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Effects of Parenteral Nutrients on Hepatic Function and Lidocaine Elimination

by

Nuzhat Zaman 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

in

Pharmacokinetics

Faculty of Pharmacy and Pharmaceutical Sciences

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Effects of Parenteral Nutrients on Hepatic Function and on Lidocaine Elimination" submitted by Nuzhat Zaman in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Pharmacokinetics).

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Date: January 3 1996

DEDICATED

TO MY

Father: Late Kazi Md. Asaduzzaman

Mother: Husnun Nahar Zaman

Husband: Sadiquzzaman Khan

&

My daughter: Nashwa Zaman

ABSTRACT

The objectives of this project were to test the hypotheses that liver function and drug metabolic enzyme activity can be altered by: (1) enterohepatic recycled secondary bile acids, (2) lipids and taurine in PN formulations, and (3) photooxidation of amino acids in PN formulations. The rat is used as the animal model for studying liver dysfunction induced by PN. Lidocaine is used as a model compound for studying metabolic alterations in perfused rat livers.

Liver histological data clearly indicated that PN treatments induced steatosis in all animals, except those that were treated with oral cholestyramine, a bile acid binder. This result suggests that secondary bile acid reabsorption may cause steatosis. The worst hepatosteatosis was observed in rats that received PN which contained photooxidized amino acids. This was followed by rats that were treated with taurine and Intralipid®.

Cholestyramine did not have any effect on LIDO metabolism in PN treated rats. Hepatic intrinsic clearance (Cl_{int}) values were reduced by more than 50% in rats that received PN alone or with cholestyramine when compared to that of chow-fed animals ($P < 0.05$). This observation suggests that PN-associated reduction in drug metabolism is independent of hepatic dysfunction.

The kinetic and metabolic profiles of LIDO were found to be significantly lower in rats receiving PN formulations that contained either Intralipid®, taurine supplementation, or photooxidized amino acids when compared to that of chow-fed. The extent of reduction was similar among these treatment groups; the Cl_{int} values were reduced to approximately one-fifth of that of the chow-fed animals ($P < 0.05$).

Metabolites to drug molar ratios indicated that N-dealkylation, ring hydroxylation and aryl methyl hydroxylation pathways of LIDO were severely impaired by PN treatments. N-Dealkylation is catalyzed by CYP2C11 and CYP2B1, ring hydroxylation by CYP2D1 and CYP2D2 and aryl methyl hydroxylation by CYP2B2 in rats. The extent of reduction in these pathways was approximately in the range of 60% to 90%. Photooxidized amino acids treated group showing the most reduction. These findings suggest that PN treatments reduce CYP enzyme activities and this reduction is multifactorial.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Glossary Of Abbreviations And Symbols

α	distribution rate constant
AAG	α_1-acid glycoprotein
AIDS	acquired immune deficiency syndrome
ANOVA	analysis of variance
AUC	area under the plasma concentration-time curve
ALP	alkaline phosphatase
ALT	alanine amino transferase
ARA	aromatic amino acid
AST	aspartate amino transferase
β	terminal elimination rate constant
BILI	bilirubin
BRA	branched chain amino acid
CG	cholylglycine
CF	chow-fed animals
C_{in}	inlet concentration
Cl_H	hepatic clearance
Cl_{int}	intrinsic clearance
Cl_s	systemic clearance
C_{out}	outlet concentration
cm	centimeter(s)
CV	coefficient of variation

CYP	cytochrome P450
°C	degree Celsius
dL	deciliter(s)
E_H	hepatic extraction ratio
EDTA	ethylenediaminotetraacetate
EMGX	2-(N-ethyl-N-methylamino)-N-(2,6-dimethylphenyl)acetamide
FADM	flavin adenine dinucleotide monooxygenase
g	gram(s)
GC/MS	gas chromatography/mass spectrometry
GX	N-dideethyl-LIDO
γ-GT	gamma glutamyl transpeptidase
Hb	hemoglobin
H & E	hematoxylin and eosin
hr	hour(s)
HPLC	high-performance liquid chromatography
I.D.	internal diameter
I.S.	internal standard
IPRL	isolated rat liver perfusion
iv	intravenous
kcal	kilocalorie(s)
kg	kilogram(s)
L	liter(s)

LCT	long chain triglycerides
LIDO	lidocaine
LFT	liver function test(s)
µg	microgram(s)
µm	micrometer
µM	micromolar
M	molar
MCT	medium chain triglycerides
MEGX	N-monodeethyl-LIDO
MEOH-LIDO	ring methyl hydroxylated LIDO
MEOH-MEGX	ring methyl hydroxylated MEGX
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm	millimeter
mmol	millimole(s)
MVI	multivitamin
n	number of observations
NADPH	nicotinamide adenine dinucleotide phosphate
nm	nanometer(s)
3-OH-LIDO	3-hydroxy-LIDO
3-OH-MEGX	3-hydroxy-MEGX

4-OH-XYL	4-hydroxy-2,6-xylidine
P	probability of rejecting the null hypothesis when it is true
PN	parenteral nutrition
PNL	lipid based parenteral nutrition
PN(+L)	light exposed parenteral nutrition
po	oral
Q	hepatic blood flow rate or buffer perfusion rate
QC	quality control
RB	riboflavin
r	coefficient of correlation
SB	sodium bisulfite
SD	standard deviation
$t_{1/2}$	half-life
%	percent
TRP	tryptophan
T_{ss}	time to reach steady state
PNT15	taurine (15 mg/dL)
PNT50	taurine (50 mg/dL)
UDPGT	uridine diphosphoglucoronyl transferase
U/L	units per liter
UV	ultra-violet
V_d	volume of distribution

V_{dss}	volume of distribution at steady state
V_{ss}	velocity of drug elimination at steady state
WBC	white blood cell

1. INTRODUCTION

1.1 Parenteral Nutrition

Parenteral nutrition (PN) is a means to supply nutrients by the intravenous route. The first successful intravenous feeding in a child was reported by Helfrick and Abelson, in 1944. Since then, recommendations for the amount of calories from protein, carbohydrate and micronutrients to be provided to pediatric patients have been developed. Then in 1966 Dudrick *et al.* demonstrated for the first time that long term parenteral feeding could support normal growth and development in beagle puppies. This idea of continuous intravenous feeding was implemented for the first time in infant by Wilmore and Dudrick in 1968. They reported that an infant with a serious form of short bowel syndrome grew normally while receiving an intravenous feeding for 44 days. Since then, the efficacy of the intravenous feeding has gained a high level of popularity. Animal research was continued aiming at developing safer and reliable techniques for continuous intravenous infusion (*Steiger et al., 1978*; *Dalton et al., 1969*; *Eve and Robinson, 1963*; *Dudrick et al., 1970*). Results of these studies formed the foundation of nutritional therapy for patients who were seriously ill and were unable to eat.

Patients suffering from a variety of illnesses, including gastrointestinal diseases, pancreatitis, major burns, cancer, acquired immune deficiency syndrome (AIDS) and more commonly, neonates who are born with a very low birth weight, are the prime candidates for PN therapy. The success of PN therapy now lies in its widespread application in surgical and many medical disorders which have been greatly improved with the incorporation of PN therapy (*Mullen et al., 1980*; *Mullen et al., 1982*). Most commonly a PN solution is composed of carbohydrate, amino acids, lipids, minerals, vitamins and

electrolytes. These components may be administered according to the patient's nutritional status, but the main intention of PN therapy for any patient, is to maintain normal growth and development (*Dudrick et al., 1970*; *Popp et al., 1982*). Despite the relative safety of PN therapy it is not yet free of complications. The most common complication, both in short-term or long-term patients is catheter-related sepsis (*Freund and Rimon, 1990*; *Mulloy et al., 1991*; *Belcastro et al., 1990*). According to an Oley Foundation annual report (*Oley Foundation: Home nutritional support patient registry, annual report 1986)*, 42% of re-admissions to the hospital were due to sepsis. With the refinement of catheter designs and improved surgical techniques, the incidence of sepsis has now reduced dramatically. Apart from sepsis, Peden *et al.* (1971) reported the first case of PN-associated liver disease in a premature infant. Liver biopsy from this infant showed evidence of cholestasis. Cholestasis, may be defined as a clinical syndrome where the bile duct gets constricted with a reduction of bile flow (*Sellinger and Boyer, 1990*). Since that time, numerous studies have documented an association between hepatobiliary complications and PN therapy (*Bell et al., 1986*; *Belli et al., 1987*; *Bowyer et al., 1985*; *Fisher, 1989*; *Merritt, 1986*). Such abnormalities associated with the liver or biliary tract may often lead to hepatic failure or even death (*Klein and Nealon, 1988*; *Sondheimer et al., 1978*; *Vileisis et al., 1982*). The spectrum of hepatobiliary abnormalities in patients may vary depending on the patient's age, metabolic status and the underlying disease entity.

1.2 Hepatic Complication in Infants

Cholestasis is most prominent in children (*Bell et al., 1986*; *Sondheimer et al., 1978*; *Merritt, 1986*; *Klein and Nealon, 1988*; *Balistreri and Bove, 1990*). The most common abnormality seen is an elevation of the serum bilirubin. Bell *et al.* (1986) defined PN-related cholestasis as a serum bilirubin level ≥ 1.5 mg/dL. Hepatobiliary disease is also detected by the slow rise in biochemical markers such as the serum amino transferases, which are aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GT), and serum bile acids (*Klein and Nealon, 1988*; *Fisher, 1989*; *Balistreri and Bove, 1990*; *Clarke et al., 1991*). The above tests are commonly referred as liver function tests (LFT).

1.3 Hepatic Complications in Adults

Hepatobiliary complications in adults are slightly different from those seen in infants. Most studies have attempted to define the incidence of hepatic dysfunction in adults receiving PN by an elevation of serum liver enzymes and bilirubin (*Lindor et al., 1979*; *Greenlaw, 1993*). This suggests that the complication is primarily due to a hepatocellular damage, and in most cases less severe than those in infants. Hepatic fat accumulation or steatosis without evidence of inflammation, cholestasis, or hepatocyte necrosis is known to occur commonly in adults receiving PN (*Balistreri and Bove, 1990*; *Eamon et al., 1993*; *Fisher, 1989*). This is the most benign lesion and perhaps results in the elevation of the (LFT) values. However liver biopsies from adult patients undergoing

PN infusion exhibited steatosis which was accompanied with also an elevation of the LFT values (*Tulikoura and Huikuri, 1982*; *Jacobson et al., 1971*). Results from studies performed at varying intervals following initiation of PN suggested that steatosis was an early, often transient effect of PN-associated cholestasis which supervenes later as duration of PN is continued (*Grant et al., 1977*; *Sheldon et al., 1978*). Some other complications that are common to both adults and infants are biliary sludge and cholelithiasis which clinically refers to development of gallstones composed predominantly of calcium bilirubinate (*Eamon et al., 1993*; *Pitt et al., 1983*; *Roslyn et al., 1983*; *Allen et al., 1981*).

These complications, the pathogeneses of which are still unknown pose serious risks to the patients, as well as problems to the physicians who intend to provide nutritionally effective and metabolically safe PN therapy. Presently, in North America an average of 10,000 patients are maintained chronically on PN (*Young et al.,1993*). The medical cost for PN therapy can mount to several thousand dollars per patient. The cost of PN therapy includes the following: solution purchase price and administration, catheter placement and maintenance, clinical and toxicity monitoring, nursing costs, physician services, nutrition support services, length of hospital stay, both PN and surgical complications and finally postdischarge care. According to a 1992 survey, the cost of providing PN for an average of 16 days before and after surgery was estimated to be US $2405 per patient; which includes the above factors, however, prolonged hospital stay would add another US $764 per patient, bringing the total to US $3169 for 16 days (*Lipman, 1993*; *Eisenberg et al., 1993*). The extra cost per additional day of

postoperative PN is estimated to be US $196 after the cost of US $3169 for 16 days (*Eisenberg et al., 1993*). It is obvious that this enormous cost per patient will rise even further with any associated complication. According to Stone *et al.* (1976) and Shapiro *et al.* (1983) an average cost of US $2000 has been estimated for each type of postoperative infectious complication including wound infections. This cost can go up to an amount of US $50,000 for the same number of patients per complication when a major complication such as anastomotic leak, renal failure or intraabdominal abscess occurs (*Couch, 1981*; *Couch et al., 1978*). Under these assumptions Twomey and Patching (1985) estimated that one would save US $1720/patient when a PN associated complication is avoided. Therefore, considering all the costs involved, it is imperative to make efforts to reduce complications associated with PN therapy. Several pathophysiological conditions have been postulated to contribute to the complications mainly to the hepatobiliary region such as steatosis and intrahepatic cholestasis.

1.4 Pathogenesis of PN-Related Hepatic Dysfunction

The most important factors that have been found to have a link with PN-related hepatic dysfunction are: a) toxic secondary bile salt reabsorption; b) sugar overload/or essential fatty acid deficiency; c) certain photo-degraded amino acid products; d) absence of taurine in the amino acid preparations; e) enteral fasting; f) bacterial overgrowth; and g) sepsis.

1.4.1 Effect of Toxic Secondary Bile Salt

During prolonged PN therapy patients are enterally fasted and this results in reduced gastrointestinal motility with a prolongation of gastrointestinal transit time and reduced fecal output (*Merritt, 1986*; *Farrell et al., 1982*). The combined effect is believed to result in an increased reabsorption of secondary bile salts thereby elevating their concentrations in the serum (*Vileisis et al., 1980*). This increase of secondary bile salt has recently been postulated to be one of the most dominant causes of cholestasis (*Farrell et al., 1982*). Secondary bile salt, particularly the sulfated form of glycine-conjugated lithocholic acid, has been found to be toxic in a number of animal species (*Miyai et al., 1977*). It has been reported that histological changes in lithocholate-treated animals match those in infants with PN-associated cholestasis (*Hofmann, 1984*; *Balistreri and Bove, 1990*). Farrell *et al.* (1982) further reported the presence of increased serum concentration of sulfated lithocholate in infants who were receiving PN and developed cholestasis.

Lithocholic acid (LCA) is a secondary bile acid, formed by bacterial dehydroxylation of the primary bile acid chenodcoxycholic acid, in the distal part of the intestine or colon (*Hofmann, 1984*). It has been reported that LCA accounts for only 1-3% of the human bile acid pool (*Macdonald et al., 1983*), and only about one-fifth of this amount reaches the liver where it undergoes biotransformation, mainly by conjugation with glycine and taurine. In addition, these conjugated LCA derivatives also undergo a sulfation step in the liver, which is believed to be incomplete. As a result, four different

species of LCA exist in bile: glycolithocholic acid, taurolithocholic acid, sulpholithocholylglycine and sulpholithocholyltaurine (*Hofmann, 1984*; *Hofmann, 1977*). Some of the sulfated conjugates of LCA may undergo desulfation by the colonic bacteria in the large intestine. Only conjugated and nonsulfated LCA can undergo enterohepatic reabsorption (*Macdonald et al., 1983*). Because of increased hydrophilicity, the sulfated form of LCA is mostly excreted in the feces. PN solutions do not usually contain taurine, but they do contain glycine and so in patients, bile acids are mainly conjugated with glycine. *In vivo* studies in rats have shown that the sulfated form glycolithocholic acid is cholestatic but the taurine conjugated form is not (*Yousef et al., 1981*). On the other hand several other researchers (*Miyai et al., 1977*; *Javitt, 1966*; *Litwin, 1972*) have found that in rats, cholestasis was induced by taurolithocholate but was reversed by taurocholate. It seems likely, therefore, that lithocholic acids are the main culprits in inducing cholestasis. LCA is well known to induce cholestasis by obstructing the bile canaliculi with the formation of crystalline precipitates within the canalicular lumen (*Miyai et al., 1975*). During PN infusion and enteral fasting, the gut remains dormant and colonic bacteria are believed to have a greater opportunity to dehydroxylate chenodeoxycholic acid to LCA (*Merritt, 1986*) and also to desulfate the sulfated forms of LCA (*Farrell et al., 1982*). Furthermore, reduced gastrointestinal motility and prolonged gastrointestinal transit time in PN-infused subjects result in reduced fecal output which may provide more time for secondary bile acid to be formed and reabsorbed through the EHC, and enrich the toxic bile acid pool (*Hofmann, 1984*). Canalicular injury is thus believed to result from an increased concentration of LCA metabolites which are not excreted through feces due to

reduced bile flow and are thus retained in the liver (*Merritt, 1986*) (Fig. 1.1). Therefore cholestasis together with other liver function abnormalities, such as elevation of serum amino tranferases, alkaline phosphatase and bilirubin, are well established as the most common complications of intravenous nutrition, and these complications can cause reduction in drug metabolism (*Knodell, 1990*).

1.4.2 Effect of Sugar Over-Load and/or Essential Fatty Acid Deficiency

Prior to November 1979 when the Food and Drug Administration approved the clinical use of an intravenous soybean oil emulsion, critically ill patients requiring prolonged parenteral nutrition were administered fat-free PN solutions comprised of glucose, amino acids, electrolytes and vitamins. Fat-free PN solutions have been well documented to be one of the factors responsible for PN-associated liver dysfunction (*Buzby et al., 1981*; *Boelhouwer et al., 1983*; *Reif et al., 1991*; *Rivera et al., 1989*; *Ikeda et al., 1979*). Riveria *et al.* (1989) in his study further pointed out that infusion of glucose alone had more deleterious effect on the liver than when present with a mixture of amino acids. In 1984 Keim and Mares-Perlman, (1984) in their study showed a good correlation between hepatic steatosis and essential fatty acid deficiency (EFAD). EFAD was present by day four of infusion, and resulted in accumulation of liver lipid or steatosis in rats which was apparent by day two of infusion with a hypercaloric glucose based fat-free PN (*Keim and Mares-Perlman, 1984*; *Ikeda et al., 1979*). EFAD is defined by a triene to tetraene ratio greater than 0.4 (*Gottschilich, 1992*; *Keim and Mares-Perlman, 1984*). Biochemically this means that in the absence of linoleic acid (essential fatty acid), the enzyme system responsible for converting linoleic acid to arachidonic acid instead

generates 5,8,11-eicosatrienoic acid from oleic acid. As the linoleic acid concentration in the body decreases, the triene (5,8,11-eicosatrienoic) concentration increases and the tetraene (arachidonic acid) concentration falls, resulting in an increased triene-to-tetraene ratio (*Gottschilich, 1992*). Apart from steatosis, EFAD during PN infusion has also been associated with poor wound healing, scaliness of the skin, thrombocytopenia, alopecia, growth retardation in infants and changes in plasma and erythrocyte fatty acids (*Gottschilich, 1992*; *Skolnik et al., 1977*; *Fleming et al., 1976*; *Richardson and Sgoulas, 1975*). To circumvent EFAD-associated hepatic dysfunctions intravenous fat emulsion came into use and are now a popular source of essential fatty acids in patients receiving PN. Initially a fat emulsion was administered separately from the amino acid and dextrose mixture to avoid catheter occlusion and solution instability. Recent studies (*Rollins et al., 1990*; *Ang et al., 1987*), however, have shown that a mixture of amino acids, dextrose and lipid or the "three-in-one" solutions are clinically safe, stable and economical. Improved patient compliance was also reported because of ease of administration (*Ang et al., 1987*). However controversial reports are found in the literature about the lipid effect on hepatic function. Reif *et al.* (1991) and Zohrab *et al.* (1973) reported reversal of hepatic steatosis with lipid infusions whereas others (*Allardyce, 1982*; *Boelhouwer et al., 1983*; *Bell et al., 1986*; *Clarke et al., 1991*; *Keim, 1987*) observed hepatic steatosis or even progressive cholestasis with lipid incorporation. Therefore further studies are required to resolve controversies regarding the effect of lipids on PN -related hepatic abnormalities.

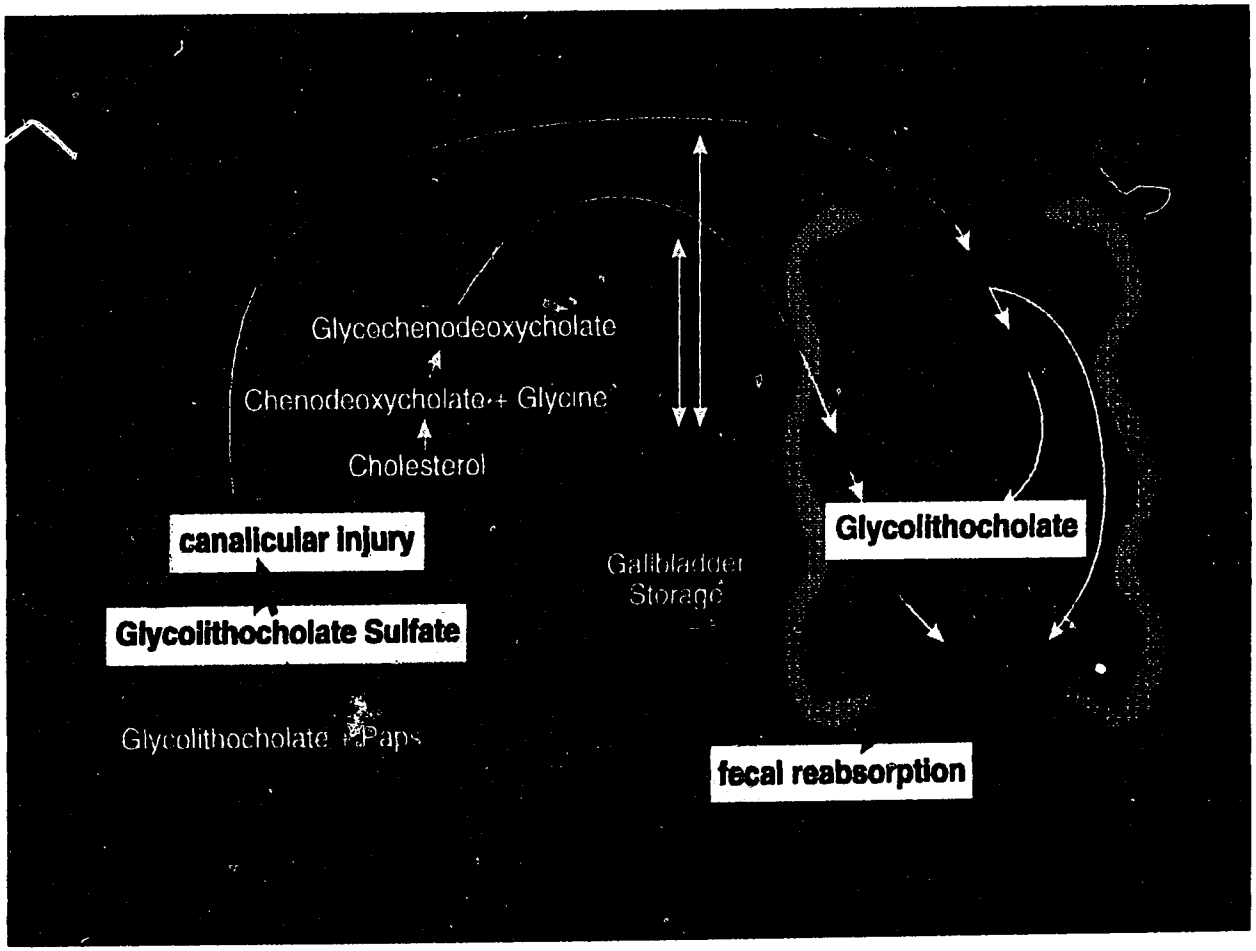

Figure 1.1 Proposed model for PN-associated hepatic injury. (Adopted from Merritt R.J. *J. Pediatr. Gastroenterol. Nutr.* 1986, 5: 9-22).

1.4.3 Effect of Taurine Deficiency

One of the several postulations of PN associated cholestasis is taurine deficiency. Data from both animal and human studies indicate that the pattern of bile acid conjugation may play an important role in its pathogenesis (*Miyai et al., 1971*; *Miyai et al., 1977*; *Miyai et al., 1975*; *Farrell et al., 1982*; *Balistreri et al., 1981*). Studies in guinea pigs (*Dorvil et al., 1983*; *Guertin et al., 1991*) revealed that taurine supplementation improved bile flow and bile acid secretion rate which are reduced when cholestasis is present. In addition, taurine administration has been shown to increase the synthesis of taurine conjugated bile acids which are important in preventing excessive accumulation of glycine conjugated secondary bile acids in guinea pigs. The latter has been shown to be cholestatic in both rats and guinea pigs (*Dorvil et al., 1983*; *Yousef et al., 1981*; *Guertin et al., 1991*; *Kibe et al., 1980*). Human neonates often have a reduced plasma level of taurine and develop cholestasis during PN infusion (*Cooper et al., 1984*; *Howard and Thompson, 1992*; *Vinton et al., 1987*). The low level of taurine is due to a limited cystathionase activity in infants that leads to a reduced synthesis of taurine from methionine (*Chesney, 1988*). As most commercially available PN solutions are devoid of taurine, it is likely that cholestasis in taurine-depleted infants may be attributable to an increase in glycine conjugated bile acids. To circumvent this situation taurine supplementation has been recommended for infants who are fed parenterally (*Howard and Thompson, 1992*).

Some controversial results have been reported on the effect of sulfated bile acids on hepatic function in rats, guinea pigs and human infants. Studies in rats showed that

sulfated forms of lithocholic and taurolithocholic acids were not cholestatic (*Yousef et al., 1981*; *Javitt, 1973*), while, sulfated lithocholic acid induced cholestasis in guinea pigs (*Dorvil et al., 1983*) and was used as a sensitive indicator of cholestasis in infants and children (*Farrell et al., 1982*; *Balistreri et al., 1981*). On the other hand findings by others (*Miyai et al., 1977*; *Javitt, 1966*; *Kakis and Yousef, 1978*; *Layden and Boyer, 1977*; *King and Schoenfield, 1995*; *Miyai et al., 1971*) showed that cholestasis in rats and hamsters was induced by the non-sulfated forms of lithocholic acid, chenodeoxycholic acid and their respective taurine conjugates but was reversed by taurocholate. The mechanisms by which cholestasis are caused by these bile acids are apparently species dependent. The reasons for this are unknown.

Despite extensive research on the role of taurine on bile salt-mediated cholestasis in animals, the exact role of taurine on the liver function of PN-treated rats remain unclear. Similarly, the beneficial effects of taurine-supplemented PN in human, are by no means definitive (*Kopple et al., 1990*). For example, taurine supplemented PN-infusion did not have any effect on hepatic dysfunctions, despite an increase in the plasma taurine levels in premature infants (*Cooke and Whitington, 1984*). Taurine does not improve the rate of growth and metabolism in the low-birth-weight infants (*Jarvenpaa et al., 1983*; *Okamoto et al., 1984*), pattern of bile acid conjugation and their synthesis, the glycine to taurine ratio (*Okamoto et al., 1984*), plasma taurine levels in sick infants (*Okamoto et al., 1984*) or in adult trauma patients suffering from hypotaurenimia (*Paauw and Davis, 1994*). The explanations for these discrepancies are not readily apparent. A more systematic appraoch is required to study the dilemma.

The rat has been proven to be a satisfactory model and has been used extensively to study the effects of different factors that influence PN-associated liver dysfunctions. Previous PN studies in animals, in our laboratory (*Ke et al., 1990b*) and by others (*Knodell et al., 1984*; *Knodell et al., 1980*; *Knodell et al., 1989*; *Ross et al., 1983*) have shown evidence of hepatic dysfunction and reduced drug metabolism. Although taurine was not present in the PN formulations used, its role on liver functions and drug metabolic enzyme activity have not been defined. This has prompted us to study the effects of taurine on hepatic function and drug metabolism.

1.4.4 Effect of Photo-Degraded Amino Acids

The link between the photo-oxidized amino acids and PN has received much attention lately, and researchers (*Bhatia et al., 1992b*; *Bhatia et al., 1983*; *Bhatia et al., 1993*; *Bhatia and Rassin, 1985*; *Merritt et al., 1984*) have demonstrated that solutions of certain amino acids, including tryptophan, methionine, tyrosine, cysteine and histidine (*Gurnani et al., 1966*; *Bhatia et al., 1983*; *Gurnani and Arifuddin, 1966*; *Bhatia et al., 1980*; *Asquith and Rivett, 1971*), in the presence of light and multivitamins, particularly riboflavin, undergo photooxidation. These photooxidized amino acid products have been found to be toxic to animal livers and also to brain tissue. Grant *et al.* (1977) demonstrated that when tryptophan was administered as a single amino acid solution or in a nutrition solution, the conversion products were toxic to both human and rat livers. Merritt *et al.* (1984) and Bhatia *et al.* (1985) have shown that tryptophan alone, and in presence of riboflavin, was deleterious to liver and caused cholestasis. These observations

are particularly important because the widely reported incidence of liver dysfunction in neonates could result from the photooxidised amino acid products. Currently most amino acids are marketed in plastic bags protected with aluminum foil, but prepared solutions containing these amino acids, dextrose, lipid, multivitamins and other additives are often infused from transparent bags and clear tubings without protection from light. Light exposure is particularly prevalent in intensive care nurseries where infants undergo phototherapy for jaundice and so in this environment solutions of amino acids are exposed to intense light. PN infusion solutions in ambulatory patients may even be subjected to direct sunlight. Exposure to these light sources can obviously promote photo-decomposition of amino acids and lead to hepatic dysfunctions.

