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# Effects of Lipid-Supplemented Total Parenteral Nutrition on Fatty Liver Disease in a Premature Neonatal Piglet Model

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**Key Words**
Liver · Total parenteral nutrition · Fatty acid profile · Lipases · Hepatic lipase · Lipoprotein lipase · Fatty liver disease · Lipids · Neonatal pig

**Abstract**
***Background:*** Routine total parenteral nutrition (TPN) in neonatal care can result in hepatic dysfunction in 40–60% of patients, most commonly as fatty liver, but little work has been conducted on the underlying mechanisms causing hepatic dysfunction. ***Objective:*** To use a piglet model for the premature human neonate on TPN, supplemented with lipid emulsions, to investigate hepatic responses. ***Method:*** Piglets were delivered 2 days prematurely. Six control piglets were fed enterally (E), whilst twelve animals were maintained on TPN. TPN piglets received the standard TPN solution plus the lipid emulsion as either ClinOleic® (C, n = 6) or Intralipid® (I, n = 6). Hepatic lipid content and the fatty acid composition of liver triacylglyercol (TAG) as well as hepatic lipase (HL) activity were determined. Lipoprotein lipase (LPL) activity was measured in the liver, muscle and adipose tissue. The plasma concentrations of choline, bilirubin, TAG and non-esterified fatty acids (NEFA) were also measured. ***Results:*** Liver lipid was significantly increased in piglets on TPN and the tissue fatty acid profiles reflected the lipid emulsion. HL and LPL activities were reduced in liver but LPL increased in adipose tissue during TPN. Plasma concentrations of choline, bilirubin, TAG and NEFA were similar across the treatments. ***Conclusions:*** The results suggest fatty liver occurs in neonates receiving TPN and the source of the accumulated lipid appears to be the lipid emulsion used. The factors regulating lipase activity during TPN require further study. The piglet can be used as a model for neonatal TPN.



## Introduction

Routine total parenteral nutrition (TPN) in neonatal care can result in hepatic dysfunction in 40–60% of patients, most commonly as fatty liver [1, 2]. Whilst mild fat infiltration of the liver is seldom symptomatic [3] progression to chronic lipid accumulation results in fibrotic changes and cirrhosis [4]. Cholestasis is the most common neonatal hepatic dysfunction [5–7], but fat infiltration is normally present too [8].

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Alpers and Sabesin [9] suggested that prevalence of fatty liver is increased by prematurity and it is known that fatty liver can be detected in children after only a few days on TPN [10, 11]. It is possible that fatty liver is under-reported in neonates because it may not be conclusively diagnosed without a liver biopsy [12], whereas cholestasis may be diagnosed readily from serum bilirubin concentrations and liver function tests and is therefore commonly reported.

Although it has been suggested that fatty liver may result from an excessive supply of calories in the form of carbohydrate [13, 14], the exact aetiology remains to be elucidated. Excess carbohydrate enhances lipogenic activity [15] and during TPN in dogs, uptake of glucose in the liver is increased [16]. Studies on a rat model of TPN have demonstrated a reduction in fat deposition in the liver by adding lipid to the TPN regime to reduce the supply of carbohydrate derived calories [17]; supplying lipid emulsions of differing composition also results in different degrees of lipid deposition in the liver of humans and rats [11, 18, 19]. The role a lipid emulsion may play in the causation or prevention of fatty liver remains unclear. Another suggestion is that fatty liver results from a decrease in lipid export from the liver and a number of studies have implicated choline deficiency as one cause [20, 21].

When modelling liver function and metabolism in human neonates, the pig has advantages over other animal models, as it has similar hepatic structure, histology and physiology [22–24] and unlike the rat, both humans and pigs possess a gall bladder [25]. The piglet is less mature at birth than the human neonate, due to reduced growth rate and maturation during the final stages of development [26]. This means that, like premature human neonates, they possess poor thermoregulatory mechanisms, high metabolic rates, and are prone to hypoglycaemia [27–29], making them an appropriate model for the premature human neonate on TPN. When delivered up to a week preterm, modelling of the different degrees of prematurity seen in human infants is possible [26]. The premature piglet has previously been used as a model for the premature infant [25, 29, 39, amongst others].

