino acids that did not meet requirements for the pregnant and lactating rat were supplemented to the diet (NRC, 1972). Table II includes the detailed calculations for the essential amino acids. Because the mineral mixture also did not meet the nutrient requirements for the rat (NRC, 1972), zinc and potassium were added to the ration (Table I). The control diet, adequate in lysine (the high-lysine diet) was prepared by supplementing the basal diet with 1.0% L-Lysine HCl, equivalent to 0.8% L-Lysine. Both diets contained 18% protein and 20% fat. Basal diets containing 20% wheat gluten as the sole protein source have been reported (Tanphaichitr and Broquist, 1973) to contain less than 0.1 u g carnitine per gram of diet as determined by carnitine acetyltransferase assay (Marquis and Fritz, 1964). Thus, the low-lysine diet contained negligible amounts of carnitine and was adequate in all nutrients except lysine.

ANIMAL EXPERIMENTS

The three animal experiments are diagrammatically depicted in Figure 3.

Table 1. Composition of Low-Lysine Diet[1]

Ingredient	Per cent of Diet
Wheat Gluten[2]	20.00
Vitamin Mixture[3]	2.20
Mineral Mixture[4]	5.00
Corn oil[5]	20.00
Cellulose[6]	5.00
Zinc Carbonate[7]	0.006
L - Histidine HCl[7]	0.32
DL - Methionine[7]	0.40
L - Threonine[7]	0.15
L - Tryptophan[7]	0.06
L - Arginine HCL[7]	0.18
Corn Starch	46.684

1 0.5% K_2PO_4 was added to the diet for the lactation studies only.

2 Wheat Gluten purchased from North American Scientific Chemicals, Vancouver, British Columbia.

3 ICN Vitamin Diet Fortification Mixture purchased from Nutritional Biochemicals, Cleveland, Ohio.

4 Bernhart Tomarelli Mineral Mixture purchased from Nutritional Biochemicals, Cleveland, Ohio.

5 Mazola Corn Oil.

6 Alfacell Non-nutritive bulk, ICN, purchased from Nutritional Biochemicals, Cleveland, Ohio.

7 ICN, purchased from Nutritional Biochemicals, Cleveland, Ohio.

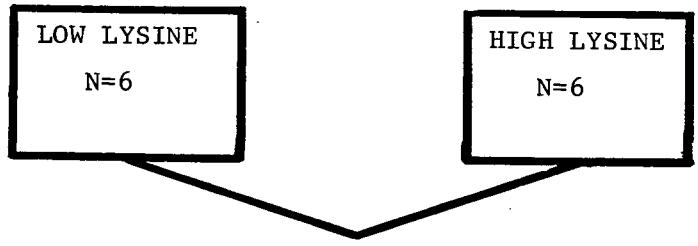
Table II. Calculated Essential Amino Acid Composition of Diets

Amino Acid	% Contributed by Wheat Gluten	% Added as Supplement	Total g/100g Diet
Aspartic Acid	0.54		0.54
Threonine	0.41	0.15	0.56
Glutamic Acid	5.99		5.99
Methionine	0.26	0.20	0.46
Isoleucine	0.67		0.67
Leucine	1.11		1.11
Tyrosine	0.59		0.59
Phenylalanine	0.83		0.83
Lysine	0.27	0.80[1]	1.07
Histidine	0.35	0.25	0.60
Arginine	0.69	0.14	0.83
Proline	2.12		2.12

1 Diets with 0.8% lysine were referred to as the high-lysine diet. The low-lysine diets received no supplement of lysine.

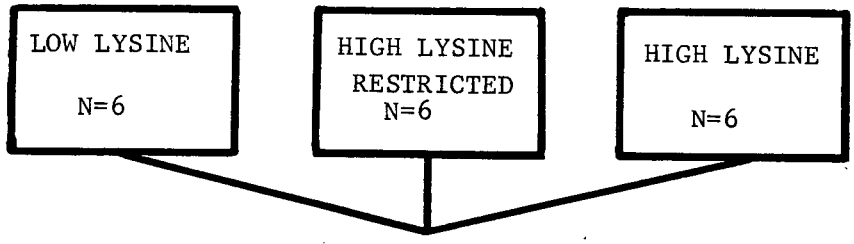
Figure 3 Diagramatic representaticn of experiments I, II, and III

EXPERIMENT I



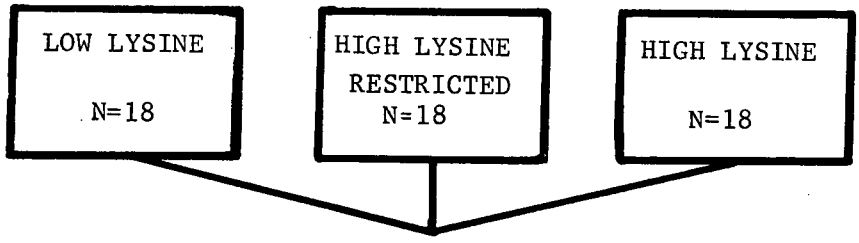
DAY 21 PREGNANCY - TISSUES (DAMS, FETUSES)

EXPERIMENT II



DAY 15 LACTATION - TISSUES (DAMS, PUPS)

EXPERIMENT III



DAY 2 LACTATION - 6/GROUP)
DAY 8 LACTATION - 6/GROUP) MILK
DAY 15 LACTATION - 6/GROUP)

Experiment I

Effect of a maternal lysine deficiency during pregnancy on tissue carnitine levels

Two groups of rats, consisting of six pregnant animals each, were fed either the low-lysine or high-lysine diets. On day 21 of pregnancy, dams were anaesthetized with ether and a sample of blood was taken by heart puncture using a heparinized syringe. The dam's heart and liver then were removed and tissue weights recorded. Fetuses with attached placenta were removed by caesarian section. Resorption sites were noted. Blood samples were obtained from decapitated fetuses using heparinized capillary tubes. Plasma was separated by centrifuging the blood samples of the dams and fetuses for 10 and 5 minutes respectively. Fetal weights were not recorded in this experiment, because the separation of the placenta from the fetus would have resulted in a loss of blood, interfering with collection of an adequate amount of blood for analysis. Heart and liver were removed from all fetuses and tissue weights recorded. Tissue samples from two fetuses within a litter were combined to provide a large enough sample for analysis. All tissues were immediately frozen and stored at -20°C.

Experiment II

Effect of a maternal lysine deficiency during pregnancy and lactation on tissue carnitine levels

After examining the results of experiment I, it was found that animals in the low-lysine group consumed significantly less food than those in the high-lysine group. Therefore a third

group of animals, the high-lysine restricted group, was added to the remaining experiments to determine the effects of decreased food intake. There were thus 3 groups of pregnant dams, 6 animals per group. Two of the groups were fed either the low-lysine or high-lysine diet ad libitum. The third group, the high-lysine restricted animals, were pair-fed the high-lysine diet to the low-lysine group on a body weight basis. Commencing on day 1 of lactation, all diets were supplemented with $ZnSO_4$. Dams were fed their respective diets until tissue samples were obtained. Pregnant dams were checked daily at twelve noon for the presence of newborns. Only those mothers with litters containing eight or more young and experiencing less than two deaths per litter were used in this experiment. This restriction on litter size was imposed due to sample size requirements for statistical analysis. At day 1 of lactation (parturition completed before twelve noon) all pups were sexed, weighed and litter size culled to eight pups. Preference was given to male offspring. The eight pups were numbered by injecting India Ink under the skin surface near the appendages. Body weights of pups were recorded on days 1, 5, 10, and 15 post partum.

On day 15 of lactation dams were anaesthetized with ether and a sample of blood was taken by heart puncture using a heparinized syringe. Heart and liver were removed and tissue weights recorded. Pups were sacrificed on day 15 of lactation by decapitation. Heparinized capillary tubes were used to collect the blood. The heart and liver of each pup were removed and tissue weights recorded. The plasma was separated by

centrifuging blood samples of the dams and their pups for 10 and 5 minutes respectively. All tissue samples were immediately frozen and stored at -20°C.

Experiment III

Effect of a maternal lysine deficiency on milk carnitine levels

In this study, there were three groups of pregnant dams, 18 animals per group. Two of the groups were fed either the low-lysine or high-lysine diet. The third group, the high-lysine restricted animals, were pair-fed the high-lysine diet to the low-lysine group on a body weight basis. Commencing on day 1 of lactation, all diets were supplemented with $ZnSO_4$. Dams were fed their respective diets until milk samples were obtained. Pregnant dams were checked daily at twelve noon for the presence of newborns. Since pups were required for stimulating milk flow rather than for tissue samples (as was the case in experiment II) dams with less than eight young per litter, or experiencing more than two deaths per litter, were permitted for use in experiment III. In those cases where litter size was less than eight, the young of foster dams (dams bred and maintained on one of the three dietary treatments) were used to maintain litter size at eight throughout the lactation period. For those litters containing more than eight young, litter size was culled to eight on day 1 of lactation (parturition completed before twelve noon). Preference was given to male offspring. The eight pups were numbered by injecting India Ink under the skin surface near the appendages. Body weights of pups, nursed by

those dams milked on day 15 of lactation, were recorded on days 1, 5, 10, and 15 post partum. Body weights of pups, nursed by those dams milked on day 8 of lactation, were recorded on days 1, 5, and 8 post partum.

Milk samples were collected from 6 dams per group on the second, eighth and fiftenth days of lactation. Litters were removed from their mothers 10-12 hours (from approximately 2300 to 0900 hours) prior to milk collection to allow accumulation of sufficient milk for analysis. Dams were anaesthetized with an intraperitoneal injection of nembutol (45 m g/kg, Grosvenor et al., 1959). Each dam was then injected intraperitoneally, with 0.5 units of oxytocin to facilitate milk flow (Grosvenor et al., 1959; Mena et al., 1974). Milk was manually expressed from the nipple and collected with a pasteur pipette into 0.5 ml containers. Milk samples were immediately frozen at -20°C. All milk samples collected were analyzed for carnitine content.

PLASMA, LIVER, AND MILK TOTAL CARNITINE ANALYSIS

Carnitine (total carnitine) extracted from plasma, liver, and milk was assayed according to the method of McGarry and Foster (1976) as modified by Parvin and Pande (1977) and Seccombe et al. (1978).

Principle of Assay

L-carnitine was incubated with (1-^{14}C)acetyl-CoA of a known specific activity and carnitine acetyltransferase. Carnitine was then transformed to the labelled acetyl-L-carnitine by the enzyme.

$$\text{L-carnitine} + (1\text{-}^{14}\text{C})\text{acetyl-CoA} \xrightarrow{\text{acetyltransferase}} (1\text{-}^{14}\text{C})\text{acetyl-L-carnitine} + \text{CoASH}$$

The labelled acetyl-L-carnitine was separated from the unreacted (1-^{14}C)acetyl-CoA by an activated charcoal slurry. After centrifugation the isotope content of the supernatant was determined.

Preparation of Plasma Carnitine Extract - Part A, Section 1

(Seccombe et al., 1978)

1) Reagents and their concentrations:

Reagent	Concentration
zinc sulfate	0.087 M
barium hydroxide	0.083 M

2) Solutions of zinc sulfate and barium hydroxide were titrated against each other such that equal volumes of each resulted in a supernatant pH of 7.3.

3) Constriction pipettes were used to measure 0.100 ml samples of plasma into separate conical polystyrene tubes (purchased from Amersham). All extractions were done in duplicate and kept on ice.

4) 0.400 ml of barium hydroxide was added to each tube and the contents mixed. The tube was then covered and heated at 37°C for 1 hour to release bound carnitine.

5) 0.400 ml of zinc sulfate was added to each tube, the contents mixed and the tube cooled on ice.

6) The precipitate was centrifuged at 2000 RPMs for 20 minutes. The supernatant was removed and frozen at -20°C until assayed for carnitine.

Preparation of Milk Carnitine Extracts - Part A, Section 2

(Robles-Valdes et al.,1976; Seccombe et al., 1978)

1) Reagents and their concentrations:

Reagent	Concentration
zinc sulfate	0.087 M
barium hydroxide	0.083 M

2) Solutions of zinc sulfate and barium hydroxide were titrated against each other such that equal volumes of each resulted in a supernatant pH of 7.3.

3) Milk samples were diluted with distilled water 1:1, 1:2, and 1:4 for days 15, 8 and 2 respectively. All samples were done in duplicate and kept on ice.

4) Samples were covered and heated at 60°C for 30 minutes and then mixed for 30 seconds to disperse any particulate material.

5) 0.400 ml of barium hydroxide was added to each tube and the contents mixed. The tube was then covered and heated at 37°C for 1 hour to release bound carnitine.

6) 0.400 ml of zinc sulfate was added to each tube, the contents mixed and the tube cooled on ice.

7) The precipitate was centrifuged at 2000 RPMs for 20 minutes. The supernatant was removed and frozen at -20°C until assayed for carnitine.

Preparation of Liver Carnitine Extracts - Part A, Section 3

(Tsai et al., 1974)

1) Reagents and their concentrations:

Reagent	Concentration
potassium hydroxide	10%
perchloric acid	72%

2) 0.2 g of tissue was hydrolyzed in 2.0 ml of potassium hydroxide at 80°C for 2 hours to release carnitine. (For combined fetal livers the total sample was hydrolyzed.) The solution was mixed twice during hydrolysis.

3) Samples were cooled to room temperature, and acidified with 72% perchloric acid and centrifuged at 2000 RPMs for 15 minutes.

4) The supernatant was removed and the pellet washed twice with 1.0 ml aliquots of water.

5) Combined washings were neutralized with potassium hydroxide, left on ice for 30 minutes, and then centrifuged at 2000 RPMs for 15 minutes.

6) The supernatant fractions were freeze dried and then rediluted to a predetermined volume using volumetric pipettes. This step was conducted to obtain a carnitine concentration that the assay was capable of detecting.

7) Rediluted supernatants were used for duplicated carnitine assays.

Total Carnitine Assay Procedure - Part E

(McGarry and Foster, 1976 ; Parvin and Pande 1977; Secccmbe et al., 1978)

1) Reagents and their concentrations:

Reagent	Concentration
a) N-2-Hydroxyethylpiperazine N-2-ethanesulfcnic acid (HEPES)	0.4 M
b) (Ethylenedinitrilo)-Tetra acetic acid (EDTA)	0.016 M
c) N-ethyl Malimide	
d) (1-^{14}C)acetyl-CoA(hot)	
e) S-acetyl-CoA (cold)	
f) sodium hydroxide	1 N
g) carnitine acetyltransferase	5 mg/ml
h) activated charcoal	
i) ethanol	100%
j) phosphoric acid	85%

Preparation of Solutions

2) HEPES/EDTA Buffer: The buffer was made by dissolving 9.532 g of HEPES and 0.666 g of EDTA in water and adjusting the pH to 7.6 with sodium hydroxide. The prepared solution was adjusted to a final volume of 100 ml.

3) 1.0 ml of the HEPES/EDTA buffer was used to dissolve 5.0 mg of N-ethyl malimide.

4) Hot and Cold Acetyl CoA Mix: 1.0 ml aliquots of (1-^{14}C) acetyl CoA (purchased from Amersham) plus cold S-acetyl-CoA were prepared such that each ml contained a total of 50 nanamoles of acetyl-CoA at a specific activity of 40,000 DPMs per nanamole.

5) 1.0 ml of the acetyl-CoA mix from step 4 was added to the 1.0 ml buffer NEM solution from step 3. The final volume of the mix was 2.0 ml and will be referred to as the assay mix.

6) Carnitine Acetyltransferase Mix: 0.05 ml of carnitine acetyltransferase enzyme solution (purchased from Sigma) plus 1.050 ml of HEPES/EDTA buffer (see step 2) were combined and mixed.

Assay

7) Constriction pipettes were used to measure 0.100 ml samples of each tissue extract into separate polystyrene tubes (purchased from Amersham). All samples were kept on ice.

8) 0.100 ml of the assay mix was added to the tissue extract.

9) The assay was started by adding 0.05 ml of the carnitine acetyltransferase mix (see step 6) and the reaction allowed to proceed at room temperature for 60 minutes.

10) The reaction was terminated by the addition of 0.600 ml of an activated charcoal slurry which was continuously stirred with a magnetic stirrer.

Charcoal Slurry	Amount
activated charcoal	4 g
ethanol	54.5 ml
phosphoric acid	1.25 ml
distilled water	4.25 ml

11) Following the addition of the charcoal mixture, the tubes were placed on ice and the contents mixed at approximately 10 minute intervals. The tubes were then centrifuged at 2000 RPMs for 20 minutes.

12) A 0.5 ml portion of the clear supernatant was transferred to mini sized scintillation vials (17 x 55 mm) containing 0.4 ml of distilled water and 5 ml of ACS Scintillant (purchased from Amersham). After mixing, and obtaining a relatively stable one-phase system, samples were counted in a Beckman LS-230 Liquid Scintillation Counter for 5 minutes each.

Determination of Carnitine Concentration

A series of blanks (distilled water) standards (25 M, 50 M, and 100 M) and quality controls (pooled plasma samples obtained from healthy Wistar males) were hydrolyzed and assayed with each batch of tissues that were analyzed. The use of these permitted determination of tissue carnitine concentrations and the reproducibility of the results. Treatment of blanks, standards, and quality controls was identical to that of the respective tissues they were used to assess, with the exception of liver. Blanks, standards and quality controls hydrolyzed with the plasma extracts were used to determine liver carnitine concentrations. The linearity of the standard curves remained good throughout the conducted analyses (average coefficient of correlation, $r \geq 0.989$), and variability of the quality controls ranged between 58.5-62.3 n mol/ml plasma indicating good reproducibility of results.