1.5 Acute Effects of Parenteral Nutrition on Pharmacokinetics

The fact that a nutrient-drug interaction may affect the pharmacokinetics of drugs is not new (*Roe, 1988*; *Roe, 1985*). There has been effort to use drugs such as metronidazole (*Kubota et al., 1990*), or gentamicin (*Spurr et al., 1989*) to ameliorate PN-associated cholestasis. In these studies the kinetics or metabolism of the respective drugs were not studied, so PN-induced drug interactions were not be determined. Reports on studies that involve a continuous infusion of drugs vary depending on the drug studied. For example, continuous infusion of lidocaine (LIDO) (*Ke et al., 1990a*) and ampicillin (*Koo et al., 1990*) in healthy adult human volunteers did not produce any significant changes in the kinetic parameters studied. However, a study by Sangrador *et al.* (1990) on continuous theophylline infusion in two groups of geriatric patients, all suffering from

chronic obstructive disease, showed a 34% decrease in the theophylline clearance. The steady state plasma concentration was two times higher only in the group of patients who received PN infusion. From the above findings it is clear that the health status of a patient does not interfere with the kinetics of a drug, it is rather the nutritional parameters that were responsible for the reduction in theophylline elimination. In order to derive a dose-schedule for continuous i.v. infusion of antibiotics, Colding *et al.* (1983) investigated the effect of continuous infusion of two antibiotics namely, ampicillin and gentamicin in newborn infants receiving PN infusion. These researchers observed a huge variability in the elimination rate constant and half-life among these infants. Clearance of these drugs was significantly lowered in neonates and the extent of lowering depended on postnatal age and weight. This finding therefore indicates that proper monitoring of plasma concentrations, especially for drugs like theophylline and gentamicin that have narrow therapeutic indices, is absolutely essential in order to verify the appropriateness of the recommended or adjusted dosage.

1.6 Short-Term Effects of Parenteral Nutrition on Pharmacokinetics

Recent studies in human and animals suggested that a short-term PN infusion can significantly alter the kinetics and elimination of drugs (*Burgess et al., 1987*; *Ross et al., 1983*; *Knodell et al., 1980*; *Knodell et al., 1984*; *Ke et al., 1990b*). These studies also indicated that the type of PN-regimen used or even the route of PN administration may also greatly influence the elimination of drugs. For example, after a 7-day infusion of amino acid-dextrose solution, Knodell *et al.* (1984) reported a significant reduction in liver

CYP content and activity. This effect is reflected by a 71% reduction in pentobarbital hydroxylation and by a 62% reduction in meperidine demethylation when compared to enterally fed animals (*Knodell et al., 1984*). Knodell *et al.* (1980) reported a 46% reduction in plasma clearance of pentobarbital when animals were fed an amino acid-dextrose solution parenterally when compared to a group of animals that received the same solution enterally. Later, Knodell *et al.* (1989) demonstrated that liver microsomes obtained from parenterally fed animals which received an amino acid-dextrose solution for 7-days exhibited a similar reduction in drug metabolic activities when compared to the group that received an identical solution enterally. In this particular study, researchers were able to demonstrate significant reductions in the apoproteins for CYP2C11 and CYP3A1 in microsomes isolated from PN-treated livers. In contrast, the amount of CYP2C6 was found to be increased significantly in liver microsomes obtained from the PN-treated animals. The levels of CYP2A1 remained unchanged between groups. These alterations were echoed by studies involving the metabolism of benzphetamine, ethylmorphine and erythromycin which are metabolized by these enzymes (*Knodell et al., 1989*). Altered gene transcription due to an absence or reduced concentrations of gastrointestinal hormones in the portal blood elicited by intravenous feeding is a possible explanation for such an observation (*Knodell et al., 1989*).

Significant reduction in the total liver CYP content (48%) was also seen in PN-treated animals (*Knodell et al., 1989*). In a separate hepatic microsomal study, Ross and colleagues (*Ross et al., 1982*) demonstrated that animal livers treated with amino acid-dextrose based PN infusion, resulted in a reduction in CYP, cytochrome b_5,

uridinediphoso-glucoronyltransferase (UDPGT) and flavin adenine dinucleotide monooxygenase (FADM).

A similar reduction in enzyme activities was observed in post-operative patients whose antipyrine clearance was reduced by 34% after an infusion of amino-dextrose mixture for 7 days. However, this metabolic reduction was reversed to the chow-fed values when 25% of the non-protein calories were replaced by 10% Intralipid (*Burgess et al., 1987*). Similar findings were also reported (*Ross et al., 1983*; *Ross et al., 1984*).

In a study published by Knodell (1990) the effects of different enteral formulae with different proportions of protein, carbohydrate and lipid content on drug metabolizing enzymes were demonstrated. Hepatic microsomal studies in rats treated with the above formulae for 7-days revealed that the total activity of CYP was significantly reduced by 46% in the animal groups containing high or low protein and carbohydrate preparations containing very little lipid, compared to the groups that contained more lipids. Microsomal meperidine demethylation and pentobarbital hydroxylation were also depressed significantly in these low lipid-fed animals.

From these studies it became evident that dietary lipid composition whether given enterally or intravenously can significantly influence human or animal drug metabolism. This observation was followed up later by Knodell *et al.* (1990) who performed another study in which the fat content of the PN solution accounted for 10% of the total intravenous calories. Lipid restored the fluidity and fatty acid composition of the membrane. The increased fluidity still failed to prevent a reduction in the total CYP content induced by PN treatment. With or without lipid, PN reduced the meperidine

demethylase activity. This particular observation was in contradiction to previous results reported by the same group of researchers where enteral lipid restored total CYP content and meperidine demethylase activity. However, in the latter parenteral study, the lipid-based PN group showed some higher trends in ethoxyresorufin deethylase activity (*Knodell et al., 1990*). The discrepancy between the studies is not well understood but could be due to the different route of administration and differences in the composition and content of lipid used.

It can be concluded that the consequences of lipid inclusion in PN are controversial. Some studies found that antipyrine metabolism was restored (*Ross et al., 1983*). In human the N-dealkylation and 3-hydroxylation of antipyrine are catalyzed by CYP1A2 and CYP2C9 respectively and 4-hydroxylation by CYP3A subfamily enzymes (*Engel et al., 1994*). Failure to prevent reduction of CYP content and demethylase activity was reported in another study (*Knodell et al., 1990*). It appears that the effect of lipid on hepatic metabolism can be selective. To resolve this issue, more studies should be performed using different probes to identify the pathways that are affected by lipid infusion.

A recent *in vitro* liver microsomal study conducted by Raftogianis *et al.* (1995) in rats after PN treatment for about 10-14 days resulted in a significant reduction in CYP content and in glutathione-S-transferase, UDP-glucuronosyltransferase and sulfotransferase activities. This observation was consistent with *in vivo* data in the same study in which another group of rats maintained on PN for the same period displayed a significant reduction in the Phase II metabolism of acetaminophen. This was indicated by

the reductions in total acetaminophen clearance and in the formation of its two conjugated metabolites, acetaminophen glucuronide and sulfate.

In summary, it is evident that PN therapy is still not free of complications particularly in the hepatobiliary region. PN-induced hepatic dysfunction could be one of the probable factors responsible for the observed reduction in the metabolism of xenobiotics. The final outcome of such interactions could be hazardous because of the selective alterations by PN on drug metabolizing enzymes. According to the above information, the effect could be both ways, that is, the concentration of one drug can be as low as to produce a sub-therapeutic effect or the concentration could be as high as to produce a toxic effect. Since patients who are receiving PN infusion will often require medications for their underlying disease, it is imperative to conduct research to evaluate the effects of PN on drug elimination. Therefore, studies should be performed to understand the pathogenesis of PN-induced hepatic dysfunction and to relate PN treatment with the reduction in drug metabolism.

1.7 Lidocaine

Lidocaine (LIDO), 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, (Fig. 1.1) is still the drug of choice for initial parenteral therapy of acute ventricular arrhythmia (*Benowitz and Meister, 1978*). LIDO is also commonly used as a local anesthetic (*Benowitz and Meister, 1978*).

1.7.1 Mechanisms of Action as an Antiarrythmic

LIDO is considered a class IB (membrane-stabilizing/sodium channel blocking) antiarrythmic agent. LIDO controls ventricular arrythmias by suppressing both automaticity in the His Purkinje system and spontaneous depolarization of ventricles during diastole. The atrial refractory period remains unchanged by LIDO therapy which makes it less efficacious for the treatment of atrial flutter or fibrillation. Since, LIDO raises the ventricular fibrillation threshold, it is useful in the treatment of ventricular arrythmias. LIDO does not interact with the autonomic nervous system (*Nolan and Otto, 1993*; *Moe and Abildskov, 1970*).

1.7.2 Mechanism of Action as a Local Anesthetic

As a local anesthetic LIDO stabilizes the neuronal membrane and prevents the initiation and conduction of nerve impulses, thereby potentiating local anesthetic action (*Moe and Abildskov, 1970*).

1.7.3 Pharmacokinetics (general considerations)

1.7.3.1 Absorption

LIDO is a weak base and has a pKa of 7.85. Maximum absorption takes place through the large surface area of the intestine. LIDO is well absorbed after an oral administration but its use as an oral anti-arrhythmic is limited because of the high first-pass effect in the liver (*Nyberg et al., 1977*; *Benowitz and Meister, 1978*). Due to extensive

hepatic extraction of LIDO (*Nyberg et al., 1977*; *Stetson et al., 1969*), oral bioavailability of LIDO is estimated to be only 30% in man (*Boyes et al., 1970*). Consequently, the oral route of administration is not preferred. The therapeutic index of LIDO ranges from 1.5 to 6 μg/mL (*Nolan and Otto, 1993*). Therefore, in patients a level below 1.5 μg/mL is likely to result in therapeutic failure and similarly, adverse manifestations become increasingly apparent with plasma levels above 6 μg/mL (*Nolan and Otto, 1993*). An oral dose of 500 mg of LIDO in human was found to be associated with gastrointestinal and central nervous system toxicity, even when the plasma concentrations were well below the therapeutic level that is achieved following an i.v. dose of LIDO (*Keenaghan and Boyes, 1972*; *Boyes et al., 1970*; *Strong et al., 1973*). These undesired effects were attributed to the formation of toxic metabolites (*Boyes et al., 1970*). The two N-dealkylated metabolites of LIDO, N-monodeethyl-LIDO (MEGX) and N-dideethyl-LIDO (GX) formed *via* first-pass metabolism, were found to be associated with CNS toxicity (*Nation et al., 1977*; *Strong et al., 1973*; *Nolan and Otto, 1993*). These observations then led to the conclusion that i.v. administration would be the preferred route of LIDO therapy.

1.7.3.2 Distribution

LIDO is widely distributed into body tissues. After rapid i.v. injection it follows multi-compartment kinetics. First, there is an early decline in plasma concentrations of the drug, with a distribution half-life (α) ranging from 5 to 10 minutes, principally associated with distribution into highly perfused tissues such as kidneys, lungs, liver and heart. This is followed by a slower elimination phase with an average elimination half-life (β) of 80 to

100 minutes in humans in which metabolism and redistribution into skeletal muscle and adipose tissues occur (*Benowitz and Meister, 1978*; *Boyes et al., 1970*; *Tucker et al., 1970*).

Binding of LIDO to plasma proteins is variable and concentration dependent. At therapeutic blood concentrations, LIDO is approximatemately 60-80% bound to plasma proteins, specifically to α_1-acid glycoprotein (AAG) (*Tucker and Boas, 1971*; *Tucker et al., 1970*). The extent of binding to AAG is dependent upon the plasma concentration of the protein present, and therefore at higher concentration of LIDO (> 10 μg/mL) binding is reduced due to saturation of the binding sites (*Benowitz and Meister, 1978*; *Nolan and Otto, 1993*). LIDO readily crosses the blood-brain barrier and the placenta. Steady state volume of distribution (Vd_{ss}) relates the amount of LIDO in the body to the LIDO concentration in the plasma or blood at steady state, during constant infusion and range from 47 L to 157 L (*Benowitz and Meister, 1978*; *Nolan and Otto, 1993*).

1.7.3.3 Metabolism

Approximately 90% of an i.v. dose of LIDO is metabolized by the liver. Hepatic metabolism of LIDO has been studied extensively in animals and human (*Keenaghan and Boyes, 1972*; *Nyberg et al., 1977*; *Tam et al., 1987*; *Saville et al., 1987*; *Gray et al., 1987*; *Beckett et al., 1966*; *Tam et al., 1990*; *Coutts et al., 1987*; *Kawai et al., 1985*). Phase I metabolism of LIDO occurs at four major sites: aromatic ring, tertiary amine group, amide linkage and at aromatic methyl groups (Fig. 1.2). The aromatic ring is susceptible to hydroxylation, the tertiary amine group to N-deethylation. The methyl groups in the

aromatic ring undergo aryl methyl hydroxylation. The ring hydroxylated metabolites undergo Phase II metabolic reactions which include glucuronidation and sulfation. Rat liver microsomal studies indicated that LIDO has a high affinity for liver microsomal CYP (*Nyberg et al., 1977*).

In rat hepatic microsomes, LIDO undergoes sequential N-dealkylation to give MEGX and GX. MEGX and GX both possess antiarrythmic and convulsant properties in animals, MEGX appearing to have antiarrythmic potencies similar to that of LIDO (*Benowitz and Meister, 1978*). GX has 10% to 26% of the antiarrythmic activity of LIDO but is much less potent as a convulsant when compared to LIDO and MEGX (*Blumer et al., 1973*; *Nolan and Otto, 1993*).

Oda *et al.* (1989) identified two pure CYP enzymes, CYP2C11 (a male specific enzyme) and CYP2B1 (phenobarbital-inducible type) which catalyzed MEGX formation extensively from LIDO. GX was also formed sparingly by CYP2C11. Aromatic hydroxylation occurs at the 3 position. Formation of 3-OH-LIDO was catalyzed by CYP1A2, CYP2D1 and CYP2D2. The aryl methyl hydroxylated metabolite MEOH-LIDO was formed by CYP2B2 in rat livers (*Masubuchi et al., 1992*; *Oda et al., 1989*; *Imaoka et al., 1990*).

Recently Tanaka *et al.* (1994) demonstrated the contribution of extrahepatic tissues in LIDO metabolism. They revealed that rat pulmonary microsomes also metabolized LIDO to MEGX to an appreciable amount under the control of CYP2B1. The turnover rate of the formation of MEGX by CYP2B1 was 74.1 nmol/min/nmol of CYP when 1 mM of substrate was used which is similar to the rate of production of by

liver CYP2C11. Renal microsomes produced MEGX and 3-OH-LIDO, although the rate of production and quantities produced were much lower than when they were produced in the lungs. LIDO was not metabolized by brain microsomes. There are no reports on the gut metabolism of LIDO, but the gut may be involved in the metabolism of LIDO in view of the fact that both human and rat intestine carries CYP enzymes such as CYP2D and CYP3A4 (*George, 1981*) that can metabolize LIDO.

Gender, species and strain variations have also been demonstrated for LIDO metabolism. Keenaghan and Boyes (1972) found that species variation exists for the hydroxylation processes. For example, in humans p-hydroxylation dominates whereas m-hydroxylation is more important in rats (*Keenaghan and Boyes, 1972*). A gender difference in LIDO metabolism has also been observed (*Keenaghan and Boyes, 1972*; *Nyberg et al., 1977*). 3-Hydroxylation is predominant in female Sprague Dawley rats; 65% of an oral dose of LIDO was recovered as 3-OH-LIDO and only 0.7% as MEGX in female rat urine (*Keenaghan and Boyes, 1972*). In contrast, 90% of LIDO was metabolized to MEGX in male rat liver microsomes of the same strain (*Nyberg et al., 1977*). Masubuchi and his coworkers (1992) revealed marked strain differences in both sexes between Wister rats and Dark Agouti rats in the formation of 3-OH-LIDO. On the other hand no sex differences were observed in MEGX formation. Species variation with respect to the enzyme involved for LIDO metabolism was later pointed out by Bargetzi *et al.* (1989) who showed that MEGX formation in human liver microsomes was actually catalyzed by CYP3A4, despite the existence of CYP2C11 in human liver (*Smith, 1991*).

In human, however the major metabolite of LIDO that has been documented so far is 4-OH-2,6-xylidine, which accounts for 73% of the total dose (*Keenaghan and Boyes, 1972*).

The CYP enzymes that are involved in LIDO metabolism are also known to catalyze the metabolism of a variety of other therapeutic compounds. Therefore, due to the involvement of the various CYP enzymes in LIDO metabolism, this drug appears to be an ideal substrate for studying the drug metabolism mechanisms.

1.7.3.4 Excretion

Less than 10% of an i.v. dose of LIDO is excreted unchanged in the urine (*Benowitz and Meister, 1978*). There is evidence of biliary recycling of ring hydroxylated metabolites of LIDO (*Keenaghan and Boyes, 1972*). However, in a single pass isolated rat liver perfusion study, LIDO concentration in bile was found to be as low as <0.4% of the total dose. This indicated that biliary excretion of LIDO is a minor eliminating pathway (*Pang and Rowland, 1977a*). There is no evidence of biliary excretion of LIDO in humans (*Gillis et al., 1989*). Species variation has also been documented in the excretory forms of LIDO. Guinea pigs and dogs have been found to excrete approximately the same total amount of dealkylated metabolites of LIDO. However, in guinea pigs the predominant form excreted is MEGX and in dogs GX was excreted abundantly (*Keenaghan and Boyes, 1972*). Another difference is that guinea pigs excretes 2,6-xylidine mainly in the unhydroxylated form, whereas, in dogs and human the form that is found more abundantly is the p-hydroxylated form. Both animal and human data so far

cannot account for all the administered LIDO which indicates that yet to be identified metabolic pathways are involved in LIDO elimination.

1.8 Rationale for Choosing Lidocaine as a Model Drug

LIDO is a highly extracted drug and is extensively metabolized during its passage through the liver (*Nyberg et al., 1977*; *Lennard et al., 1983*; *Pang and Rowland, 1977b*). LIDO is used extensively in drug metabolism studies because the metabolism of this drug involves a number of very common enzymatic reactions, including N-dealkylation, aromatic hydroxylation, aryl methyl hydroxylation and N-oxidation. The primary metabolites can undergo sequential metabolism or conjugate with glucuronide or sulfate. Recent studies have been successful in isolating and characterizing specific CYP enzymes involved in the Phase I metabolism of LIDO (*Oda et al., 1989*; *Imaoka et al., 1990*).

Therefore, any changes in the metabolic profile of LIDO will give a clue as to the alteration of the respective metabolic pathways.

A previous study in our laboratory has shown that a short course of PN resulted in a reduction in the rate of LIDO metabolism (*Ke et al., 1990b*). Having this in mind we designed the following studies in order to identify the mechanisms and quantify the alteration in LIDO metabolism associated with PN infusion.

LIDO has been found not to interfere with the inherent physiological processes of the liver and therefore is not hepatotoxic (*Lennard et al., 1983*). This provides an added advantage of using LIDO in our PN studies because interference from our drug of choice with the PN-induced liver functions would be unlikely.

Finally, in human, formation kinetics of MEGX by CYP3A4 has been documented as a novel approach for the assessment of liver function for pre-transplant liver donors and recipients (*Oellerich et al., 1995*; *Oellerich et al., 1990*; *Rossi et al., 1992*; *Potter et al., 1992*). Therefore, a reduction of LIDO clearance is used as a relatively sensitive indicator of liver function. Although the same reaction in rat is mediated by CYP2C11, but rat CYP enzymes have similar substrate specificity similar to those of human (*Smith, 1991*), therefore, our animal data may be useful to predict a similar clinical situation.

1.9 Rationale for Choosing the *In Vitro* Animal Models

1.9.1 Choice of Male Sprague Dawley Rat

Rat is the most common animal model used to evaluate effects of PN on liver function because of its small size and cost and its availability, ease of handling and maintenance. In addition, a large literature data base is available for comparison purposes. Moreover, the metabolic pathways of LIDO are qualitatively similar in rats and humans (*Keenaghan and Boyes, 1972*). Therefore, the animal model chosen, although not ideal, appears to be appropriate. Due to gender specificity, only male Sprague Dawley rats were used in particular.

1.9.2 *In Vtro* Single-Pass Isolated Rat Liver Perfusion

The isolated rat liver perfusion (IPRL) technique is an efficient method for studying drug metabolism. A single-pass method in particular offers added advantage in that it allows a direct assessment of material balance across the liver. Steady state can be

reached easily and this enables researchers to isolate the parameters affecting drug metabolism and then to study them individually. For this reason, this design is most suitable for measuring the kinetics of enzymatic reactions. Other advantages include the option of taking a large number of samples for both inlet and outlet measurements and the ease of varying experimental conditions such as the inlet concentration, temperature, flow rate, pH and cosubstrates required for the study. A single-pass system is also free from interference by accumulated endogenous metabolites, which can inhibit the elimination of LIDO (*Lennard et al., 1983*). In contrast to other *in vitro* techniques such as microsomal preparations or isolated and cultured hepatocytes, the hepatic architecture, cell polarity and integrity are better preserved in the IPRL technique which is a better representation of the *in vivo* situation. Finally, single-pass IPRL studies are best suited for studying high first-pass hepatic extracted drugs such as LIDO (*Tam et al., 1987*; *Pang and Rowland, 1977a*; *Ahmad et al., 1983*; *Saville et al., 1987*). This is because the system was found to be linear and stable at a particular flow rate and varying input concentration between 0.95 to 7 mg/L. The extraction ratio of LIDO was found to be 90% in rats (*Tam et al., 1987*; *Ngo et al., 1995*), which was constant up until 100 min after perfusion (*Pang and Rowland, 1977a*). In contrast the clearance and extraction ratio of LIDO decreased with time in a recirculating system (*Lennard et al., 1983*). Our studies were conducted using an inlet concentration of 3.5 mg/L for LIDO and the perfusion time was up to a period of 70 min.

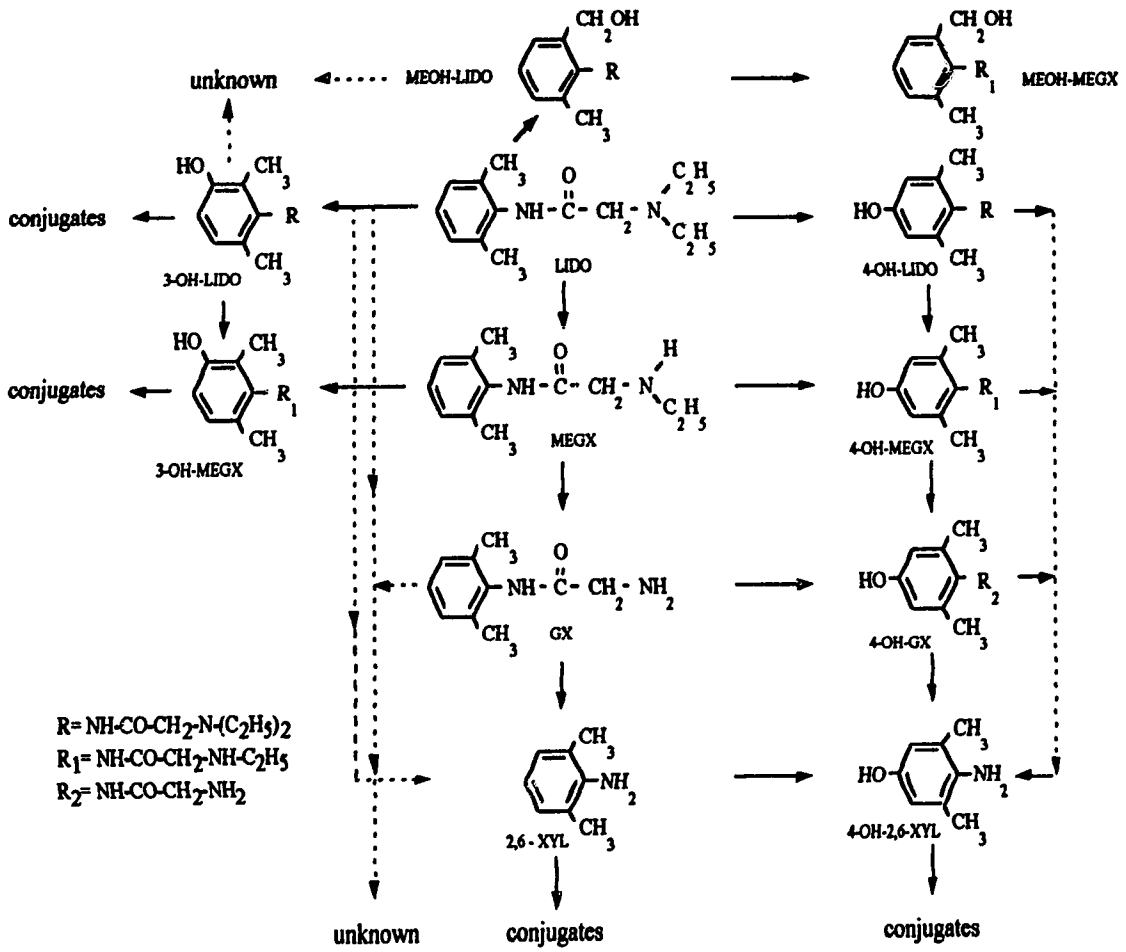

Figure 1.2 Schematics of lidocaine metabolism in humans and animals. (Adopted from Keenaghan and Boyes, *J. Pharmacol. Exp. Ther.* 1972; 180: 454-463).

1.10 Hypotheses

1. Increased reabsorption of bile salts are responsible for hepatic dysfunction and reduction in lidocaine metabolism.
2. Lipid substitution for dextrose will restore lidocaine metabolism.
3. Taurine supplementation will affect lidocaine metabolism.
4. Photo-degraded amino acid products cause cholestasis and reduce drug metabolism.

1.11 Research Objectives:

1. To evaluate how the interruption of bile salt reabsorption by cholestyramine (CH), a bile salt binder would affect liver function and lidocaine metabolism.
2. To evaluate the effect of lipid substitution for dextrose on lidocaine metabolism.
3. To evaluate lidocaine metabolism with taurine supplementation in PN.
4. To study the effects of photo-oxidized amino acids on liver morphology and lidocaine metabolism.

2. MATERIALS AND METHODS

2.1 Animals

Male Sprague-Dawley rats weighing 200-230 g were supplied by Biosciences Animal Services, University of Alberta. All animals were housed in the Dentistry-Pharmacy Building Animal Services Facility and were fed conventional rodent chow (Richmond Standard, PMI Feed Inc., St. Lois, MO). The protocol of this study met the guidelines of the Canadian Council on Animal Care and the use of animals was approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

2.2 Composition of PN solution and Rate of PN Solution Administration

The composition of the PN solution was identical to that reported by Ke *et al.* (1990b). Each litre of PN contained 242 g dextrose, 52 g amino acids (10% Travasol blend B with electrolytes; Baxter-Travenol Lab., Malton, Canada), 2 mL of multivitamin (MVI 1,000, Aqueous Multivitamin IV infusion, USV Canada Inc.) and 2.25 mmol of calcium gluconate (10% calcium gluconate injection, Squibb Canada Inc., Montreal, Canada). Solutions were prepared aseptically in a laminar flow hood and containers were immediately covered with aluminum foil to avoid exposure to light. Chow-fed rats received intravenous saline at a rate of 3 mL/hr and had free access to rat chow and water. All infusions were delivered by a volumetric infusion pump (model 927, IMED Corporation, San Diego, CA). The rate of PN infusion in all the animals was 3 mL/hr, the same as that of the chow-fed animals, but PN animals were only allowed free access to water and not rat chow. Each rat was weighed just prior to sacrifice (on the eighth day) and its liver was isolated.

2.3 Experimental Protocols

The project consists of two major experimental protocols. The first protocol was designed to test the first objective, which is to evaluate the effect(s) of the interruption of enterohepatic reabsorption of bile salts by CH on hepatic function and LIDO metabolism. This was evaluated using three groups of animals. They were: chow-fed animals (CF; n=6); those that received dextrose/amino acid (PN; n=6); and those that were administered dextrose/amino acid plus CH (PNC; n=6).

The second protocol was designed to test the second, third and fourth objectives, which were to evaluate the effects of lipid substitution for dextrose, taurine supplemented PN on hepatic function and LIDO elimination and photooxidation of amino acids.

2.3.1 Experimental Protocol 1:

2.3.1.1 Cholestyramine Study

The effects of secondary bile salts on liver function and LIDO metabolism were studied using the following protocol. Male Sprague-Dawley rats (n=6) were randomly assigned to one of the three treatment groups: CF, PN and PNC. Chow-fed animals received saline infusions and had free access to rat chow and water, while those in the PN group received infusions of dextrose and amino acids, and those in the PNC group were treated in the same manner as the animals in the PN group but also received a daily oral dose (0.34 g/kg) of CH. A 16 gauge feeding needle (Fine Science Tools Inc., N. Vancouver, Canada) was used for the oral administration of CH to animals in the PNC

group. Animals in the chow-fed and PN groups were also administered water orally with a 16 gauge feeding needle. All animals were infused for 7 days after which livers were isolated. The same protocol was conducted in a preliminary study (n = 3 for each group) where the infusion period was prolonged to 14 days.

2.3.2 Experimental Protocol 2:

The effects of lipid, light exposed amino acid preparations and taurine supplementation on PN-associated hepatic dysfunction and LIDO elimination were tested using six groups of animals. These were: 1) chow-fed animals (CF; n=6); 2) animals that received dextrose/amino acid (PN; n=6); 3) PN animals that also received lipid (PNL; n=6); 4) animals that were administered PN plus 15 mg/dL of taurine (PNT15; n=4); 5) a similar group of animals that were given PN plus 50 mg/dL of taurine (PNT50; n=4); 6) animals that received PN with light exposure [PN(+L); n=6].