Little work has been reported on the prevalence or causes of fatty liver in the premature neonate and there are very few established models that can be used to elucidate these points. This study sets out to use a piglet model for the premature human neonate on TPN to study the incidence of fatty liver and the possible role of the lipid emulsion in its aetiology.

## Materials and Methods

*Experimental Design*

Four nulliparous sows (25% Meishan, 12.5% Duroc and 62.5% Large White X Landrace) were entered into the study. A routine caesarean section was carried out under anaesthesia at 113 days of gestation (term = 115 days). Anaesthesia was induced by administration of 2–5 mg/kg ketamine i.v. (Ketaset, Fort Dodge, Southampton, UK) and maintained using isoflurane 4–6% (Schering-Plough Animal Health, Harefield, UK) oxygen (BOC, Manchester, UK). Following delivery, the piglets were given oxygen and an intramuscular injection of vitamin K (0.25 ml, Konakion MM 10 mg $ml^{-1}$, Roche Products Ltd., Lewes, UK) to assist blood clotting and allowed to recover from anaesthesia.

Piglets were selected so as to balance experimental groups for sex, body weight at birth and maternal influences (i.e. equal numbers of piglets from each of the 4 sows were represented in each treatment group). Six piglets were assigned to each of three treatment groups, fed either enterally (E) with a commercially available sow milk replacer (4.5 kJ $ml^{-1}$, Primary Diets, Ripon, UK), or parenterally with TPN solution (1690 J/ml; 99.34 mg $ml^{-1}$ glucose; 17.89 mg $ml^{-1}$ amino acids; Portsmouth Hospital NHS Trust, Portsmouth, UK), plus one of the following commercially available lipid emulsions: Intralipid® 20% (I: 8.36 MJ $l^{-1}$; Fresenius Kabi Ltd., Runcorn, UK) or ClinOleic® 20% lipid emulsion (C: 8.36 MJ $l^{-1}$; Baxter Healthcare Ltd., Northampton, UK). The TPN solution was formulated according to the prescription used by the local neonatal care centre (The William Harvey Hospital, Ashford, Kent). As humans and pigs have similar nutritional requirements [27] it is possible to provide adequate nutritional support in the pig using clinical TPN regimes, which is useful in establishing an animal model with clinical relevance [25].

In the initial experiments a further group of piglets (n = 4) were enterally fed the TPN solution plus the lipid emulsion, Intralipid 20%, in the same ratio and rate as used for the TPN infused Intralipid group. These animals failed to thrive and consequently they were humanely slaughtered using pentobarbitone sodium (Euthatal, Rhone Merieux, Harlow, UK) at 2–3 days of age. Data from these pigs have not been presented, although it should be noted that they did not show any signs of hepatic fat accumulation (3.16 ± 0.12 g lipid/100 g fresh weight liver) at that age.

Two hours post-delivery, piglets were anaesthetised using isoflurane 0.5–2% (v:v; Schering-Plough Animal Health, Harefield, UK) and bilateral jugular catheters inserted, one for daily blood sampling and administration of intravenous antibiotics, the other catheter to administer TPN in the C and I groups. Piglets were administered intramuscular analgesic (0.05 ml $kg^{-1}$ tolfenamic acid 4% (w:v), Tolfine, Vetoquinol UK Ltd., Buckingham, UK) and housed in individual open top cages (75 × 100) with 75 cm high walls.

After recovering from surgery, E piglets were given initially 10 ml of milk replacer and subsequently 100 ml of milk replacer at 4-hour intervals. TPN solution was infused into C and I animals using a volumetric infusion pump (IVAC 598, Alaris medical, Basingstoke, UK) at 2.9 ml $h^{-1}$ $kg^{-1}$ and lipid emulsion, via the same infusion line, using a syringe pump (IVAC P7000, Alaris medical, Basingstoke, UK) at 0.2 ml $h^{-1}$ $kg^{-1}$ for the first 12 h. After 12 h the infusion rate of the TPN solution was increased to 5.8 ml $h^{-1}$ $kg^{-1}$ and lipid emulsion at 0.5 ml $h^{-1}$ $kg^{-1}$ and, after 24 h,

piglets were receiving TPN solution at 5.8 ml $h^{-1}$ $kg^{-1}$ and lipid emulsion at 1.0 ml $h^{-1}$ $kg^{-1}$ (4.8 g $kg^{-1}$ $day^{-1}$). These rates were maintained throughout the remainder of the study.