The following formula was used to determine tissue carnitine concentration:

$$x = \frac{100\text{-dmp(blank)} \times \text{dpm(sample)} \times 100\%}{\text{dpm(100 M standard)} - \text{dpm(blank)}} = \text{n mol/ml or g}$$

Recovery

A known quantity of DL-carnitine was added to duplicate sets of plasma, milk and liver samples to determine the percent recovery of carnitine from tissues. Recovery of carnitine was 93% for plasma, 91% for milk, and 95% for liver. Tsai et al. (1974) reported recovery of ^{14}C-carnitine added to liver tissue to be 97 to 100%. Seccombe et al. (1978) calculated the recovery of acylcarnitine added to plasma to be greater than 95% of theoretical. Percent recovery of carnitine from milk has not, to the authors knowledge, been reported in the literature.

STATISTICS

All statistical procedures were performed by programs from the Statistical Package for the Social Sciences (SPSS, Nie et al., 1975) on the University of British Columbia's Michigan Terminal System (MTS) computer program. Data obtained from fetuses and pups were expressed as the means of litters rather than as individual samples. These means of litters were used to calculate the standard deviations. This method was used rather than the measurements of individual offspring to eliminate the problem of correlation of observations within litters (Abbey and Howard 1973).

Experiment I

The t-test, at 0.05 level, was used to determine whether there was any significant differences between treatment groups.

The analysis of variance with repeated measures, at the 0.05 level was used to test whether there was any significant differences in weight gain and/or food intake during pregnancy.

Experiment II and III

Unless otherwise stated, all analyses for experiments II and III were conducted at the 0.05 level of significance. The analysis of variance was used to determine if there were any significant differences among treatment groups. To explore the source of the effect, Duncan's multiple range test was used. The analysis of variance with repeated measures was used to test whether there was any significant difference in weight of dams and pups and food intake of dams, during pregnancy and/or lactation.

CHAPTER IV

RESULTS

EXPERIMENT I

A low-lysine diet, when fed to pregnant rats, resulted in significantly lower maternal body weights, food intakes and, liver weights when compared to animals fed the high-lysine diet (Tables III, IV, and Figure 4).

Dams fed the high-lysine diet consumed 17.3 g food/day during gestation, whereas the animals in the low-lysine group ate an average of 14.9 g/day, a significant decrease in food intake. However, when food consumption was expressed as g/100 g body weight there was no significant effect of diet on food intake (Table III).

Dams fed the low-lysine diet gained significantly less weight (83.8 g) during gestation than dams consuming the high-lysine diet (147.1 g, Table III). The weekly weight gain of dams during pregnancy (Table III, Figure 4) was also significantly affected by dietary treatment ($p<0.005$). A significant difference in body weights between the two dietary groups was observed as early as day 7 of gestation and continued throughout pregnancy. Body weights of dams in the high-lysine and low-lysine groups were 376.3 g and 308.3 g respectively, on day 21 of gestation.

The effect of dietary treatment on maternal liver and heart weight is presented in Table IV. Liver weights for the low-

Table III. Food Intake and Weight Gain of Dams Fed a High-Lysine or Low-Lysine Diet during Gestation

	Experimental[A] Group	Initial Body Weight	Week of Gestation[B] 1	2	3	Daily Food Intake	Total Weight Gain
Feed (g/day)	High-Lysine		6.5±0.2	6.7±0.4	6.6±0.3	17.3±0.6	
	Low-Lysine		6.3±0.3	6.5±0.5	5.9±0.3	14.9±0.5[C]	
Body Weight (g)	High-Lysine	229.2±7.9	262.9±6.7	300.8±7.2	376.3±9.5		147.1±10.0
	Low-Lysine	224.6±5.2	239.0±4.9[C]	259.4±8.8[D]	308.3±10.0[E]		83.8±7.5[E]

A Mean ± SEM for 6 dams

B Food intake is expressed as g/100 g body weight

C Mean value differs significantly from high-lysine group ($p<0.05$)

D Mean values differ significantly from high-lysine group ($p<0.01$)

E Mean values differ significantly from high-lysine group ($p<0.001$)

Figure 4 Effect of a high-lysine or low-lysine diet on weight gain of dams during gestation

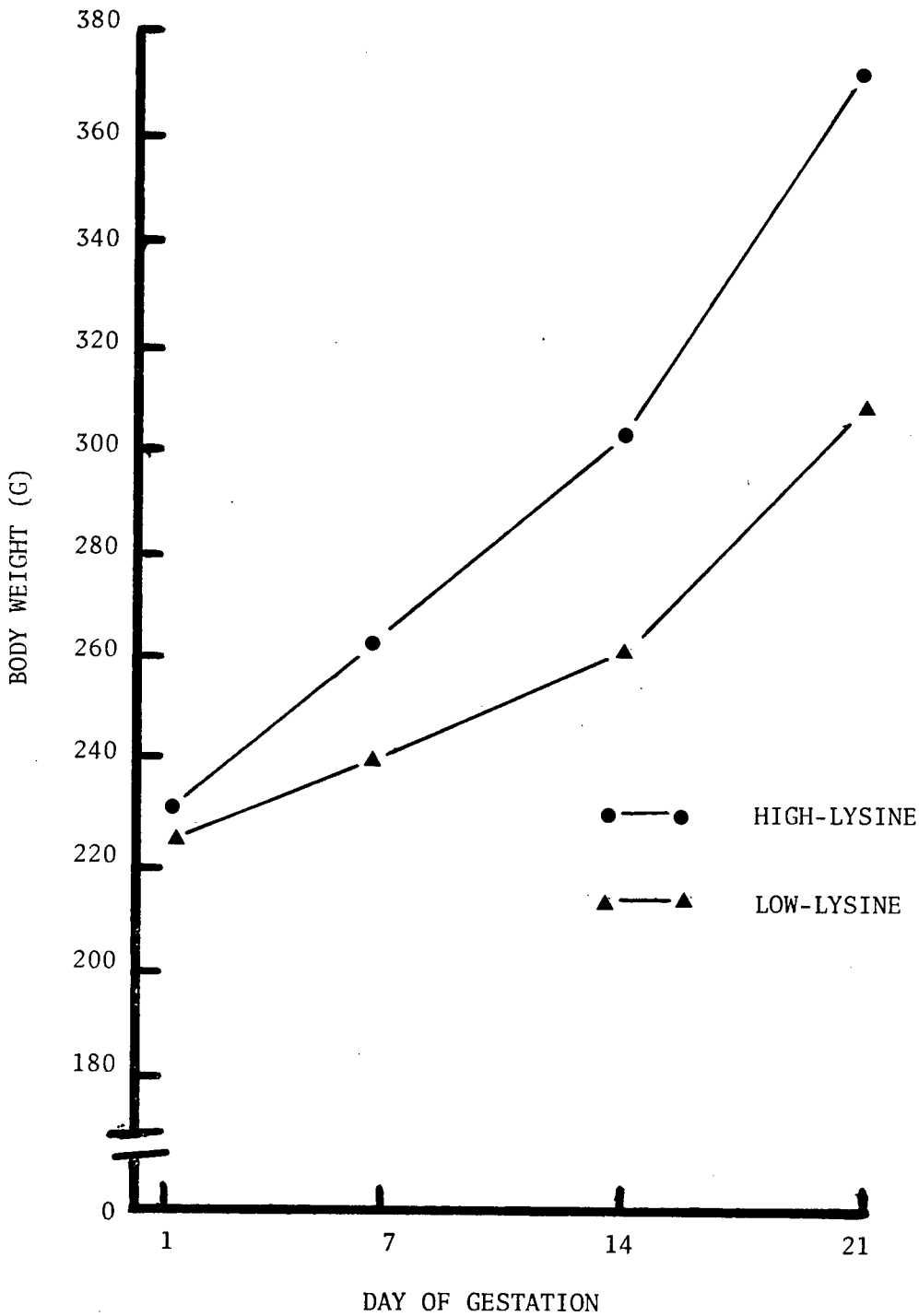

380
360
340
320
300
280
260
240
220
200
180
0
BODY WEIGHT (G)
1
7
14
21
DAY OF GESTATION
HIGH-LYSINE
LOW-LYSINE

Table IV. Heart and Liver Weights of Dams Fed a High-Lysine or Low-Lysine Diet until Day 21 of Gestation

Experimental[A] Group	Tissue Weight g		Tissue Weight % of Body Weight	
	Heart	Liver	Heart	Liver
High-Lysine	0.7430±0.01	14.5071±0.48	0.20±0.01	3.86±0.09
Low-Lysine	0.7035±0.04	11.4096±0.71[B]	0.22±0.03[C]	3.71±0.23

A Mean ± SEM for 6 dams

B Mean values differ significantly from high-lysine group ($p<0.005$)

C Mean values differ significantly from high-lysine group ($p<0.05$)

lysine group (11.4096 g) were significantly lower than in the high-lysine animals (14.5071 g). However, when liver weights were expressed as a percent of body weight, there was no significant difference between the two dietary groups. Lysine deficiency did not affect maternal heart weights which were 0.7430 g and 0.7035 g respectively, for the high-lysine and low-lysine groups.

Although the low-lysine diet resulted in a 14% decrease in food intake compared to the high-lysine group, litter size was not affected by dietary treatment (Table V). Number of fetuses for the high-lysine group was 11.7, and 11.8 for the low-lysine group (Table V). Fetal liver weights were significantly lower in the low-lysine group (0.2033 g) than in the high-lysine group (0.2523 g). Fetal heart weights however, were significantly greater in the low-lysine group (0.0150 g) than the high-lysine group (0.0142 g). Only one resorption site was identified for the dams fed the low-lysine diet, and no evidence of resorptions were noted for the high-lysine group.

The dietary lysine level did not have a significant effect on maternal plasma or liver carnitine levels (Table VI and Figure 5). Plasma carnitine levels averaged 43.97 and 37.70 n mol/ml for dams fed the low lysine and high-lysine diets respectively. Liver carnitine levels were much higher than plasma levels, averaging 168.87 and 166.56 n mol/g tissue in the low-lysine and high-lysine groups respectively.

Fetal plasma carnitine levels were significantly higher in the low-lysine (22.85 n mol/ml) group than the high-lysine (18.04 n mol/ml) group (Table VI, Figure 6). Fetal liver

Table V. Effect of a High-Lysine or Low-Lysine Diet on the Number of Fetuses, and Fetal Heart and Liver Weight on Day 21 of Gestation

Experimental[A] Group	Number of Fetuses	Fetal Heart Weight g	Fetal Liver Weight g
High-Lysine	11.8±1.4	0.0142±0.000	0.2523±0.005
Low-Lysine	11.7±0.9	0.0150±0.001[B]	0.2033±0.014

A Mean ± SEM for 6 litter means

B Mean values differ significantly from high-lysine group ($p<0.005$)

C Mean values differ significantly from high-lysine group ($p<0.05$)

Table VI. Effect of a High-Lysine or Low-Lysine Diet on Plasma and Liver Carnitine of Dams and Fetuses on Day 21 of Gestation

	Experimental Group	Plasma Carnitine nmol/ml	Liver Carnitine nmol/g
Dams[A]	High-Lysine	37.70±8.60	166.56±10.80
	Low-Lysine	43.97±2.40	168.87±9.50
Fetuses[B]	High-Lysine	18.04±0.50	174.62±16.70
	Low-Lysine	22.85±1.40[C]	189.12±19.90

A Mean ± SEM for 6 dams

B Mean ± SEM for 6 litter means

C Mean values differ significantly from high-lysine group ($p<0.05$)

Figure 5 Effect of a high-lysine or low-lysine diet on plasma and liver carnitine in dams on day 21 of gestation

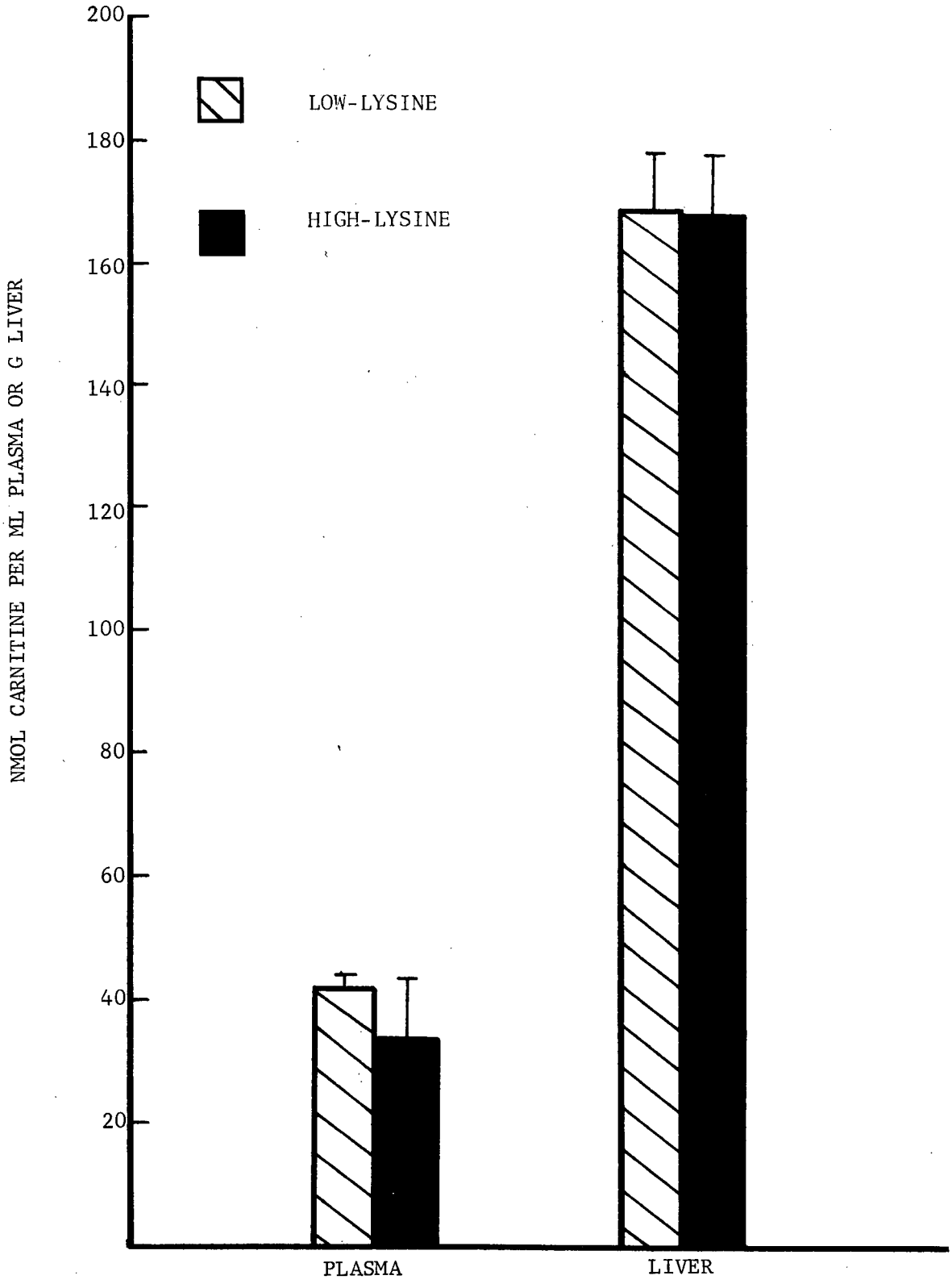
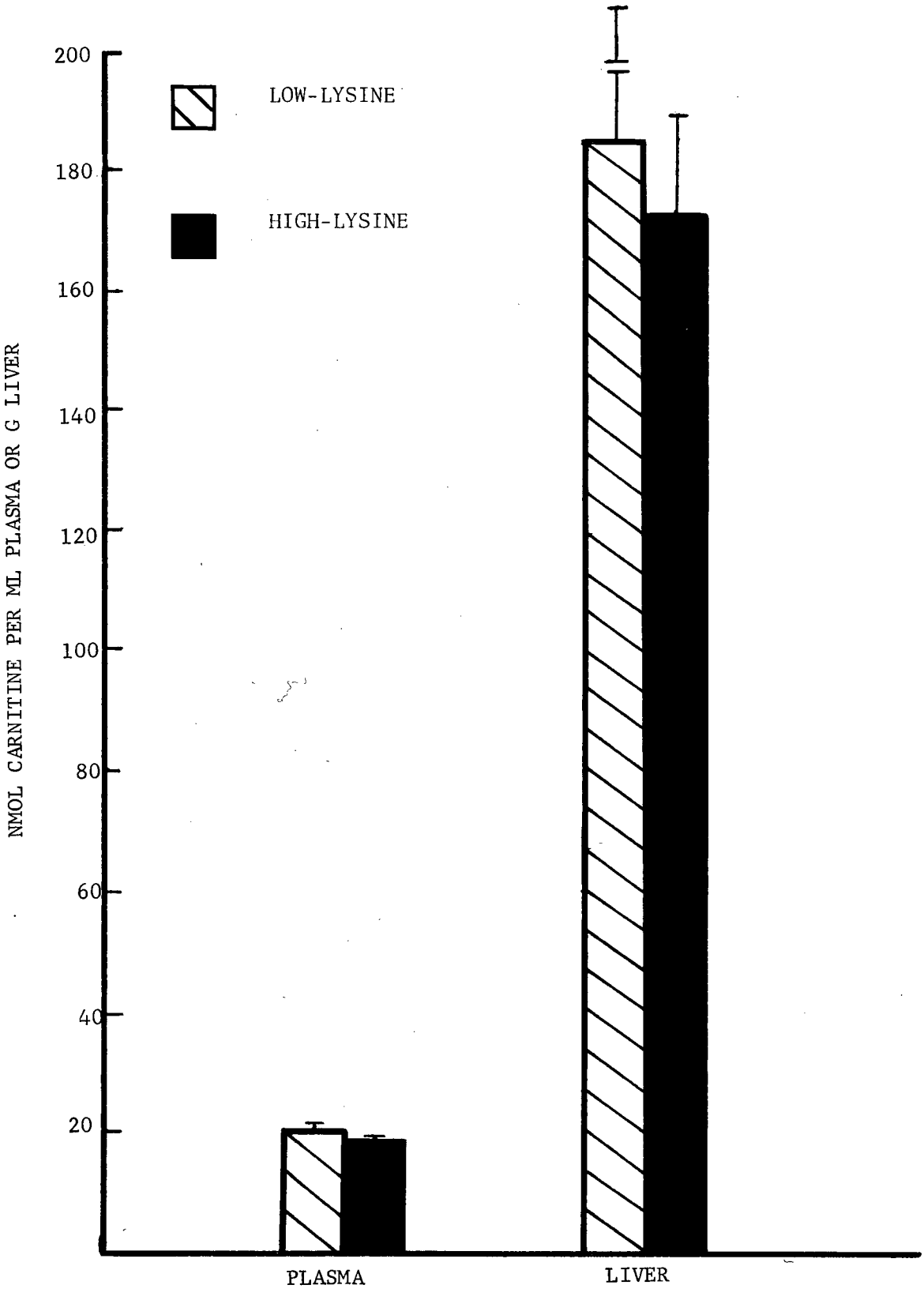

NMOL CARNITINE PER ML PLASMA OR G LIVER
200
180
160
140
120
100
80
60
40
20
LOW-LYSINE
HIGH-LYSINE
PLASMA
LIVER

Figure 6 Effect of a high-lysine or low-lysine diet on plasma and liver carnitine in fetuses on day 21 of gestation


200
180
160
140
120
100
80
60
40
20
NMOL CARNITINE PER ML PLASMA OR G LIVER
LOW-LYSINE
HIGH-LYSINE
PLASMA
LIVER

carnitine levels tended to be higher in the low-lysine (189.12 n mol/g) group than the high-lysine (174.62 n mol/g) group, however the difference was not significant. Fetal plasma carnitine levels were 52% lower than maternal levels, whereas liver carnitine levels were similar in both the dams and fetuses.