The CF and PN groups were used commonly in the following studies for comparison purposes.

2.3.2.1 Lipid Study

The effect of lipid was studied by substituting 30% of non-protein calories, which is dextrose, with 20% Intralipid® (Kabi Pharmacia Canada Inc.), keeping the solution isocaloric to that received by the PN group (mentioned in section 2.2). The results of CF, PN and PNL in the second experimental protocol were compared. In order to avoid any fat emulsion instability, the order of mixing the components were as follows: to the

dextrose solution was added in sequence Travasol, calcium gluconate, multi-vitamin and finally lipid. The pH of the final emulsion was between 5.5 and 6.6 to provide optimal emulsion stability (*Warshawsky, 1992*). Freshly prepared total nutrient mixtures, especially those containing multi-vitamins, were off-white or banana colored and were found to be devoid of any signs of creaming or cracking even when infusion bags were stored at 4°C for up to a period of two weeks.

2.3.2.2 Taurine Study

The effect of taurine supplemented PN solution on hepatic function and LIDO elimination was tested. The results from CF and PN animals and the two taurine treated groups, PNT15 and PNT50 were compared. The taurine treated PNT15 group received the same dextrose/amino acid solution as in the PN group plus 15 mg/dL. The other taurine treated PNT50 group received the same PN solution plus 50 mg/dL taurine. Taurine solutions were made in water for injection (Water for injections B.P. Technilab Inc., Montreal, Canada) and then mixed with the final PN solutions.

2.3.2.3 Amino Acid Photo-Oxidation Study

The effects of photooxidized amino acids on the hepatic function and LIDO elimination, was studied by comparing the results of CF, PN and PN(+L) groups in the second protocol. The PN(+L) group received the same dextrose/amino acid solution as in PN group, but the solution was placed approximately 18 cm below a bank of fluorescent lamps for 24 hr prior to infusion to animals. The light exposure was maintained at 6 μW/ cm^2/ nm (425-475 nm) identical to that reported by Bhatia *et al.* (1992b). This procedure

simulates the light intensities of infant intensive care units. Under these conditions, infusate solutions turned brown after a 24 hr exposure. Infusion bags were then protected from light by covering them with aluminum foil during the infusion period to avoid further decomposition. Samples were withdrawn before and after light exposure to determine the extents of decomposition.

2.3.2.4 Effects of Photooxidized Amino Acids

The amino acid concentrations of each PN solution used to infuse animals in PN(+L) group were determined before and after solutions were exposed to the light source. Individual solutions of four amino acids, tryptophan, methionine, histidine and tyrosine, previously reported to be photosensitive (*Kleinman et al., 1973*; *Kanner and Fennema, 1987*; *Gurnani et al., 1966*; *Asquith and Rivett, 1971*; *Gurnani and Arifuddin, 1966*), were evaluated individually and in conjunction with three common photosensitizers present in a PN solution, namely riboflavin (RB), multivitamin (MVI) and sodium bisulfite (SB), using the same illuminating technique as described in section 2.3.2.2. The initial concentrations of the four amino acids were identical to those found in the PN solution. Solutions were prepared in glass beakers. An aliquot of each solution was first analyzed to confirm the exact initial concentration of each amino acid before light exposure for 24 hr while the top of the beaker was covered with parafilm™. The concentrations of riboflavin: 0.2 mg/dL, multivitamin: 0.2 mL/dL and sodium bisulfite: 0.14 g/dL were the same as present in the PN solution. Since the antioxidant sodium bisulfite is present in all amino acid mixtures it was preferred to add sodium bisulfite to each prepared solution containing the individual amino acid with riboflavin or multivitamin. The effect of sodium

bisulfite on each amino acid solution and in the absence of riboflavin and multivitamin was determined to see whether amino acid decomposition occurred upon light exposure. The amino acids solutions were analyzed by the same HPLC technique that was used for the serum samples. An aliquot of each solution was also analyzed by UV spectrophotometer before and after light exposure at 200-600 nm to determine color changes.

2.4 Jugular Vein Cannulation

Each rat was anesthetized with methoxyflurane and its right jugular vein was catheterized with a silastic tubing (o.d., 0.047" and i.d., 0.025", Dow Corning Corporation, Midland MI). The catheter was then tunneled subcutaneously and exteriorized in the scapular region where it was connected to a coiled metallic spring mounted on a swivel (Rodent Single Channel Swivel, Alice King Chatham Medical Arts, Los Angeles, CA). This connection permitted free mobility of the subject. Surgical equipment was sterilized prior to each surgery and the entire surgical procedure was carried out aseptically in a laminar flow hood. After each surgery, the animal was weighed and placed in a metabolic cage which was housed in a well ventilated room where a 12 h light and dark cycle was maintained. Rats were allowed to recover for at least three days before infusion during which time they had free access to a standard laboratory rodent chow (Richmond Standard, PMI Feed Inc., St. Lois, MO) and water. On day four, the weight of each animal was recorded.

2.5 Isolated Rat Liver Perfusion

A "one-pass" liver perfusion method was used in this study. Details of the isolated liver perfusion procedure were reported previously (*Tam et al., 1987*; *Miller, 1973*;

Hussain et al., 1994). Concisely, the portal vein was cannulated with an intravascular "over-the-needle" 16 G Teflon® catheter (Quick-cath, Baxter Healthcare Co., Deerfield, IL, U.S.A.) which was used to provide oxygenated Kreb's bicarbonate buffer. The effluent perfusate sample was collected from a catheter located in the thoracic inferior vena cava. Liver function was monitored by measuring oxygen consumption, aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) levels in the effluent perfusate at zero and 70 minutes, the hydrostatic pressure, and by the organ's general physical appearance. The rate of oxygen consumption was 2-3 mL/hr/g and was within the normal range reported (*Bloxam, 1973*). AST, ALT, ALP levels and the hydrostatic pressure were constant throughout the experiment. It has been reported that liver will remain viable for at least 3 to 4 hours after its removal from the animal (*Gores et al., 1986*). Our experiment was completed within 90 minutes. The viability of the liver was further confirmed by the stable concentrations of LIDO and its metabolites at steady state during the infusion period. Each liver was infused with LIDO (3.5 μg/mL at a rate of 30 mL/min) *via* a Vario pump (Cole Parmer Instrument Co., Chicago IL) for 70 minutes. This period was found to be sufficient for LIDO and its metabolites to approach a steady state (*Tam et al., 1987*). The inlet concentration of LIDO (C_{in}) was determined by obtaining 6 samples (2 mL each) directly from the inlet reservoir at 0, 12, 22, 33, 44 and 70 minutes after the initiation of LIDO infusion. The outlet concentration of LIDO and its metabolites (C_{out}) were measured in the effluent samples at 0, 1, 3, 5, 7, 10 minutes and at 5 minute intervals up to 50 minutes and then at a 10 minute interval up to 70 minutes. After each experiment the liver was blotted dry and its weight was recorded.

2.6 Histological Examination

A portion of each liver was stored in 10% formalin prior to being stained with hematoxylin-eosin, trichrom stain for connective tissue and evaluated by Perl's reaction

for iron. Histological examination was done in a blinded fashion in the Department of Laboratory Medicine and Pathology, University of Alberta. The degree of steatosis was rated as none, mild, moderate or severe.

2.7 Serum Enzymes and Amino Acid Assays

Blood (~1.5 mL) was collected from the posterior vena cava of all the animals immediately after catheterizing the portal vein and the serum was separated by centrifugation. Serum levels of AST, ALT, ALP, gamma glutamyl transpeptidase (γ-GT), total bilirubin (BILI) were determined in all the studies. In cholestyramine study, besides the above tests two other tests, namely cholylglycine (CG) and total bile acid (TBA) determinations were performed. Levels of AST, ALT, ALP, γ-GT and BILI were determined using a Dri-Stat™ diagnostic kit (Beckman Instrumentation, Inc.) and a Multistat 3 Micro Centrifugal Analyzer (Instrumentation Laboratory Inc.). The tests were performed by the Surgical Medical Research Institute laboratory personnel. The coefficient of variation of each assay was within ± 3%. Individual serum amino acid concentrations were quantified on a Varian 5000 high performance liquid chromatograph (HPLC) coupled to a Varian Fluorichrom detector (*Sedgwick et al., 1991*). Determination of amino acid levels were conducted by the staff of the Department of Animal Sciences. The reproducibility of the assay was within ± 2%. Serum CG was measured using a radio immunoassay method (CG RIA, Abbott Laboratories, Diagnostic Division Abbott Park, IL). Serum TBA was measured using the fluorometric method developed by Osuga *et al.* (1977). The coefficient of variation of the assay was less than ± 5%. A posterior vena cava blood sample was also collected into a sterile vacutainer containing ethylenediaminotetraacetic acid for white blood cell count (WBC) and hemoglobin (Hb) measurements using a Coulter Counter M 430 (Coulter Electronics Inc., FL).

2.8 Chemicals and Reagents

Samples of LIDO hydrochloride and four of its metabolites, N-monodeethyl-LIDO [MEGX: 2-(ethylamino)-N-(2,6-dimethylphenyl)acetamide], N-dideethyl-LIDO [GX: 2-amino-N-(2,6-dimethylphenyl)acetamide], 3-hydroxy-LIDO [3-OH-LIDO: 2-(diethylamino)-N-(2,6-dimethyl-3-hydroxyphenyl)acetamide], 3-hydroxy-MEGX [3-OH-MEGX: 2-(ethylamino)-N-(2,6-dimethyl-3-hydroxyphenyl)acetamide] and the internal standard [EMGX: 2-(N-ethyl-N-methylamino)-N-(2,6-dimethylphenyl)acetamide] were gifts from Astra Pharmaceuticals (Mississauga, Canada). The remaining two metabolites, ring-methyl hydroxylated LIDO [MeOH-LIDO: 2-(diethylamino)-N-(2-hydroxymethyl-6-methylphenyl)acetamide] and ring-methyl hydroxylated MEGX [MeOH-MEGX: 2-(ethylamino)-N-(2-hydroxymethyl-6-methylphenyl)acetamide] were synthesized in our laboratory based on the methods of Nelson *et al.* (1977). Their chemical structures and purity were confirmed using NMR, elemental analysis and GC/MS. HPLC grade solvents and reagents were obtained from BDH Inc., Toronto, Ontario.

2.9 High Performance Liquid Chromatography

The HPLC system consisted of two M-45 pumps, a model 441 UV detector (set at 214nm), a Model 840 data processing station (Waters Associate, Mississauga, Ontario) and a Shimadzu automatic sampler (Model SIL-9A Shimadzu Corp., Kyoto, Japan). Separation of LIDO and its six metabolites was achieved on a C_{18} LiChrospher® 60 RP-select B column (5 μm, 125mm × 4 mm, Chromatography Merck, Darmstadt, F.R., Germany).

2.10 Sample Analysis

An extraction procedure was required before solutions of LIDO and its metabolites were injected into the HPLC for quantitation. One mL of standard solutions containing known quantities of LIDO and its metabolites or perfusate samples containing unknown quantities of LIDO and its metabolites were first alkalinized with excess potassium bicarbonate (~ 1 g) and extracted with methylene chloride (8 mL). Six milliliters of the organic phase was then removed and dried using a Savant Speed Vac SC 100 (Savant Instruments Inc., VG-5). The residue was reconstituted in 250 to 300 μL of 0.005 M (pH 2.2) HCl. Between 100 to 150 μL of the sample was injected onto the column. A gradient elution technique at a constant flow of 1.6 mL/min was used. The aqueous buffer (pH 3.5) contained 6.55 g potassium dihydrogen phosphate, 150 μL of phosphoric acid and 1.011 g of sodium n-heptanesulfonate as ion-pairing agent in 1 L double distilled deionized water. The final mobile phase before use was filtered through a 0.45 μM filter, type HA for the aqueous phase and type GV for acetonitrile (Millipore). The composition of the mobile phase was initially maintained at aqueous buffer 91% (v/v): acetonitrile 9% (v/v) from 0 to 5 minutes, then programmed to change linearly over 10 minutes to buffer 82% (v/v): acetonitrile 18% (v/v) which was maintained from 15 to 26.5 minutes before returning to the initial conditions within one minute. The column was then allowed to equilibrate for 7.5 minutes before the next run was started, the total run time was 35 minutes.

2.11 Standard Curves

Standard solutions were prepared by spiking 1 mL volumes of the blank perfusate with various concentrations (0.02-3.5 μg/mL) of LIDO and its six metabolites. Calibration curves, constructed by plotting the peak area ratio of LIDO and each of its

metabolites to that of the internal standard versus the respective concentration of the drug or metabolite, were linear over the range studied ($r > 0.99$). Quality control samples, prepared by non-involved personnel in the laboratory were used to validate the assay. The quantifiable limit of this assay was 0.02 μg/mL for LIDO and its six metabolites when a 1 mL aliquot of perfusate sample was used. The percent deviation from expected values as a measure of accuracy was less than 15 % for the standards and less than 10% for the quality control samples. The intra-day and inter-day coefficients of variation for standard and quality control samples of LIDO and its metabolites were within 10%. A stability study revealed that stock solutions of LIDO and its metabolites were stable for 8 weeks when stored at -20°C.

2.12 Pharmacokinetic Analysis

The times for LIDO and its metabolites to reach steady state (T_{ss}) were statistically determined according to the method reported (*Saville et al., 1989*). The efficiency of the liver in removing LIDO at steady state was expressed by the following equations:

$$E_H = \frac{(C_{in} - C_{out})}{C_{in}} \quad (1)$$

$$Cl_{int} = \frac{Q E_H}{(1 - E_H)} \quad (2)$$

where Q is the buffer perfusion rate, C_{in} is the inlet drug concentration, C_{out} is the effluent drug concentration, E_H is the drug extraction ratio and Cl_{int} is hepatic intrinsic clearance.

Percent of dose recovered as unchanged LIDO and as metabolites was computed by as the molar ratio of steady state effluent LIDO or metabolite concentration C_{out} to inlet LIDO concentration C_{in} times 100. Mass balance was the sum of these values.

2.13 Statistical Analysis

2.13.1 Experiment Protocol 1

Shapiro-Wilks test and Kolmogorov-Smirnov Goodness of Fit test were first used to test for the normality of the data (*Montgomery, 1991a*). Parameters deviating from normal distribution were evaluated using non-parametric Kruskal-Wallis one way ANOVA to detect the difference (*Montgomery, 1991b*). When a significant difference was detected by the F statistics, Duncan's multiple comparison test was used to evaluate the differences among the groups. Significance was set at P = 0.05 level. Values are expressed as means ± S.D.

2.13.2 Experimental Protocol 2

In this protocol rats were randomized to six groups; chow-fed, PN, PNL, PN(+L), PNT15 and PNT50 groups. The PNL, PNT15, PNT50 and PN(+L) groups were designed to test the effects of lipid substitution for dextrose, photo-oxidation of amino acids and two different strengths of taurine supplementation respectively, on PN-associated hepatic function and LIDO elimination. In order to do so, the results of each of the above four different treatment groups were compared individually with the results of chow-fed and the PN animals which did not receive any one of the above treatments. Comparisons were made from a set of three experimental units, when results of PNL or

PN(+L) animals were compared with the results of PN and chow-fed animals respectively, and a set of four experimental units when results of PNT15 and PNT50 animals were compared with the same chow-fed and the PN group. Since the chow-fed and PN group were common for each set of studies, the following statistical tests were performed to justify this experimental procedure. In addition an overall comparison among the six groups were also performed.

At first the power of the test was calculated as described (*Montgomery, 1991a*). It was found that with the treatment means, a power of the test (1-β) of 0.80 was reached when the sample size was 4 (P = 0.05), and 0.95 when the sample size was 6 (P = 0.05), in order to detect a difference between any two groups at the calculated variance and when the means differed anywhere between two to six times from each other. Subsequently, all parameters in the six groups were then tested for normality using tests similar to those mentioned in section 2.14.1. When it was found that data were not normally distributed, non-parametric Kruskal-Wallis One Way Anova was performed on the test parameters obtained from these groups to evaluate the difference. The level of significance was set at P = 0.05. When a significance was obtained, the Two Independent Sample Mann-Whitney U Rank Sum Test was performed on that variable by comparing the values of two groups at a time, such as the chow-fed group *vs* PN, chow-fed *vs* PNL, chow-fed *vs* PN(+L), chow-fed *vs* PNT15 and chow-fed *vs* PNT50 groups. Similarly, the results in the PN group were compared with those of another group taking two groups at a time such as the PN *vs* PNL, PN *vs* PN(+L), PN *vs* PNT15 and PN *vs* PNT50 groups, which makes nine comparisons in total. In this case the observed level of significance was 0.05/9, or 0.005,

for the difference to be significant at the 0.05 significance level. This whole procedure is known as Bonferroni test (*Norusis, 1992*). Following these tests when chow-fed, PN plus any one of the groups such as PNL, PN(+L), PNT15 and PNT50 groups were compared (as a set of 3 or 4 experimental units) similar statistical measures were carried out as mentioned in section 2.14.1.

In order to make comparisons among all six groups, first non-parametric Kruskal-Wallis One Way Anova was performed on the parameters of the six groups. When a difference was detected, Duncan's multiple comparison test was used to evaluate the differences among the groups. The level of significance was set at $P = 0.05$. The SPSS® for windows™ computer program was used for these statistical calculations.

3. RESULTS

3.1 Cholestyramine Study

3.1.1 Body and Liver Weight Changes

All animals that participated in this study had similar body weights (200-230 g) at the beginning of the experiment. Although all animals appeared to be healthy at the end of each experiment, only animals in the chow-fed group gained weight (7.55 ± 1.52 g/day). Animals in the PN and PNC groups did not show any changes of weight throughout the experiment. Actual liver weights (Table 3.1) and liver weights expressed as percent of body weight were significantly lower in all PN treated animals compared to chow-fed values (PN, 3.34 ± 0.33 %; PNC, 3.41 ± 0.29 %; *vs* CF, 3.86 ± 0.30 %; $P < 0.05$).

3.1.2 Liver Functions and Serum Amino Acids

The serum liver function test values: ALP, ALT, AST, γ-GT, CG, BILI and TBA, for all the animals studied are within normal ranges (see appendix 1). The variability of these values are consistent with that reported in the literature (*Farkas and Hyde, 1992*). Interestingly, lowest LFT values were found in the PNC group. Levels of BILI in PNC animals were significantly lower than those of the PN group. Significantly lower CG levels relative to the values in PN and chow-fed groups were observed in the PNC group (Table 3.2). The WBC and Hb values for all rats (Table 3.2) were also within the normal ranges (*Guide to the care and use of experimental animals,* 1984), suggesting an absence of sepsis.

Most serum amino acid levels were similar among the three treatment groups; however, significantly higher levels of serine, glycine, histidine, threonine and methionine were found in the PN group relative to that of the chow-fed (Table 3.3).

The steatosis scores obtained from the preliminary study are shown in Table 3.4. All rats in the chow-fed and CH treated groups had a score of 1, indicating absence of steatosis. In contrast, two rats in the PN group exhibited mild to moderate steatosis.

3.1.3 Lidocaine Elimination

Representative concentration *vs* time profiles of LIDO and its metabolites in the three treatment groups are shown in Figure 3.1. The effluent LIDO concentration was highest in the animals in the PN group, lower in the PNC group and lowest in chow-fed animals; the steady state C_{out} values for LIDO in the two PN groups were also significantly different from those of the chow-fed group (Table 3.1).

The kinetic parameters (Table 3.1) reveal that the Cl_{int} values were significantly reduced in the PN and PNC animals; these values were one-half and one-third respectively, of that of the chow-fed group ($P < 0.05$). This observation clearly suggests that LIDO metabolism is severely compromised during PN treatments. CH has a tendency to reverse this trend.

Steady state percent recovery of LIDO and its metabolites are shown in Table 3.5. A significantly lower GX level and an absence of MeOH-MEGX in the effluent were observed in the PN group. The levels of these two metabolites were similar between the PNC group and chow-fed animals. Levels of MeOH-LIDO were the highest in the chow-fed group. Molar ratios of metabolites to their precursors (Table 3.6) revealed that N-dealkylation, as indicated by MEGX/LIDO, was reduced in the PN treated animals. This reduction is mainly due to an increase in the steady state effluent LIDO concentration.

Interestingly, MEGX levels were not reduced in the PN [illegible] animals. This phenomenon may be due to a reduced formation of sequential metabolites such as GX, MeOH-MEGX and unknowns. The latter is substantiated by a significant reduction in the level of unknown metabolites (PN, 38.42 ± 6.76; PNC, 44.92 ± 7.22; *vs* CF, 58.16 ± 9.26 μM; $P < 0.05$) indicating lower rates of metabolism for these unknown pathways. N-deethylation of MEGX and aryl methyl hydroxylation of LIDO, as indicated by the GX/MEGX and the MeOH-LIDO/LIDO ratios, were similar between the PN and PNC groups; both ratios were significantly reduced when compared to chow-fed values ($P < 0.05$). Although the 3-OH-LIDO/LIDO ratio appeared to be halved in both the PN and PNC groups, a statistical difference for these ratios, relative to that of chow-fed group, was not achieved.

The results of the 14-day preliminary study (Figure 3.2) also indicate an elevation of steady state effluent LIDO concentration in the PN and PNC groups. This is reflected by a reduction of Cl_{int} values in the PN group. Due to a small sample size and variability in the data, statistical significance was not reached.

TABLE 3.1 Steady state kinetic parameters of LIDO from perfused rat liver after the 7-day treatment[a]

Kinetic Parameters	PN	PNC	CF
Q (mL/min/g)	4.38 ± 0.47^{b}	4.1 ± 0.55^{b}	3.12 ± 0.54
C_{in} (μM)	14.84 ± 0.82	14.26 ± 1.24	14.1 ± 0.87
C_{out} (μM)	4.71 ± 1.60^{b}	3.71 ± 1.02^{b}	1.68 ± 0.81
Liver (g)	7.36 ± 0.78^{b}	7.68 ± 0.94^{b}	10.35 ± 1.33
Cl_{int} (mL/min/g)	10.90 ± 4.79^{b}	13.26 ± 7.41^{b}	28.20 ± 17.06

[a]Values are represented as mean ± SD,(n = 6, in each group).

[b]$P < 0.05$ *vs* CF group.

PN = dextrose/amino acid; PNC = dextrose/amino acids and CH; CF = chow-fed.

TABLE 3.2 Concentrations of serum liver function test values in three treatment groups[a]

Tests	PN	PNC	CF
AST (U/L)	100.18 ± 22.03	83.25 ± 18.41	108.43 ± 44.84
ALT (U/L)	26.50 ± 8.31	25.17 ± 3.28	31.82 ± 17.46
ALP (U/L)	258.23 ± 111.85	226.45 ± 82.11	396.23 ± 200.36
BILI (mg/dL)	0.62 ± 0.22[c]	0.36 ± 0.13	0.53 ± 0.36
CG (μg/dL)	56.16 ± 73.11	19.10 ± 5.50[b]	187.40 ± 236.52
TBA (μmol/L)	30.33 ± 14.28	28.93 ± 22.92	31.73 ± 7.98
WBC ($x10^3/mm^3$)	11.00 ± 3.50	11.60 ± 3.80	10.45 ± 1.09
Hb (g/dL)	13.25 ± 1.55	12.95 ± 1.25	11.53 ± 1.63

[a] Values are represented as mean ± SD (n = 6, in each group).

[b] $P < 0.05$ *vs* CF group.

[c] $P < 0.05$ *vs* PNC group.

PN = dextrose/amino acid; PNC = dextrose/amino acids and CH; CF = chow-fed.

TABLE 3.3 Serum amino acid concentrations in μmol/L on day 8 in three groups[a]

Amino Acid	PN	PNC	CF
Aspartic acid	18 ± 15	13 ± 2	31 ± 25
Glutamic acid	88 ± 46	66 ± 17	74 ± 40
Asparagine	59 ± 35	67 ± 22	76 ± 41
Serine	317 ± 93[b]	244 ± 69	203 ± 94
Glutamine	548 ± 228	507 ± 113	436 ± 108
Histidine	100 ± 34[b]	93 ± 34	64 ± 16
Glycine[d]	572 ± 177	328 ± 94	304 ± 74
Threonine	354 ± 103[c]	219 ± 47	91 ± 135
Citrulline	46 ± 19	43.± 18	54 ± 28
Arginine	188 ± 68	186 ± 63	189 ± 86
Taurine	442 ± 412	249 ± 83	140 ± 84
Alanine	705 ± 526	316 ± 141	546 ± 207
Tyrosine	98 ± 55	90 ± 21	76 ± 28
Tryptophan	75 ± 33	62 ± 25	51 ± 31
Methionine	101 ± 31[b]	61 ± 18	58 ± 36
Valine	190 ± 101	166 ± 98	161 ± 90
Phenylalanine	98 ± 54	84 ± 31	54 ± 29
Isoleucine	90 ± 44	75 ± 35	93 ± 51
Leucine	135 ± 87	122 ± 72	136 ± 66
Ornithine	156 ± 147	91 ± 28	104 ± 40
Lycine	637 ± 365	563 ± 186	809 ± 241

[a] Value represented as mean ± SD (n = 6, in each group).

[b] $P < 0.05$ *vs* CF group.

[c] $P < 0.05$ *vs* PNC group.

[d] $P < 0.05$ significantly different in each group.

PN = dextrose/amino acid; PNC = dextrose/amino acids and CH; CF = chow-fed.

TABLE 3.4 Steatosis scores observed in the three group of rats in the 14-day pilot st

	Steatosis Score			
	1 = none	2 = mild	3 = moderate	4 = severe
CF1	1			
CF2	1			
CF3	1			
PN1	1			
PN2		2		
PN3			3	
PNC1	1			
PNC2	1			
PNC3	1			

CF = chow-fed;

PN = amino acid and dextrose group;

PNC = amino acid, dextrose and CH group.

TABLE 3.5 **Mean recoveries as % LIDO dose for unchanged LIDO and its metabolites in effluent perfusate sample at steady state in the three treatment groups[a]**

Compound	PN	PNC	CF
LIDO	31.68 ± 10.46[b]	26.39 ± 8.58[b]	11.93 ± 5.91
MEGX	19.13 ± 3.65	18.66 ± 5.08	18.66 ± 4.61
GX	1.25 ± 0.17[b]	1.51± 0.19	2.04 ± 0.39
3-OH-LIDO	7.75 ± 2.20	6.53 ± 2.23	4.84 ± 1.26
MeOH-LIDO	0.55 ± 0.14[b]	0.64 ± 0.13[b]	2.28 ± 0.89
3-OH-MEGX	1.48 ± 0.56	1.06 ± 0.55	1.26 ± 0.54
MeOH-MEGX	ND	0.96 ± 0.54	0.98 ± 0.72
TOTAL	61.58 ± 6.76[b]	55.06 ± 7.22[b]	41.84 ± 9.26

[a] Values are represented as mean ± SD (n = 6, in each group).

[b] $P < 0.05$ *vs* CF group.

PN = dextrose/amino acid; PNC = dextrose/amino acids and CH; CF = chow-fed.

TABLE 3.6 Molar ratios of metabolites to their respective precursors in the three treatment groups on day 8 [a]

Molar ratio	PN	PNC	CF
MEGX/LIDO	0.67 ± 0.25[b]	0.80 ± 0.39[b]	1.88 ± 0.92
GX/MEGX	0.07 ± 0.02[b]	0.08 ± 0.03[b]	0.12 ± 0.03
3-OH-LIDO/LIDO	0.27 ± 0.13	0.28 ± 0.16	0.58 ± 0.43
MeOH-LIDO/LIDO	0.02 ± 0.01[b]	0.03 ± 0.01[b]	0.26 ± 0.18

[a] Values are represented as mean ± SD (n = 6, in each group).

[b] $P < 0.05$ *vs* CF group.

PN = dextrose/amino acid; PNC = dextrose/amino acids and CH; CF = chow-fed.