Piglets were weighed daily and given antibiotics i.v. (50 mg benzylpenicillin: Crystapen, Brittannia Ltd., Redhill, UK) twice daily. On the final day of treatment, crown-to-rump length, femur length, head and abdominal circumference were recorded, and a blood sample was obtained prior to their being humanely killed as described above. The fresh weights of the major organs were recorded. Samples of liver, subcutaneous adipose tissue and muscle tissues were taken, snap frozen in liquid nitrogen and stored at –80°C. Blood samples were taken into EDTA tubes (Teklab, Durham, UK) and centrifuged at 1,400 *g* in a bench centrifuge for 15 min at 4°C, and plasma was removed and stored at –20°C for subsequent analysis.

Experimental protocols were carried out as defined by the regulations of the Animals (Scientific Procedures) Act 1986, followed local ethical standards and were approved by the Home Office, UK.

*Laboratory Analyses*

The concentrations of plasma metabolites (non-esterified fatty acids (NEFA), triacylglycerol (TAG), choline, bilirubin) were determined using commercially available enzymatic assays (Randox, UK). A reference sample was included in all assays and the intra- and inter-assay coefficient of variation was always below 10 and 15%, respectively.

To assess the fatty acid profile of the hepatic TAG, lipids were first extracted and purified in chloroform-methanol [30]. The lipids were separated by thin layer chromatography and TAG was eluted from the plate. Aliquots of the TAG extracts were saponified and the fatty acids methylated following the method of Lepage and Roy [31, 32]. Fatty acid methyl esters were separated on a 30 m × 0.25 mm Omegawax capillary column (Supelco, Bellefonte, Pa., USA) in a Perkin-Elmer gas chromatograph (Autosystem; Norwalk, Conn, USA) and quantified with a hydrogen flame ionization detector. Nitrogen was used as the carrier gas, and the fatty acid methyl esters were identified by comparison of their retention times with genuine standards (Sigma Chemical Co., St Louis, Mo., USA).

Hepatic lipid content was assayed gravimetrically following a modified Folch extraction [30, 33]. Lipoprotein lipase (LPL) activity in the liver, adipose tissue and skeletal muscle was determined by the conversion of 1–$^{14}$C-triolein to 1–$^{14}$C-oleic acid [34]. Hepatic lipase (HL) activity in the liver was measured by the conversion 1–$^{14}$C-triolein to 1–$^{14}$C-oleic acid [35]. Liver sections were examined by light microscopy after being stained with 1% Oil Red O and Erlich's haematoxylin.

*Statistics*

Values are presented as mean ± SEM, unless otherwise stated. A general linear model, analysis of variance in Minitab (version 14 for Windows, Minitab Ltd., Coventry, UK) was used to assess differences between the treatments. A few animals (E, n = 2; I, n = 2; C, n = 3) were slaughtered prior to the end of the study due to the varying ability of individual piglets to tolerate parenteral feeding. Any pig slaughtered after 5 days of treatment was included in the data. Age at end of study was, therefore, included as a covariate in the statistical analysis. Correlations were analysed using Pearson correlation and regression analysis.

**Table 1.** Effect of enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20% on organ weight as a percentage of bodyweight

| Organ | Weight of organ, % of body weight | | |
|---|---|---|---|
| | enteral | ClinOleic | Intralipid |
| Gut | 5.34 ± 1.10[a] | 2.87 ± 0.14[b] | 3.15 ± 0.45[b] |
| Liver | 3.33 ± 0.30 | 4.03 ± 0.30 | 4.14 ± 0.30 |
| Lungs | 1.55 ± 0.11 | 2.07 ± 0.23 | 1.95 ± 0.22 |
| Brain | 1.59 ± 0.07 | 1.80 ± 0.08 | 1.71 ± 0.05 |
| Stomach | 0.74 ± 0.04 | 0.92 ± 0.16 | 0.78 ± 0.05 |
| Heart | 0.61 ± 0.02 | 0.78 ± 0.08 | 0.74 ± 0.03 |
| Left kidney | 0.43 ± 0.01 | 0.46 ± 0.03 | 0.45 ± 0.03 |
| Right kidney | 0.41 ± 0.02 | 0.43 ± 0.04 | 0.43 ± 0.04 |
| Spleen | 0.15 ± 0.01 | 0.24 ± 0.03 | 0.27 ± 0.08 |
| Pancreas | 0.18 ± 0.04 | 0.13 ± 0.04 | 0.11 ± 0.02 |

The organs were weighed after 7 days of treatment. All treatment groups n = 6. Values are given as mean ± SEM. Results marked with different letters within a row are significantly different $p < 0.001$. Within rows without superscript letters, there are no significant differences between the groups.