EXPERIMENT II

In experiment I food intake of the low-lysine dams was significantly less than that of the high-lysine dams. Therefore a third group of animals, high-lysine restricted, was added to determine if the observed effects were due to a lysine deficiency or a restricted food intake. In experiment II dams fed the low-lysine or high-lysine restricted diets consumed significantly less food, gained significantly less weight, and had significantly lower liver weights than animals fed the high-lysine diet. There were no significant differences between the low-lysine and the high-lysine restricted groups in any of these parameters (Table VII, VIII, IX, and Figure 7).

Food intake during gestation was significantly greater in the high-lysine females (15.0 g/day) than in the low-lysine (13.0 g/day) or high-lysine restricted (13.2 g/day) females. However, when food consumption during pregnancy was expressed as g/100 g body weight there was no significant effect of diet on food intake (Table VII).

Dams fed the high-lysine diet gained significantly more weight (124.6 g) during pregnancy than either the low-lysine

Table VII. Food Intake and Weight Gain of Dams Fed a High-Lysine, Low-Lysine or High-Lysine Restricted Diet during Gestation in Experiment II

	Experimental Group[A]	Initial Body Weight	Week of Gestation[B] 1	2	3	Daily Food Intake	Total Weight Gain
Feed (g/day)	High-Lysine		6.9±0.3	6.3±0.3	5.9±0.2	15.0±0.5[a]	
	Low-Lysine		6.1±0.2	6.1±0.5	4.9±0.4	13.0±0.4[b]	
	High-Lysine Restricted		5.8±0.2	5.9±0.3	5.0±0.4	13.2±0.7[b]	
Body Weight (g)	High-Lysine	217.2±7.1	244.1±4.4	275.8±5.4	341.8±5.6		124.6±5.2[a]
	Low-Lysine	228.4±1.2	238.4±1.2	259.4±8.5	299.3±5.7		70.8±8.3[b]
	High-Lysine Restricted	231.3±5.3	238.8±4.4	268.8±5.0	317.3±7.4		86.0±9.2[b]

C

A Mean ± SEM for 6 dams

B Food intake is expressed as g/100 g body weight

C Means in a column with different superscripts are significantly different ($p<0.05$)

(70.8 g) or high-lysine restricted (86.0 g) groups (Table VII). Weekly weight gains of dams during pregnancy (Table VII and Figure 7) were not significantly affected by dietary treatment.

Food intake of dams during lactation was significantly greater in the high-lysine (27.1 g/day) group than in the low-lysine (14.2 g/day) or high-lysine restricted groups (13.7 g/day, Table VIII). When food consumption during lactation was expressed as g/100 g body weight, a significant effect of diet on food intake was indicated ($p<0.001$). Food intake of the high-lysine dams was significantly greater than the low-lysine and high-lysine restricted dams during weeks 1 and 2 of lactation.

Dams fed the high-lysine diet gained 20.1 g during lactation whereas the low-lysine and high-lysine restricted groups lost 47.0 g and 45.9 g, respectively (Table VIII and Figure 11). There were no significant differences in weight between the low-lysine and high-lysine restricted groups of animals however, both groups differed significantly from that of the high-lysine group. The effect of diet on weekly weight changes during lactation was also significant ($p<0.001$). When compared to the high-lysine group, the average body weights of dams fed the low-lysine and high-lysine restricted diets were significantly lower on days 1, 7, and 14. Body weights of dams on day 15 for the high-lysine, low-lysine, and high-lysine restricted groups were 296.8 g, 190.2 g, and 202.2 g respectively (Table VIII).

The effect of dietary treatment on maternal liver and heart weight in the dams is presented in Table IX. Livers of the low-

Figure 7 Effect of a high-lysine, low-lysine, or high-lysine restricted diet on weight gain of dams during gestation in Experiment II

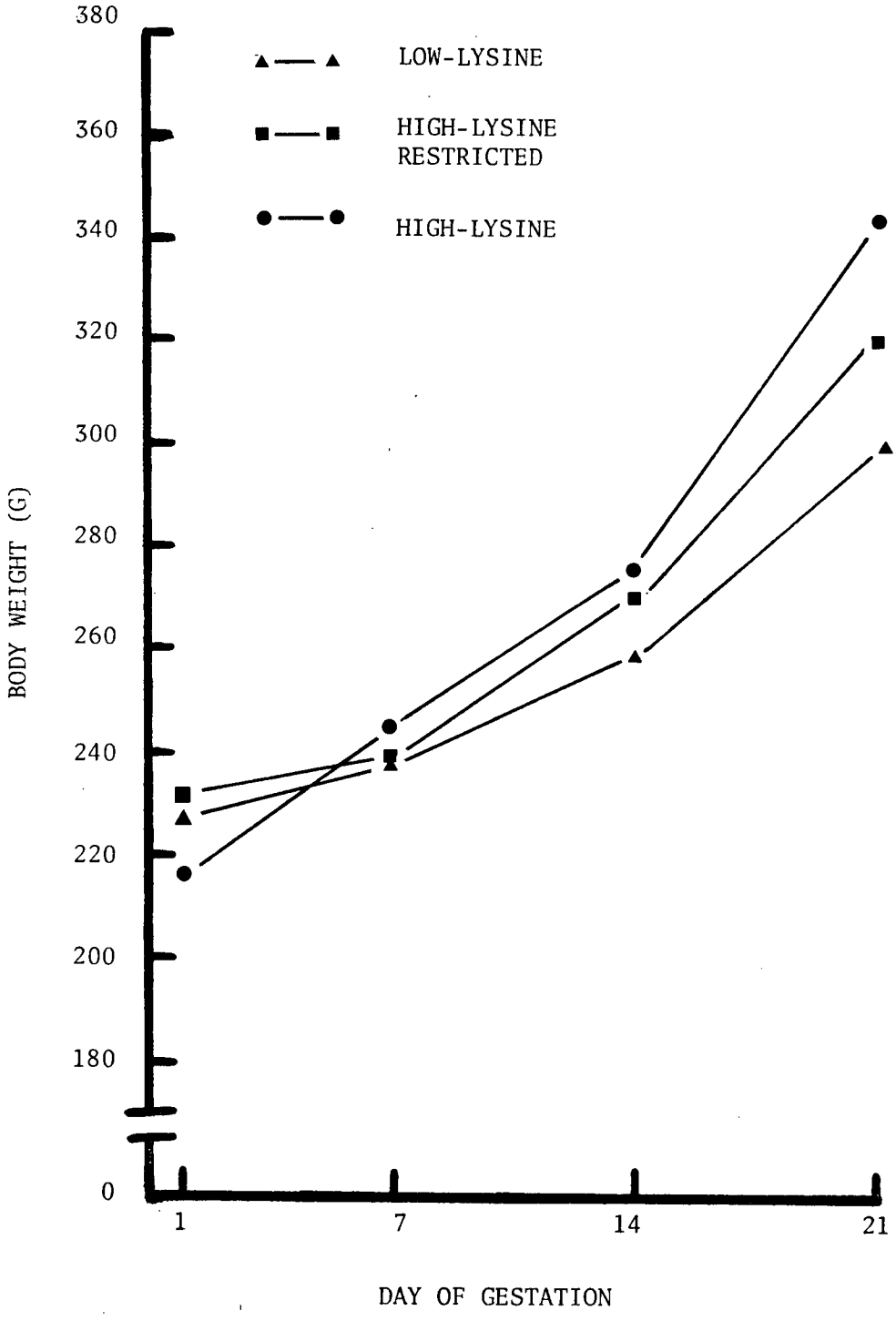

380
360
340
320
300
280
260
240
220
200
180
0
BODY WEIGHT (G)
LOW-LYSINE
HIGH-LYSINE RESTRICTED
HIGH-LYSINE
1
7
14
21
DAY OF GESTATION

Table VIII. Food Intake and Weight Changes of Dams Fed a High-Lysine, Low-Lysine or High-Lysine Restricted Diet during Lactation

	Experimental[A] Group	Initial Weight	Week of Lactation[B] 1	2	Daily Food Intake	Total Weight Gain
Feed (g/day)	High-Lysine		7.4±0.5[a][C]	12.6±1.1[a]	27.1±1.1[a]	
	Low-Lysine		5.4±0.7[b]	6.9±0.9[b]	14.2±1.5[b]	
	High-Lysine Restricted		5.1±0.4[b]	7.0±1.0[b]	13.7±1.5[b]	
Body Weight (g)	High-Lysine	276.6±11.1[a]	278.4±11.1[a]	296.8±5.6[a]		+20.1±10.7[a]
	Low-Lysine	236.1± 4.6[b]	208.3± 3.9[b]	190.2±5.7[b]		-47.0± 8.8[b]
	High-Lysine Restricted	249.2± 4.9[b]	221.4± 5.1[b]	202.2±8.5[b]		-45.9± 4.7[b]

A Mean ± SEM for 6 dams

B Food intake is expressed as g/100 g body weight

C Means in a column with different superscripts are significantly different ($p<0.05$)

Table IX. Heart and Liver Weights of Dams Fed a High-Lysine, Low-Lysine or High-Lysine Restricted Diet until Day 15 of Lactation

Experimental Group[A]	Tissue Weight g		Tissue Weight % of Body Weight	
	Heart	Liver	Heart	Liver
High-Lysine	4.9148±0.30	14.2202±0.62[a B]	1.70±0.10	4.80±0.24
Low-Lysine	3.9336±0.30	8.6252±0.93[b]	2.07±0.10	4.55±0.22
High-Lysine Restricted	4.8560±0.30	8.0036±1.02[b]	2.43±0.20	3.90±0.32

A Mean ± SEM for 6 dams

B Means in a column with different superscripts are significantly different ($p<0.05$)

lysine (8.6252 g) and high-lysine restricted (8.0036 g) dams weighed significantly less than those of the high-lysine (14.2202 g) dams. Heart weights did not differ significantly among the three dietary groups. When tissue weights were expressed as a percentage of body weight, dietary treatment had no significant effect on either liver or heart weight.

Effects of dietary treatment on reproduction as assessed by litter size and birth weight of pups, is presented in Table X. Litter size for the high-lysine (11.0), low-lysine (10.3), and high-lysine restricted (13.0) groups was not significantly different. When compared with offspring of animals maintained on the high-lysine control diet (6.0 g), birth weight of pups was however, significantly lowered by either a lysine deficiency (5.5 g) or food restriction (5.4 g). There was no difference in birth weight between the low-lysine and high-lysine restricted groups.

The lactation performance of dams, as measured by the growth of pups, is presented in Table XI and Figure 8. The effect of maternal diet on weight gain of pups during lactation was significant ($p<0.001$). The high-lysine group of pups weighed significantly more than the high-lysine restricted group which in turn weighed significantly more than low-lysine group on days 5, 10, and 15 of lactation. Pups of high-lysine, low-lysine, and high-lysine restricted dams weighed 28.9 g, 12.6 g, and 17.8 g respectively, on day 15 of lactation.

The effect of maternal diet on pups liver and heart weight is presented in Table XII. Livers of pups in the high-lysine group (0.9184 g) weighed significantly greater than those of the

Table X. Effect of a High-Lysine, Low-Lysine or High-Lysine Restricted Diet on Reproductive Performance of Dams in Experiment II

Experimental[A] Group	Litter Size	Birth Weight of Pups g
High-Lysine	11.0±1.2	6.0±0.2[a][B]
Low-Lysine	10.3±0.4	5.5±0.2[b]
High-Lysine Restricted	13.0±0.3	5.4±0.8[b]

A Mean ± SEM for 6 litter means

B Means in a column with different superscripts are significantly different ($p<0.05$)

Table XI. Effect of a High-Lysine, Low-Lysine or High-Lysine Restricted Maternal Diet on Weight Gains of Pups in Experiment II

	Experimental[A] Groups	Days Post Partum 1	5	10	15
Body Weight (g)	High-Lysine	6.1±0.3[a][B]	10.1±0.3[a]	18.8±0.5[a]	28.9±0.9[a]
	Low-Lysine	5.5±0.2[b]	8.4±0.3[b]	10.8±0.5[b]	12.6±0.7[b]
	High-Lysine Restricted	5.5±0.1[b]	9.2±0.3[c]	14.1±0.8[c]	17.8±1.3[c]

A Mean ± SEM for 6 litter means

B Means in a column with different superscripts are significantly different ($p<0.05$)

Figure 8 Effect of a high-lysine, low-lysine, or high-lysine restricted maternal diet on weight gain of pups in Experiment II

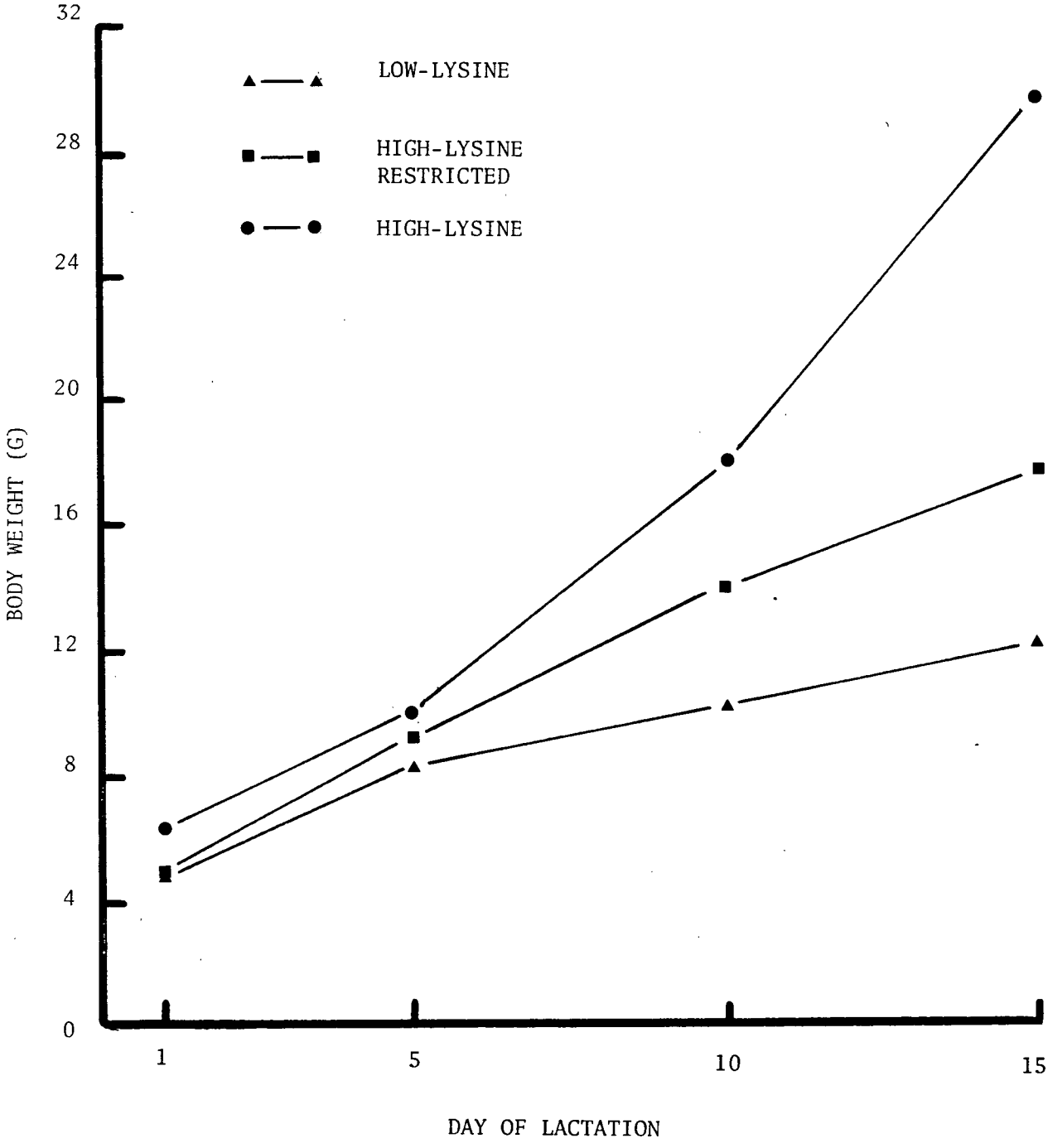

32
28
24
20
16
12
8
4
0
BODY WEIGHT (G)
LOW-LYSINE
HIGH-LYSINE
RESTRICTED
HIGH-LYSINE
1
5
10
15
DAY OF LACTATION

Table XII. Effect on a High-Lysine, Low-Lysine or High-Lysine Restricted Maternal Diet on Heart and Liver Weights of Pups on Day 15 of Lactation

Experimental Group[A]	Tissue Weight g		Tissue Weight % of Body Weight	
	Heart	Liver	Heart	Liver
High-Lysine	0.1234±0.010[a] [B]	0.9184±0.042[a]	0.40±0.02	2.90±0.60
Low-Lysine	0.0639±0.005[b]	0.3528±0.028[b]	0.51±0.03	2.82±0.02
High-Lysine Restricted	0.1146±0.054[a]	0.5205±0.050[c]	0.60±0.10	3.20±0.10

A Mean ± SEM for 6 litter means

B Means in a column with different superscripts are significantly different ($p<0.05$)

high-lysine restricted group (0.5205 g) which were also significantly greater than those of the low-lysine group (0.3528 g). Heart weights of pups in the high-lysine (0.1234 g) and high-lysine restricted groups (0.1146 g) were significantly greater than thcse of the low-lysine group (0.0639 g). When tissue weights were expressed as a percentage of body weights, there were no significant differences among the three dietary groups.