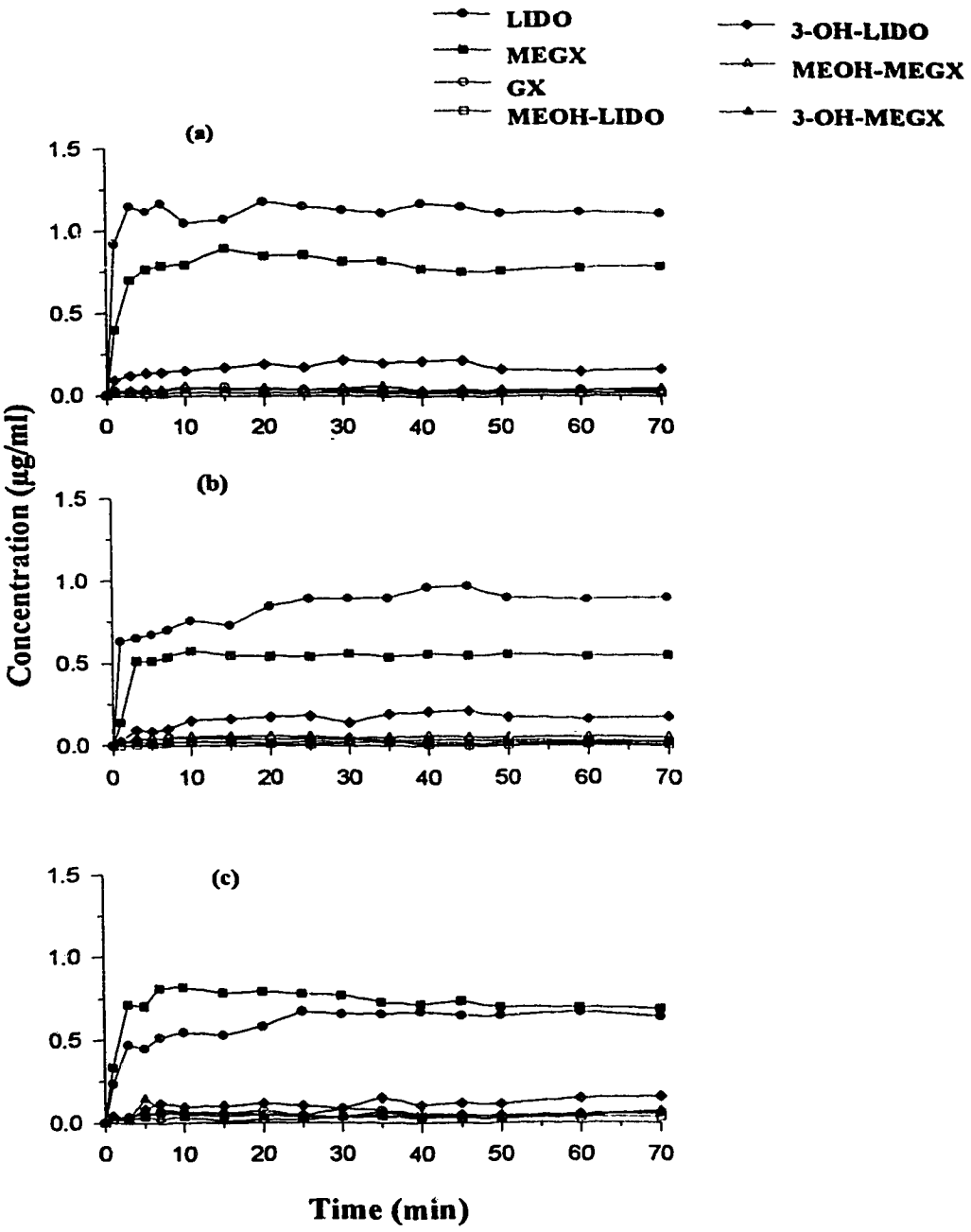

Figure 3.1 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused rat livers from the three treatment groups: (a) group PN: C_{in} = 15.51 µM; (b) group PNC: C_{in} = 13.58 µM; (c) chow-fed group CF: C_{in} = 13.03 µM.

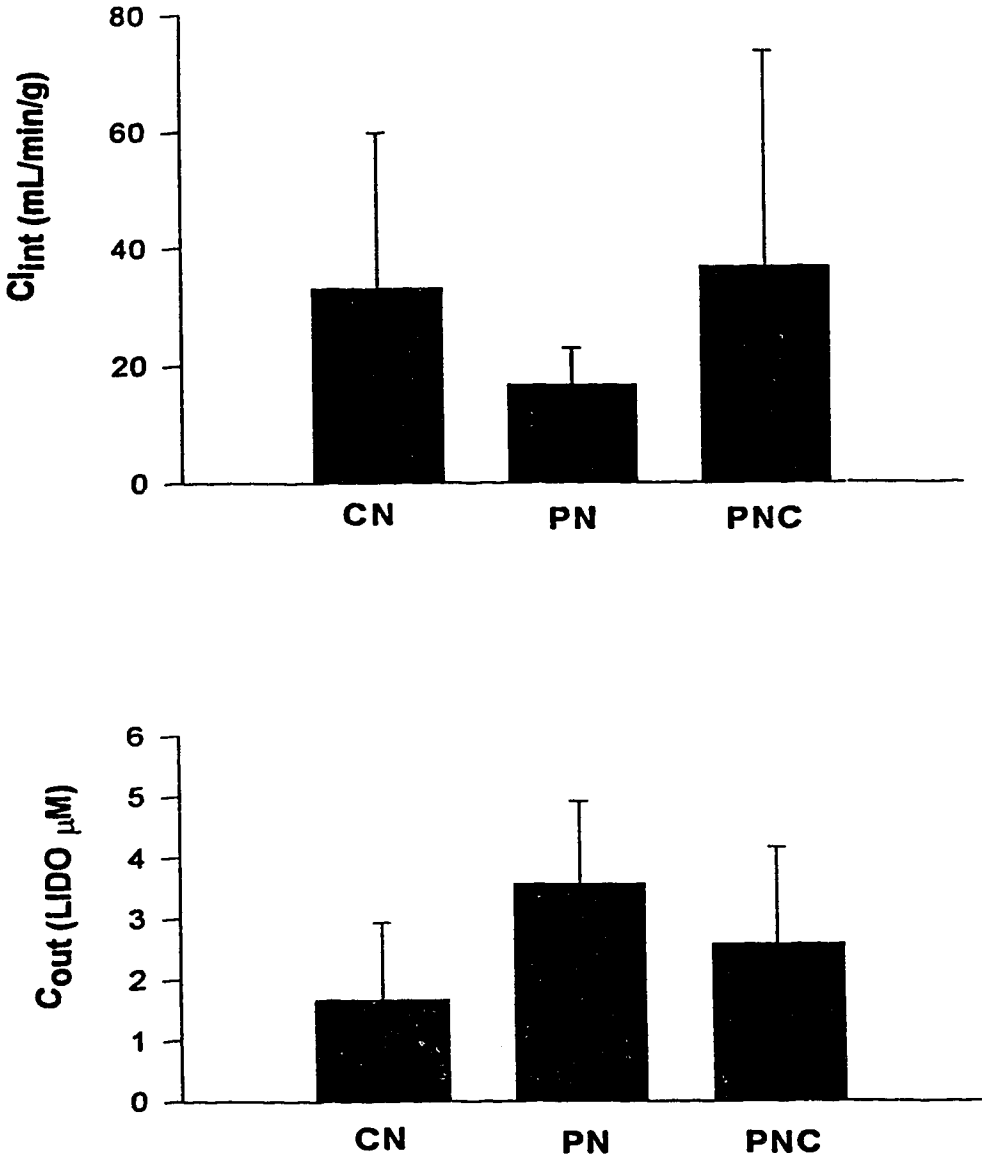

Figure 3.2 Bar diagrams of liver weight normalized intrinsic clearance (Cl_{int} mL/min/g) and steady state effluent LIDO concentration (C_{out}) in the 14-day cholestyramine study (n = 3).

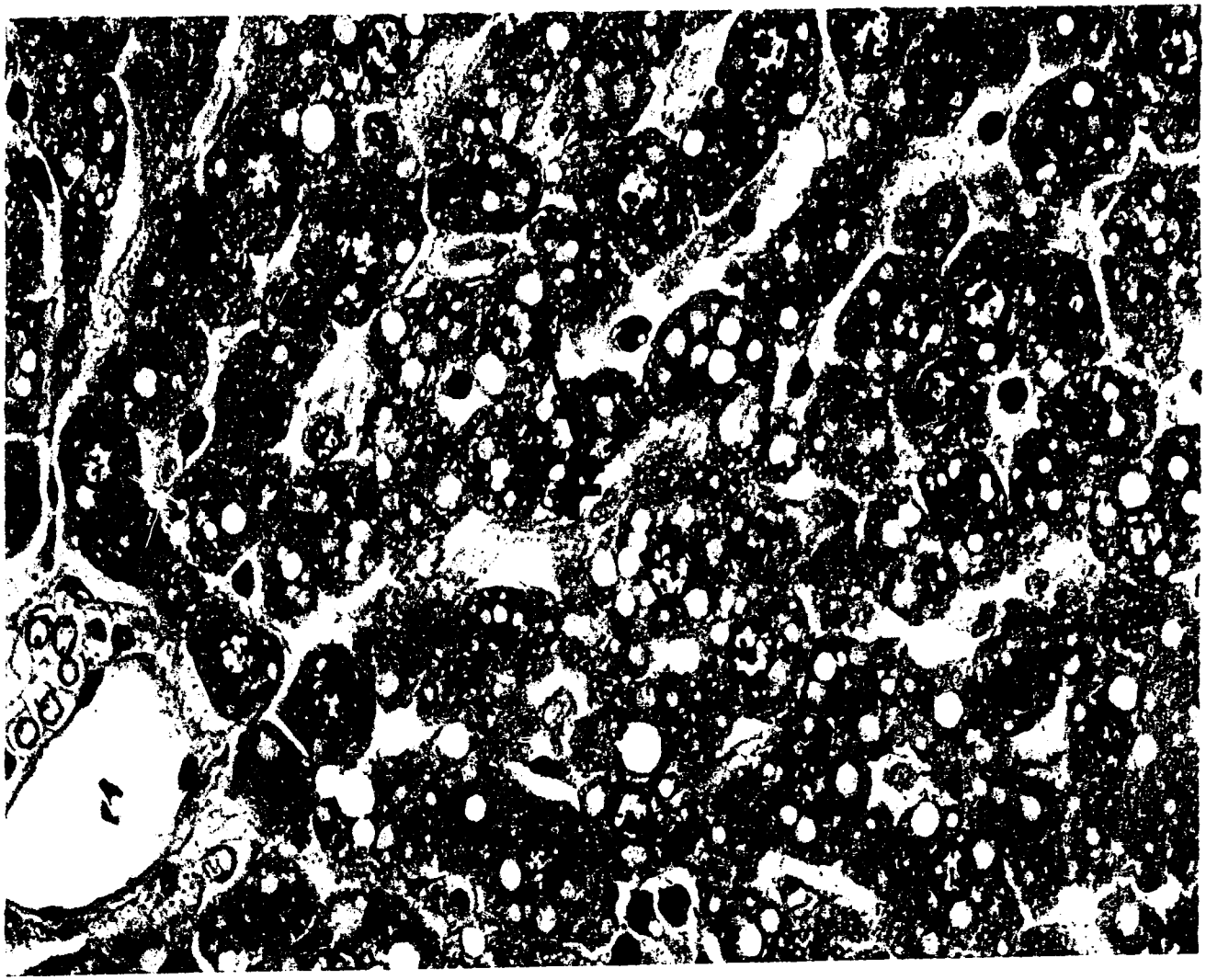

Figure 3.3 Representative photomicrograph of a rat liver from the PN group (dextrose/amino acids). Severe steatosis is evident in the form of both large and small fat droplets (F) throughout the hepatocytes. (Hematoxylin & eosin, original magnification, x 880).

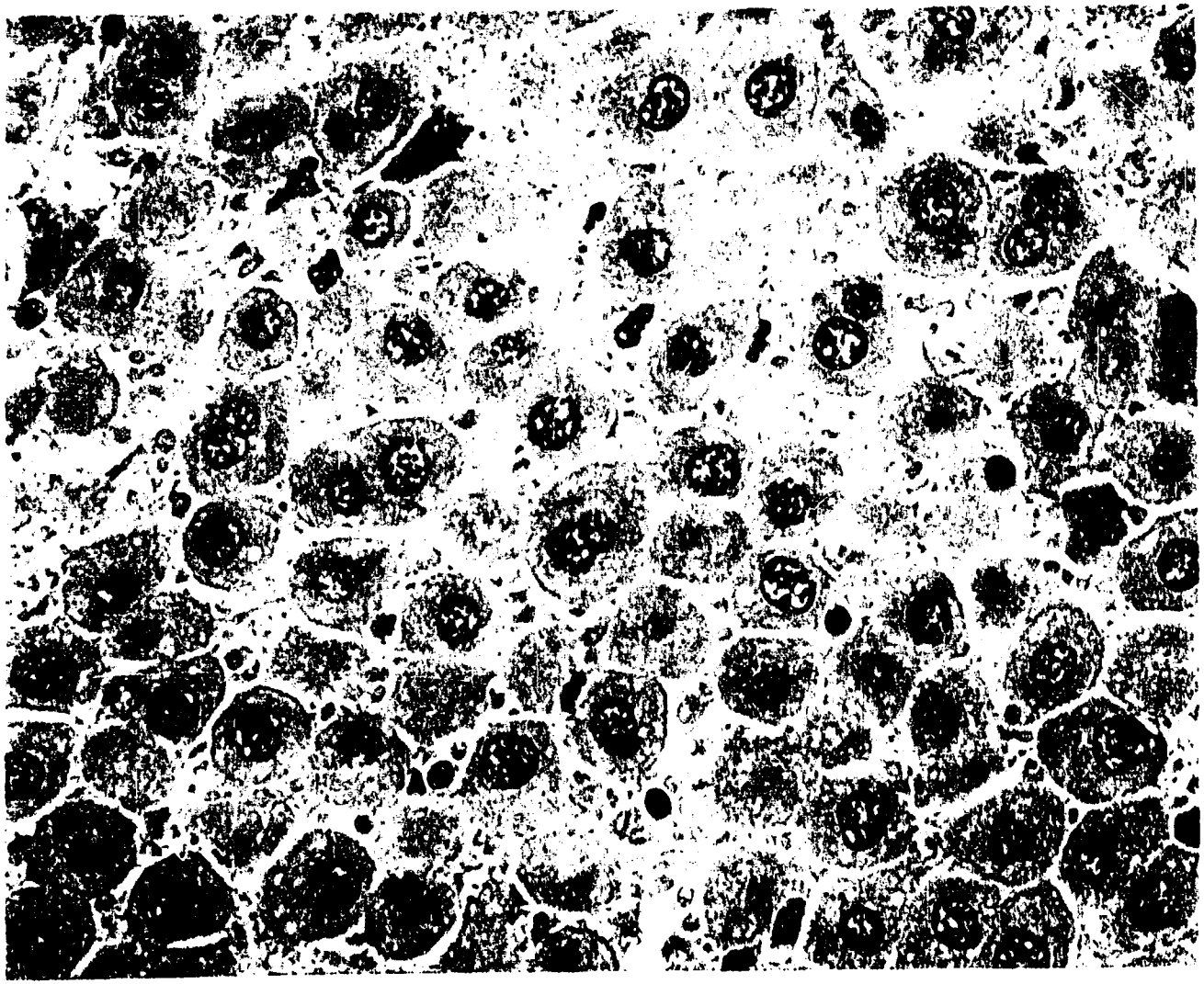

Figure 3.4 Representative photomicrograph of a rat liver from the PNC group (dextrose/amino acids plus CH). A normal cellular architecture with no signs of steatosis is evident. (Hematoxylin & eosin, original magnification, x 880).

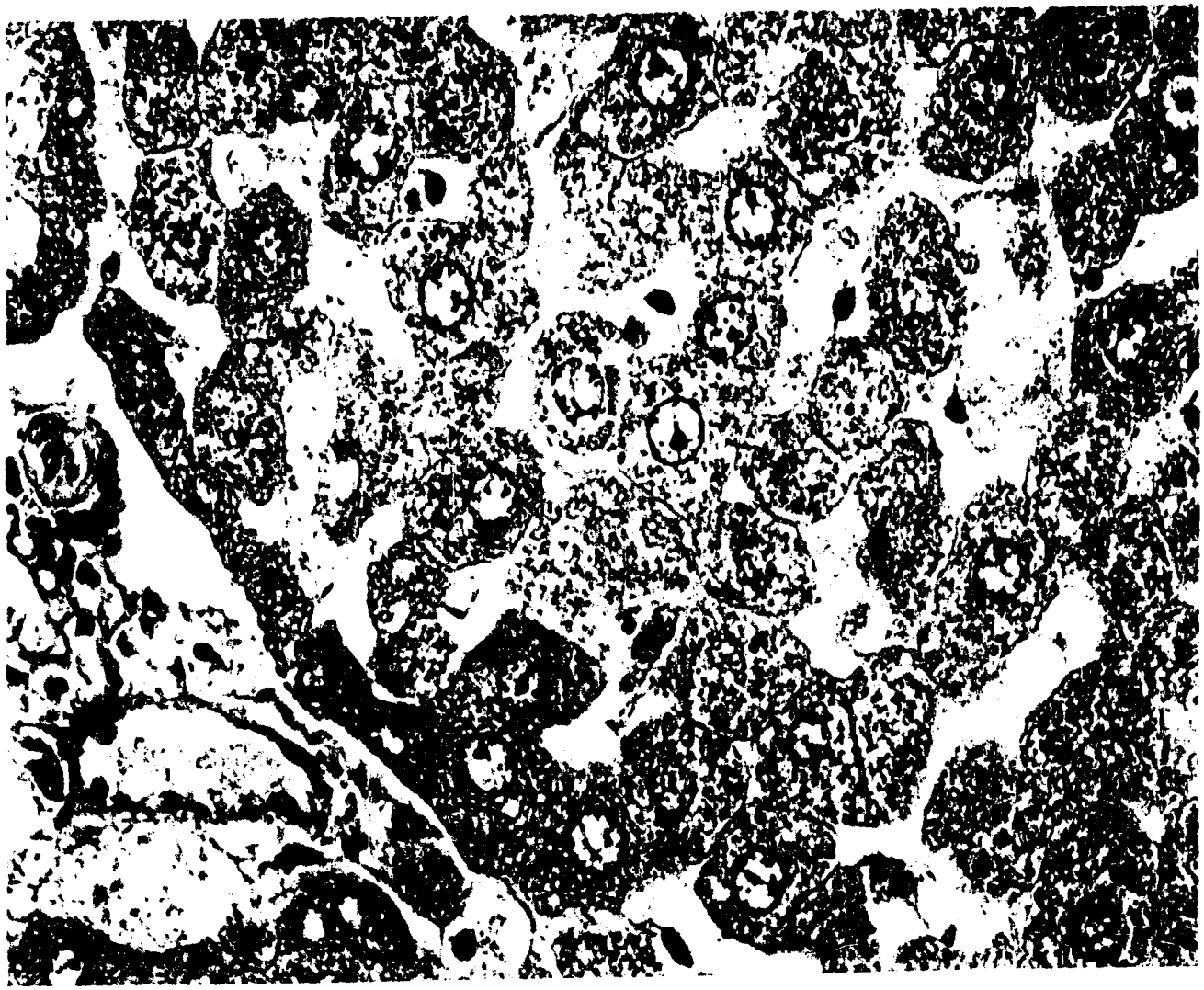

Figure 3.5 Representative photomicrograph of a rat liver from the CF group (chow-fed) showing a normal cellular architecture. (Hematoxylin & eosin, original magnification, x 880).

3.2. Lipid Study

3.2.1 Body and Liver Weight Changes

Animals in the PNL, PN and CF groups had similar body weights (PNL, 220.33 ± 15.42; PN, 213.80 ± 19.59; *vs* CF, 224.50 ± 22.06 g) before initiation of the experiment. Despite a healthy appearance of all the animals at the end of each experiment, only animals in group CF exhibited a significant weight gain of 7.55 ± 1.52 g/day. Animals in group PNL and PN on the other hand only maintained their initial body weight until the end of each experiment. The liver weights, were significantly lower in groups PNL and PN, group PN being the lowest when compared to chow-fed (Table 3.7). A difference in liver weights in terms of percent body weight was found only between group PNL and PN (PNL, 4.49 ± 0.68 %; PN, 3.69 ± 0.31; % *vs* CF, 3.98 ± 0.30 %; $P < 0.05$).

3.2.2 Liver Functions and Serum Amino Acids

There were no significant differences in any of the serum LFT values, AST, ALT, ALP, BILI and γ-GT, among the groups (Table 3.8) which were within the normal ranges reported (see appendix 1).

The most dramatic changes in serum amino acid concentrations (μmol/L) of histidine, glycine, methionine and phenylalanine were observed in groups PNL and PN relative to group CF (Table 3.9).

3.2.3 Liver Histology

The histological data with the steatosis scores are shown in Table 3.10. Examination of liver specimens from group PNL animals revealed that five out of six had developed significant steatosis and their severity was rated between 2 to 3. Steatosis was characterized by fatty degeneration of hepatocytes and numerous, clear intracytoplasmic fat globules, extended diffusely from the perilobular regions to the central veins (Fig. 3.6). Two out of five liver specimens in group PN received a score of 2 and exhibited a few fat droplets (Fig. 3.7); however there was no evidence of cell injury or inflammation in either of the groups. Liver specimens from group CF animals received a score of 1 showing absence of steatosis and a normal cellular architecture (Fig. 3.8).

3.2.4 Lidocaine Elimination

Representative concentration *vs* time profiles of LIDO and its metabolites in the three treatment groups are shown in Figures 3.9 to 3.11. The effluent LIDO concentration is the highest in group PNL (Fig. 3.9), followed by groups PN (Fig. 3.10) and CF (Fig. 3.11); the values are also significantly different from each other (Table 3.7).

The kinetic parameters are shown in Table 3.7. The significant reduction in Cl_{int} values in the two PN groups, particularly in the PNL group which has a mean value approximately equal to one-sixth of that of the CF group, suggesting LIDO metabolism is severely compromised after lipid treatment.

Steady state percent recoveries of LIDO and its metabolites are shown in Table 3.11. A significant increase in the recovery of unchanged LIDO was observed in groups PNL and PN; the most drastic change occurred in group PNL. Other than MEGX, which

is significantly lower in group PNL, MeOH-LIDO, which shows the highest value in group CF, and MeOH-MEGX, which was not detected in group PN but was similar between groups PNL and CF, there was no obvious trend in a change of the known metabolite levels. However, the levels of unknown metabolites were found to be the highest in CF group and lowest in the PNL group (PNL, 36.36 ± 6.75; PN, 46.54 ± 6.02; *vs* CF, 55.18 ± 5.44; μM $P < 0.05$) indicating a lower rate of metabolism in these unknown pathways. When ratios of metabolites to LIDO were used for comparison (Table 3.12), significant trends towards reductions in N-dealkylation, ring hydroxylation and aryl methyl hydroxylation were observed. Consistent with the kinetic parameters obtained for LIDO, group PNL had the most pronounced reduction in LIDO metabolism.

TABLE 3.7 Steady state kinetic parameters of LIDO in perfused rat livers after 7-day parenteral nutrient (with and without lipids) treatments[a]

Kinetic parameters	PNL	PN[1]	CF
Q (mL/min/g) [d]	3.23 ± 0.23	3.95 ± 0.40	2.83 ± 0.35
C_{in} (μM)	16.04 ± 1.86	15.35 ± 1.48	15.53 ± 1.62
C_{out} (μM) [d]	6.26 ± 1.41	3.99 ± 0.47	1.77 ± 0.81
Liver (g) [d]	9.63 ± 0.81	7.95 ± 0.84	11.1 ± 1.51
Cl_{int} (mL/min/g) [d]	5.46 ± 2.40	11.33 ± 1.62	28.06 ± 16.40

[a] Values are represented as mean ± S.D.

[d] Values of the three groups are significantly different ($P < 0.05$) from each other.

PNL = amino acids, dextrose and lipid group n = 6;

PN = dextrose/amino acids group n = 5;

CF = chow-fed n = 6.

[1] One rat in the dextrose/amino acid:PN group was tested to be an outlier, and was discarded from the studies in the second protocol (*Montgomery, 1991a*; *Norusis, 1992*).

TABLE 3.8 Serum concentrations of liver function values in the three treatment groups on day 8[a]

TESTS	PNL	PN	CF
AST (IU/L)	191.33 ± 129.38	120.9 ± 21.99	115.17 ± 21
ALT (IU/L)	50.55 ± 31.49	29.4 ± 11.07	35.1 ± 4.19
ALP (IU/L)	222.88 ± 122.37	295.78 ± 219.11	364.98 ± 226.73
BILI (mg/dL)	2.43 ± 2.84	1.22 ± 0.71	0.74 ± 0.38
γ-GT (IU/L)	31.30 ± 29.84	35.26 ± 20.91	57.03 ± 37.91

[a] Values represented as mean ± SD.

PNL = amino acids, dextrose and lipid group n = 6.

PN = dextrose/amino acids group n = 5.

CF = chow-fed n = 6.

TABLE 3.9 Serum amino acid concentrations in μmol/L on day 8 in the three groups[a]

Amino acid	PNL	PN	CF
Aspartic acid	35 ± 32	21 ± 5	15 ± 2
Glutamic acid	95 ± 67	89 ± 15	74 ± 22
Asparagine	44 ± 6[b]	59 ± 26	102 ± 34
Serine	529 ± 216[b]	551 ± 92[b]	276 ± 83
Glutamine	371 ± 40[bc]	750 ± 133	662 ± 40
Histidine [e]	118 ± 13	218 ± 61	70 ± 4
Glycine [e]	828 ± 330	1439 ± 319	272 ± 36
Threonine	413 ± 186	463 ± 269	272 ± 56
Citrulline	55 ± 15	46 ± 31	53 ± 5
Arginine	131 ± 88	269 ± 89	221 ± 27
Taurine	282 ± 73	475 ± 205[b]	208 ± 57
Alanine	719 ± 377	1286 ± 616	631 ± 102
Tyrosine	106 ± 25	99 ± 74	91 ± 15
Tryptophan	83 ± 7	125 ± 14[bd]	78 ± 14
Methionine [e]	119 ± 9	210 ± 52	58 ± 7
Valine	207 ± 51	224 ± 33	187 ± 31
Phenylalanine	119 ± 23[b]	141 ± 49[b]	60 ± 8
Isoleucine	110 ± 46	101 ± 31	98 ± 18
Leucine	166 ± 80	133 ± 44	153 ± 17
Ornithine	523 ± 63[b]	281 ± 205	128 ± 60
Lycine	1117 ± 342	948 ± 430	1060 ± 402

[a] Values are represented as mean ± S.D. (n = 4, in each group).
[b] $P < 0.05$ *vs* CF group; [c] $P < 0.05$ *vs* PN group.
[d] $P < 0.05$ *vs* PNL group; [e] Values significantly different ($P < 0.05$) from each other.
PNL = amino acids, dextrose and lipid group; PN = dextrose/amino acids group; CF = chow-fed.

TABLE 3.10 Steatosis score of the three treatment groups of the lipid study

	Steatosis Score			
	1 = none	2 = mild	3 = moderate	4 = severe
CF1	1			
CF2	1			
CF3	1			
CF4	1			
CF5	1			
CF6	1			
PN1	1			
PN2		2		
PN3	1			
PN4		2		
PN5	1			
PNL1			3	
PNL2		2		
PNL3		2		
PNL4	1			
PNL5		2		
PNL6		2		

CF = chow-fed;

PN = amino acid and dextrose group;

PNL = amino acid, dextrose and lipid group.

TABLE 3.11 Mean steady state recoveries (% LIDO dose) of LIDO and its metabolites in the three treatment groups[a]

Compound	PNL	PN	CF
LIDO[c]	39.75 ± 11.36	25.92 ± 2.09	11.35 ± 5.28
MEGX	13.32 ± 3.9[bc]	19.85 ± 5.48	21.34 ± 3.16
GX	1.07 ± 0.33	1.58 ± 0.69	1.74 ± 0.76
3-OH-LIDO	7.34 ± 1.66	5.23 ± 2.70	5.83 ± 1.70
MEOH-LIDO	0.58 ± 0.14[b]	0.65 ± 0.48[b]	2.48 ± 0.83
3-OH-MEGX	1.07 ± 0.34	1.76 ± 1.01	1.31 ± 0.35
MEOH-MEGX	0.51 ± 0.43	ND	0.76 ± 0.64
TOTAL[c]	**63.64 ± 6.75**	**53.46 ± 6.02**	**44.82 ± 5.44**

[a] Values are represented as mean ± SD.

[b] $P < 0.05$ *vs* CF group.

[c] Values of the three groups are significantly different ($P < 0.05$) from each other.

ND = not detected.

PNL = amino acids, dextrose and lipid group n = 6;

PN = amino acids and dextrose group n = 5; CF = chow-fed.

TABLE 3.12 **Molar ratios of MEGX, 3-OH-LIDO and MeOH-LIDO to LIDO in the three treatment groups**[a]

Molar ratio	PNL	PN	CF
MEGX/LIDO [c]	0.38 ± 0.19	0.76 ± 0.21	2.32 ± 1.32
3-OH-LIDO/LIDO	0.20 ± 0.08[b]	0.20 ± 0.11[b]	0.64 ± 0.42
MeOH-LIDO/LIDO	0.02 ± 0.01[b]	0.024 ± 0.018[b]	0.26 ± 0.14

[a] Values are represented as mean ± S.D.

[b] $P < 0.05$ *vs* CF group.

[c] Values of the three groups are significantly different ($P < 0.05$) from each other.

PNL = amino acids, dextrose and lipid group n = 6;

PN = amino acids and dextrose group n = 5;

CF = chow-fed n = 6.