## Results

The number of piglets surviving to day 7 of age was comparable between treatment groups (E, n = 4; I, n = 4; C, n = 3). Regardless of lipid emulsion used, piglets on TPN had lower ($p < 0.001$) body weights at slaughter than enterally fed piglets (E: 1,700 ± 29.84; C: 1,480 ± 29.84; I: 1,614 ± 29.97 g). Crown-to-rump length (28.2 ± 0.5 cm), femur length (20.6 ± 0.22 cm) and head circumference (49.2 ± 0.3 mm) were similar between groups. Abdominal circumference (E, 21.5 ± 0.3; I, 20.2 ± 0.3; C, 19.8 ± 0.7 cm) and ponderal index (E, 82 ± 2; I 68 ± 4; C, 66 ± 7 kg/$m^3$) were higher ($p < 0.05$) in E compared with TPN piglets. Conversely, head:abdominal circumference was lower ($p < 0.05$) in the E group (E, 2.31 ± 0.04; I, 2.49 ± 0.09; C, 2.43 ± 0.02). Growth rate was lowest ($p < 0.05$) in C piglets, but there was also a reduction in growth of I piglets when compared with E piglets (E: 59 ± 6; C: 19 ± 7; I: 43 ± 6 g $day^{-1}$). The total energy intake, per kg of final weight, of the piglets in the study was highest ($p < 0.001$) in the enterally fed group (E: 10.31 ± 0.94; C: 3.20 ± 0.94; I: 2.63 ± 0.94 MJ $kg^{-1}$ final weight).

Liver weight as a percentage of body weight, was increased by TPN ($p < 0.1$), whilst the weight of the gut as a percentage of body weight, was decreased ($p < 0.001$; table 1). TPN had no significant effect on the weight of

**Table 2.** Composition of key fatty acids (% by weight) within the nutritional treatment and the triacylglycerol extracted from the liver of piglets maintained on total parenteral nutrition for 5–7 days

| Fatty acid | Enteral | | ClinOleic 20% | | Intralipid 20% | |
|---|---|---|---|---|---|---|
| | enteral diet | liver TAG | TPN | liver TAG | TPN | liver TAG |
| 12:0 | 19.18 ± 0.15 | 0.13 ± 0.13 | 0.04 ± 0.00 | 0.10 ± 0.05 | 0.04 ± 0.01 | 0.05 ± 0.02 |
| 14:0 | 8.55 ± 0.01 | 2.72 ± 0.55[a] | 0.04 ± 0.00 | 1.13 ± 0.14[b] | 0.05 ± 0.04 | 0.96 ± 0.09[b] |
| 16:0 | 23.17 ± 0.08 | 25.09 ± 0.94[a] | 12.98 ± 0.09 | 20.01 ± 0.49[b] | 12.25 ± 0.12 | 16.42 ± 0.32[c] |
| 18:0 | 6.46 ± 0.03 | 6.73 ± 1.14[a] | 3.82 ± 0.01 | 3.67 ± 0.46[b] | 3.56 ± 0.04 | 3.65 ± 0.16[b] |
| 18:1 (n–9) | 33.34 ± 0.36 | 40.95 ± 2.39[a] | 60.32 ± 0.14 | 49.17 ± 0.96[b] | 23.49 ± 0.02 | 21.73 ± 0.89[c] |
| 16:1 (n–7) | 0.74 ± 0.06 | 5.95 ± 0.90[a] | 0.97 ± 0.02 | 5.06 ± 0.56[a] | 0.18 ± 0.00 | 2.10 ± 0.48[b] |
| 18:2 (n–6) | 6.45 ± 0.13 | 10.78 ± 2.33[a] | 18.39 ± 0.05 | 13.98 ± 1.03[a] | 52.80 ± 0.22 | 44.19 ± 1.26[b] |
| 20:4 (n–6) | 0.05 ± 0.00 | 2.20 ± 0.96 | 0.20 ± 0.00 | 1.24 ± 0.19 | 0.21 ± 0.01 | 1.52 ± 0.13 |
| 18:3 (n–3) | 0.94 ± 0.02 | 0.72 ± 0.24[a] | 2.09 ± 0.01 | 0.89 ± 0.10[a] | 5.57 ± 0.06 | 3.20 ± 0.16[b] |
| 20:5 (n–3) | 0.78 ± 0.39 | 0.27 ± 0.05 | 0.15 ± 0.07 | 0.21 ± 0.03 | 0.62 ± 0.08 | 0.19 ± 0.05 |
| 22:6 (n–3) | 0.00 ± 0.00 | 0.81 ± 0.30 | 0.12 ± 0.00 | 0.44 ± 0.08 | 0.16 ± 0.02 | 0.00 ± 0.06 |