When compared to high-lysine control values, plasma and liver carnitine levels were significantly higher at day 15 of lactation, in both the pups and dams consuming either the low-lysine diet ad libitum , or the restricted high-lysine diet (Table XIII, Figures 9 and 10). Heart and liver carnitine for both the pups and dams did not differ significantly between the low-lysine and high-lysine restricted grcups.

The low-lysine diet resulted in maternal plasma carnitine levels of 38.22 n mol/ml compared tc 34.79 n mol/ml for the high-lysine restricted dams and 19.22 n mol/ml for the high-lysine dams. Liver carnitine values of dams in the high-lysine, high-lysine restricted and low-lysine groups were, 120.98, 187.60, and 176.24 n mol/ml respectively. The plasma carnitine levels in dams fed the high-lysine diet were higher at day 21 of pregnancy (37.70 n mol/ml, Table VI) than at day 15 of lactation (19.22 n mol/ml, Table XIII). Liver carnitine levels in animals fed the same high-lysine diet averaged 166.56 n mol/g at the end of gestation and 120.98 n mol/g on day 15 of lactation (Table VI and XIII respectively).

Plasma carnitine levels for the low-lysine (61.02 n mol/ml)

Table XIII. Effect of a High-Lysine, Low-Lysine or High-Lysine Restricted Maternal Diet on Plasma and Liver Carnitine of Dams and Pups on Day 15 of Lactation

	Experimental Group	Plasma Carnitine nmol/ml	Liver Carnitine nmol/g
Dams[A]	High-Lysine	19.22±3.86[a] [C]	120.98±32.39[a]
	Low-Lysine	38.22±7.56[b]	176.24±13.23[b]
	High-Lysine Restricted	34.79±5.14[b]	187.60± 9.84[b]
Pups[B]	High-Lysine	51.10±1.78[a]	253.99±11.68[a]
	Low-Lysine	61.02±4.13[b]	370.71±85.44[b]
	High-Lysine Restricted	62.07±3.75[b]	360.78±40.82[b]

A Mean ± SEM for 6 dams

B Means in a column with different superscripts are significantly different ($p<0.05$)

C Mean ± SEM for 6 litter means

Figure 9 Effect of a high-lysine, low-lysine, or high-lysine restricted diet on plasma and liver carnitine of dams on day 15 of lactation

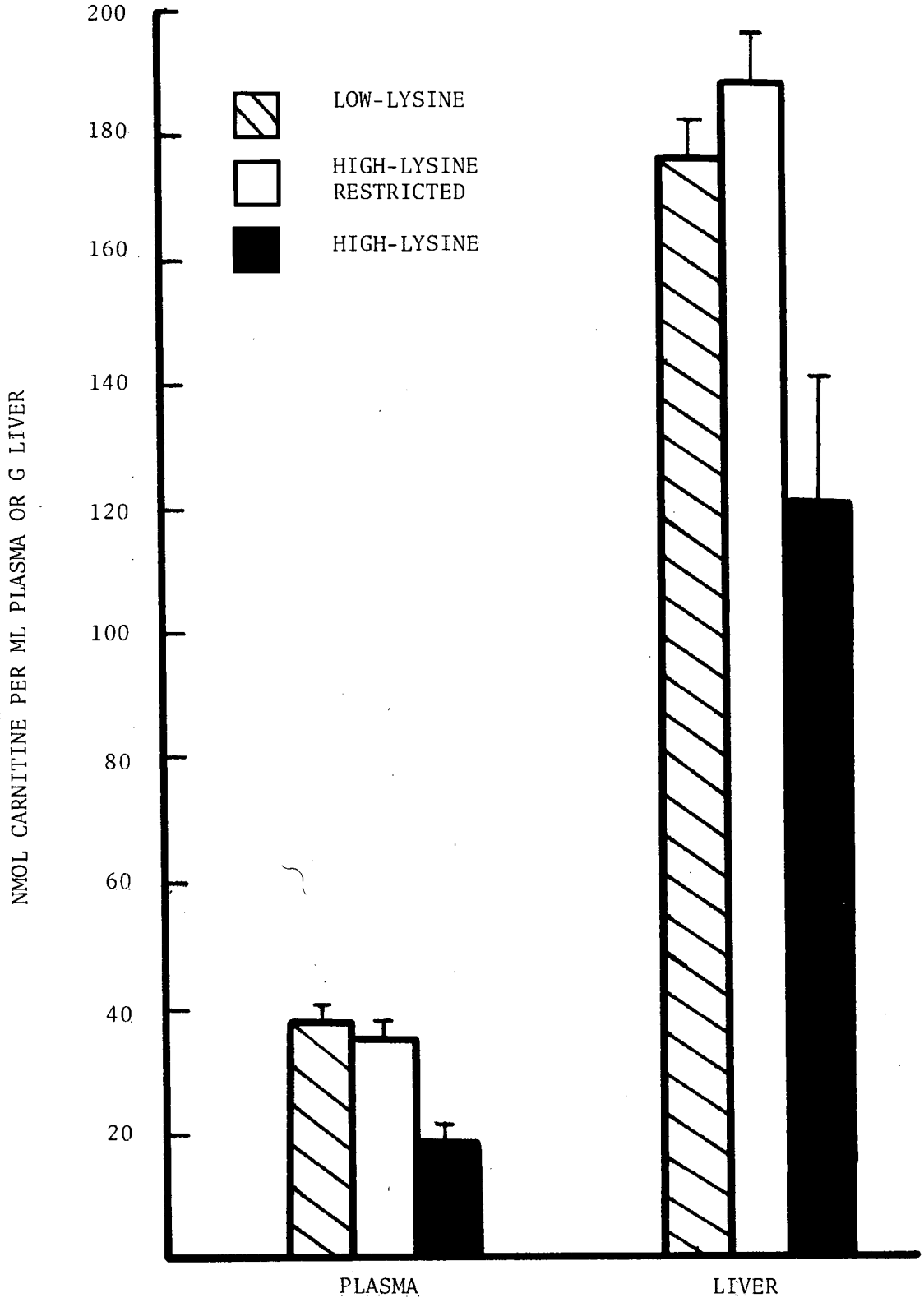
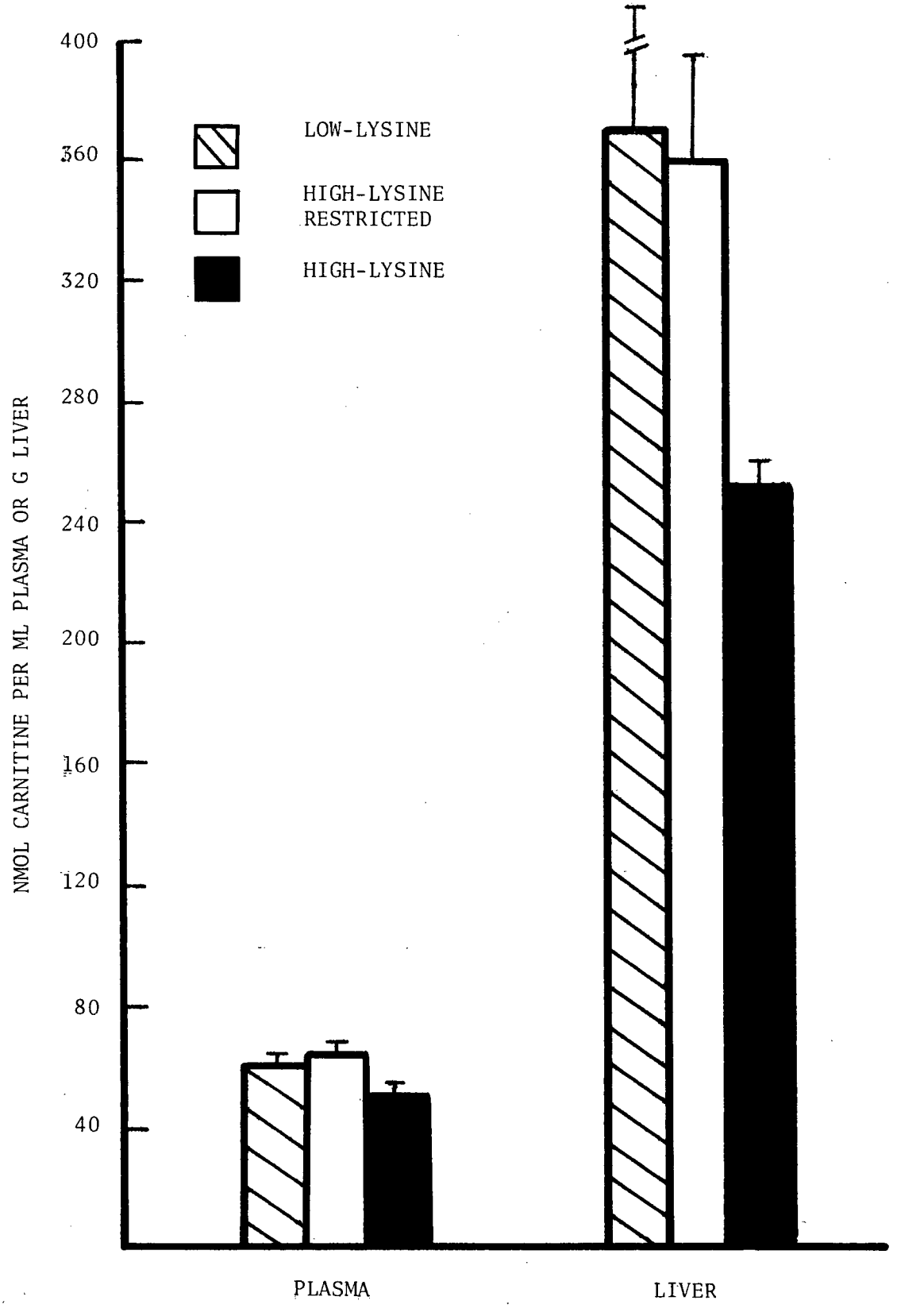

200
180
160
140
120
100
80
60
40
20
NMOL CARNITINE PER ML PLASMA OR G LIVER
LOW-LYSINE
HIGH-LYSINE RESTRICTED
HIGH-LYSINE
PLASMA
LIVER

Figure 10 Effect of a high-lysine, low-lysine, or high-lysine restricted maternal diet on plasma and liver carnitine of pups on day 15 of lactation


400
360
320
280
240
200
160
120
80
40
NMOL CARNITINE PER ML PLASMA OR G LIVER
LOW-LYSINE
HIGH-LYSINE RESTRICTED
HIGH-LYSINE
PLASMA
LIVER

and high-lysine restricted (62.07 n mol/ml) groups of pups were significantly higher than those of the high-lysine pups (51.10 n mol/ml). Likewise, liver carnitine levels for the low-lysine (370.71 n mol/g) and high-lysine restricted (360.78 n mol/g) pups were significantly higher than those for the high-lysine pups (253.99 n mol/g). Plasma and liver carnitine levels of pups on day 15 of lactation (Table XIII) were almost double the levels of carnitine in fetal tissues (Table VI).

Weight changes of dams during pregnancy and lactation are depicted in Figure 11. The histogram emphasizes the similarity in weight gains that occurred in the low-lysine and high-lysine restricted groups during pregnancy. During lactation both the low-lysine and high-lysine restricted dams lost a significant amount of weight whereas the high-lysine control animals gained weight.

EXPERIMENT III

The dams in experiment III fed the low-lysine or high-lysine restricted diets consumed significantly less food and gained significantly less body weight than those animals fed the high-lysine diet (Tables XIV, XV, XVI, and Figure 12).

The food intake of dams during gestation was significantly greater in the high-lysine group (16.5 g/day) than in the low-lysine (13.5 g/day) or high-lysine restricted groups (13.5 g/day, Table XIV). Food intake between the low-lysine and high-lysine restricted females did not differ significantly. When food consumption during pregnancy was expressed as g/100 g body

Figure 11. Effect of a high-lysine, low-lysine, or high-lysine restricted diet on weight changes of dams during pregnancy and lactation in Experiment II

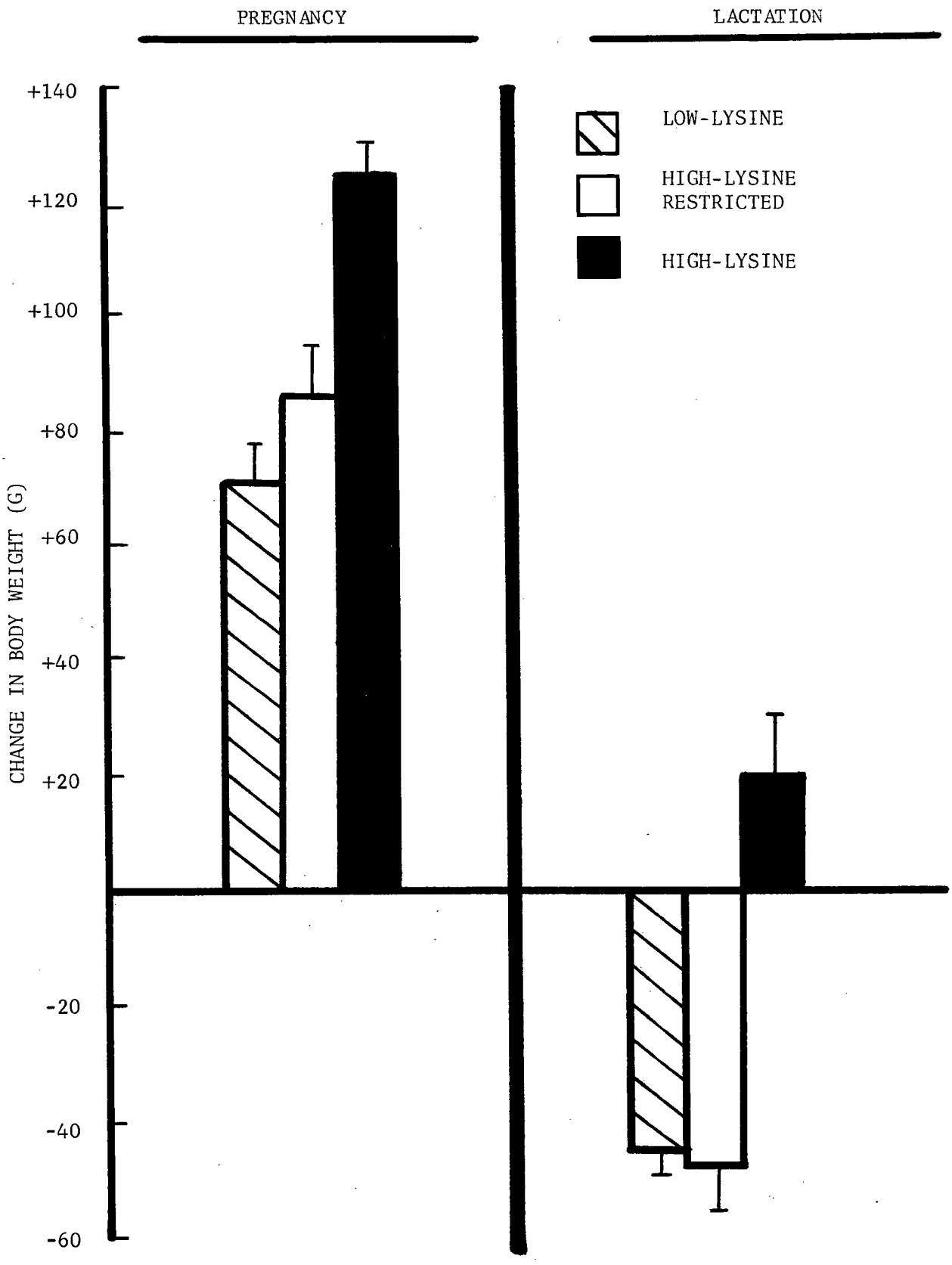

PREGNANCY
LACTATION
LOW-LYSINE
HIGH-LYSINE RESTRICTED
HIGH-LYSINE
CHANGE IN BODY WEIGHT (G)
+140
+120
+100
+80
+60
+40
+20
-20
-40
-60

Table XIV. Food Intake and Weight Gain of Dams Fed a High-Lysine, Low-Lysine or High-Lysine Restricted Diet during Gestation in Experiment III

	Experimental[A] Group	Initial Body Weight	Week of Gestation[B] 1	2	3	Daily Food Intake	Total Weight Gain
Feed (g/day)	High-Lysine		7.2±0.3	7.4±0.3	6.1±0.3	16.5±0.4[a C]	
	Low-Lysine		6.4±0.2	5.9±0.2	5.2±0.3	13.5±0.3[b]	
	High-Lysine Restricted		6.1±0.2	5.6±0.1	5.0±0.2	13.5±0.4[b]	
Body Weight (g)	High-Lysine	217.5±5.2	248.1±4.8	276.6±4.3	336.3±5.4		118.8±4.1[a]
	Low-Lysine	232.0±3.3	239.1±4.8	244.2±3.8	281.4±3.5		49.5±1.7[b]
	High-Lysine Restricted	232.0±3.4	244.0±3.6	267.0±4.5	318.2±6.5		86.8±4.4[c]

A Mean ± SEM for 18 dams

B Food intake is expressed as g/100 g body weight

C Means in a column with different superscripts are significantly different ($p<0.05$)

weight, there was no significant effect of diet on food intake (Table XIV). These results were similar to those obtained in experiment II.