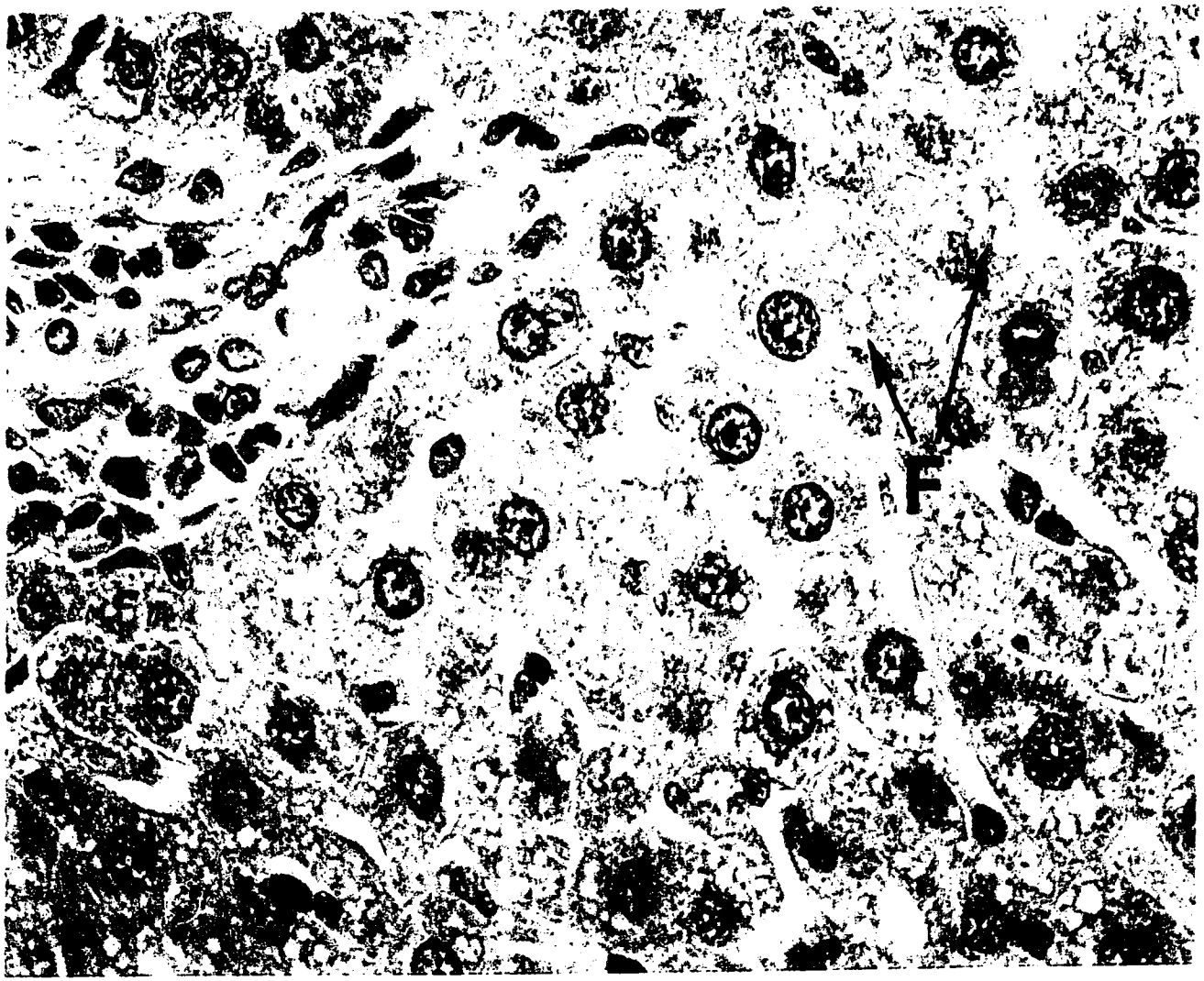

Figure 3.6 Representative photomicrograph of a rat liver from the Intralipid® treated group. Steatosis was indicated by intracytoplasmic fat globules (F) in the hepatocytes. (Hematoxylin & eosin, original magnification, x 880).

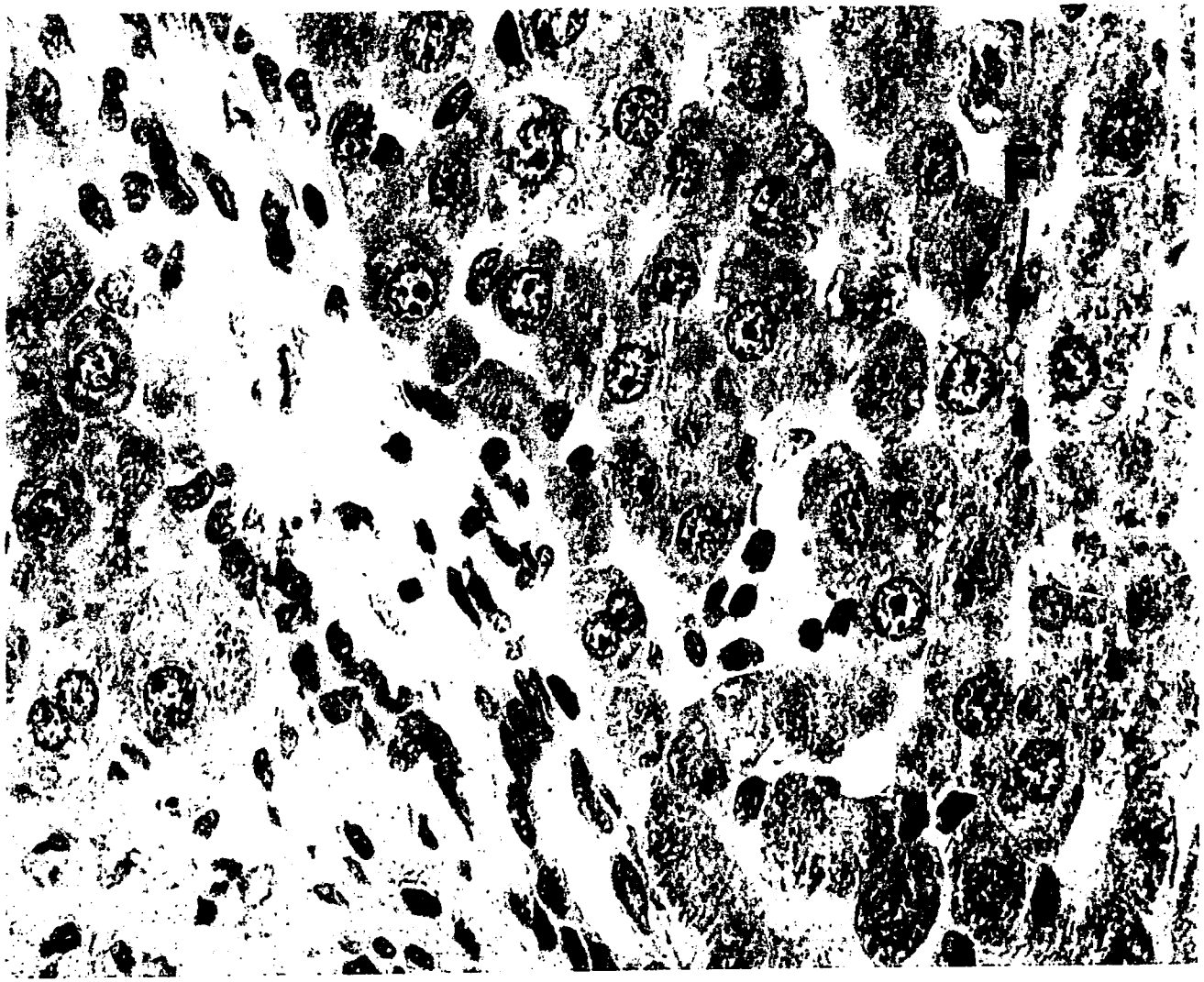

Figure 3.7 Representative photomicrograph of a rat liver from an animal in the PN (dextrose/amino acids protected from light). Mild steatosis as indicated by the fat droplets (F) is evident in hepatocytes of acinar zone 3 (pericentral region). (Hematoxylin & eosin, original magnification, x 880).

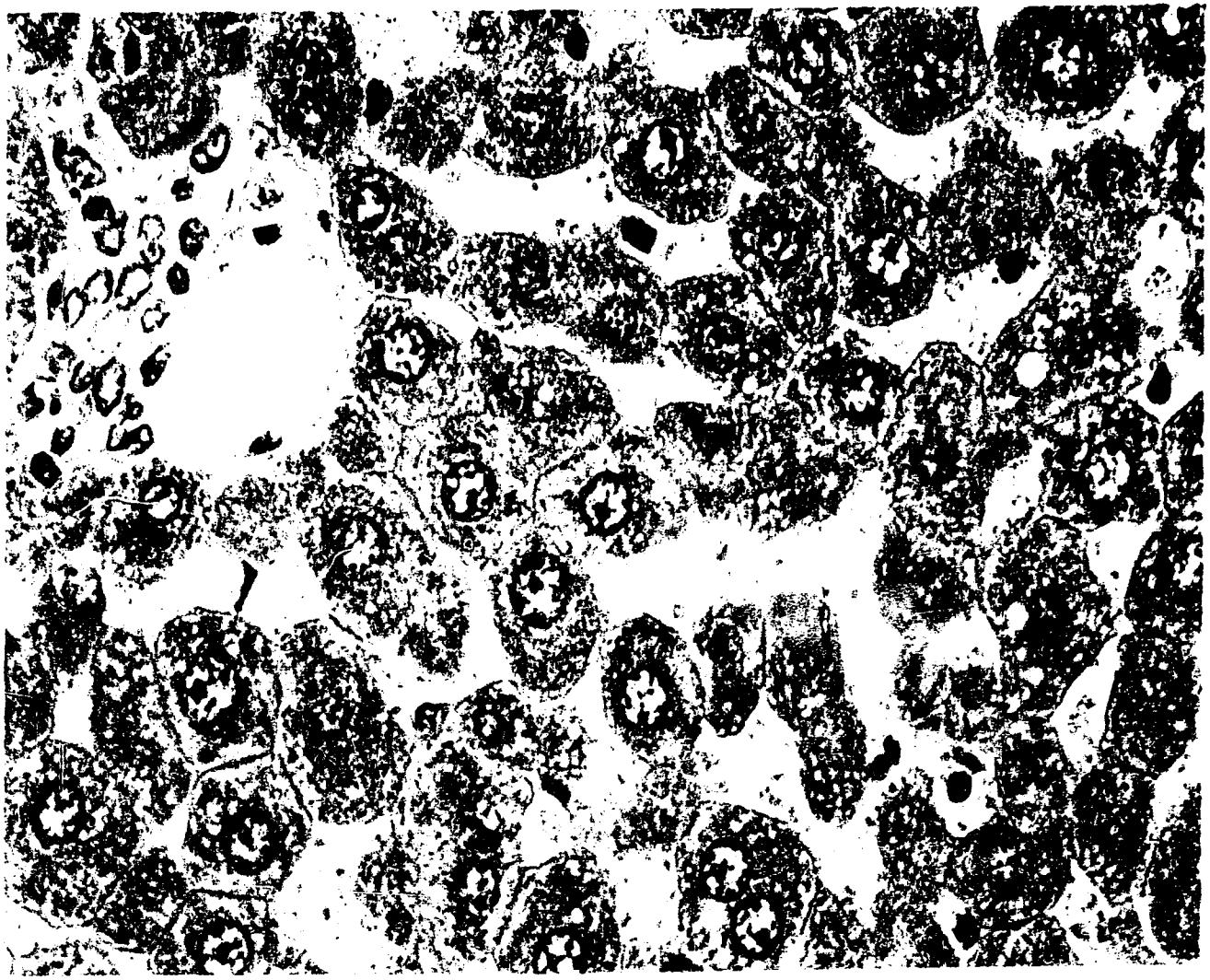

Figure 3.8 Representative photomicrograph of a rat liver from the CF group (chow-fed), showing normal cellular architecture. (Hematoxylin & eosin, original magnification, x 880).

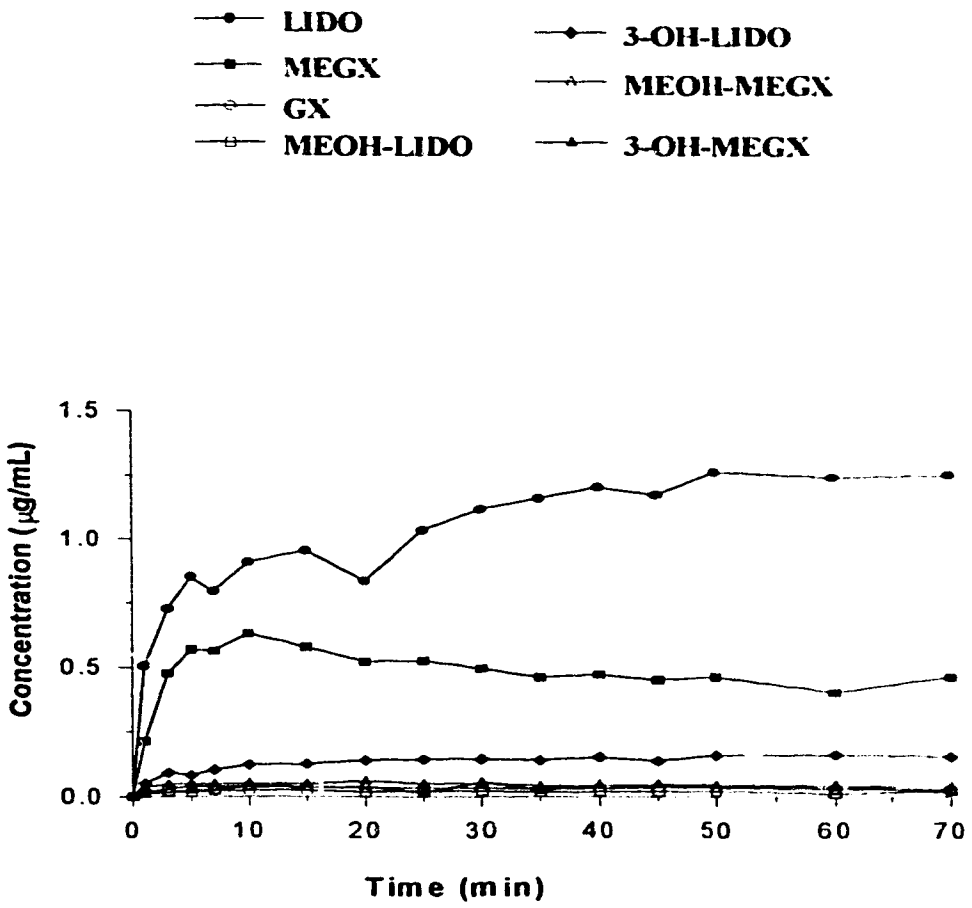

Figure 3.9 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused livers of rats in the PNL group (amino acid, dextrose and lipid treated animals), C_{in} = 13.92 µM.

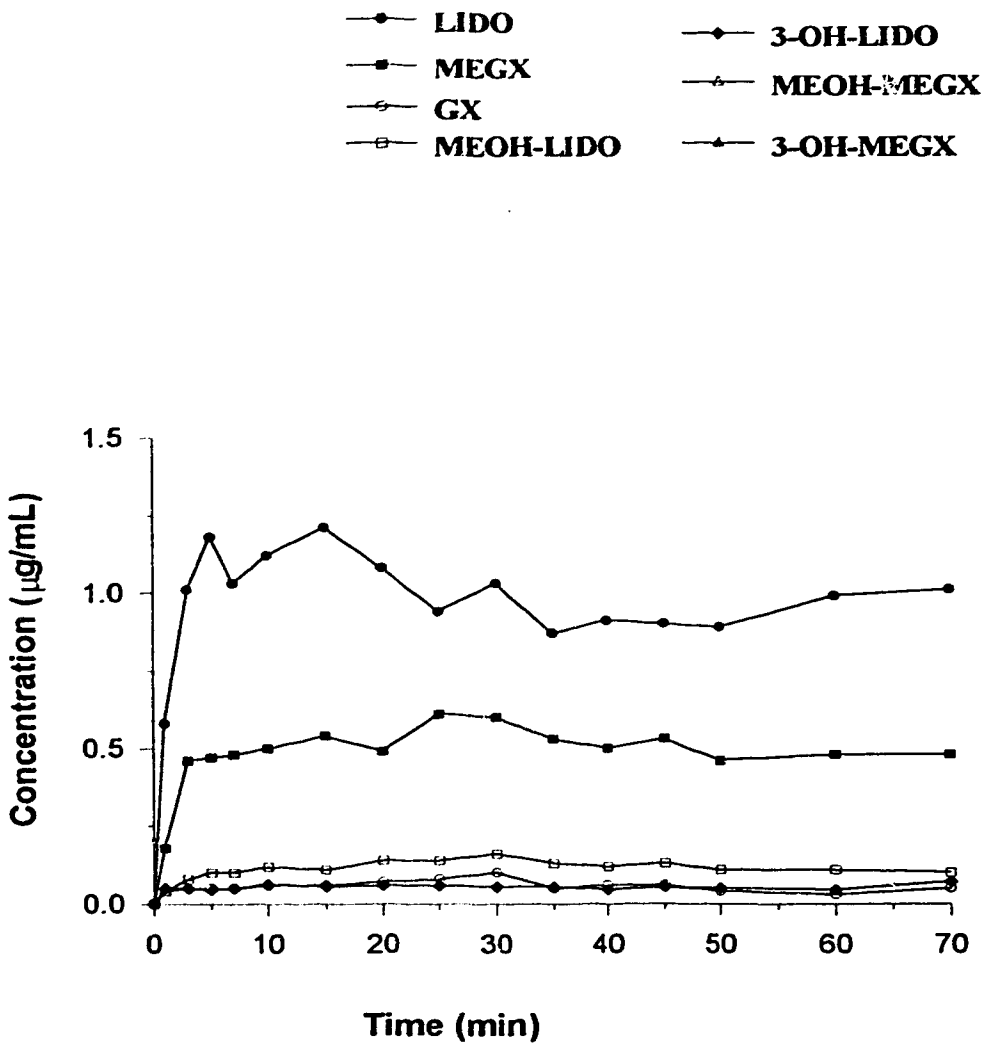

Figure 3.10 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused rat livers from PN animals (dextrose/amino acid treated animals), C_{in} = 14.56 µM.

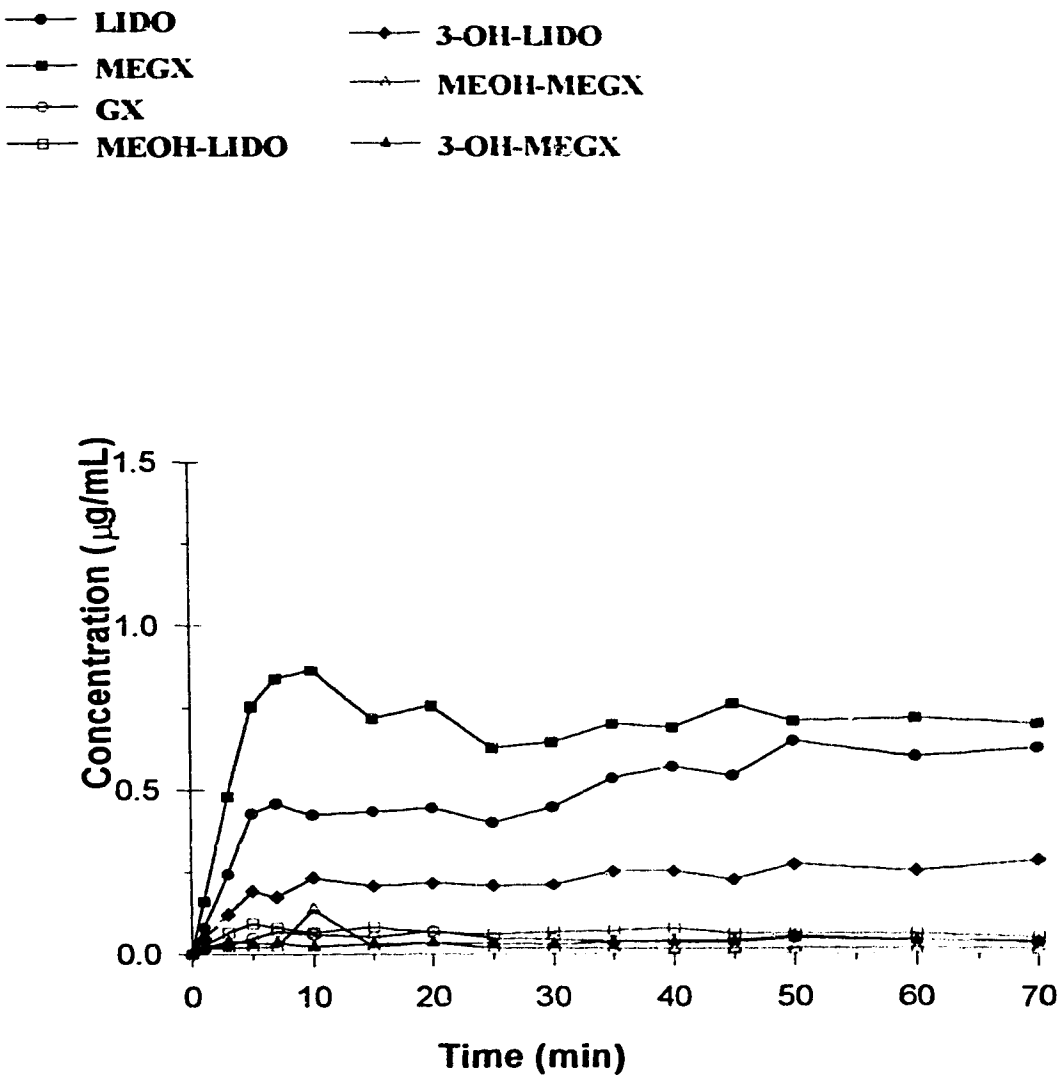

Figure 3.11 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused rat livers from chow-fed animals (CF group), C_{in} =16.21 μM.

Taurine Study

3.3.1 Body and Liver Weight Changes

All animals had similar body weights (CF, 224.50 ± 22.06 g; PN, 213.80 ± 19.59 g; PNT15, 224.25 ± 10.72 g; PNT50; 211.50 ± 11.82 g) before initiation of the experiment. Despite healthy appearances of all animals at the end of each experiment, only animals in the chow-fed group exhibited a significant weight gain of 7.55 ± 1.52 g/day, whereas animals in all the PN groups showed no marked changes (CF, 277.33 ± 21.79 g *vs* PN, 216.20 ± 19.23 g; PNT15, 215.75 ± 6.65 g; PNT50, 218.75 ± 23.78 g; $P < 0.05$). The liver weights in groups PN and PNT15 animals were similar and they were significantly lower than those of groups CF and PNT50 animals (Table 3.13). When liver weight was expressed as a percent of body weight, there was no significant differences among the groups (CF, 3.98 ± 0.30 %; PN, 3.69 ± 0.31 %; PNT15, 3.82 ± 0.23 %; PNT50, 4.56 ± 1.08 %).

3.3.2 Liver Functions and Serum Amino Acids

Serum LFT values among the groups were within the normal ranges reported for rats (see appendix 1) despite a significant reduction of serum AST and BILI levels in the PNT50 group (Table 3.14). Although the WBC value in the PNT15 group was found to be significantly higher than that of the chow-fed, the values in all the groups were within normal ranges (*Guide to the care and use of experimental animals*, 1984), indicating an absence of sepsis. Serum levels of serine, glycine, and phenylalanine were significantly higher in all the PN-treated groups than those of chow-fed. In addition, levels of alanine,

methionine and tryptophan in groups PN and PNT50, taurine in the PN group, aspartic acid and glutamine in the PNT15 group, valine and isoleucine in the PNT50 group, were also found to be significantly increased when compared to that of chow-fed animals ($P < 0.05$) (Table 3.15).

3.3.3 Liver Histology

The steatosis scores of the four treatment groups are shown in Table 3.16. Examination of liver specimens revealed that the incidence of steatosis was higher in taurine treated rats. Three out of four PNT50 animals developed significant steatosis, two displayed mild steatosis with a score of 2 and one had moderate steatosis with a score of 3. Two out of four rats in the PNT15 group developed mild steatosis and received a score of 2. Two out of five liver specimens from the PN group were also mildly steatotic. All liver specimens from CF animals had no sign of steatosis (Fig. 3.8). In the photomicrographs, steatosis was visually characterized by fatty degeneration of hepatocytes and the presence of numerous, clear intracytoplasmic vacuoles representing fat globules. Steatosis was found in the pericentral region in the PN liver samples (Fig. 3.7), whereas this condition was predominant in the periportal regions of the livers obtained from PNT15 group (Fig. 3.12). Steatosis was present in all the zones in the liver specimens of PNT50 animals (Fig. 3.13). There was no evidence of hepatocellular injury or inflammation in all three PN-treated rat livers.

3.3.4 Lidocaine Elimination

LIDO elimination was impaired in all PN-treated animals and the extent of impairment was highest in the PNT50 group. Representative concentration *vs* time profiles of LIDO and its metabolites in the four animal groups are shown in Figures 3.10, 3.11, 3.14, and 3.15. Despite an unchanged C_{in} of LIDO, the steady state C_{out} of LIDO was the highest in PNT50 animals (Fig. 3.15), followed by groups PNT15 (Fig. 3.14) and PN (Fig. 3.10), and lowest in chow-fed animals (Fig. 3.11 & Table 3.13). Similarly, a comparison of kinetic parameters (Table 3.13) reveals that Cl_{int} values were the lowest in the PNT50 group, followed by the PNT15 and PN groups. The significant reduction in Cl_{int} values in the three PN groups particularly in the PNT50 group, which has a mean value approximately one-seventh of that of group CF, clearly suggest that LIDO metabolism is severely compromised. Although the rate of LIDO elimination was the lowest in PNT50 animals, the values did not differ significantly from those of the PNT15 group (Table 3.13).

Steady state percent recoveries of LIDO and its metabolites are shown in Table 3.17. A reduction in the rate and extent of LIDO metabolism was most prominent in the PNT50 group. Three metabolites, MEOH-LIDO, 3-OH-MEGX and MEOH-MEGX were not detected in this group. LIDO metabolism was also reduced in the PNT15 and PN groups. Although levels of MEGX were the lowest in the PNT50 group, they were not significantly different from the other animal groups. A significant rise in the levels of GX was found in the PNT50 group when compared to values of the PNT15 group ($P < 0.05$). Levels of MEOH-LIDO were significantly lower in the animals belonging to the PN and

PNT15 groups. MEOH-MEGX was not detected in the PN group but levels of this metabolite were similar between the CF and PNT15 animals. The ring hydroxylated metabolite, 3-OH-LIDO was produced in similar amounts in all animal groups (Table 3.17). Besides the changes in the known metabolite levels, the unknown metabolite levels were also found to be the highest in the CF group and the lowest in the PNT50 group (PNT50, 29.81 ± 14.41; PNT15, 34.76 ± 14.96; PN, 46.54 ± 6.02; *vs* CF, 55.18 ± 5.44; μM $P < 0.05$) indicating a lower rate of metabolism in these unknown pathways. Despite an unchanged MEGX level in the three PN groups, the calculation of molar ratios of metabolites to their precursors (Table 3.18) revealed that N-dealkylation, as indicated by MEGX/LIDO was reduced in all PN treated groups, PNT50 being the lowest. Similarly, ring hydroxylation as indicated by the 3-OH-LIDO/LIDO was significantly reduced in PN and PNT50 groups when compared to chow-fed animals ($P < 0.05$); the PNT50 group has a significantly lower value than that of the PN group. Aryl methyl hydroxylation as indicated by the MeOH-LIDO/LIDO ratio was similar between the PN and PNT15 groups; this ratio was significantly reduced when compared to chow-fed values ($P < 0.05$). Consistent with the kinetic parameters obtained for LIDO, PNT50 animals had the most pronounced reduction in the rate and extent of LIDO metabolism.

TABLE 3.13 **Steady state kinetic parameters of LIDO in perfused rat livers that were harvested after short term parenteral nutrition containing taurine supplementation[a]**

Kinetic Parameters	CF	PN	PNT15	PNT50
Q (mL/min/g)	2.83 ± 0.35	3.95 ± 0.40[b]	3.76± 0.30[b]	3.26± 0.50[c]
C_{in} (μM)	15.53 ± 1.62	15.35 ± 1.48	15.45 ± 1.00	15.62 ± 1.30
C_{out} (μM)	1.77 ± 0.81	3.99 ± 0.47[b]	4.78 ±0.95[bc]	7.39 ± 3.08[bc]
Liver (g)	11.1 ± 1.51	7.95 ± 0.84[bd]	8.24 ±0.50[bd]	9.87 ± 2.00
Cl_{int} (mL/min/g)	28.06 ±16.40	11.33 ± 1.62[b]	8.82 ± 1.99[bc]	4.87 ± 3.62[bc]

[a] Values are represented as mean ± SD.

[b] $P < 0.05$ *vs* CF group

[c] $P < 0.05$ *vs* PN group.

[d] $P < 0.05$ *vs* PNT50 group

CF = chow-fed (n = 6); PN = dextrose/amino acid group (n = 5).

PNT15 = PN + taurine 15 mg/dL (n = 4); PNT50 = PN + taurine 50 mg/dL (n = 4).

TABLE 3.14 Concentrations of serum liver function test values in the four treatment groups[a]

Tests	CF	PN	PNT15	PNT50
AST (U/L)	115.17 ± 21.01	120.9 ± 21.99	111.23 ± 27.52	42.60 ± 26.05[bce]
ALT (U/L)	35.10 ± 4.19	29.40 ± 11.07	25.80 ± 11.28	39.28 ± 26.45
ALP (U/L)	364.98 ± 226.73	295.78 ± 219.11	271.68 ± 277.89	208.03 ± 194.34
BILI (mg/dL)	0.74 ± 0.38	1.22 ± 0.71	0.93 ± 0.91	0.27 ± 0.09[bce]
α-GT (U/L)	57.03 ± 37.91	35.26 ± 20.91	30.48 ± 19.97	20.50 ± 6.08
WBC (x $10^3/mm^3$)	10.42 ± 1.55	12.76 ± 1.21	12.88 ± 1.74[b]	12.65 ± 0.95
Hb (g/dL)	11.92 ± 1.92	12.68 ± 1.36	10.78 ± 1.31	13.65 ± 0.70

[a] Values are represented as mean ± SD.