Liver triacylglycerol samples were obtained from 6 animals in each treatment group. Fatty acid composition of the diets is based on results from the analysis of three individual samples of the diet. Values are least-squares means ± SEM. Results for liver TAG marked with different letters within a row are significantly different. Within rows without superscript letters, there are no significant differences in the Liver TAG between the groups.

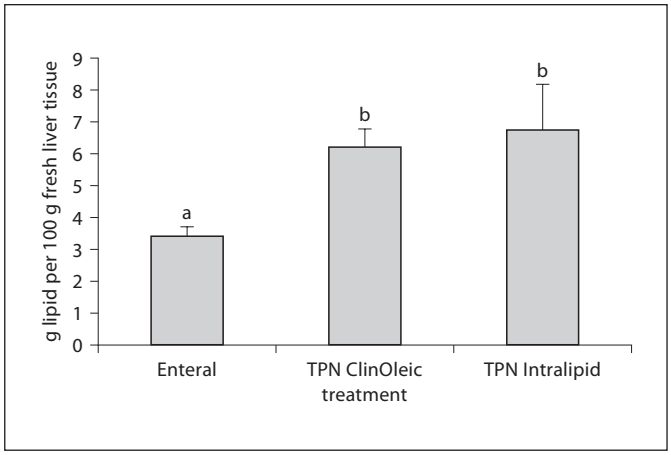


**Fig. 1.** The effect of enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20% on hepatic lipid content at the end of treatment. For all treatment groups n = 6. Values are least-squares means, error bars show SEM. Bars with different letters differ significantly at $p < 0.05$.

the other organs. Increased liver weight during TPN was paralleled by a significant increase ($p < 0.05$) in hepatic lipid content in TPN piglets when compared to the control E piglets (fig. 1). This finding was supported by the histological finding which showed larger and more numerous lipid droplets in the livers of TPN piglets than in the E group (fig. 2). The fatty acid profile of the hepatic TAG can be seen to follow closely that of the lipid emulsion given during TPN and was markedly altered by treatment (table 2). There was a correlation between the proportion of each fatty acid in the liver and the proportion of the same fatty acid in the diet in all treatment groups (fig. 3).

The activity of HL was ($p < 0.001$) reduced during TPN (fig. 4). This was mirrored by a similar decrease in the activity of LPL in the liver of piglets on TPN (fig. 5). A smaller decrease in the activity of skeletal muscle LPL was also seen during TPN. In contrast, a twofold increase was seen in adipose tissue LPL during TPN (fig. 5). The LPL activity in the TPN groups was very variable, probably due to different time lags between the end of infusion and slaughter [36], consequently the LPL results did not reach significance.

During the trial, piglets receiving TPN showed signs of transient, or in some cases permanent, jaundice. TPN had little effect on the plasma concentrations of bilirubin, choline, NEFA and TAG which were similar between treatment groups (table 3).

## Discussion

Most infants on TPN suffer clinical complications, therefore metabolic requirements are complex and data interpretation is difficult [25]. Animal models allow co-