Dams fed the high-lysine diet gained significantly more weight (118.8 g) during gestation than either the low-lysine (49.5 g) or high-lysine restricted (86.8 g) groups (Table XIV and Figure 12). These results differed from those in experiment II. The difference between the absolute weight gain by the low-lysine and high-lysine restricted dams was not significant in experiment II whereas the difference was significant in experiment III (Tables VII and XIV respectively).

Food intake and weight changes during lactation, for those dams used for milk carnitine analysis on day 15 of lactation, are presented in Table XV. As previously reported in experiment II (Table VII), food intake of dams was again significantly lower in the low-lysine (17.5 g/day) and high-lysine restricted (20.3 g/day) groups than in the high-lysine (29.0 g/day) group. When food consumption during lactation was expressed as g/100 g body weight, a significant effect of diet on food intake was observed ($p<0.001$). Food intake for the high-lysine dams was significantly greater than for the low-lysine and high-lysine restricted groups. Body weight changes for these three groups of dams during lactation were of a smaller magnitude than, but in the same direction as those obtained in experiment II (Table VIII). Dams consuming the high-lysine diet gained an average of 17.1 g body weight whereas, the dams fed the low-lysine or high-lysine restricted diets lost an average of 16.4 and 29.2 g respectively. Weight change between the low-lysine and high-

Figure 12 Effect of a high-lysine, low-lysine, or high-lysine restricted diet on weight gain of dams during gestation in Experiment III

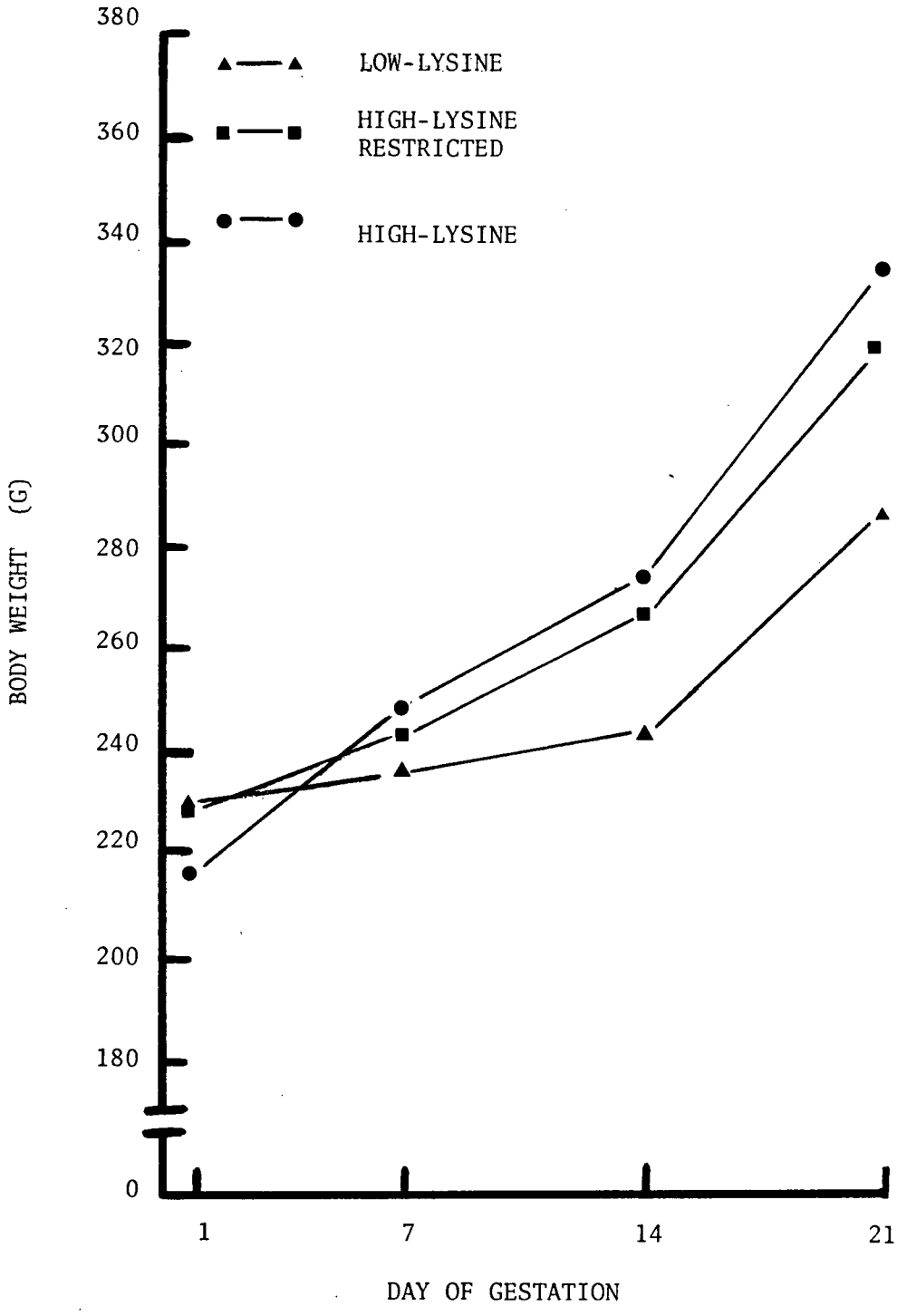

380
360
340
320
300
280
260
240
220
200
180
0
LOW-LYSINE
HIGH-LYSINE
RESTRICTED
HIGH-LYSINE
BODY WEIGHT (G)
1
7
14
21
DAY OF GESTATION

TABLE XV. Food Intake and Weight Changes of Dams Milked on Day 15 of Lactation and Fed a High-Lysine, Low-Lysine or High-Lysine Restricted Diet during Lactation

	Experimental[A] Group	Initial Body Weight	Week of Lactation[B] 1	Week of Lactation[B] 2	Daily Food Intake	Total Weight Gain
Feed (g/day)	High-Lysine		8.2±0.3	12.6±0.2[a]	29.0±1.5[a]	
	Low-Lysine		7.6±0.5	9.2±0.4[b]	17.5±0.5[b]	
	High-Lysine Restricted		7.0±0.2	9.0±0.1[b]	20.3±1.5[b]	
Body Weight (g)	High-Lysine	280.9±6.0[a]	279.9±5.4[a]	297.9±5.7[a]		+17.1±7.8[a]
	Low-Lysine	217.6±5.4[b]	204.4±6.9[b]	201.2±7.1[b]		-16.4±5.4[b]
	High-Lysine Restricted	259.0±10.0[b]	248.0±10.7[c]	229.8±10.2[c]		-29.2±11.4[b]

A Mean ± SEM for 6 dams

B Food intake is expressed in g/100g body weight

C Means in a column with different superscripts are significantly different ($p<0.05$)

lysine restricted groups were similar however both values differed significantly from the high lysine group. The effect of diet on weekly weight changes during lactation was also significant ($p<0.001$). The high-lysine dams weighed significantly more than the high-lysine restricted and low-lysine dams on days 7 and 15 of lactation. The body weight of the low-lysine and high-lysine restricted animals on days 7 and 15 were also significantly different, as was the case in experiment II.

Food intake and weight changes during lactation, for those dams used for milk carnitine analysis on day 8 of lactation (Table XVI) are similar to those obtained for dams used for milk carnitine analysis on day 15 of lactation. Food intake was significantly lower in the low-lysine (15.8 g/day) and high-lysine restricted (17.7 g/day) dams than in the high-lysine (24.2 g/day) females. When food consumption was expressed as g/100 g body weight, a significantly greater food intake was found in those dams fed the high-lysine diet than dams consuming the low-lysine or high-lysine restricted diets. Dams fed the high-lysine diet gained an average of 14.8 g during the first week of lactation, whereas the low-lysine and high-lysine restricted females lost an average of 12.1 g and 10.8 g respectively. Weight gain of the low-lysine and high-lysine restricted dams differed significantly from high-lysine dams. On day 8 of lactation the high-lysine group weighed significantly more than the high-lysine restricted group, which in turn weighed significantly more than the low-lysine group.

Maternal dietary treatment effects on reproduction, as

Table XVI. Food Intake and Weight Changes of Dams Milked on Day 8 of Lactation and Fed a High-Lysine, Low-Lysine or High-Lysine Restricted Diet during Lactation

	Experimental[A] Group	Initial Body Weight	Week of Lactation[B] 1	Daily Food Intake	Total Weight Gain
Feed (g/day)	High-Lysine		8.9±0.3[a][C]	24.2±1.4[a]	
	Low-Lysine		7.0±0.3[b]	15.8±0.4[b]	
	High-Lysine Restricted		7.0±0.2[b]	17.7±0.9[b]	
Body Weight (g)	High-Lysine	274.1±9.4[a]	288.9±6.9[a]		+14.8±6.0[a]
	Low-Lysine	220.5±7.3[b]	210.5±5.0[b]		-12.1±6.5[b]
	High-Lysine Restricted	254.7±11.6[a]	243.9±8.2[c]		-10.8±4.4[b]

A Mean ± SEM for 6 dams

B Food intake is expressed as g/100g body weight

C Means in a column with different superscripts are significantly different ($p<0.05$)

assessed by litter size and birth weight of pups, is presented in Table XVII. Litter size for the high lysine (10.7), low-lysine (11.9), and high-lysine restricted (12.3) groups was similar. Birth weight of pups in the high-lysine group (6.1 g) was significantly greater than that of the high-lysine restricted (5.5 g) or the low-lysine (4.9 g) groups. Although birth weights of pups did not differ significantly between the low-lysine and high-lysine restricted groups in experiment II (Table X), the birth weight of pups did differ significantly between groups in experiment III.

The mortality rate of pups in the high-lysine, high-lysine restricted, and low-lysine groups was 0.7%, 0.7%, and 1.2% respectively. The difference between groups was not significant (Table XVII).

The lactation performance of dams, as measured by the growth of pups, in experiment III (Table XVIII and Figures 13, 14) was similar to that reported in experiment II (Table XI). On both days 8 ($p<0.001$) and 15 ($p<0.001$) of lactation, the pups in the high-lysine group weighed significantly more than the high-lysine restricted group which in turn weighed significantly more than the low-lysine group. The average weight of pups on day 15 was 27.7 g in the high-lysine group and 10.9 and 20.7 g in the low-lysine and high-lysine restricted groups respectively.

The data obtained in experiment II indicated the the high-lysine restricted and low-lysine animals had higher tissue carnitine levels than the high-lysine control animals. The effect of diet on milk carnitine levels, as presented in Table

Table XVII. Effect of a High-Lysine, Low-Lysine or High-Lysine Restricted Diet on Reproductive Performance of Dams in Experiment III

Experimental[A] Group	Litter Size	Birth Weight of Pups g	% Mortality
High-Lysine	10.7±0.9	6.1±0.2[a B]	0.7
Low-Lysine	11.9±0.6	4.9±0.2[b]	1.2
High-Lysine Restricted	12.3±0.6	5.5±0.2[c]	0.7

A Mean ± SEM for 16 litter means

B Means in a column with different superscripts are significantly different ($p<0.05$)

Table XVIII. Effect of a High-Lysine, Low-Lysine or High-Lysine Restricted Maternal Diet on Weight Gains of Pups in Experiment III

	Experimental[A] Group	Days Post Partum 1	5	8	10	15
Body Weight[B] (g)	High-Lysine	6.8±0.2[a][C]	11.4±0.3[a]		20.1±0.6[a]	27.7±1.3[a]
	Low-Lysine	5.1±0.2[b]	7.4±0.5[b]		9.2±0.7[b]	10.9±1.1[b]
	High-Lysine Restricted	5.8±0.2[c]	9.4±0.7[c]		16.0±1.0[c]	20.7±2.0[c]
Body Weight[D] (g)	High-Lysine	6.4±0.3[a]	11.1±0.5[a]	14.9±0.5[a]		
	Low-Lysine	5.0±0.3[ab]	7.6±0.5[b]	8.4±0.7[b]		
	High-Lysine Restricted	5.6±0.2[b]	9.5±0.5[c]	12.7±0.5[c]		

A Mean ± SEM for 6 litter means

B Pups of dams milked on day 15

C Means in a column with different superscripts are significantly different ($p<0.05$)

D Pups of dams milked on day 8

Figure 13 Effect of a high-lysine, low-lysine, or high-lysine restricted maternal diet on weight gains of pups born to those dams milked on day 15 of lactation

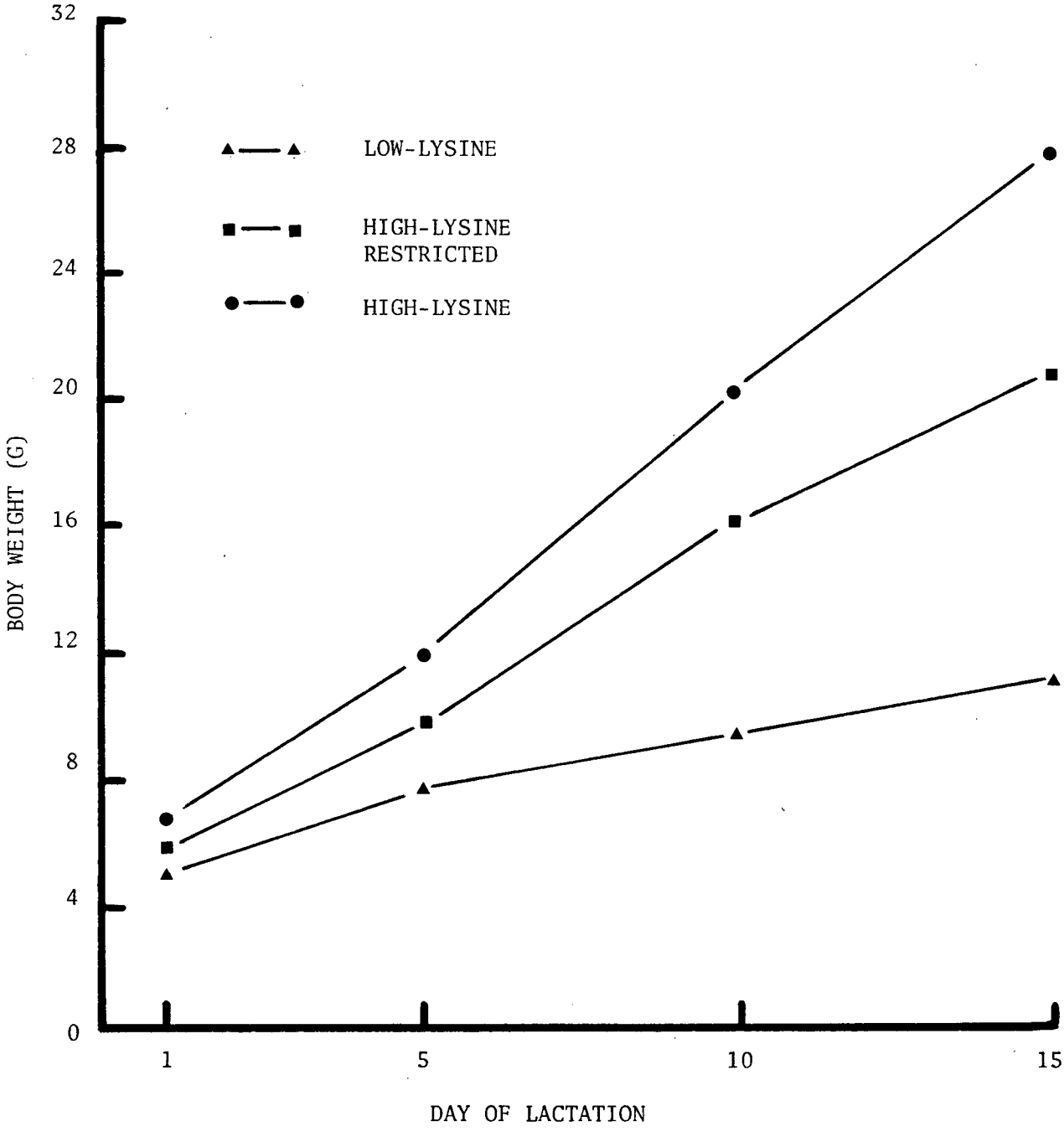
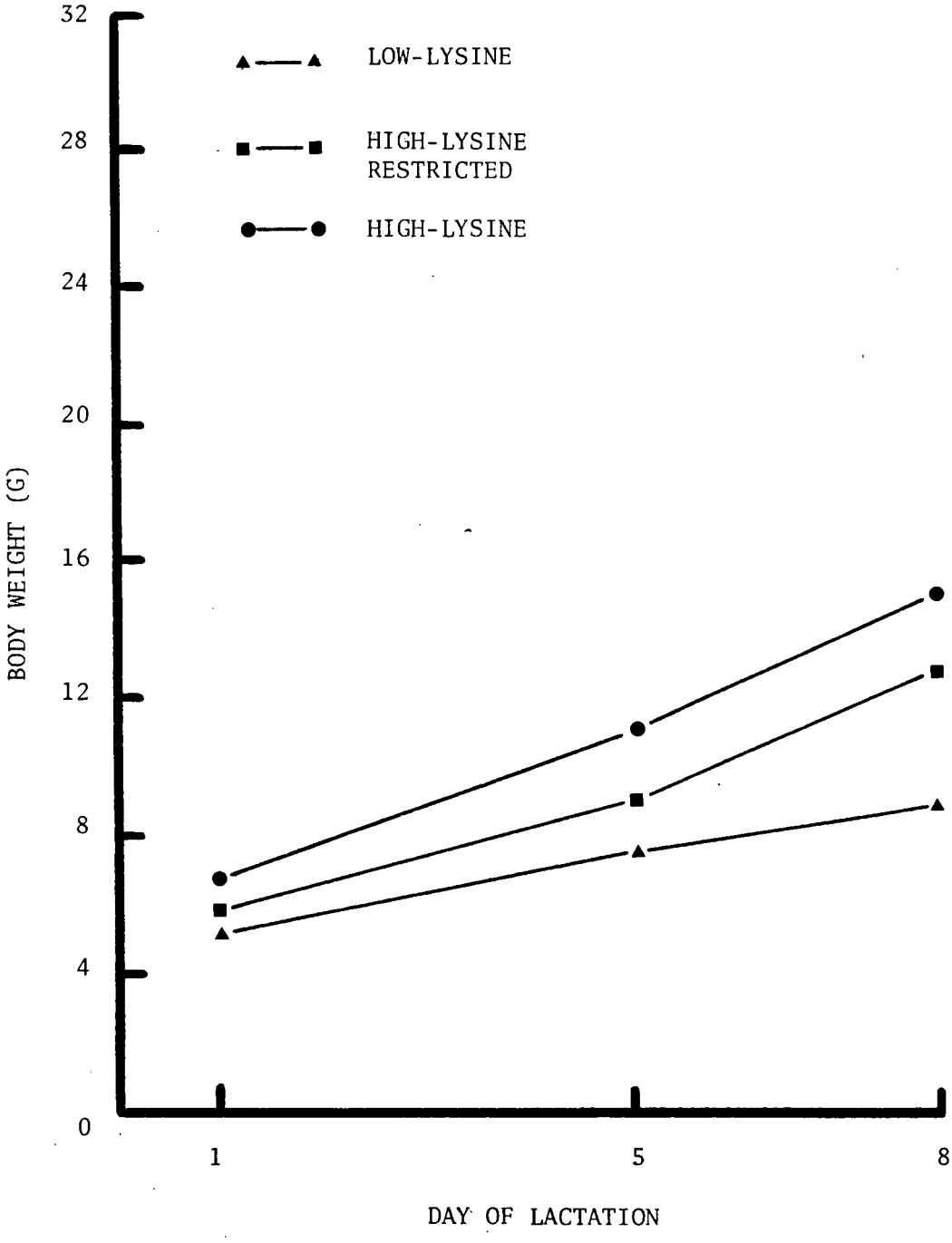