[b] $P < 0.05$ *vs* CF group.

[c] $P < 0.05$ *vs* PN group.

[e] $P < 0.05$ *vs* PNT15 group.

CF = chow-fed (n = 6); PN = dextrose/amino acid group (n = 5).

PNT15 = PN + taurine 15 mg/dL (n = 4); PNT50 = PN + taurine 50 mg/dL (n = 4).

TABLE 3.15 Serum amino acid concentration in μmol/litre on day 8 in four treatment groups[a]

Amino acid	CF	PN	PNT15	PNT50
Aspartic acid	15 ± 2	21 ± 5	27 ± 6[b]	12 ± 5[ce]
Glutamic acid	75 ± 23	89 ± 15	105 ± 20	94 ± 20
Asparagine	102 ± 34	59 ± 26	56 ± 19	90 ± 54
Serine	276 ± 84	551 ± 92[bc]	440 ± 33[b]	509 ± 41[bc]
Glutamine	662 ± 22	750 ± 133	551 ± 61[bc]	587 ± 79
Histidine	71 ± 4[c]	218 ± 62	118 ± 57[c]	109 ± 9[c]
Glycine	272 ± 36	1439 ± 319[b]	745 ± 211[bc]	980 ± 82[bc]
Threonine	272 ± 57	463 ± 27	351 ± 80	362 ± 28
Citrulline	53 ± 5	46 ± 31	52 ± 20	37 ± 5[c]
Arginine	222 ± 28	269 ± 89	192 ± 43	224 ± 27
Taurine	209 ± 58	475 ± 205[b]	261 ± 108	275 ± 52
Alanine	632 ± 102	1286 ± 616[be]	514 ± 61	1276 ± 324[be]
Tyrosine	91 ± 15	99.0 ± 74	83 ± 5	103 ± 52
Tryptophan	78 ± 14	125 ± 14[b]	94 ± 29	117 ± 8[b]
Methionine	58 ± 7	210 ± 52[b]	103 ± 53[c]	124 ± 4[bc]
Valine	188 ± 31	224 ± 33	175 ± 74	259 ± 21[bc]
Phenylalanine	60 ± 8	141 ± 49[b]	92 ± 21[bc]	131 ± 43[b]
Isoleucine	98 ± 18	101 ± 31	90 ± 26	133 ± 5[be]
Leucine	153 ± 17	133 ± 45	117 ± 29	168 ± 37
Ornithine	128 ± 60	281 ± 205	231 ± 146	207 ± 57
Lycine	1060 ± 403	948 ± 430	642 ± 104	596 ± 215

[a] Values are represented as mean ± SD (n = 4 in each group).

[b] $P < 0.05$ *vs* CF group; [c] $P < 0.05$ *vs* PN group; [d] $P < 0.05$ *vs* PNT50 group; [e] $P < 0.05$ *vs* PNT15 group.

CF = chow-fed; PN = dextrose/amino acid group; PNT15 = PN + taurine 15 mg/dL; PNT50 = PN + taurine 50 mg/dL.

TABLE 3.16 **The steatosis scores of livers obtained from the four treatment groups in the taurine supplemented PN study**

	Steatosis Score			
	1 = none	**2 = mild**	**3 = moderate**	**4 = severe**
CF1	1			
CF2	1			
CF3	1			
CF4	1			
CF5	1			
CF6	1			
PN1	1			
PN2		2		
PN3	1			
PN4		2		
PN5	1			
PNT151	1			
PNT152		2		
PNT153		2		
PNT154	1			
PNT501			3	
PNT502		2		
PNT503	1			
PNT504		2		

CF = chow-fed; PN = amino acid and dextrose group; PNT15 = amino acid, dextrose and taurine (15 mg/dL); PNT50 = amino acid, dextrose and taurine (50 mg/dL).

TABLE 3.17 Mean steady state recoveries (% LIDO dose) of LIDO and its metabolites in the four treatments[a]

Compound	CF	PN	PNT15	PNT50
LIDO	11.35 ± 5.28	25.92 ± 2.09[b]	30.71 ± 4.37[b]	47.07 ± 18.60[bc]
MEGX	21.34 ± 3.16	19.85 ± 5.48	15.56 ± 7.43	12.54 ± 8.46
GX	1.74 ± 0.76	1.58 ± 0.69	1.17 ± 0.54	4.78 ± 3.20[c]
3-OH-LIDO	5.83 ± 1.70	5.23 ± 2.70	9.52 ± 4.04	5.28 ± 2.80
MEOH-LIDO	2.48 ± 0.83	0.65 ± 0.48[b]	0.80 ± 0.25[b]	ND
3-OH-MEGX	1.71 ± 1.01	1.76 ± 1.01	1.09 ± 0.37	ND
MEOH-MEGX	0.92 ± 0.58	ND	0.62 ± 0.05	ND
TOTAL	**44.82 ± 5.44**	**53.46 ± 6.02[b]**	**59.11 ± 6.93[b]**	**66.47 ± 13.61[bc]**

[a]Values are represented as mean ± SD.

[b] $P < 0.05$ *vs* CF group.

[c] $P < 0.05$ *vs* PN group.

[c] $P < 0.05$ *vs* PNT15 group.

ND = not detected.

CF = chow-fed (n = 6); PN = dextrose/amino acid group (n = 5).

PNT15 = PN + taurine 15 mg/dL (n = 4); PNT50 = PN + taurine 50 mg/dL (n = 4).

TABLE 3.18 Metabolic ratios of the four treatment groups[a]

Molar ratio	CF	PN	PNT15	PNT50
MEGX/LIDO	2.32 ± 1.32	0.76 ± 0.21[b]	0.52 ± 0.26[b]	0.36 ± 0.32[bc]
3-OH-LIDO/LIDO	0.64 ± 0.42	0.20 ± 0.11[b]	0.33 ± 0.13	0.12 ± 0.02[bc]
MEOH-LIDO/LIDO	0.26 ± 0.14	0.024 ± 0.018[b]	0.03 ± 0.02[b]	ND

[a] Values are represented as mean ± SD.

[b] $P < 0.05$ *vs* CF group.

[c] $P < 0.05$ *vs* PN group.

[e] $P < 0.05$ vs PNT15 group.

CF = chow-fed (n = 6); PN = dextrose/amino acid group (n = 5).

PNT15 = PN + taurine 15 mg/dL (n = 4); PNT50 = PN + taurine 50 mg/dL (n = 4).

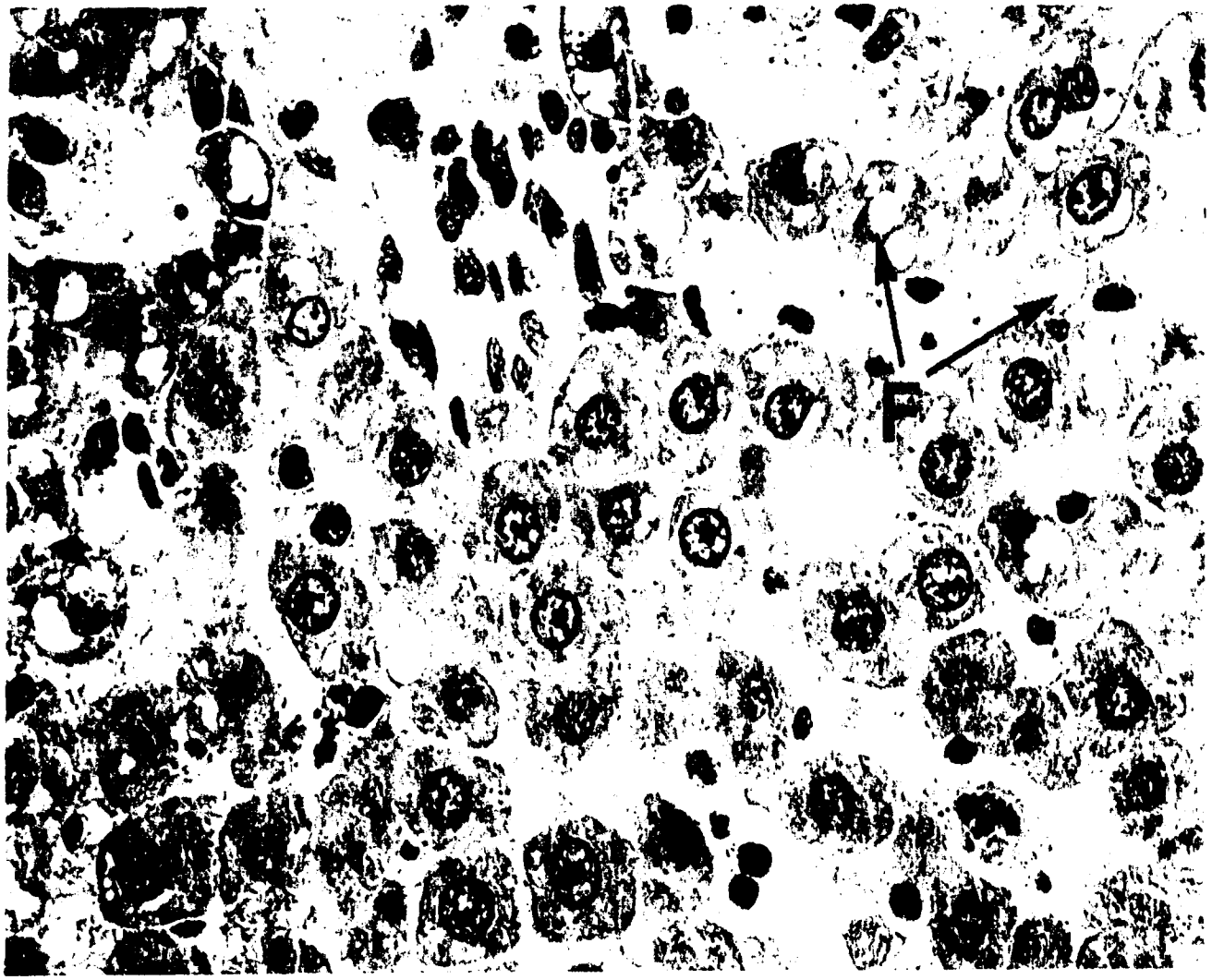

Figure 3.12 Representative photomicrograph from PNT15 group (dextrose/amino acids, and taurine 15 mg/dL). Fat droplets (F) is evident in acinar zone 1 (periportal region). (Hematoxylin & eosin, original magnification, x 880).

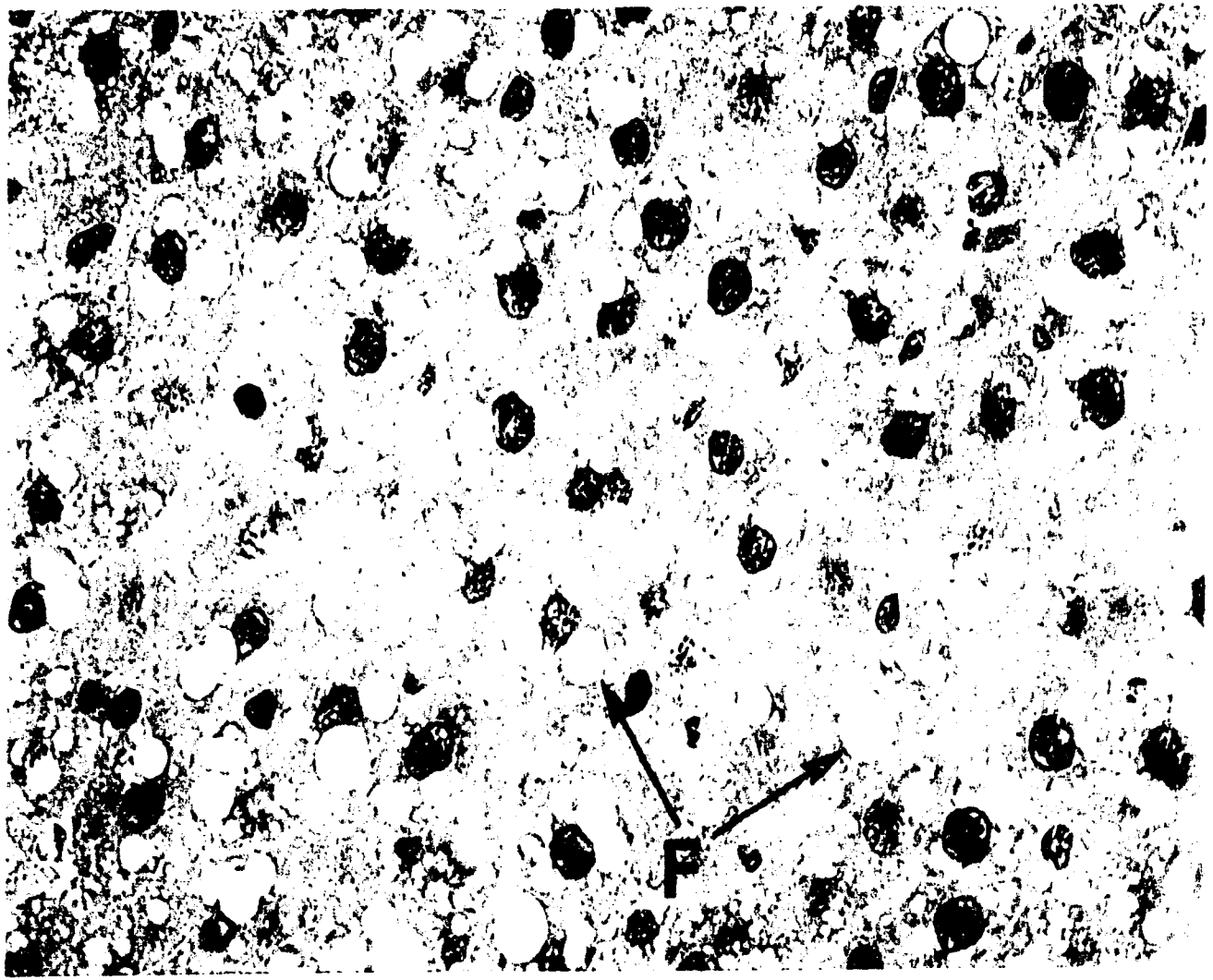

Figure 3.13 Representative photomicrograph from PNT50 group (amino acids, dextrose and taurine 50 mg/dL). Fat droplets (*F*) are evident in hepatocytes of all zones. (Hematoxylin & eosin, original magnification, x 880).

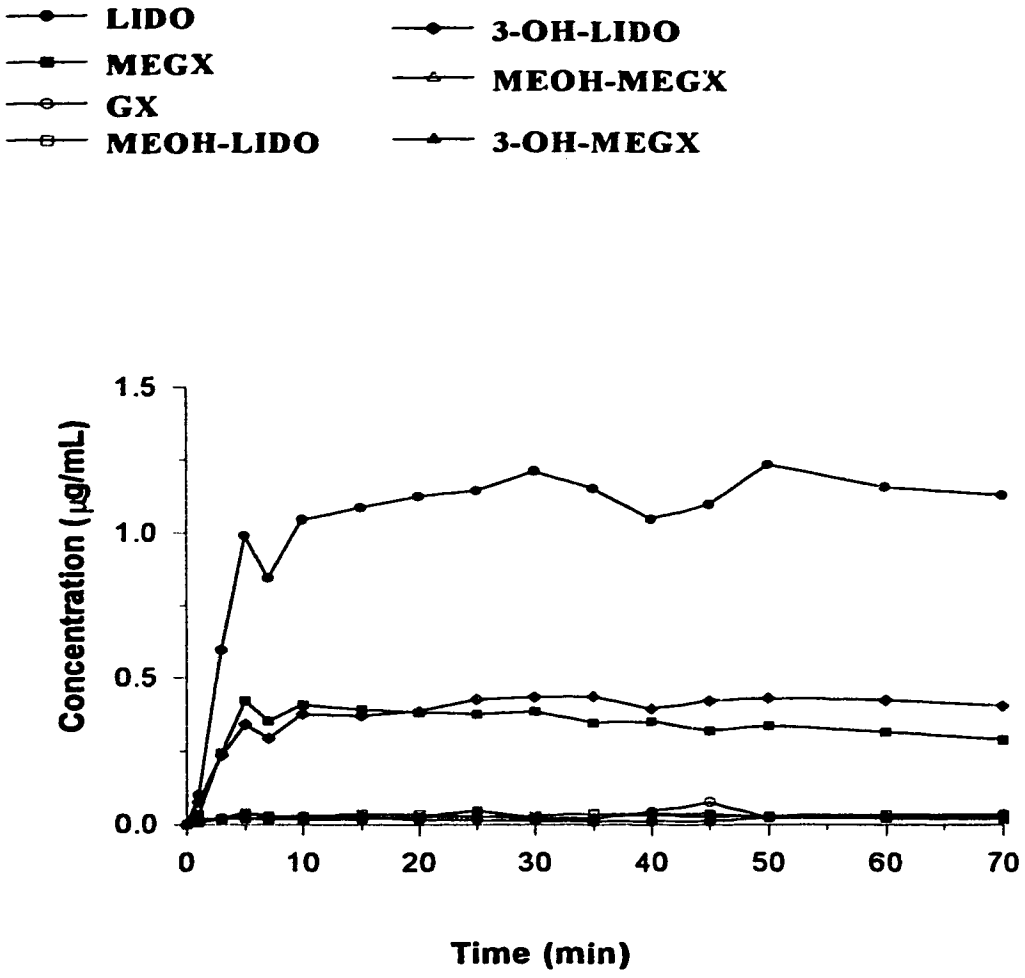

Figure 3.14 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused liver from a rat in the PNT15 group (dextrose/amino acid, and taurine 15 mg/dL treated animals, C_{in} = 15.55 µM.

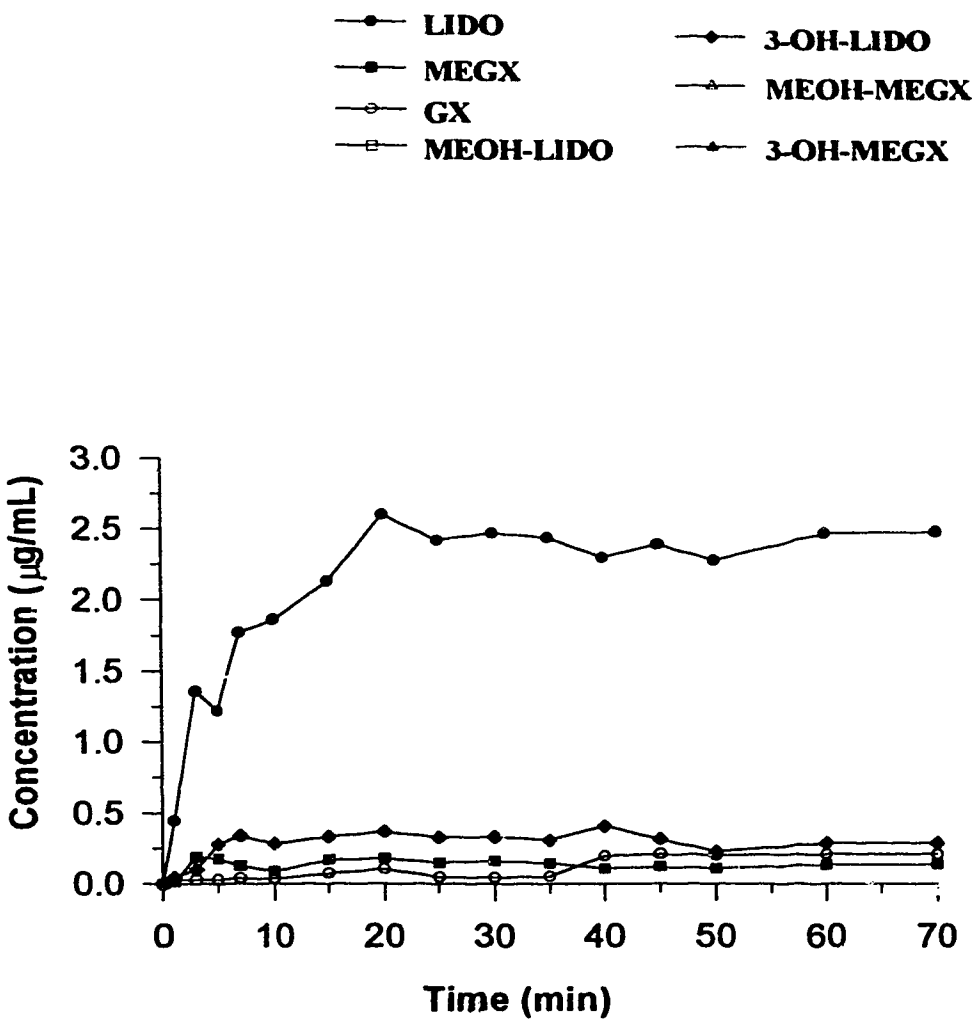

Figure 3.15 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused liver from a rat in the PNT50 group (dextrose/amino acid, and taurine 50 mg/dL treated animals, C_{in} = 15.63 μM.

3.4 Amino Acid Photooxidation Study

3.4.1 Body and Liver Weight Changes

All animals had similar body weights [PN(+L), 220.58 ± 13.29 g; PN, 213.80 ± 19.59 g; CF, 224.50 ± 22.06 g] before initiation of the experiment. Despite a healthy appearance of all animals at the end of each experiment, only chow-fed animals exhibited a significant weight gain of 7.55 ± 1.52 g/day. In contrast, animals in the PN(+L) and PN groups did not show any changes in body weight throughout the study. Liver weights were significantly lower in PN animals when compared to the other two groups (Table 3.19). When liver weights were expressed as percent body weights, PN(+L) animals were found to have the highest value [PN(+L), 4.72 ± 0.58 %; *vs* PN, 3.69 ± 0.31 %; and CF, 3.98 ± 0.30 %, $P < 0.05$].

3.4.2 Liver Functions and Serum Amino Acids

There were no significant differences among the groups in any of the serum LFT values, despite a lower total BILI value in the PN(+L) group (Table 3.20). All values were within the normal ranges reported (see appendix 1). WBC value was found to be significantly higher in the PN group but there was no indication of sepsis as the WBC and Hb values among groups were within the normal ranges reported for rats (5-13 x $10^3/mm^3$) (*Guide to the care and use of experimental animals,* 1984).

Serum concentrations of glycine, serine, taurine, tryptophan, methionine, phenylalanine and histidine in both PN groups were significantly higher than chow-fed values (Table 3.21) ($P < 0.05$).

3.4.3 Photooxidation of Amino Acids

The color of the mixed PN solution changed from a yellow to brown after light exposure. This was attributed to the Millard reaction (*Merritt et al., 1984*) in which dextrose and amino acid solutions turn brown on heating. The light source used in this experiment generated heat; the infusion bags felt warm after the 24 hr light exposure. This might be a sufficient explanation of the browning of the solution. In contrast, solution of all four amino acids changed from yellow to almost colorless in the presence of riboflavin and multivitamin after 24 hr of light exposure. When no additives were present, only tryptophan solutions changed from colorless to yellow after light exposure.

The extent of amino acid decomposition was studied in the PN mixtures and in solutions containing single amino acids. Only one amino acid, methionine, was significantly reduced (8.3% to 20%) in all test conditions except when no sensitizer was present (Table 3.22). The percent decomposition of methionine in the presence of sodium bisulfite alone was not significantly different from the observed changes in the presence of riboflavin or multivitamin (Table 3.22). Among the four amino acids, tryptophan showed a small but consistent 5-6% reduction in all of the six test conditions (Table 3.22). Histidine showed a significant decomposition in the presence of riboflavin. Tyrosine concentration remained unaffected in all tests performed.

From this study, it is apparent that components present in multivitamin other than riboflavin do not induce changes in the rates of amino acid decomposition. Interestingly, histidine seemed to have been stabilized when present in the PN solutions or in presence of

multivitamin (Table 3.22). The UV spectrum of tryptophan after light exposure of solution (with or without sensitizers) revealed the presence of a new small absorbance peak at 324 nm, which did not appear to affect the magnitude of the absorbance peak of tryptophan at 298 nm. The presence of an adduct which absorbed at 324 nm may explain the small (5%) decomposition of tryptophan taking place after light exposure in each of the test conditions. Significant alterations to the characteristic UV absorption band of riboflavin in the 300-450 nm range were observed in all four individual amino acid solutions analyzed after light exposure. This change is consistent with the formation of amino acid-riboflavin adducts as previously reported (*Merritt et al., 1984*; *Donoso et al., 1988*; *Grant et al., 1977*; *Bhatia et al., 1985*), and may be responsible for the loss of the yellow color of the riboflavin- or multivitamin-containing amino acid solutions after light exposure.

3.4.4 Liver Histology

The steatosis scores of the three treatment groups are shown in Table 3.23. Examination of liver specimens from PN(+L) rats revealed that significant steatosis had developed in five out of six liver specimens. Two livers in the PN(+L) animals developed severe steatosis, and the remainder had mild steatosis. Severe steatosis was evident in the hepatocytes throughout the liver (Fig. 3.16). Two out of five liver specimens obtained from PN animals exhibited mild steatosis (Fig 3.7), but there was no evidence of cell injury or inflammation. Liver specimens from chow-fed animals were normal (Fig. 3.8).

3.4.5 Lidocaine Elimination

Representative concentration *vs* time profiles of LIDO and its metabolites in the three treatment groups are shown in Figures 3.10, 3.11, & 3.17. The effluent LIDO concentration was the highest in PN(+L) group, and lowest in chow-fed animals. Values are also significantly different in all three groups (Table 3.19).

A comparison of the kinetic parameters provided in Table 3.19 reveals that the Cl_{int} value was the lowest in PN(+L) animals ($P < 0.05$), which had a mean value of approximately one-sixth of that of CF group. This observation clearly suggests that LIDO metabolism is severely compromised, when animals are given PN solutions that had been exposed to light prior to infusion.

Steady state percent recoveries of LIDO and its metabolites are shown in Table 3.24. A significant increase in the recovery of unchanged LIDO was observed in both PN groups relative to chow-fed values. MEGX levels were the lowest in the PN(+L) group, although a significance was not achieved due to a large variability in the MEGX data of the PN(+L) group. Levels of MEOH-LIDO were significantly lower in both PN(+L) and PN groups. Two metabolites, 3-OH-MEGX and MEOH-MEGX, were not detected in the PN(+L) group and MEOH-MEGX was not detected in the PN group. Levels of 3-OH-MEGX were similar in the PN and chow-fed groups (Table 3.24). Besides the alterations in the levels of known metabolites, the unknown metabolites were formed in the lowest amounts in the PN(+L) group and were formed in the highest quantities in the CF group [(PN(+L), 31.64 ± 10.24; PN, 46.54 ± 6.02; *vs* CF, 55.18 ± 5.44; μM $P < 0.05$)

indicating a lower rate and extent of metabolism in these unknown pathways. Molar ratios of metabolites to their precursors revealed that N-dealkylation, as indicated by MEGX/LIDO ratio, was reduced in both PN and PN(+L) groups (Table 3.25). Similarly, aryl methyl hydroxylation as indicated by the MeOH-LIDO/LIDO ratio was similar between the PN and PN(+L) groups and ring hydroxylation as indicated by the 3-OH-LIDO/LIDO was the lowest in PN(+L) group; both ratios were significantly reduced when compared to chow-fed values ($P < 0.05$). Consistent with the kinetic parameters obtained for LIDO, PN(+L) animals had the most pronounced reduction in the rate of LIDO metabolism.

TABLE 3.19 Steady state kinetic parameters of LIDO in perfused rat livers that were harvested after short term parenteral nutrient treatments (with and without light exposure) [a]

Kinetic parameters	PN(+L)	PN	CF
Q (mL/min/g)	3.01 ± 0.58	3.95 ± 0.40[bc]	2.83 ± 0.35
C_{in} (μM)	16.07 ± 2.14	15.35 ± 1.48	15.53 ± 1.62
C_{out} (μM) [d]	7.88 ± 3.47	3.99 ± 0.47	1.77 ± 0.81
Liver (g)	10.29 ± 174	7.95 ± 0.84[bc]	11.08 ± 1.51
Cl_{int} (mL/min/g)	5.29 ± 5.09[b]	11.33 ± 1.62[b]	28.06 ± 16.40

[a] Values are represented as mean ± SD.

[b] $P < 0.05$ *vs* CF group.

[c] $P < 0.05$ *vs* PN(+L) group.

[d] Significantly different ($P < 0.05$) from each other groups.

PN(+L) (n = 6); PN (n = 5); CF (n = 6).

TABLE 3.20 Serum concentrations of liver function values in the three treatment groups on day 8 [a]

TEST	PN(+L)	PN	CF
AST (U/L)	136.2 ± 107.33	120.9 ± 21.99	115.17 ± 21.0
ALT (U/L)	25.03 ± 7.63	29.40 ± 11.07	35.10 ± 4.19
ALP (U/L)	357.80 ± 254.55	295.78 ± 219.11	364.98 ± 226.73
BILI (mg/dL)	0.44 ± 0.31[bc]	1.22 ± 0.71	0.74 ± 0.38
γ-GT (U/L)	18.83 ± 10.24[b]	35.26 ± 20.91	57.03 ± 37.91
WBC (x 10^3/mm^3)	10.40 ± 0.73	12.76 ± 1.21[bc]	10.42 ± 1.55
Hb (g/dL)	13.38 ± 0.71	12.68 ± 1.36	11.92 ± 1.92

[a] Values are represented as mean ± SD.
[b] $P < 0.05$ vs CF group.
[c] $P < 0.05$ vs PN(+L).
[e] $P < 0.05$ *vs* PN group.
PN(+L) (n = 6); PN (n = 5); CF (n = 6).

TABLE 3.21 Serum amino acid concentrations in μmol/litre on day 8 in three groups[a]

Amino acid	PN(+L)	PN	CF
Aspartic acid	40 ± 49	21 ± 5	15 ± 2
Glutamic acid	281 ± 393	89 ± 15	74 ± 22
Asparagine	80 ± 68	59 ± 26	102 ± 34
Serine	632 ± 351[b]	551 ± 92[b]	276 ± 83
Glutamine	725 ± 557	750 ± 133	662 ± 40
Histidine	266 ± 243[b]	218 ± 61[b]	70 ± 4
Glycine	2014 ± 1697[b]	1439 ± 319[b]	272 ± 36
Threonine	680 ± 559	463 ± 269	272 ± 56
Citrulline	92 ± 75	46 ± 31	53 ± 5
Arginine	260 ± 68	269 ± 89	221 ± 27
Taurine	585 ± 517[b]	475 ± 205[b]	208 ± 57
Alanine	1813 ± 2135	1286 ± 616	631 ± 102
Tyrosine	263 ± 362	99 ± 74	91 ± 15
Tryptophan	153 ± 77[b]	125 ± 14[b]	78 ± 14
Methionine	259 ± 222[b]	210 ± 52[b]	58 ± 7
Valine	467 ± 488	224 ± 33	187 ± 31
Phenylalanine	309 ± 373[b]	141 ± 49[b]	60 ± 8
Isoleucine	242 ± 280	101 ± 31	98 ± 18
Leucine	377 ± 491	133 ± 44	153 ± 17
Ornithine	621 ± 873	281 ± 205	128 ± 60
Lycine	1221 ± 1357	948 ± 430	1060 ± 402

[a] Values are represented as mean ± S.D.

[b] $P < 0.05$ *vs* CF group.

PN(+L) (n = 6); PN (n = 5); CF (n = 6).

TABLE 3.22 **Percent reduction of the selected amino acids after light exposure (420-470 nm) and in the presence of different additives[a]**

Amino acids	Initial concentration (μmol/mL)	no additives	RB+SB	MVI+SB	SB	PN	dextrose/amino acids
Tryptophan	5.87 ± 0.31	6% (NS)	6% (NS)	5% (NS)	5% (NS)	5% (NS)	5% (NS)
Methionine	22.07 ± 1.23	< 5%	13%[b]	13%[b]	20%[b]	8.3%[b]	< 5%
Histidine	16.44 ± 1.73	< 5%	18%[b]	< 5%	< 5%	5%	< 5%
Tyrosine	1.79 ± 0.43	< 5%	< 5%	< 5%	< 5%	< 5%	< 5%

[a] Values are expressed as mean ± S.D. (n = 6)

[b] Significantly different *vs* initial value ($P < 0.05$).

RB = riboflavin; MVI = multivitamin.

SB = sodium bisulfite; NS = not significant.

TABLE 3.23 The steatosis scores of the three treatment group in the light exposed PN study

	Steatosis Score			
	1 = none	2 = mild	3 = moderate	4 = severe
CF1	1			
CF2	1			
CF3	1			
CF4	1			
CF5	1			
CF6	1			
PN1	1			
PN2		2		
PN3	1			
PN4		2		
PN5	1			
PN(+L)1				4
PN(+L)2				4
PN(+L)3		2		
PN(+L)4	1			
PN(+L)5		2		
PN(+L)6		2		

CF = chow-fed;
PN = amino acid and dextrose group;
PN(+L) = amino acid and dextrose exposed to light.

TABLE 3.24 **Mean steady state recoveries (% LIDO dose) of LIDO and its metabolites in the three treatment groups [a]**

Compound	PN(+L)	PN	CF
LIDO [d]	47.93 ± 17.59	25.92 ± 2.09	11.35 ± 5.28
MEGX	14.52 ± 12.99	19.85 ± 5.48	21.34 ± 3.16
GX	1.95 ± 1.25	1.58 ± 0.69	1.74 ± 0.76
3-OH-LIDO	3.97 ± 1.54	5.23 ± 2.70	5.83 ± 1.70
MEOH-LIDO	0.41 ± 0.09[b]	0.65 ± 0.48[b]	2.48 ± 0.83
3-OH-MEGX	ND	1.76 ± 1.01	1.71 ± 1.01
MEOH-MEGX	ND	ND	0.92 ± 0.58
TOTAL[d]	**68.36 ± 10.24**	**53.46 ± 6.02**	**44.82 ± 5.44**

[a] Values are represented as mean ± SD.
[b] $P < 0.05$ *vs* CF group.
[d] Significantly different ($p < 0.05$) from each other group.
PN(+L) (n = 6); PN (n = 5); CF (n = 6).

TABLE 3.25 Metabolic ratios in the PN(+L), PN, and CF treatment groups[a].

Molar ratio	PN(+L)	PN	CF
MEGX/LIDO	0.48 ± 0.72[b]	0.76 ± 0.21[b]	2.32 ± 1.32
3-OH-LIDO/LIDO [d]	0.10 ± 0.05	0.20 ± 0.11	0.64 ± 0.42
MEOH-LIDO/LIDO	0.008 ± 0.002[b]	0.024 ± 0.018[b]	0.26 ± 0.14

[a] Values are represented as mean ± SD.

[d] Significantly different ($P < 0.05$) from each other groups.

[b] $P < 0.05$ *vs* CF group.

PN(+L) (n = 6); PN (n = 5); CF (n = 6).

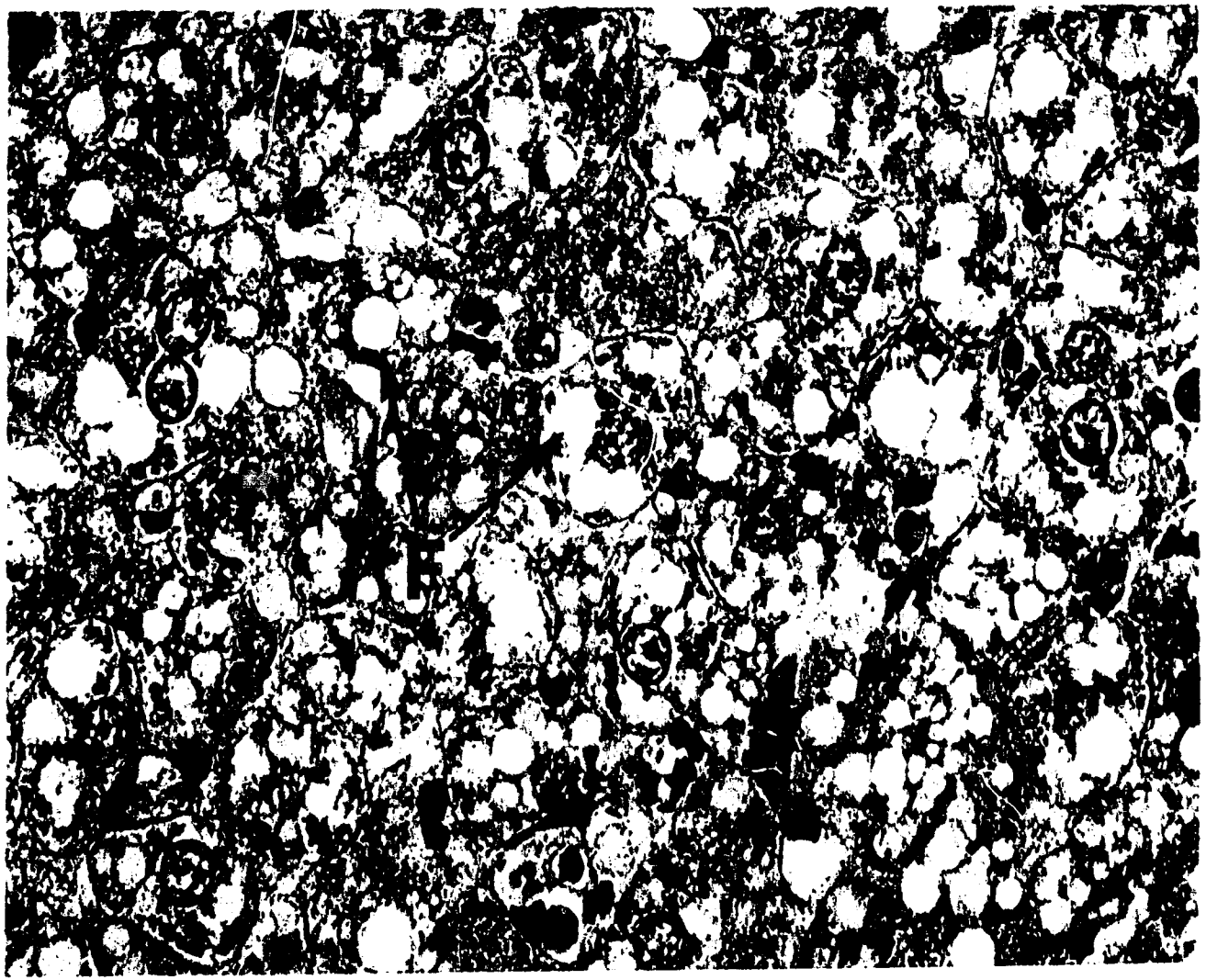

Figure 3.16 Representative photomicrograph of a liver from a rat in the PN(+L) group (dextrose/amino acids, exposed to light). Fat droplets (F) were evident in hepatocytes of all three zones. (Hematoxylin & eosin, original magnification, x 880).

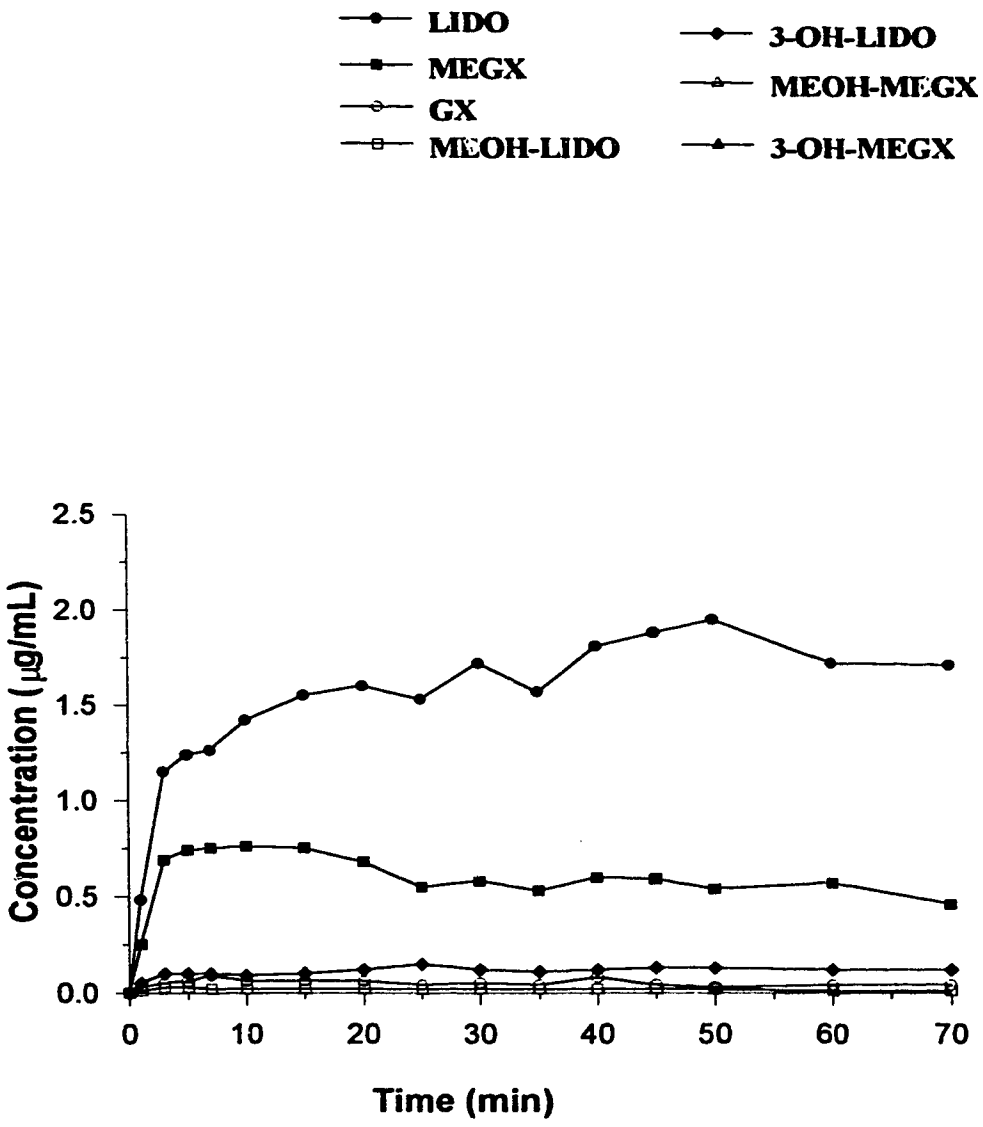

Figure 3.17 Representative concentration *vs* time profiles of LIDO and its metabolites in the effluent of perfused liver from a rat in the PN(+L) group (dextrose/ amino acid exposed to light treated animals, C_{in} = 15.51 µM.

TABLE 3.26 Steady state kinetic parameters of LIDO in perfused rat livers that were harvested after short term parenteral nutrition in six animal groups[a]

Kinetic Parameters	CF	PN	PNL	PN(+L)	PNT15	PNT50
Q (mL/min/g)	2.83 ± 0.35	3.95 ± 0.40[bdeg]	3.23 ± 0.23[cf]	3.01 ± 0.58[cf]	3.76± 0.30[bde]	3.26± 0.50[c]
C_{in} (μM)	15.53 ± 1.62	15.35 ± 1.48	16.04 ± 1.86	16.07 ± 2.14	15.45 ± 1.00	15.62 ± 1.30
C_{out} (μM)	1.77 ± 0.81	3.99 ± 0.47[b]	6.26 ± 1.41[bc]	7.88 ± 3.47[bc]	4.78 ±0.95[b]	7.39 ± 3.08[bc]
Liver (g)	11.1 ± 1.51	7.95 ± 0.84[bdeg]	9.63 ± 0.81	10.29 ± 174	8.24 ±0.50[bdeg]	9.87 ± 2.00
Cl_{int} (mL/min)	302.65 ±166.86	89.03 ± 6.14[b]	52.29 ± 22.59[bc]	43.24 ± 38.49[bc]	71.65 ± 12.70[b]	44.45 ± 30.82[bc]

[a] Values are expressed as mean ± S.D.

[b] $P < 0.05$ *vs* CF group; [c] $P < 0.05$ *vs* PN group.

[d] $P < 0.05$ *vs* PNL group; [e] $P < 0.05$ *vs* PN(+L) group.

[f] $P < 0.05$ *vs* PNT15 group; [g] $P < 0.05$ *vs* PNT50 group.

TABLE 3.27 Mean steady state recoveries (% LIDO dose) of LIDO and its metabolites in six treatment groups[a]

Compound	CF	PN	PNL	PN(+L)	PNT15	PNT50
LIDO	11.35 ± 5.28	25.92 ± 2.09[b]	39.75 ± 11.36[bc]	47.93 ± 17.59[bc]	30.71 ± 4.37[b]	47.07 ± 18.60[bc]
MEGX	21.34 ± 3.16	19.85 ± 5.48[b]	13.32 ± 3.9[b]	14.52 ± 12.99[b]	15.56 ± 7.43[b]	12.54 ± 8.46[b]
GX	1.74 ± 0.76	1.58 ± 0.69	1.07 ± 0.33	1.95 ± 1.25	1.17 ± 0.54	4.78 ± 3.20[df]
3-OH-LIDO	5.83 ± 1.70	5.23 ± 2.70	7.34 ± 1.66	3.97 ± 1.54[df]	9.52 ± 4.04	5.28 ± 2.80
MEOH-LIDO	2.48 ± 0.83	0.65 ± 0.48[b]	0.58 ± 0.14[b]	0.41 ± 0.09[bf]	0.80 ± 0.25[b]	ND
3-OH-MEGX	1.71 ± 1.01	1.76 ± 1.01	1.07 ± 0.34	ND	1.09 ± 0.37	ND
MEOH-MEGX	0.92 ± 0.58	ND	0.51 ± 0.43	ND	0.62 ± 0.05	ND
TOTAL	**44.82 ± 5.44**	**53.46 ± 6.02**	**63.64 ± 6.75[bc]**	**68.36 ± 10.24[bc]**	**59.11 ± 6.93[b]**	**66.47 ± 13.61[bc]**

[a] Values are expressed as mean ± S.D.

[b] $P < 0.05$ *vs* CF group; [c] $P < 0.05$ *vs* PN group.

[d] $P < 0.05$ *vs* PNL group; [f] $P < 0.05$ *vs* PNT15 group.

TABLE 3.28 Metabolic ratios in the six treatment groups[a]

Molar ratio	CF	PN	PNL	PN(+L)	PNT15	PNT50
MEGX/LIDO	2.32 ± 1.32	0.76 ± 0.21	0.38 ± 0.19[bc]	0.48 ± 0.72[bc]	0.52 ± 0.26[b]	0.36 ± 0.32[bc]
3-OH-LIDO/LIDO	0.64 ± 0.42	0.20 ± 0.11[b]	0.20 ± 0.08[b]	0.10 ± 0.05[bcd]	0.33 ± 0.19[eg]	0.12 ± 0.02[b]
MEOH-LIDO/LIDO	0.26 ± 0.14	0.024 ± 0.018[b]	0.02 ± 0.01[b]	0.008 ± 0.002[b]	0.03 ± 0.02[b]	ND

[a] Values are expressed as mean ± S.D.

[b] $P < 0.05$ *vs* CF group; [c] $P < 0.05$ *vs* PN group.

[d] $p < 0.05$ *vs* PNL group; [e] $P < 0.05$ *vs* PN(+L) group.

[f] $P < 0.05$ *vs* PNT15 group; [g] $P < 0.05$ *vs* PNT50 group.

4. DISCUSSION

4.1 Cholestyramine Study

There are two major findings in the 7 day and the preliminary 14 day studies: 1). Liver histological studies clearly indicate that PN administration is associated with hepatosteatosis (Table 3.4 and Fig. 3.3). CH treatment reversed the hepatoabnormality (Fig. 3.4). This result is consistent with our hypothesis that absorption of toxic secondary bile salts contributes significantly to the development of liver malfunction. Serum liver function indicators such as the AST, ALP, ALT, γ-GT, CG, BILI and TBA values were all within normal ranges (*Guide to the care and use of experimental animals,* 1984), suggesting infiltrative processes like an abcess or neoplasia within the liver (*Farkas and Hyde, 1992*) or cell necrosis and hepatobiliary functions like cholestasis caused by PN were not present (*Ke et al., 1990b*; *Knodell et al., 1984*; *Knodell et al., 1980*; *Ross et al., 1983*). The results of these studies clearly show that these LFT values are not sensitive in detecting steatosis. 2). PN infusion after a short term period reduces hepatic drug metabolism; CH treatment tends to alleviate this reduction; however, this effect was not significant.

Although the reason for the discrepancy between the LFT results of the present study and that of the literature was not readily apparent, sepsis may have played an important role in the previous studies. Lack of sepsis may be a reason for not observing signs of cholestasis in any of our animals; it is well documented that sepsis plays a role in the induction of cholestasis in PN-treated animals or humans (*Freund and Rimon, 1990*; *Wolf and Pohlandt, 1989*; *Shu et al., 1991*). Based on our results, it is likely that cholestasis may be avoided by preventing sepsis; the latter can be achieved by using strict aseptic surgical techniques.

LFT values and kinetic parameters of the 7-day and the 14-day studies were within 10% of each other. As indicated by the Cl_{int} values (Fig. 3.2), there is a clear trend in the

14-day study towards lowered drug metabolizing enzyme capacities, particularly in the PN group. This observation is mirrored by the elevation of the steady state effluent LIDO concentration (Fig. 3.2). These results are qualitatively similar to that of the 7-day study, however due to a small sample size, statistical significance was not reached among the three treatment groups.

Our kinetic and metabolic data clearly suggest that drug metabolizing enzyme activities are reduced with a 7-day PN treatment; this observation is consistent with findings by others (*Knodell et al., 1989*; *Knodell et al., 1984*; *Knodell et al., 1980*; *Ke et al., 1990b*). In the present study, reduced metabolic efficiency was indicated by lower Cl_{int} values: 61% in the PN group and 53% in the PNC group ($P < 0.05$). Reduced metabolic clearance by PN treatment resulted in an increase in the material balance by 47% in the PN group and by 31% in the PNC group compared to that of the chow-fed group (Table 3.4).

The molar ratio data (Table 3.5) reveal that a reduction in the aryl methyl hydroxylation pathway was the highest, 88% to 92% in groups PN and PNC, respectively, followed by N-dealkylation and ring hydroxylation pathways. N-Dealkylation of LIDO to MEGX is catalyzed in rats by the CYP enzymes, CYP2C11 and CYP2B1 (*Oda et al., 1989*; *Imaoka et al., 1990*). From its contribution to the mass balance, N-dealkylation appears to be a major metabolic pathway of LIDO metabolism. Reduction of CYP2C11 enzyme activity by PN treatment has also been observed *in vitro* (*Knodell et al., 1989, Knodell et al. 1987)*. These investigators found that N-dealkylation of drugs mediated by CYP2C11, such as benzphetamine, ethylmorphine and erythromycin, was significantly reduced in the intravenously fed animals, compared to intragastrically fed ones. Since LIDO metabolism involves unidentified pathway(s), the huge increase in the unchanged LIDO levels in both PN groups may not be due only to the inhibition of the two known enzymatic pathways; it is likely that some unidentified pathways may also be inhibited.

The results obtained from the CH rats are interesting because liver function and histology were apparently normal and yet LIDO metabolism was significantly reduced. The mechanism behind this observation is unknown. One possibility could be that CH alters the synthesis and turnover rate of CYP enzymes. This may be linked to the therapeutic effect of CH in treating hypercholesterolemia (*Einarsson et al., 1991*; *Carrells et al., 1991*). The effect of CH on cholesterol metabolism could alter CYP enzyme activities because cholesterol is required for the production of the lipoidal structure of cell membranes and is a constituent of endoplasmic reticulum (*Schachter, 1984*; *Gibson and Skett, 1986*). This postulation may be invalid, because CH in rat diets at a much higher dose than used in the present study did not produce any significant changes in microsomal free or serum cholesterol levels (*Pandak et al., 1994*). CH also did not change the total hepatic secretion rates of biliary lipids in human at the normal hyperlipidimic dose (*Carrells et al., 1991*). In fact, improvement of cell membrane fluidity by lipid infusion has little effect on multiple forms of CYP enzymes (*Knodell et al., 1990*). Moreover, there has been no reports in the literature to show that CH alters CYP enzyme levels. Therefore, CH is an unlikely causative factor on the observed reduction of LIDO metabolism. Drug interactions with CH have been restricted to the absorption level because of the ability of CH to bind drugs (*Bailey et al., 1995*; *Malloy et al., 1994*). Furthermore, a potential drug interaction between CH and LIDO would not occur because CH was not used during LIDO infusion. This study also clearly indicates that hepatic metabolism during a PN infusion can be independent of hepatosteatosis.

The observed reduction in LIDO metabolism that results from PN infusion may be caused by: 1) amino acid over-load/imbalance, 2) excessive sugar intake, 3) hormonal imbalance, and 4) stress or trauma. Amino acid load during PN therapy is known to reduce drug metabolism (*Knodell, 1990*; *Ke et al., 1990b*). In an *in vitro* guinea pig liver explant study Cohen (1975) observed that certain amino acids, including leucine, threonine, isoleucine and glycine, were hepatotoxic. The link between an elevation of

amino acid levels and a reduction in drug metabolism (*Ke et al., 1990b*) is not supported by the data of the present study (Table 3.3). Rats receiving CH treatment have amino acid levels similar to those of chow-fed animals and yet metabolism of LIDO is reduced.

Excess carbohydrate in the PN solution has been associated with the reduction in metabolism of a number of drugs (*Burgess et al., 1987*; *Hartshorn et al., 1979*). Carbohydrate-based PN solutions, in conjunction with an excessive amino acid supply, are believed to decrease protein synthesis and increase the process of lipogenesis (*Hartshorn et al., 1979*; *Heger and Frydrych, 1989*). Both CYP enzyme activity and lipogenesis require NADPH, (*Pantuck et al., 1995*) and a possible competition between CYP enzyme and lipogenesis for NADPH might exist, which could be partly responsible for the observed reduction in enzyme activity as observed in previous studies (*Burgess et al., 1987*; *Hartshorn et al., 1979*).

Expression and regulation of CYP enzymes are known to be influenced by different gastrointestinal and gonadal steroid hormones (*Morgan et al., 1985*; *Dannan et al., 1986*; *Fang and Strobel, 1981*). The importance of testosterone on levels of expression of male specific CYP2C11 and CYP3A1 in adult male rats have been demonstrated (*Dannan et al., 1986*; *Morgan et al., 1985*). Farrel and Koltai (1988) further demonstrated that portal bypass caused either by portacaval shunt or portal hypertension induced by portal vein inclusion is associated with testicular atrophy and low serum levels of testosterone. Such portal vein ligated male rats exhibited lowered hepatic microsomal CYP content and testosterone 16α- and 2α hydroxylation activities catalyzed almost exclusively by male specific CYP2C11 (*Farell et al., 1986*). Since the mode of PN administration also bypasses the portal circulation it may give rise to a similar situation and may help to explain our observation of reduced CYP2C11 activities. Chronic administration of pentagastrin a gastrin analogue, has also been reported to increase the levels of total CYP content (*Fang and Strobel, 1981*), and this hormone and other gut and pancreatic hormones have been found to be either reduced during a PN infusion (*Johnson*

et al., 1975; *Zamir et al., 1994*) or remain unaffected (*Knodell et al., 1984*; *Greenberg et al., 1981*) indicates towards further studies to resolve such controversy.

One other factor responsible for reduced enzymatic activity may be a poor weight gain and a reduction in liver weight in the PN animals. An argument against this possibility is that PN-treated animals that gained the same weight as chow-fed animals also exhibited a reduction in drug metabolism (*Ke et al., 1990b*). It has been observed (*Lickley et al., 1978*) that intravenously fed animals gained less weight than intragastrically fed animals. This finding cannot be explained in terms of total calorie intake, which was ~ 33 kcal/100g/day in our study, and was found to be nutritionally sufficient (*Knodell, 1990*; *Ross et al., 1984*; *Ross et al., 1983*). The lack of appreciable weight gain by PN animals observed by us has also been reported by others (*Lickley et al., 1978*). Goodgame *et al.* (1978) demonstrated that body weight gain is not an adequate parameter of nutritional completeness in the parenterally fed rats. It is likely that bypassing the portal system during the PN administration reduces the efficiency with which these nutrients are ultimately metabolized (*Goodgame et al., 1978*). This hypothesis can be substantiated by the fact that orally fed animals even when they are fed the same glucose/amino acid mixture as the parenterally fed rats results in an increase in the serum levels of insulin and glucagon, the hormones that are believed to contribute largely to the disposal of nutrients and thus results in greater weight gain in the intragastrically fed animals (*Lickley et al., 1978*).

Finally, the likely contribution from any stress ar surgery related trauma in the PN-related reduction of drug metabolism in the present study could be eliminated on the basis that an optimum recovery period of three to four days were provided for these animals which is important and has been found to eliminate the artifact caused by the metabolic stress response from operative trauma (*Popp et al., 1982*). All rats in this study grew normally and gained weight (5 g/day) during the postoperative period. During PN

infusion, the rats ceased to gain weight, whereas the chow-fed rats maintained their rate of growth.

In conclusion, it has been shown that oral administration of CH prevents hepatic dysfunctions including steatosis induced by PN, possibly by interrupting secondary bile acid reabsorption, and that CH has very little effect in preventing a reduction in LIDO metabolism in rats that were receiving PN.

4.2 Lipid Study

PN is associated with hepatic dysfunction (*Merritt, 1986*; *Klein and Nealon, 1988*) and may also affect hepatic drug elimination (*Knodell et al., 1980*; *Knodell et al., 1984*; *Ross et al., 1983*; *Ke et al., 1990b*). The underlying mechanisms by which PN may alter drug elimination are still not well understood. Ke *et al.* (1990b) have shown that a dextrose and amino acid-based PN produced a significant alteration in LIDO metabolism after a short term PN treatment. Ross *et al.* (1983) in another study observed unchanged antipyrine pharmacokinetics when a fat-based PN was used.

The current objective was to evaluate the effect of Intralipid® on hepatic functions and on elimination of LIDO after a short term treatment of PN in an animal model.