32
28
24
20
16
12
8
4
0
BODY WEIGHT (G)
LOW-LYSINE
HIGH-LYSINE
RESTRICTED
HIGH-LYSINE
1
5
10
15
DAY OF LACTATION

Figure 14 Effect of a high-lysine, low-lysine, or high-lysine restricted maternal diet on weight gain of pups born to those dams milked on day 8 of lactation


32
28
24
20
16
12
8
4
0
BODY WEIGHT (G)
LOW-LYSINE
HIGH-LYSINE
RESTRICTED
HIGH-LYSINE
1
5
8
DAY OF LACTATION

XIX and Figure 15, is not clear. On the 2nd day of lactation, milk carnitine levels of dams fed the high-lysine diet (426.04 n mol/ml), were significantly higher than the low-lysine (300.65 n mol/ml) females, which in turn were significantly higher than the high-lysine restricted (151.21 n mol/ml) females. On day 8 of lactation there was no significant difference between the milk carnitine levels in those animals consuming either the high-lysine (122.42 n mol/ml) diet ad libitum, or the high-lysine restricted (128.52 n mol/ml) diet. The same pattern prevailed on day 15 of lactation for the two dietary groups, i.e., there was no significant difference in milk carnitine levels between the high-lysine (107.15 n mol/ml) and high-lysine restricted (134.19 n mol/ml) groups. Dams fed the low-lysine diet had significantly higher milk carnitine levels on day 8 (277.46 n mol/ml) and day 15 (194.20 n mol/ml) than animals in the two other dietary groups. Milk carnitine values for the low-lysine and high-lysine restricted groups did not change significantly during the course of lactation. Milk carnitine values, for dams fed the high-lysine diet, were significantly greater on day 2 of lactation than on days 8 and 15 post partum.

Plasma carnitine levels of high-lysine, low-lysine, and high-lysine restricted dams and their offspring are compared with milk carnitine values in Figure 16. Plasma and liver carnitine values are for day 15 of lactation. Plasma carnitine concentrations for dams and their offspring were significantly higher in both the low-lysine and high-lysine restricted groups, when compared with their respective control values. Plasma carnitine concentrations for the low-lysine and high-lysine

Table XIX. Effect of a High-Lysine, Low-Lysine or High-Lysine Restricted Diet on Milk Carnitine on Days 2, 8 and 15 Post Partum

	Experimental[A] Group	Days Post Partum 2	8	15
Milk Carnitine (n mol/ml)	High-Lysine	426.04±29.50[a,d][B]	122.42±16.23[a,d][C]	107.15±12.56[a,d]
	Low-Lysine	300.65±43.21[b,d]	277.46±38.21[b,e]	194.20±12.63[b,e]
	High-Lysine Restricted	151.21±37.76[c,d]	128.52±27.68[a,e]	134.19±22.43[a,e]

A Mean ± SEM for 6 dams

B Means in a column with different superscripts are significantly different (a, b, c - $p<0.05$)

C Means in a row with different superscripts are significantly different (d, e - $p<0.05$)

Figure 15 Effect of a high-lysine, low-lysine, or high-lysine restricted diet on milk carnitine on days 2, 8, and, 15 of lactation

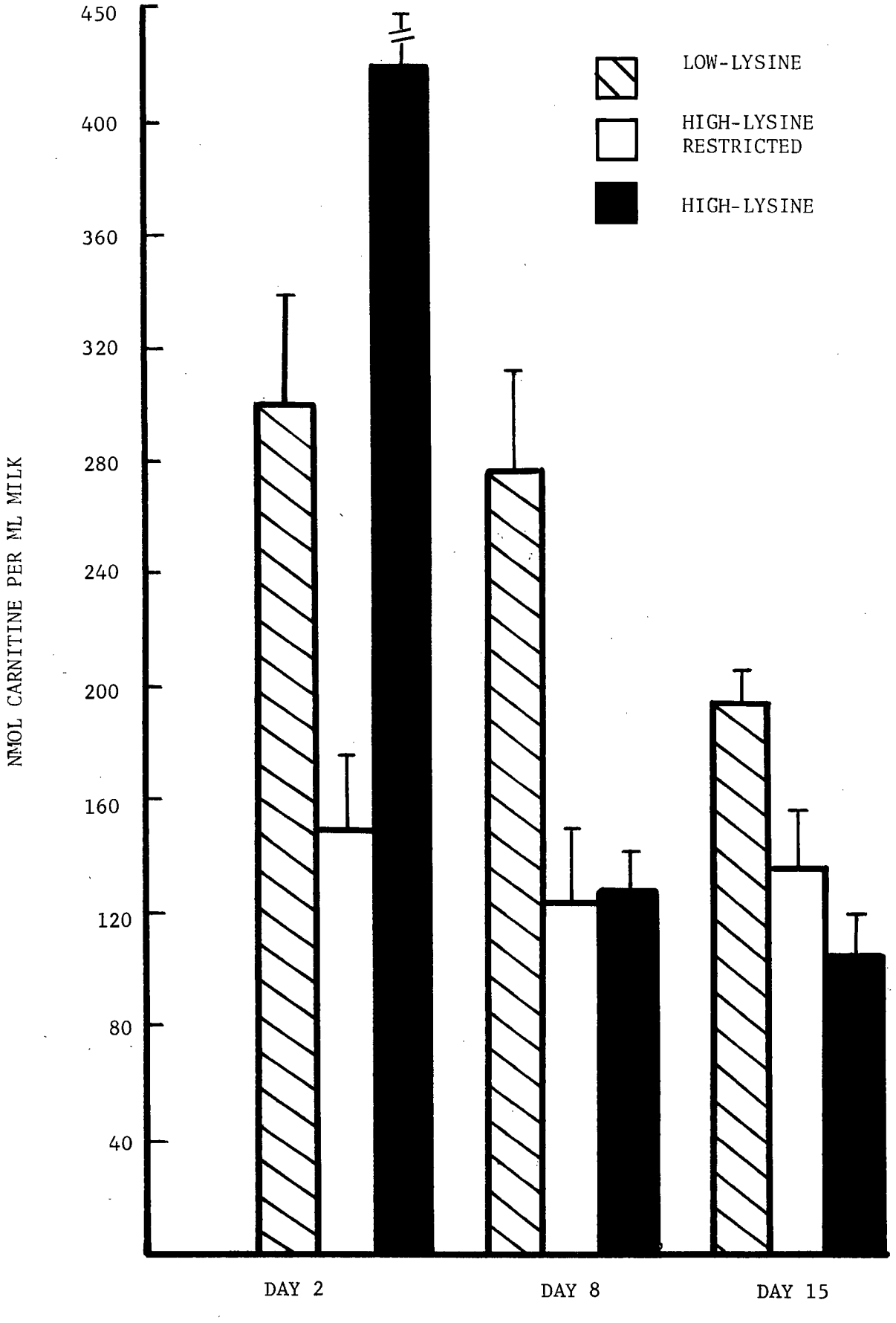
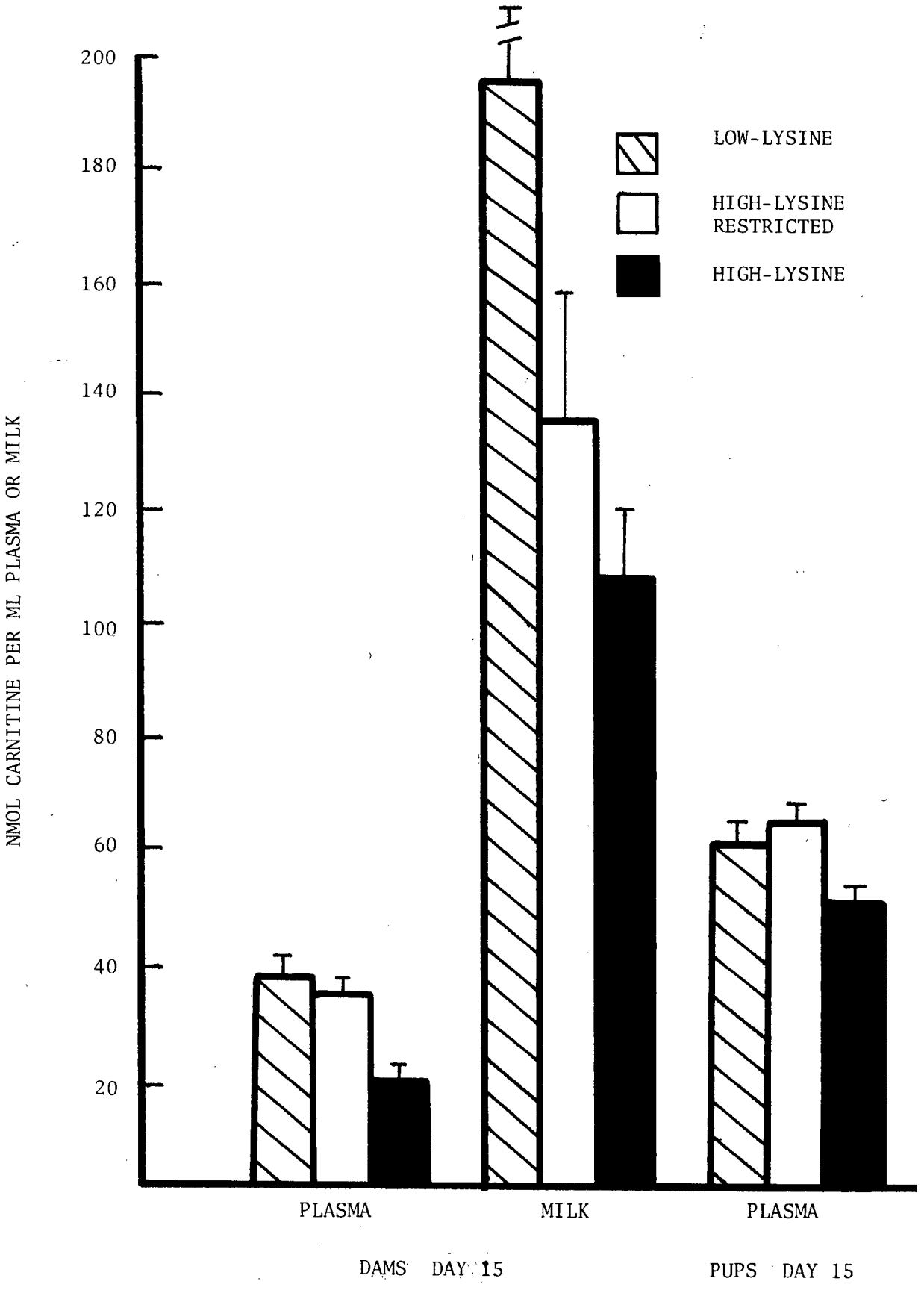

450
400
360
320
280
240
200
160
120
80
40
NMOL CARNITINE PER ML MILK
LOW-LYSINE
HIGH-LYSINE RESTRICTED
HIGH-LYSINE
DAY 2
DAY 8
DAY 15

Figure 16 Effect of a high-lysine, low-lysine, or high-lysine restricted diet on maternal plasma and milk carnitine in dams, and pup plasma carnitine on day 15 of lactation


NMOL CARNITINE PER ML PLASMA OR MILK
200
180
160
140
120
100
80
60
40
20
LOW-LYSINE
HIGH-LYSINE RESTRICTED
HIGH-LYSINE
PLASMA
MILK
PLASMA
DAMS DAY 15
PUPS DAY 15

restricted groups did not differ significantly. Milk carnitine levels, on day 15 of lactation, were also higher in the low-lysine and high-lysine restricted groups than the high-lysine control group. In addition, milk carnitine was significantly different between the low-lysine and high-lysine restricted groups, on day 15 of lactation, whereas no significant differences between the high-lysine restricted and high-lysine control groups were observed.

CHAPTER V
DISCUSSION

DIETARY LYSINE AND REPRODUCTION

The results of this study confirm those of Stapleton and Hill (1972,1980), who reported that a maternal lysine deficiency had no significant effect on litter size but significantly decreased the birth weight of offspring. Niiyama et al. (1970,1973) documented an adverse effect of a lysine free diet on the birth weight of pups. Litter size however was not affected by feeding a lysine free diet. They suggested that animals fed the lysine deficient diet may have maintained pregnancy because of the lysine sparing mechanism (Yamashita and Ashita, 1968) shown to exist in non-pregnant adult rats (Canfield and Chytil, 1978; Chu and Hegsted, 1976). Stapleton and Hill (1972) reported a dramatic drop in plasma lysine levels during the last week of pregnancy for those animals consuming a lysine deficient diet. The authors suggested that the decreased birth weight of the lysine deficient pups may have resulted from a decreased supply of lysine to the fetus during the last week of gestation.

The quantity of food consumed by dams affects reproductive performance of dams. Offspring born to dams fed the low-lysine or high-lysine restricted diets weighed significantly less than those born to dams fed the high-lysine diet. There was no significant difference between the birth weight of pups from the

lysine deficient and calorie restricted groups in experiment II, the birth weight of pups for the low-lysine and high-lysine restricted groups differed significantly in experiment III. The results of the third experiment only, suggest that both food restriction and lysine deficiency of dams during gestation affect birth weight of offspring.

Previous reports have associated a decrease in birth weight of offspring with decreased food intake by dams during gestation (Barry, 1920; Niiyama et al., 1973). Chow and Lee (1964) have reported a reduction in fetal body weight associated with a 25% decrease in food intake by rats during pregnancy. These investigators expressed food intake by dams during pregnancy as the average daily for the total period of gestation.

Food consumption and weight gain during pregnancy were affected by dietary treatment. Lysine deficient animals and their respective pair-fed groups consumed significantly less food (g/day) than control animals fed the lysine supplemented diet. The reduced food intake of the low-lysine group of dams is thought to be due to decreased appetite as a result of plasma amino acid imbalance caused by the lysine deficiency (Sanahuja and Harper, 1963; Leung et al., 1968).

Dams consuming significantly less food than controls during pregnancy also gained significantly less weight than controls. Although there was no significant difference between the amount of weight gained by the low-lysine and high-lysine restricted groups, dams fed the high-lysine restricted diet gained more weight than those dams consuming the low-lysine diet, suggesting that weight gain of dams during pregnancy was affected by total

food intake and dietary lysine level. Stapleton and Hill (1980) previously reported that the weight gain of lysine deficient dams during pregnancy was significantly less than that of lysine supplemented controls.

DIETARY LYSINE AND LACTATION PERFORMANCE

Several authors are of the opinion that nursing rats should increase their body fat and weight by as much as non-lactating control rats during the same period, since a loss in body weight indicates that maternal body tissues rather than dietary supplies are used for milk production (Kon and Cowie, 1961; Munro and Allison, 1964). In this study the high-lysine controls gained weight during lactation while dams in the low-lysine and high-lysine restricted groups lost weight, such results confirm earlier reports by Stapleton and Hill (1980), and suggest utilization of body tissues by the lysine deficient and calorie restricted groups.

Change in body weight during lactation appears to be dependent on the amount of food consumed during this period. Absolute food intake was greatest for the high-lysine controls again confirming the results of Stapleton and Hill (1972,1980). Food intake during lactation, when expressed in proportion to body weight, was also significantly greater for the high-lysine animals than for the the low-lysine cr high-lysine restricted groups.

Jansen and Chase (1976) also noted a significant reduction in food intake during lactation in dams fed a basal bread diet,

deficient in lysine. It is interesting to note that lysine fortification of the basal bread diet increased food intake and decreased maternal weight loss during lactation.

Weight loss which occurs during lactation, when dietary intake is restricted, is probably due to increased utilization of body fat. Naismith (1971) reported that lactating rats fed 25% protein diets lost no body protein but lost 70% of the fat accumulated during pregnancy. Rats fed a low protein diet lost 10% of their body protein.

It is highly unlikely that a lactating dam with small fat stores or a reduced food intake would be able to produce milk to the same extent as a dam with large body stores of fat (accumulated during pregnancy) and a sufficient food intake. This may be reflected in the deficient animals which not only weighed significantly less than the high-lysine controls on day 1 of lactation but also consumed significantly less food during lactation.

The lower body weight of the lysine deficient dams at the end of gestation in experiment III compared to the lysine deficient dams in experiment II, indicates a lower accumulation of body fat by the former group. However growth rates of offspring of both groups were similar. Animals with the lower body fat stores compensated by increasing their food intake during lactation. Therefore, even though energy reserves were lower for one group of lysine deficient animals, these dams were able to maintain the same lactation performance by increasing slightly their food intake during lactation.

A relationship between food intake during lactation and

successful milk production has often been reported. Growth impairment was observed in offspring of dams whose food intake was restricted during both pregnancy and lactation (Chow and Lee, 1964). Lactating rats placed on a low protein diet produced significantly less milk than those fed a high protein diet (Mueller and Cox, 1946). This decrease in milk volume caused lower weaning weights in offspring. Stapleton and Hill (1980) examined the effects of a lysine deficient diet on milk production. When compared with milk produced by dams fed a lysine supplemented diet, there was a significant reduction in the amount of milk produced and in the quantity of protein in the milk, of dams fed a low-lysine diet. Similarly weaning weights of pups from dams fed the lysine deficient diet were significantly lower than that of controls.

In the present study, pups of mothers fed the low-lysine rations weighed significantly less than pups from the high-lysine control females on day 15 of lactation. Food intake was also significantly reduced for the low-lysine dams, as was the case in the studies reported by Stapleton and Hill (1972,1980). Thus it appears that a decrease in the amount of milk produced and in the protein content of the milk produced by dams fed the low-lysine diet also occurred in this study. Body weight of the third group of pups, high-lysine restricted, on day 15 of lactation was significantly greater than the low-lysine pups yet significantly less than the high-lysine control pups, indicating that both food intake and lysine deficiency affects milk production of the mother rat.

DIET AND TISSUE WEIGHTS

Liver weight in dams, neonates, and fetuses was higher in the high-lysine control group than in the low-lysine and high-lysine restricted groups. However, when liver weights were expressed as a percentage of body weight there was no significant difference among the dietary groups. These results suggest that the animal's liver weight was proportional to the total body weight.

Lysine deficiency did not retard heart weight of dams or their fetuses during pregnancy. However, on day 15 of lactation heart weight of the low-lysine pups was significantly less than control values whereas, heart weights of mothers were not affected by lysine deficiency. When heart weights were expressed in proportion to body weight, for both dams and their offspring, there was no significant effect of dietary treatment on heart weight on day 15 of lactation.

DIETARY LYSINE AND PLASMA AND LIVER CARNITINE

Dams

The diets containing 0.27% lysine did not limit plasma and liver carnitine levels of dams during gestation or lactation, under the experimental conditions of this study. On day 21 of pregnancy there was a small but insignificant increase in both plasma and liver carnitine concentrations in those dams fed the low-lysine diet. The increase in plasma and liver carnitine in

the lysine deficient dams and their pair-fed controls on day 15 of lactation was significant.

Plasma carnitine during pregnancy and liver carnitine during lactation, in the high-lysine control animals, were in the same range as those reported earlier by Robles-Valdes et al. (1976). However, liver carnitine during pregnancy and plasma carnitine during lactation, were approximately half that of control values previously reported. The reason for the contradictions in plasma and liver carnitine levels of controls animals between the present work and that reported by Robles-Valdes et al. (1976) is not understood, and to this author's knowledge, there is no other source with which tc compare normal maternal tissue carnitine levels for rats.

Tissue carnitine levels did not differ significantly between the low-lysine and high-lysine restricted groups on day 15 of lactation, suggesting that total food intake was a dominant factor in the regulation of plasma and liver carnitine levels. The carnitine acyltransferase system is required for the intramitocondrial transport and oxidation of long-chain fatty acids (Fritz and Yue, 1963) and interestingly, oxidation of palmitate by heart muscle has been shown to increase when food intake is restricted (Khan and Bamji, 1979). Furthermore, McGarry et al., (1975) reported that the ketogenic capacity of hepatic tissue of fasted weaned rats correlated with carnitine concentrations. Therefore, if dietary lysine is not limiting carnitine synthesis by the liver, an increase in tissue carnitine levels may be expected under conditions of increased fat oxidation.

Tanphaichitr et al. (1976) previously noted a significant increase in plasma carnitine levels in non-pregnant, post weaning female rats consuming a lysine deficient diet. When compared with lysine supplemented controls, liver carnitine levels were also increased in the deficient females. Only liver carnitine levels were increased in male weanling rats fed a low-lysine diet. This difference in response to dietary lysine between male and female rats suggests that other factors, i.e., sex of the animal, are involved in the regulation of tissue carnitine values.

The results of the present study suggest that tissue carnitine levels for dams, at least during lactation, were a reflection of alterations in fat metabolism caused by depressed food intake. Animals fed diets that restricted food intake, lost body weight during this period, presumably due to increased oxidation of body fat. Plasma and liver carnitine levels were significantly higher in these animals than in control animals who, in fact gained weight during lactation.

Fetus and Neonates

A maternal lysine deficiency did not limit plasma or liver carnitine levels in either fetuses or offspring. In fact tissue carnitine levels were higher in the low-lysine and high-lysine restricted groups than in the controls, indicating that food intake rather than dietary lysine levels affects tissue carnitine levels.

Although fetal plasma and liver carnitine levels were

higher in the low-lysine group than in the high-lysine group, only the plasma level was significantly higher. It is interesting to note that the carnitine levels in fetal tissues are a reflection of maternal plasma carnitine levels, even though placental transfer of carnitine is limited in the rat (Hahn and Skala, 1975).

There is no evidence at present to indicate fetal synthesis of carnitine in mammals. The primary metabolic fuel for the fetal tissue is maternally derived glucose. Fetal carnitine requirements are low, as is the capacity for the rat fetus to oxidize long chain fatty acids (Drahota et al., 1964; Augenfeld and Fritz, 1970; Lockwood and Bailey, 1971). One would expect carnitine levels to be lower in the fetus than in animals utilizing fat as a major source of energy. Therefore, it is not surprising, that fetal plasma and liver carnitine in high-lysine control animals was approximately 50% lower than that of control neonates on day 15 of lactation. These results confirm previously reported plasma and liver carnitine concentrations during pre- and postnatal development of the rat (Robles-Valdes et al., 1976; Borum, 1978; Ferre et al., 1978; Seccombe et al., 1978).

Immediately after birth the neonatal rat receives a diet rich in fat, which acts as the main energy yielding substrate (Dymsza, 1964; Luckey et al., 1954). Rat milk contains about 14% fat and adequate amounts of carnitine are needed to allow for fatty acid oxidation to proceed at normal rates. This period of increased fatty acid utilization is characterized by raised plasma concentrations of ketone bodies (Drahota et al.,

1964; Lockwood and Bailey, 1971; Ferre et al., 1978) and carnitine, and increased liver carnitine concentration (Robles-Valdes et al., 1976; Borum, 1978; Ferre et al., 1978). Plasma ketone and carnitine levels and liver carnitine concentrations decrease when the rat pup is weaned from the high fat milk diet to the high carbohydrate diet (Lockwood and Bailey, 1971; Seccombe et al., 1978).

In the present study differences in tissue carnitine levels between low-lysine and high-lysine restricted pups were insignificant. However, both groups had significantly higher liver carnitine levels compared to controls, which suggested increased fatty acid oxidation. In addition, pups from the low-lysine and high-lysine restricted groups weighed significantly less than the high-lysine controls on day 15 of lactation. One can speculate that the deficient groups of pups were forced to utilize a larger percentage of the high fat diet for immediate energy demands therefore increasing their carnitine requirements.

The suckling rat's ability to synthesize carnitine from its precursors reaches adult values by the second week of lactation (Hahn, unpublished data). Until then, the neonate depends on milk as its major source of carnitine (Hahn and Skala, 1975; Robles-Valdes et al., 1976). The results of this study suggest that increased carnitine synthesis during lactation occurs in those dams having increased utilization of fat stores. In turn higher circulating levels of carnitine are available for transportation via their milk to nursing offspring whose carnitine requirements are greater. Plasma carnitine levels in

lysine deficient dams during lactation and pregnancy were similar whereas, plasma carnitine values in controls during lactation were 50% of those in controls during pregnancy. A similar trend was observed for liver carnitine values, i.e., there was little change between pregnancy and lactation in the lysine deficient dams but control values dropped by 73% in controls.

MILK CARNITINE

The primary source of carnitine in neonatal tissue, at least 24 hours postpartum is the milk from the mother rat (Ferre et al., 1978). Milk carnitine concentrations in the high-lysine control animals were highest on day 2 of lactation and decreased by 71% and 75% on days 8 and 15 post partum respectively. This decline in carnitine concentration during lactation is in agreement with the results of Robles-Valdes et al. (1976), and may occur because the aging pup is less dependent on milk carnitine supplies because of *in situ* carnitine synthesis.

Dietary lysine deficiency increased maternal plasma levels of carnitine, which appears to increase milk carnitine levels. The decrease in milk carnitine levels in lysine deficient dams was small and insignificant during the course of lactation. Milk carnitine levels in the lysine deficient group on days 8 and 15 were significantly higher than those in high-lysine controls. With the exception of day 2, milk carnitine values in the high-lysine restricted dams were similar to those in the controls. Thus milk carnitine levels appear to respond to

dietary lysine levels rather than to total food intake. However, on day 15 of lactation, milk carnitine and plasma carnitine, in both dams and offspring, within a dietary group, responded in the same way to that dietary treatment (see Figure 16).

The higher milk carnitine levels in lysine deficient dams probably contributed to the higher plasma and liver levels in their offspring. Movement of carnitine from maternal liver to maternal plasma into maternal milk and finally into neonatal liver has been reported after mothers were injected with of ^{14}C butyrobetaine, the immediate precursor of carnitine (Robles-Valdes et al., 1976).

A problem exists when determining nutrient concentrations in milk. The volume of milk obtained varies and small quantities of milk may have a concentrating effect on the carnitine levels, for example, at day 2 of lactation the control rats yielded the lowest quantity of milk, whereas at days 8 and 15 the low-lysine fed animals yielded the smallest volume of milk. The significance of the low milk carnitine concentrations in the high-lysine restricted dams on day 2 cannot be explained at this time.

The results of this study indicate that maternal lysine deficiency does not impair plasma and liver carnitine levels in the dam, fetus or offspring. Plasma and liver carnitine was actually higher in deficient animals, the higher level being in

response to a general food restriction rather than to a lysine deficiency per se. Further study regarding the metabolic alterations associated with changes in tissue carnitine levels in both the dams and their offspring during maternal lysine deficiency is warranted.

The significance of dietary carnitine for human newborns is unknown, but several reports (Hahn and Skala, 1975; Robles-Valdes et al., 1976), have indicated the importance of carnitine in the newborn mammal. Carnitine is synthesized from the essential amino acid lysine. One of the main objectives of this study was to examine the effect of a maternal lysine deficiency in the rat on neonatal tissue carnitine levels. A deficiency of carnitine may not develop in the offspring of animals fed a diet low in lysine and containing minimal carnitine because of the supply of carnitine in the milk. When compared to human newborns fed human breast milk or cow's milk, plasma carnitine and acetylcarnitine levels in infants receiving a soybean based formula are significantly reduced (Novak et al., 1979). Soyabean based formulas have no detectable carnitine, whereas human breast milk and cow's milk contain a significant amount of carnitine (Schmidt-Sommerfeld et al., 1978). It is probable that a carnitine deficiency in the rat could be induced under conditions of artificial feeding. The implication for the human infant is that a baby fed human breast milk or cow's milk is protected against a carnitine deficiency.

Since fatty acid oxidation is the main pathway by which energy is produced by the neonate, dietary intake of carnitine may be more important during this period than prenatally or in the adult. Thus the results of this study suggest that carnitine may only be an essential dietary nutrient for the neonate, if artificial feeding practices are used. Further study may indicate whether or not the effect of decreased tissue carnitine levels in the neonate has a detrimental effect on the newborn's ability to utilize fatty acids as a source of energy.

CHAPTER VI

SUMMARY OF RESULTS

1) A maternal lysine deficiency significantly decreased weight gain and daily food intake (g/day) of dams during pregnancy. Weight gain of restricted control dams was also significantly lower.

2) A maternal lysine deficiency or maternal food restriction during pregnancy did not affect litter size. However, birth weight of offspring was significantly decreased.

3) A maternal lysine deficiency did not retard heart weight in dams or their fetuses during pregnancy. However, liver weights of dams and fetuses were significantly lower in the lysine deficient group.

4) A maternal lysine deficiency during pregnancy tended to increase plasma and liver carnitine levels of dams on day 21 of pregnancy. However, fetal plasma carnitine levels were significantly increased.

5) A maternal lysine deficiency resulted in a significantly reduced food intake (g/day) and weight loss during lactation, whereas lysine supplemented dams gained weight during this period. High-lysine restricted dams also lost a significant amount of weight during lactation.

6) A maternal lysine deficiency during pregnancy and lactation resulted in a significantly lower body weight of pups on day 15 of lactation. High-lysine restricted pups also weighed significantly less than lysine supplemented controls, but significantly more than the low-lysine pups.

7) A maternal lysine deficiency during pregnancy and lactation significantly reduced liver weight but not heart weight of dams on day 15 of lactation. Liver weights of high-lysine restricted dams were also significantly reduced.

8) Livers of high-lysine restricted pups weighed significantly less than those of lysine supplemented controls, but significantly more than those of the lysine deficient group. Heart weights of lysine deficient pups were significantly less than those in the high-lysine and high-lysine restricted pups.

9) A maternal lysine deficiency during pregnancy and lactation significantly increased plasma and liver carnitine on day 15 of lactation in both dams and pups. High-lysine restricted dams and their offspring also had significantly higher plasma and liver carnitine levels than lysine supplemented controls.

10) A maternal lysine deficiency during pregnancy and lactation significantly increased milk carnitine concentrations on days 8 and 15 of lactation when compared to milk of lysine supplemented controls and high-lysine restricted animals. On day 2 of lactation, milk of lysine supplemented controls contained

significantly more carnitine than milk cf lysine deficient dams, which in turn had a higher milk carnitine concentration than high-lysine restricted dams.

BIBLIOGRAPHY

Abbey, H., and Howard, E. (1973). Statistical procedure in developmental studies on species with multiple offspring. Developmental psychobiology 6:329-335.

Augenfeld, J., and Fritz, I. B. (1970). Carnitine-palmityltransferase activity and fatty acid oxidation by livers from fetal and neonatal rats. Can. J. Biochem. Physiol. 48:288-294.

Barry, L. W. (1920). The effects of inanition in the pregnant albino rat with special reference tc the changes in the relative weights of the various parts, systems and organs of the offspring. Contrib. Embroyl. Carneg. Inst. 11:91-136.

Beattie, D. S. (1968). The submitochondrial distribution of the fatty acid oxidizing system in the rat liver mitochondria. Biochem. Biophys. Res. Commun. 30:57-62.

Borum, P. R. (1978). Variation in tissue carnitine concentrations with age and sex in the rat. Biochem. J. 176:677-681.

Borum, P. R., and Broquist, H. P. (1977). Lysine deficiency and carnitine in male and female rats. J. Nutr. 107:1209-1215.

Bremer, J. (1977). Carnitine and its role in fatty acid metabolism. TIBS 2:207-209.

Bremer, J. (1962). Carnitine precursors in the rat. Biochem. Biophys. Acta. 57:327-335.

Bremer, J. (1968). In Cellular Compartmentalization and Control of Fatty Acid Metabolism. ed. F. C. Gran. pp.65-88. Academic Press, New York.

Bressler, R. (1970). In Physiological-Chemical Aspects of Fatty Acid Oxidation. ed. S. J. Wakil. pp.49-77. Academic Press, New York, London.

Bressler, R., and Wittles, B. (1965). The effect of diptheria

toxin on carnitine metabolism in the heart. Biochem. Biophys. Acta. 104:39-45.