The collected data showed that the C_{out} of LIDO at steady state (Table 3.7) increased almost four times in the PNL group and twice as much in the PN group relative to the CF group, suggesting impaired drug elimination. The metabolic reduction was indicated by the a decrease in Cl_{int} by 82% in the PNL group and by 70% in the PN group ($P < 0.05$). Reduced metabolic clearance also resulted in an increase in the material balance of LIDO by 42% in the PNL group and by 19% in the PN group compared to the CF group. The

formation of the N-dealkylated metabolite of LIDO, MEGX, is catalyzed by CYP2C11 in rats (*Oda et al., 1989*; *Imaoka et al., 1990*), and accounts approximately for about 40 to 45% of the measured mass balance. The molar ratio data (Table 3.12) reveals a 83% reduction in the PNL group and a 67% reduction in the PN group ($P < 0.05$). This is consistent with the findings of the *in vitro* microsomal study, conducted by Knodell *et al.* (1989), who showed that N-dealkylations of benzphetamine, ethylmorphine and erythromycin, which are also mediated by the CYP2C11, were significantly reduced in the intravenously fed animals, compared to those fed intragastrically. In addition to a reduced N-dealkylation pathway, significant reductions in ring hydroxylation and aryl methyl hydroxylation also occurred in both PNL and PN animals. The fact that LIDO metabolism also involves unidentified pathway(s), and sequential metabolic pathways, it is not possible to deduce the rate or extent of reduction of each metabolic pathway from this study. It is quite possible that one or more unknown pathways may also have been inhibited. However, the data clearly suggest that PN reduces LIDO elimination, and inclusion of lipid in PN exacerbates the reduction. The findings in PN group animals were consistent with those observed by Ke *et al.* (1990b). In a study, Knodell *et al.* (1990) revealed that lipid-based PN normalized microsomal membrane fluidity and lipid profile, and yet this change failed to reverse the reduction of meperidine demethylase activity. On the other hand, deethylation of ethoxyresorufin was returned to the normal values in the lipid group. These findings suggest that the effect of lipid on membrane composition may have selective effects on the drug metabolizing enzymes. This observation could explain the discrepancy between the study by Ross *et al.* (1983) and our findings with lipid. It may be

argued that the reduced enzyme activities in PNL and PN groups could be due to poor total body weight gain and lower liver weights, but this possibility may be ruled out because the liver weight in terms of percent body weight of groups PNL and PN did not differ from the CF group value. The calorie intake in all animals were 330 kcal/kg/day, which is sufficient to provide the right nutrition (Ross et al., *1983*; *Ross et al., 1984*; *Knodell, 1990*). The gains in weight of intragastrically fed animals were greater than those of intravenously fed ones. This observation was also made by previous researchers .

Mild to moderate hepatosteatosis was observed in five out of six rat livers in PNL animals, although LFT values were not altered. This observation was consistent with those of previous investigators (*Zamir et al., 1994*; *Keim and Mares-Perlman, 1984*; *Yeh and Zee, 1976*). There have been controversial reports both in animal and human data on the effect of lipid on hepatic morphology and the test markers. Various studies by (*Reif et al., 1991*; *Zohrab et al., 1973*; *Buzby et al., 1981*), have shown a reversal of steatosis with lipid administration, whereas other studies (*Allardyce, 1982*; *Keim, 1987*; *Boelhouwer et al., 1983*; *Tayek et al., 1990*; *Clarke et al., 1991*; *Black et al., 1981*) have shown the opposite. In a recent study, Balderman *et al.* (1991) compared ultrasound results of two groups of patients receiving PN with either a mixed lipid emulsion of medium chain triglycerides and long chain triglycerides (MCT/LCT) or just LCT. Liver ultrasound results after 7 days of PN therapy indicated a significant increase in liver size and gray-scale value only in those patients who received LCT. These changes are not apparent in the group that received MCT/LCT. The increased liver size in the LCT group could be due to an increased deposition of glycogen and fat in the liver. Potential benefits of using

MCT/LCT over LCT in preventing hepatomegaly and fatty liver infiltration was also demonstrated by Clarke *et al.* (1991). However, the pathogenesis of PN-induced steatosis in the rat is multifactorial and still remains inconclusive. In this study animals in the PN group did show some lipid deposition or displayed evidence of steatosis but the result was less prominent than that observed in the PNL group.

Possible explanations of the observed steatosis in PN group animals are: a) the instability of the emulsion; b) the procedure of lipid incorporation; c) the amount of lipid infused; d) the type of lipid infused. A major argument against emulsion instability is that infusions were freshly prepared. No marked changes in their color or consistency were observed in lipid-containing PN infusions. The "three-in-one" media used are reported to be clinically safe, stable and economical (*Ang et al., 1987*; *Rollins et al., 1990*). The amount of lipid that was used provided 30% of non-protein calories and this is also consistent with amounts used in previous studies (*Ross et al., 1984*; *Nakagawa et al., 1991*; *Steiger et al., 1978*; *Martins et al., 1985*).

The cause of the steatosis observed mainly in PNL animals may be attributable to the type of fatty acid infused, which is a long chain triglyceride (LCT) of linoleic acid. This is the major constituent of Intralipid® which is widely used in clinical practice. A LCT preparation poses some problems; their water insolubilty means that they cannot be easily hydrolyzed in the intestinal lumen and may require bile salts and pancreatic lipase for their digestion. Furthermore, LCTs require carnitine to enter the cell mitochondria (*Gottschilich, 1992*). Since, carnitine is often found to be depleted in PN-treated patients, this situation may eventually lead to a reduced capacity to metabolize LCTs (*Gottschilich,*

1992). In contrast, MCTs can enter mitochondria directly and can be oxidized quickly to provide energy (*Gottschilich, 1992*; *Bremer, 1980*; *Sailer and Muller, 1981*; *Ball and White, 1989*). Therefore, it may be advantageous to use an MCT or a mixture of LCT/MCT in place of LCT to prevent liver dysfunction and reduce the risk of PN-associated reduction in hepatic drug metabolism.

Cohen (1975) performed an *in vitro* guinea pig liver explant study and observed that the amino acids, leucine, threonine, isoleucine and particularly glycine, were hepatotoxic. This observation may have some relevance to current findings that serum levels of glycine were elevated almost five fold in PN animals, were doubled in PNL animals relative to animals in the CF group, although the PNL group showed more hepatic insufficiency. Photo-decomposition of amino acids results in products which are hepatotoxic (*Grant et al., 1977*; *Merritt et al., 1984*) but formation of such products was prevented in this study by consistently protecting infusions liquids from the damaging effects of light. The exact mechanism(s) by which amino acids in PN solutions interact with hepatic drug metabolism is(are) still not understood.

In summary, it has been determined that PN with and without lipid, produces a selective reduction in LIDO metabolism in rats, and that incorporation of lipid in PN solutions promotes steatosis and exacerbates the reduction further. Therefore, this animal data may be helpful in the design and interpretation of human PN studies.

4.3 Taurine Study

The adverse effect on hepatic drug metabolism of PN solutions that were supplemented with taurine has been demonstrated for the first time. Two different strengths of taurine (15 mg/dL and 50 mg/dL) in PN solutions were evaluated in the present study. Both differed significantly in terms of their metabolic, kinetic and liver histologic profiles when compared with chow-fed animals and with animals receiving a PN solution that did not contain taurine. All of the kinetic and metabolic parameters were consistently lower in the group that received the higher rate of taurine infusion. This clearly indicates that taurine supplementation can have deleterious effects on liver function. Animals in the higher taurine-fed group exhibited the most severe hepatic dysfunction accompanied by steatosis, and the greatest reduction in LIDO metabolism., compared to the other groups. Reduction in LIDO metabolism appeared to parallel the liver histological findings in this higher taurine-fed group. Mean Cl_{int} value obtained from the PNT50 group was reduced to 1/5 of the chow-fed value (Table 3.13). Metabolic pathways that were severely affected were: N-dealkylation, ring hydroxylation and aryl methyl hydroxylation. These LIDO metabolic pathways are catalyzed by CYP enzymes that are involved in the transformation of a numerous other xenobiotics (*Oda et al., 1989*; *Imaoka et al., 1990*; *Wrighton et al., 1989*; *Coutts et al., 1994*; *Smith, 1991*; *Cholerton et al., 1992*). This implies that drug therapy in conjunction with PN infusion should be treated with caution.

With regard to steatosis, the group that received a lower amount of taurine infusion (15 mg/dL) developed steatosis only in the periportal area, while this condition

was present in the pericentral area in the PN group that received no taurine. It is known that distribution of Phase 1 and Phase 2 metabolic enzymes are uneven across the liver acinus (*Pang et al., 1983*; *Katz, 1995*; *Gascon-Barre et al., 1995*). In general, CYP and glucuronidation enzymes occur predominantly in the pericentral zone, whereas sulfate conjugation and other oxidative metabolic reactions such as gluconeogenesis, amino acid metabolism and bile acid synthesis are associated with the periportal area (*Katz, 1995*). However, metabolism of some drugs are the same in both pericentral and periportal regions, whereas some drugs are predominantly metabolized in the pericentral area (*Gascon-Barre et al., 1995*). There is no evidence in the literature about the zonal preference of LIDO metabolizing enzymes in the liver. According to our findings, it appears that the reduction of LIDO metabolism was more severe in livers with periportal steatosis. Since periportal steatosis was secondary to the taurine effect, the exacerbated reduction of LIDO metabolism may be a direct effect of excess taurine in these animals. Therefore, no conclusive remark can be made regarding the regional preference of LIDO metabolism from this study.

There is evidence that taurine is a membrane stabilizer because it can restore membrane fluidity and function (*Zelikovic and Chesney, 1989*; *Guertin et al., 1993*). Guertin *et al.* (*1993 and 1991*) revealed that when taurine (15 mg/dL) was used as a supplement, the liver sinusoidal membrane fluidity, function and bile flow were restored in guinea pigs after receiving a 3-day PN infusion. This concentration of taurine was also able to stabilize bile canilicular membrane composition which was significantly altered in animals that did not receive any taurine. Whether these taurine-fed animals would have a

normal metabolic enzyme capacity is not clear because drug metabolism was not evaluated. There is indirect literature evidence (*Knodell et al., 1990*) that a reversal of cell membrane fluidity by lipid infusion in PN rats had very little effect on liver CYP enzymes. Although changes in membrane fluidity was not measured in this study, the observations by Knodell *et al.* (1990) support our data that the membrane stabilizing effect of taurine (if present) had no effect on CYP enzymes. This hypothesis is further supported by the fact that, both *in vitro* and *in vivo,* taurine conjugated bile acids have been found to produce marked morphological changes particularly of the endoplasmic reticulum (*Priestly et al., 1971*; *Hutterer et al., 1970b*). When rats were injected with sodium taurolithocholate, it caused a prolongation of zoxazolamine paralysis time indicative of a reduced metabolism of this drug (*Priestly et al., 1971*). Other studies in rats showed that taurochenodeoxycholate, at a concentration found *in vivo,* produce a Type I binding spectrum with CYP enzymes, and competitively inhibited the metabolism of Type I substrates like aminopyrine and hexobarbital (*Hutterer et al., 1970c*; *Hutterer et al., 1970b*; *Hutterer et al., 1970a*).

LIDO metabolism was also reduced in the PN group that did not receive taurine. This indicates that some other factors are involved in this PN-related reduced drug metabolism. Reduction in drug metabolizing enzyme activity after PN infusion has been documented by others (*Ke et al., 1990b*; *Zaman and Tam, 1993*; *Zaman and Tam, 1994*; *Ross et al., 1983*; *Knodell et al., 1989*) but the precise mechanism(s) involved have not been identified. It has been speculated that PN-induced metabolic and liver dysfunction may be due to a number of factors: 1) photooxidized amino acid products 2) bacterial

overgrowth or sepsis 3) amino acid over-load/imbalance. These factors are not relevant in the present study because the photooxidation of amino acids was prevented by shielding the PN solution from light, and sepsis was absent as indicated by the WBC values and the reported hepatotoxic amino acids (*Cohen, 1975*), such as glycine, showed an inverse relationship with the observed hepatotoxic effects in the present study. The findings in the cholestyramine study further support this observation (see section 4.1).

Interestingly, in this study a lack of correlation between the amount of taurine infused and the serum concentration was found (Table 3.15). At this point, it is not clear as to why the taurine levels in the PN group did not differ significantly from the supplemented groups. Similar observation was made by others in rats (*Guertin et al., 1991*). Although speculative, but it could be a possibility that when an excess supply of taurine was provided it was being conjugated more with the bile acids in rats. Given that bile acids are conjugated mainly with taurine in rats, and certain taurine conjugated bile acids are hepatotoxic (*Miyai et al., 1977*; *Javitt, 1966*; *Kakis and Yousef, 1978*; *Layden and Boyer, 1977*), this partially explains our observation in the taurine-fed animals.

Similar unrewarding findings of taurine administration were reported previously in human studies (*Cooke and Whitington, 1984*; *Paauw and Davis, 1994*; *Okamoto et al., 1984*). Nonetheless, we have to bear in mind that factors such as animal species (*Schersten, 1970*), age (*Poley et al., 1964*), availability of glycine or taurine (*Dorvil et al., 1983*) as well as disease states (*Anderson et al., 1979*; *Bruusgaard and Thaysen, 1981*) may greatly influence the pattern of bile acid conjugation. Therefore, the extent of hepatotoxicity after taurine administration observed in the present model may also differ

depending on one or more of the above factors. One should be careful while extrapolating animal data into a clinical situation.

In conclusion, the findings in the present study clearly indicate that taurine supplementation in PN solutions has deleterious effect on liver function and drug metabolism in the rat model. These animal data can be useful in assessing whether or not taurine should be an essential component of infant formula.

4.4 Photo-Oxidation Study

Solutions of various amino acids, particularly tryptophan, methionine, tyrosine, cysteine and histidine, have been reported to be highly photosensitive (*Kleinman et al., 1973*; *Kanner and Fennema, 1987*; *Gurnani et al., 1966*; *Asquith and Rivett, 1971*; *Gurnani and Arifuddin, 1966*). Light exposure of solutions, especially those that also contain vitamins, particularly riboflavin, accelerates the photooxidation of these amino acids (*Gurnani et al., 1966*; *Bhatia et al., 1983*; *Gurnani and Arifuddin, 1966*; *Bhatia et al., 1980*; *Asquith and Rivett, 1971*).

Research so far has been focused mainly on tryptophan, and studies have revealed that when tryptophan was administered as a single amino acid solution or in a nutrition solution which contained riboflavin, the conversion products formed were toxic to both human and rat livers (*Grant et al., 1977*; *Merritt et al., 1984*; *Bhatia et al., 1985*). These observations are particularly important because of the widely reported incidence of liver dysfunction in neonates and in adults that could result from the presence of photo-oxidized amino acid products.

The present study demonstrates for the first time that photooxidized amino acids not only cause hepatosteatosis but also affect hepatic metabolic enzyme activity. This was indicated by a severe reduction in various metabolic pathways of the model drug, LIDO, in animals that received light-exposed PN solutions. LIDO was chosen as the model drug because the metabolism of this drug is mediated by a wide variety of CYP enzymes. Metabolic pathways include N-dealkylation, aromatic hydroxylation and aryl methyl hydroxylation (*Oda et al., 1989*; *Imaoka et al., 1990*).

Liver histological examinations showed that severe steatosis was present in livers of all the animal that received a PN solution that was exposed to light (Fig. 3.16). Similar observations have been reported in previous studies (*Bhatia et al., 1993*; *Grant et al., 1977*). It is possible that the hepatotoxicity observed in neonates is due to the prolonged parenteral infusion of illuminated PN solutions.

The current study have shown that LIDO metabolism was impaired in both PN-treated groups but the most dramatic reduction was observed in the PN(+L) animals which displayed significantly reduced enzymatic activities. Cl_{int} values were lower by almost 86% in the PN(+L) group and by 70% in the PN group, when compared to chow-fed values ($P < 0.05$). The reduced metabolic activity led to a much higher recovery of unchanged LIDO particularly in the PN(+L) group. N-Dealkylated, ring-hydroxylated and aryl methyl hydroxylated metabolic pathways of LIDO were most affected in the PN(+L) group. Ring-hydroxylation of LIDO to 3-OH-LIDO and N-dealkylation to MEGX are major pathways that contribute significantly to the mass balance of LIDO metabolism. MEGX formation is catalyzed by CYP2C11 and CYP2B1 in rats (*Oda et al., 1989*;

Imaoka et al., 1990) and by CYP3A4 in humans (*Bargetzi et al., 1989*). Ring-hydroxylation in rats is mediated by CYP1A2 and CYP2D enzymes (*Imaoka et al., 1990*; *Oda et al., 1989*; *Masubuchi et al., 1992*) and CYP2B2 is involved in the formation of MEOH-LIDO (*Oda et al., 1989*; *Imaoka et al., 1990*). All of these enzymes, particularly the ones belonging to the CYP2D subfamily and CYP3A4 are involved in the metabolism of a wide variety of commonly used drugs (*Coutts et al., 1994*; *Smith, 1991*; *Cholerton et al., 1992*). These observations imply that drug treatment during PN infusion should be carefully monitored.

Reductions of drug metabolism in PN-treated animals have been observed previously (*Ke et al., 1990b*; *Zaman and Tam, 1993*; *Zaman and Tam, 1994*; *Knodell et al., 1984*; *Ross et al., 1983*). The finding that photo-decomposition of amino acids were prevented in the PN animals was consistent with observations by others (*Ke et al., 1990b*). Therefore, the pronounced reduction of LIDO elimination in the PN(+L) group may be attributable to the presence of toxic photooxidized amino acid products in this group. The data suggest that more than one mechanism may be involved in PN-induced metabolic reduction. Some postulated mechanisms described in the literature are: 1) amino acid overload/imbalance (*Ke et al., 1990b*; *Knodell et al., 1984*; *Grant et al., 1977*), 2) sugar load (*Buzby et al., 1981*; *Rivera et al., 1989*; *Hartshorn et al., 1979*; *Pantuck et al., 1995*) and 3) sepsis (*Shu et al., 1991*; *Gimmon et al., 1981*; *Gimmon et al., 1981*).

Despite a small reduction in levels of some amino acids after light exposure in the PN(+L) group, the serum levels of most amino acids were higher in the light-exposed PN group (Table 3.21). This elevation is presumably a secondary effect of the toxic amino

acid products on the liver that perturbed the efficiency by which the amino acids would ultimately get utilized *in vivo* (*Heger and Frydrych, 1989*). Significant elevations of certain serum amino acids during PN infusion with or without light exposure is very common (*Ke et al., 1990b*; *Bhatia et al., 1992b*).

Potential toxicities of tryptophan decomposition have been well documented (*Kanner and Fennema, 1987*; *Bhatia et al., 1985*; *Donoso et al., 1988*; *Grant et al., 1977*; *Merritt et al., 1984*; *Bhatia and Rassin, 1985*). However, the decomposition of tryptophan is minimal in the current study (5-6% in all six test conditions; Table 3.22) and yet a severe liver toxicity was observed. This raises a doubt about whether tryptophan plays any role in the photooxidized PN-induced hepatotoxicity. The discrepancy between our data and those reported by others may be due to various factors: a) the decomposed component(s) may have eluted at similar retention times as tryptophan itself and could not be detected by the HPLC assay used or b) the extent of photooxidation is dependent upon the dose of tryptophan, quantity of sensitizer present (*Kanner and Fennema, 1987*), and the duration of light exposure (*Donoso et al., 1988*). The first possibility can be ruled out for three reasons: 1) a solution of kynurenine, a common decomposed product of tryptophan (*Hirata et al., 1967*; *Kanner and Fennema, 1987*) was found to have an HPLC retention time different from that of tryptophan. Kynurenine was not detected in our assay; apparently it did not form in the light exposed tryptophan sample, 2) the adduct of tryptophan that may have formed in presence of 0.005 μmol/mL of riboflavin would be less than 0.1% of total tryptophan; 3) the UV spectrum of a photooxidized tryptophan sample contained a weak absorption peak at 324 nm, that was indicative of tryptophan

decomposition, but the intensity of this peak was insignificant relative to the size of the tryptophan peak at 298 nm. The peak of the decomposed product was present in both the light exposed tryptophan solution in the presence or absence of riboflavin. This observation may also explain the consistent small reduction (5%) in tryptophan concentration in all test conditions. The complete conversion of riboflavin to its adduct was also shown by significant changes in the absorption of riboflavin in the 300 to 450 nm range.

The second possibility that the photo-decomposition of tryptophan and riboflavin is dose-dependent is more likely for two reasons: 1) decomposed tryptophan products have been reported to increase the level of cholylglycine (CG; marker of cholestasis) in a dose-dependent manner (*Merritt et al., 1984*). In this study a daily dose of 4 mmol/kg tryptophan solution previously exposed for 7 days to roof-top sunlight, resulted in a significant rise of CG after 7 days. This would result in a much higher degree of decomposition when compared to the 24 hr light exposure used in the current study. In another study (*Bhatia and Rassin, 1985*) a similar dose (4 mmol/kg/day) of light-exposed tryptophan solution along with 2.14 μmol/kg/day of riboflavin resulted in a significant rise of γ-GT after 4 days. In contrast, the daily intake of tryptophan and riboflavin from the PN solution in our study was calculated to be 1.92 mmol/kg/day and 1.6 μmol/kg/day respectively.

2) Donoso *et al.* (1988) proposed the formation of a photoinduced toxic tryptophan-riboflavin adduct when they irradiated a solution containing 48 mM of tryptophan and 0.26 mM of riboflavin. These concentrations are several folds higher than

what was used in our study. The highest degree of hepatotoxicity also resulted when their solution was irradiated for 48 hr.

It is concluded that drastic hepatic damage in our PN(+L) animals cannot be attributed to the minor decomposition of tryptophan that occurred.

Present results clearly indicate that the extent of methionine decomposition was the highest when sensitizers were present than with other amino acids (Table 3.22). Since the quantities of the sensitizers used were identical in all amino acid solutions the methionine-riboflavin adduct that formed would theoretically account approximately for only 0.02% of total methionine. The larger (13%) decomposition of methionine clearly indicates that other products besides an adduct had formed. Studies (*Weil et al., 1951*; *Bhatia et al., 1980*) have shown that photooxidation of methionine yields methionine sulfoxide which is hepatotoxic (*Litwin, 1972*; *Nixon and Wang, 1977*; *Hirata et al., 1967*). Therefore, the possibility that decomposed methionine product(s) induced the hepatotoxicity observed in the present study should not be overlooked. Further studies should be conducted to isolate and identify decomposition products of methionine. It is important to mention at this point that solutions of amino acids that also contained the same sensitizers did not undergo any decomposition during a period of 10 days when protected from light.

Some investigations have shown a correlation between the ratio of aromatic amino acids (ARA) to branched chain amino acids (BRA) and the severity of hepatic encephalopathy (HE). When the ratio of BRA/ARA drops to one, HE prevails, and HE improves when the ratio is close to three (*Fischer et al., 1976*; *Fischer et al., 1975*). In the present study, the BRA/ARA ratio was found to be significantly lower in the PN(+L)

and PN groups compared to chow-fed [PN(+L): 2.53 ± 0.6; PN: 2.1 ± 0.5; *vs* CF: 2.91 ± 0.3]. This amino acid imbalance could be partly responsible for the observed hepatic abnormalities in the two PN groups but it is premature to make any conclusion at this point.

Bhatia *et al.* (1992a) reported a depletion of biliary glutathione in animals which developed hepatotoxic effects after having received PN solutions that had been exposed to light. Glutathione is essential for the detoxification of a number of xenobiotics. Levels of glutathione were not measured but it is possible that its depletion partly contributed to the observed hepatotoxicity in our PN(+L) animals.

Sepsis and bacterial infection due to an indwelling catheter have been shown to be associated with PN-induced hepatotoxicity (*Shu et al., 1991*; *Wolf and Pohlandt, 1989*) which could contribute to a reduction in hepatic drug metabolism (*Gimmon et al., 1981*). However, our chow-fed animals with indwelling catheters had similar rates of LIDO metabolism when compared to those animals that did not receive any surgery (*Ngo et al., 1995*). Furthermore, none of our PN-treated or chow-fed animals showed any evidence of sepsis since all had normal WBC and LFT values (Table 3.20). In addition, our observed serum amino acid levels were not compatible with sepsis (*Freund et al., 1979*).

When a sugar-load is given in association with a PN solution, a reduction in drug metabolism is observed (*Burgess et al., 1987*; *Hartshorn et al., 1979*). This has been attributed to an increase in the rate of lipogenesis, a process that would compete with CYP enzymes for NADPH (*Hartshorn et al., 1979*; *Pantuck et al., 1995*) and be partly responsible for the observed hepatotoxic effect observed in our study.

In conclusion, the results indicate the following: a) PN infusion after light exposure caused severe hepatic damage and a reduction in LIDO metabolism in rats; b) Of all the amino acids investigated, methionine underwent greatest decomposition after light exposure. It is possible that the significant steatosis observed in rats and the pronounced reduction in LIDO metabolism that occurred could be attributed to methionine toxicity.

These results suggest that PN solutions with or without vitamins should always be protected from light during infusion to minimize hepatotoxicity in human subjects. It may even be a good practice to add vitamins just prior to infusion.

5. SUMMARY

Our studies indicate that PN administration is associated with hepatic dysfunction, particularly hepatosteatosis. This condition was found to have no correlation with the serum liver function test markers. PN treatment was also found to be associated with a reduction in the activities of drug metabolizing enzyme(s). In LIDO metabolism a significant reduction was observed in N-dealkylation, aryl methyl hydroxylation and ring hydroxylation. Reduction of these processes indicate a reduced activity of several CYP enzymes including CYP2C11, CYP2B2 and CYP2D subfamilies which respectively catalyze these three metabolic reactions during PN therapy.

Interestingly, with cholestyramine treatment, hepatosteatosis was not seen and all liver function test values were normal, yet LIDO metabolism was reduced. This observation suggests that the etiology of PN-associated reduction in CYP enzyme activity and hepatobiliary abnormalities could be of different origin. With this in mind, the effect of lipid, an essential fatty acid replenisher, was evaluated. PN solutions that are deficient in essential fatty acids have been documented to be responsible for the PN-associated hepatic dysfunction. Our data indicate, however, that lipid incorporation had no beneficial effect on LIDO metabolism. Infact, the reduction that was observed with PN treatment was even lowered significantly (Tables 3.26 to 3.28). The animals in the lipid group (PNL) also developed marked steatosis along with exacerbated metabolic reduction. From the lipid data, it is clear that commercially available LCT emulsions may not be a good choice as replenishers of essential fatty acids. Some of the problems posed by LCTs are water insolubility, poor hydrolyzing properties and carnitine dependence for entry into

cell mitochondria. Since carnitine levels are depleted in PN treated patients, there is a reduced rate of LCT mobilization. Therefore, MCT or a mixtures of LCT/MCT should be made available for clinical use in North America.

Taurine has been reported to be a good cell membrane stabilizer and enhancer of cell fluidity (*Kendler, 1989*; *Wright et al., 1986*; *Zelikovic and Chesney, 1989*). This amino acid is absent in most of the commercially available amino acid mixtures used in PN preparations. Lack of taurine has been linked to PN-related hepatic disorders in adults and children, but our results indicate that in a rat model there is no advantage to taurine supplementation of PN administration as far as hepatic drug metabolism (Table 3.13, Table 3.15 and Table 3.16) and liver histology are concerned. This taurine data may be useful in any evaluation of whether taurine should be regarded as an essential component in PN mixtures used for humans.

Our findings in animals that received photooxidized PN infusions revealed that light exposure of PN solution had drastic effects on drug metabolism (Table 3.24 and Table 3.25) and liver histological status. These findings suggest that light exposure during a PN therapy can be very harmful to both adults and infants. In particular, low birth-weight newborn infants may be at a higher risk due to their undeveloped detoxification capabilities.

At this point the exact mechanism(s) underlying the cause for PN-related reduced drug metabolism is(are) not clear. The observed selective alteration of drug metabolizing enzymes with PN infusion is worthy of more investigations. However, the toxic effects of PN on the liver can be certainly be minimized by protecting the PN solution from light.

Finally, our studies warrant future experiments designed to improve the efficiency of PN solutions and minimize their adverse effects on drug metabolism.

6. CONCLUSIONS

- Cholestyramine prevents hepatic dysfunction including steatosis induced by PN administration, possibly by interruption of secondary bile salt reabsorption.

- Intralipid® administration as a non-protein calorie source in the PN solution results in hepatosteatosis and reduction of LIDO metabolism.

- Taurine supplementation to PN solutions has deleterious effects on liver function and on drug metabolizing enzymatic activity in rats. This information may be useful to decide whether or not taurine should be regarded as an essential component in PN solutions especially for infants.

- Exposure of PN solution to light results in photooxidized amino acid products that are toxic to liver, cause severe steatosis and reduction in LIDO metabolism.

- The four photosensitive amino acids, histidine, tryptophan, methionine and tyrosine all showed evidence of forming adducts with the riboflavin that was present in the multivitamin component in the PN solutions. Only methionine showed a significant reduction from its initial concentration indicating the presence of other unidentified photodecomposed products that may be responsible for the toxic effect to the liver.

- Significant hepatosteatosis may be present without causing any elevations in the serum transaminases.

7. RECOMMENDATIONS

- PN solutions during infusion should be protected from light exposure to minimize the production of toxic products and their adverse effects on the liver.

- It would be a good practice to add multivitamins to the PN infusion bag just prior to infusion.

- When drug therapy is needed during a course of PN treatment proper drug monitoring should be exercised, especially for those drugs which have narrow therapeutic windows, to determine whether dosage adjustment is necessary.

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9. APPENDIX 1

Normal ranges of the liver function test values obtained from the laboratory of Surgical Medical Research Institute*.

TESTS	Normal Ranges
AST (U/L)	45-572
ALT (U/L)	19.3-196
ALP (U/L)	15.6-410
γ-GT (U/L)	10-60
BILI (mg/dL)	0.18-1.43

* The tests were performed using a Dri-Stat™ diagnostic kit (Beckman Istrumentattion, Inc.) and a Multistat 3 Micro Centrifugal Analyzer (Istrumentation Laboratory Inc.).