Broquist, H. P. (1976). Amino acid metabolism. Nutr. Rev. 34:289-293.

Broquist, H. P., Horne, D. W., and Tanphaichitr, V. (1975). In Lysine Metabolism in Protein-Calorie Malnutrition with Attention to the Synthesis of Carnitine. ed. R. E. Olson. pp.50-60. Academic Press, New York.

Brosman, J. T., Kopec, B., and Fritz, I. B. (1973). The localization of carnitine palmityltransferase on the inner membrane of bovine liver mitochondria. J. Biol. Chem. 248:4075-4082.

Canfield, L. M., and Chytil, W. F. (1978). Effect of low lysine diet on rat protein metabolism. J. Nutr. 108:1343-1347.

Capek, K., and Jelinek, J. (1956). The development of the control of water metabolism. Physiol. Bohemastov. 5:94-96.

Carter, H. E., Bhattacharyya, P. K., Neidman, K. R., and Fraenkel, G. (1952). Chemical studies on vitamin BT. Isolation-characterization as carnitine. Arch. Biochem. Biophys. 38:405-416.

Casillas, E. R., and Erickson, B. J. (1975). Studies on carnitine in the rat epididymis. J. Reprod. Fert. 44:287-291.

Cederblad, G., and Lindstedt, S. (1971). Excretion of L-carnitine in man. Clin. Chim. Acta. 33:117-123.

Cederblad, G., and Lindstedt, S. (1976). Metabolism of labelled carnitine in the rat. Arch. Biochem. Biophys. 175:173-180.

Chow, B. F., and Lee, C. J. (1964). Effect of dietary restriction in pregnant rats on body weight of the offspring. J. Nutr. 82:10-18.

Chu, S. H. W., and Hegsted, D. M. (1976). Adaptive response of

lysine and threonine degrading enzymes in adult rats. J. Nutr. 106:1089-1096.

Cox, R. A., and Hoppel, C. L. (1973a). Biosynthesis of carnitine and 4-N-trimethylaminobutyrate from lysine. Biochem. J. 136:1075-1082.

Cox, R. A., and Hoppel, C. L. (1973b). Biosynthesis of carnitine and 4-N-trimethylaminobutyrate from 6-N-trimethyllysine. Biochem. J. 136:1083-1090.

Cox, W. M., and Mueller, A. J. (1937). The composition of milk from stock rats and an apparatus for milking small laboratory animals. J. Nutr. 13:249-261.

Crnic, L. S., and Chase, H. P. (1978). Models of infantile undernutrition in rats: effects on milk. J. Nutr. 108:1755-1760.

Dole, V. P. (1956). A relationship between non-esterified fatty acids in plasma and the metabolism of glucose. J. Clin. Invest. 35:150-154.

Drahota, Z., Hahn, P., Kleinzeller, A., and Kostolanska, A. (1964). Acetoacetate formation by liver slices from adult and infant rats. Biochem. J. 93:61-65.

Dymasa, H. A., Czajka, D. M., and Miller, S. A. (1964). Influence of artificial diet on weight gain and body composition of the neonatal rat. J. Nutr. 84:100-106.

FAO Nutritional Studies (1970). In Amino Acid Content of Foods and Biological Data on Proteins. Rome.

Farstad, M., Bremer, J., and Norum, K. R. (1967). Long-chain acyl-CoA synthesis in rat liver. A new assay procedure for the enzyme, and studies on its intracellular localization. Biochem. Biophys. Acta. 132:492-502.

Ferre, P., Pegorier, J. P., Williamson, D. H., and Girard, J. R. (1978). The development of ketogenesis at birth in the rat. Biochem. J. 176:759-765.

Fraenkel, G. (1954). The distribution of BT (carnitine)

throughout the animal kingdom. Arch. Biochem. Biophys. 50:486-495.

Fraenkel, G. (1957). In Vitamins and Hormones. Vol. 15 pp. 73-80. Academic Press, New York.

Fraenkel, G., and Blewett, M. (1946). Folic acid in the nutrition of certain insects. Nature 157:697.

Fredrickson, D. S., and Gordon, R. S., Jr. (1958). The metabolism of albumin-bound C^{14}-labelled unesterified fatty acids in normal human subjects. J. Clin. Invest. 37:1504-1515.

Fritz, I. B. (1955). The effect of muscle extracts on the oxidation of palmitic acid by liver slices and homogenates. Acta. Physiol. Skand. 34:367-385.

Fritz, I. B. (1963). Carnitine and its role in fatty acid metabolism. Advan. Lipid Res. 1:285-234.

Fritz, I. B. (1967). An hypothesis concerning the role of carnitine in the control of interrelations between fatty acid and carbohydrate metabolism. Perspect. Biol. Med. 10:643-678.

Fritz, I. B., Kaplan, E., and Yue, K. T. N. (1962). Specificity of carnitine action on fatty acid oxidation by heart muscle. Amer. J. Physiol. 202:117-121.

Fritz, I. B., and Yue, K. T. N. (1963). Long-chain carnitine acetyltransferase and the role of acylcarnitine derivatives in the catalytic increases of long-chain fatty acid oxidation. Lipid Res. 4:279-288.

Gordon, R. S., Jr. (1957). Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty Acid. J. Clin. Invest. 36:810-815.

Gordon, R. S., Jr., and Cherkes, A. (1958). Production of unesterified fatty acids from isolated rat adipose tissue incubated in vitro. Proc. Soc. Exp. Biol. Med. 97:150-151.

Grosvenor, C. E., and Turner, C. W. (1959). Effect of growth

hormone and oxytocin upon milk yield in the lactating rat. Proc. Soc. Exp. Biol. Med. 100:158-161.

Hahn, P. unpublished data

Hahn, P., and Koldovsky, O. (1966). Nutrition and Postnatal Development. pp. 100. Pergammon Press, Oxford.

Hahn, P., and Skala, J. P. (1975). The role of carnitine in brown adipose tissue of suckling rats. Comp. Biochem. Physiol. 51B:507-515.

Hahn, P., Skala, J. D., Seccombe, D. W., Frohlich, J., Penn-Walker, D., Novak, M., Hynie, I., Towell, M. E. (1977). Carnitine content of blood and amniotic fluid. Pediat. Res. 11:878-880.

Haigler, H. T., and Broquist, H. P. (1974). Carnitine synthesis in rat tissue slices. Biochem. Biophys. Res. Commun. 56:676-681.

Hochalter, J. B., and Henderson, L. M. (1976). Carnitine biosynthesis. The formation of glycine from carbons 1 and 2 of 6-N-trimethyl-L-lysine. Biochem. Biophys. Res. Commun. 70:364-366.

Hoppel, C. L., and Tomac, R. J. (1972). Carnitine palmityltransferase. Location of two enzymatic activities in rat mitochondria. J. Biol. Chem. 247:832-841.

Horne, D. W., and Broquist, H. P. (1973). Role of lysine and E-N-trimethyllysine in carnitine biosynthesis. J. Biol. Chem. 218:2170-2175

Horne, D. W., Tanphaichitr, V., and Broquist, H. P. (1971). Role of lysine in carnitine biosynthesis in Neurospora crassa. J. Biol. Chem. 246:4373-4375.

Hulse, J. D., Ellis, S. R., and Henderson, L. M. (1978). Carnitine biosynthesis. B-hydroxylation of trimethyllysine by an -ketoglutarate-dependent mitochondrial dioxygenase. J. Biol. Chem. 253:1654-1659.

Isaacs, H., Heffron, J. J. A., Badenhorst, M., and Pickering, A. (1976). Weakness associated with the pathological presence of lipid in skeletal muscle: a detailed study of a patient with carnitine deficiency. J. Neurol. Neurosurg. Psych. 39:1114-1123.

Jansen, G. R., and Chase, H. P. (1976). Effect of feeding lysine and threonine fortified bread during gestation and lactation on growth of offspring in rats. J. Nutr. 106:33-40.

Khan, L., and Bamji, M. S. (1979). Tissue carnitine deficiency due to dietary lysine deficiency: triglyceride accumulation and concomitant impairment in fatty acid oxidation. J. Nutr. 109:24-31.

Klingenberg, M., and Pfaff, E. (1966). In Regulation of Metabolic Processes in Mitochondria. eds. J. M. Tager, S. Papa, E. Z. Quagliarello, and E. C. Slater. pp. 180-201. Elsevier, Amsterdam, New York.

Kon, S. K., and Cowie, A. T., ed. (1961). Milk: Mammary Gland and its Secretion. Vol. I. Academic Press, New York, London.

Kopec, B., and Fritz, I. B. (1973). Comparison of properties of carnitine palmitoyltransferase I with those of carnitine palmitoyltransferase II, and preparation of antibodies to carnitine palmityltransferases. J. Biol. Chem. 248:4069-4074.

LaBadie, J., Dunn, W. A., and Aronson, N. N., Jr. (1976). Hepatic synthesis of carnitine from protein-bound trimethyl-lysine. Lysosomal digestion of methyl-lysine-labelled asialofetuin. Biochem. J. 160:85-95.

Latifa, K., and Bamji, M., S. (1977). Plasma carnitine levels in children with protein calorie malnutrition before and after rehabilitation. Clin. Chem. Acta. 75:163-167.

Lee, L. P. K., and Fritz, I. B. (1971). Hepatic ketogenesis during development. Canad. J. Biochem. 49:599-605.

Leung, P. M. B., Roger, Q. R., and Harper, A. E. (1968). Effect of amino acid imbalances on plasma and tissue free amino acids in the rat. J. Nutr. 96:303-318.

Lindstedt, G. (1967). Hydroxylation of -butyrobetaine to carnitine in rat liver. Bicchemistry 6:1271-1282.

Lindstedt, G., and Lindstedt, S. (1965). Studies on the biosynthesis of carnitine. J. Biol. Chem. 240:316-321.

Lockwood, E. A., and Bailey, E. (1970). Fatty acid utilization during development of the rat. Biochem. J. 120:49-54.

Lockwood, E. A., and Bailey, E. (1971). The course of ketosis and the activity of key enzymes of ketogenesis and ketone body utilization during development of the posnatal rat. Biochem. J. 124:249-254.

Luckey, T. D., Meade, T. J., and Pleasants, J. (1954). The physical and chemical characteristics of rat milk. J. Nutr. 54:345-359.

Maebashi, M., Kawamura, N., Sato, N., Yashinaga, K., and Suzuki, M. (1976). Uninary excretion of carnitine in man. J. Lab. Clin. Med. 87:760-766.

Marquis, N. R., and Fritz, I. B. (1964). Enzymological determination of free carnitine concentrations in rat tissues. J. Lipid Res. 5:184-187.

McGarry, J. D., and Foster, D. W. (1971). The regulation of ketogenesis from oleic acid and the influence of antiketogenic agents. J. Biol. Chem. 246:6247-6253.

McGarry, J. D., and Foster, D. W. (1974). The metabolism of (-)-octanoylcarnitine in perfused livers from fed and fasted rats. Evidence for a possible regulatory role of carnitine acyltransferase in the control of ketogenesis. J. Biol. Chem. 249:7984-7990.

McGarry, J. D., Meier, J. M., and Foster, D. W. (1973). Effects of starvation and refeeding on carbohydrate and lipid metabolism *in vivo* and in the perfused rat liver. J. Biol. Chem. 248:270-278.

McGarry, J. D., Wright, P. H., and Foster, D. W. (1975). Hormonal control of ketogenesis. Rapid activation of hepatic ketogenic capacity in fed rats by anti-insulin serum and glucagon. J. Clin. Invest. 55:1202-1209.

McGarry, J. D., and Foster, D. W. (1976). An improved and simplified radioisotopic assay for the determination of free and esterified carnitine. J. Lipid Res. 17:277-281.

Mena, F., Beyer, C., and Grosvenor, C. E. (1974). On the mechanism by which oxytocin depresses milk ejection and milk secretion in rats. Amer. J. Physiol. 227:1249-1254.

Mikhail, M. M., and Mansour, M. M. (1976). The relationship between serum carnitine levels and the nutritional status of patients with schistosomiasis. Clin. Chim. Acta. 71:207-214.

Mueller, A. J., and Cox, W. M. (1946). The effect of changes in diet on the volume and composition of rat milk. J. Nutr. 31:249-259.

Munro, H. N., and Allison, J. B., eds. (1964). Mammalian Protein Metabolism. Vol. I and II. Academic Press, New York, London.

Niiyama, Y., Kishi, K., and Inoue, G. (1970). Effect of ovarian steriods on maintenance of pregnancy in rats fed diets devoid of one essential amino acid. J. Nutr. 100:1461-1470.

Niiyama, Y., Kishi, K., Endo, S., and Incue, G. (1973). Effects of diets devoid of one essential amino acid on pregnancy in rats maintained by ovarian steriods. J. Nutr. 103:207-212.

Naismith, D. J. (1971). Role of body fat,accumulated during pregnancy, in lactation in the rat. Proc. Nutr. Soc. 30:93a.

Nie, N. H., Hull, C. H., Jenkins, J. G., Steinbrenner, K., Bent, D. H. (1975). Statistical Package for the Social Sciences. McGraw-Hill, U.S.A.

Norum, K., and Bremer, J. (1963). Acyl coenzyme A as an intermediate in the mitcchondrial acylation of carnitine by -keto acids. Biochem. Biophys. Acta. 78:77-84.

Norum, K. R., Farstad, M., and Bremer, J. (1966). The submitochondrial distribution of acid: CoA ligase (AMP) and palmityl-CoA: carnitine palmityltransferase in rat. Biochem. Biophys. Res. Commun. 24:797-804.

Novak M., Wieser, P. B., Buch, M., and Hahn, P. (1979). Acetylcarnitine and free carnitine in body fluids before and after birth. Pediat. Res. 13:10-15.

NRC (1972). Nutrient Requirements of Laboratory Animals. National Academy of Sciences. No. 10 2nd ed. Washington, D.C.

Page, M. A., Krebs, H. A., and Williamson, D. A. (1971). Activities of enzymes of ketone body utilization in brain and other tissues of the suckling rat. Biochem. J. 121:49-53.

Pande, S. V. (1975). A mitochondrial carnitine acylcarnitine translocase system. Proc. Nat. Acad. Sci. U.S.A. 72:883-887.

Panter, R. A., and Mudd, J. B. (1969). Carnitine levels in some higher plants. FEBES Letters 5:167-170.

Parvin, R., and Pande, S. V. (1977). Microdetermination of (-) carnitine and carnitine acetyltransferase activity. Analytical 23:460-465.

Parvin, R., and Pande, S. V. (1979). Enhancement of mitochondrial carnitine and carnitine acylcarnitine translocase-mediated transport of fatty acids into liver mitochondria under ketogenic conditions. J. Biol. Chem. 254:5423-5429.

Pearson, D. J., and Tubbs, P. K. (1967). Carnitine and its derivatives in rat tissues. Biochem. J. 105:953-963.

Robles-Valdes, C., and McGarry, J. D., and Foster, D. W. (1976). Maternal-fetal carnitine relationships and neonatal ketosis in the rat. 251:6007-6012.

Rossi, C. R., and Gibson, D. M. (1964). Activation of fatty acids by a guanosine triphosphate-specific thiokinase from liver mitochondria. J. Biol. Chem. 239:1694-1699.

Sanahiya, J. C., and Harper, A. E. (1963). Effect of dietary amino acid pattern on plasma amino acid pattern and food intake. Am. J. Physiol. 204:686-690.

Schmidt-Sommerfeld, E., Novak, M., Penn, D., Wieser, P., Buch, M., and Hahn, P. (1978). Carnitine and development of newborn adipose tissue. Pediat. Res. 12:660-664.

Seccombe, D. W., Hahn, P., and Novak, M. (1978). The effect of diet and development on blcod levels of free and esterified carnitine in the rat. Biochim. Biophys. Acta. 528:483-489.

Solberg, H. E. (1970). Carnitine octanolytransferase. Evidence for a new enzyme in mitochondria. FEBS Letters 12:134-136.

Stapleton, P., and Hill, D. C. (1972). Plasma amino acid levels and offspring viability of rats fed a diet low in lysine during pregnancy and lactation. Nutr. Rep. Internat. 6:199-207.

Stapleton, P., and Hill, D. C. (1980). Effect of maternal dietary lysine and methionine levels on pregnancy and lactation in the rat. Nutr. Rep. Internat. (in press).

Tanphaichitr, V., and Broquist, H. P. (1973). Lysine deficiency in the rat: concomitant impairment in carnitine biosynthesis. J. Nutr. 103:80-87.

Tanphaichitr, V., and Broquist, H. P. (1974). Site of carnitine biosynthesis in the rat J. Nutr. 104:1669-1673.

Tanphaichitr, V., Zaklama, M. S., and Broquist, H. P. (1976). Dietary lysine and carnitine: relation to growth and fatty livers in rats. J. Nutr. 106:111-117.

Taylor, C. B., Bailey, E., Bartley, W. (1967). Changes in hepatic lipogenesis during development of the rat. Biochem. J. 105:717-722.

Tsai, A. C., Romsos, D. R., and Leveille, G. A. (1974). Significance of dietary carnitine for growth and carnitine turnover in rats. J. Nutr. 104:782-791.

Wittles, B., and Bressler, R. (1964). Biochemical lesion of diptheria toxin in the heart. J. Clin. Invest. 43:630-637.

Yamashita, K., and Ashida, K. (1969). Lysine metabolism in rats fed lysine-free diet. J. Nutr. 99:267-273.

Yates, D. W., and Garland, D. B. (1966). The partial latency and intramitochondrial distribution of carnitine-palmityltransferase (e.c.2.3.1.-), and the CoASH and carnitine permiable space of rat liver mitochondria. Biochem. Biophys. Res. Commun. Biochem. 79:190-201.