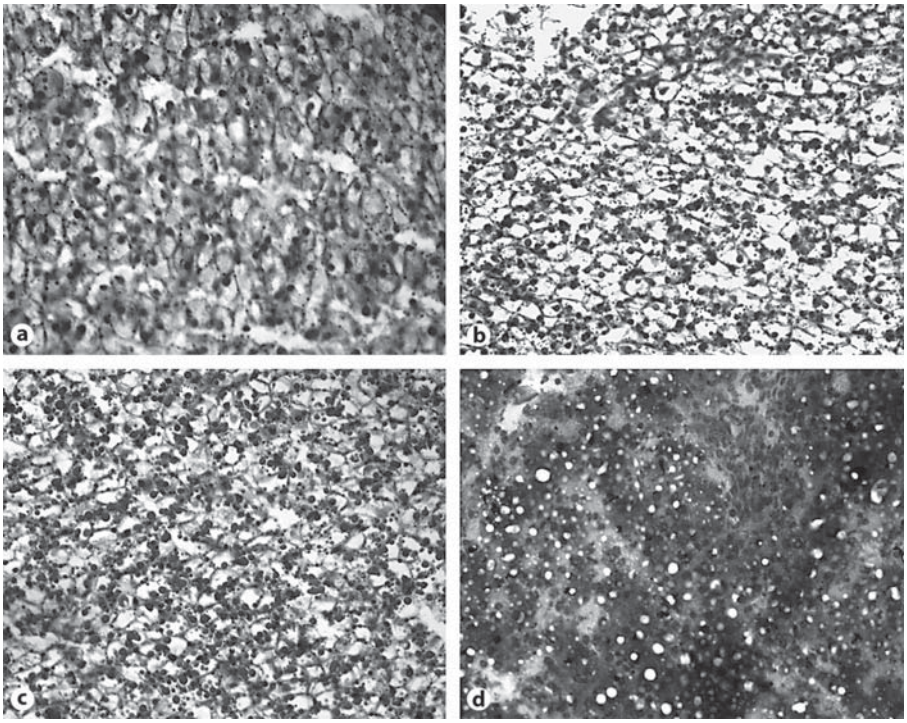


**Fig. 2.** Liver sections taken from piglets maintained for 7 days on enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20%. Sections were stained with Oil Red O and counterstained with Haematoxylin. ×150. The sections were taken from the liver having hepatic lipid content nearest to the mean for the treatment group. **a** From a piglet in the enterally fed group having 3.5 g lipid/100 g fresh liver tissue. **b** From a piglet in the TPN ClinOleic group having 5.8 g lipid/100 g fresh liver tissue. **c** From a piglet in the TPN Intralipid group having 6.3 g lipid/100 g fresh liver tissue. **d** The extreme example from the 18 piglets, it was from the TPN Intralipid group and had 13.7 g lipid/100 g fresh liver tissue.

**Table 3.** Effect of enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20% on plasma bilirubin, choline, non-esterified fatty acid and triacylglycerol concentrations at the end of treatment

| Parameter | Treatment group | | | Population SEM |
|---|---|---|---|---|
| | enteral | ClinOleic 20% | Intralipid 20% | |
| Plasma bilirubin, mM | 27.24 | 30.88 | 31.41 | 1.96 |
| Plasma choline, μM | 1.791 | 3.503 | 6.814 | 2.948 |
| Plasma NEFA, mM | 0.252 | 0.272 | 0.383 | 0.092 |
| Plasma TAG, mM | 0.215 | 0.298 | 0.230 | 0.036 |

The blood samples analysed were collected after 7 days of treatment. All treatment groups n = 6. Values expressed as least-squares means.

trolled experimental development of TPN. We have shown that piglets can be maintained for up to 7 days on the same TPN regimes used for human neonates, something that cannot be done in the rat [25]. During TPN, the piglets showed typical responses previously reported in human infants.

Growth rate was lower in TPN piglets compared to enterally fed piglets, possibly due to the unavoidable lower energy intake with TPN. The discrepancy in the energy intakes of TPN and control animals could not be controlled due to the differing availability of metabolisable energy from dietary sources of diverse composition and alternative routes of administration. Any attempts to do so would have nutritionally compromised the control groups and been to the detriment of the piglets health in both nutritional groups. Our attempts to increase the energy intake of the TPN piglets to improve growth rates resulted in fluid retention and/or pulmonary and cardiac weakness. Whilst differences in energy intake are not desirable in a controlled experiment, it does emulate the situation in the human neonate where TPN generally results in a lower energy intake [55] and consequently lower growth rates. Growth rates in the TPN groups are at the lower end of those seen in piglets of the same postnatal age, but prematurity may have contributed to this. The reasons for the lower growth rate seen in the TPN-ClinOleic group when compared with that seen in the TPN-Intralipid group cannot be explained from the results of

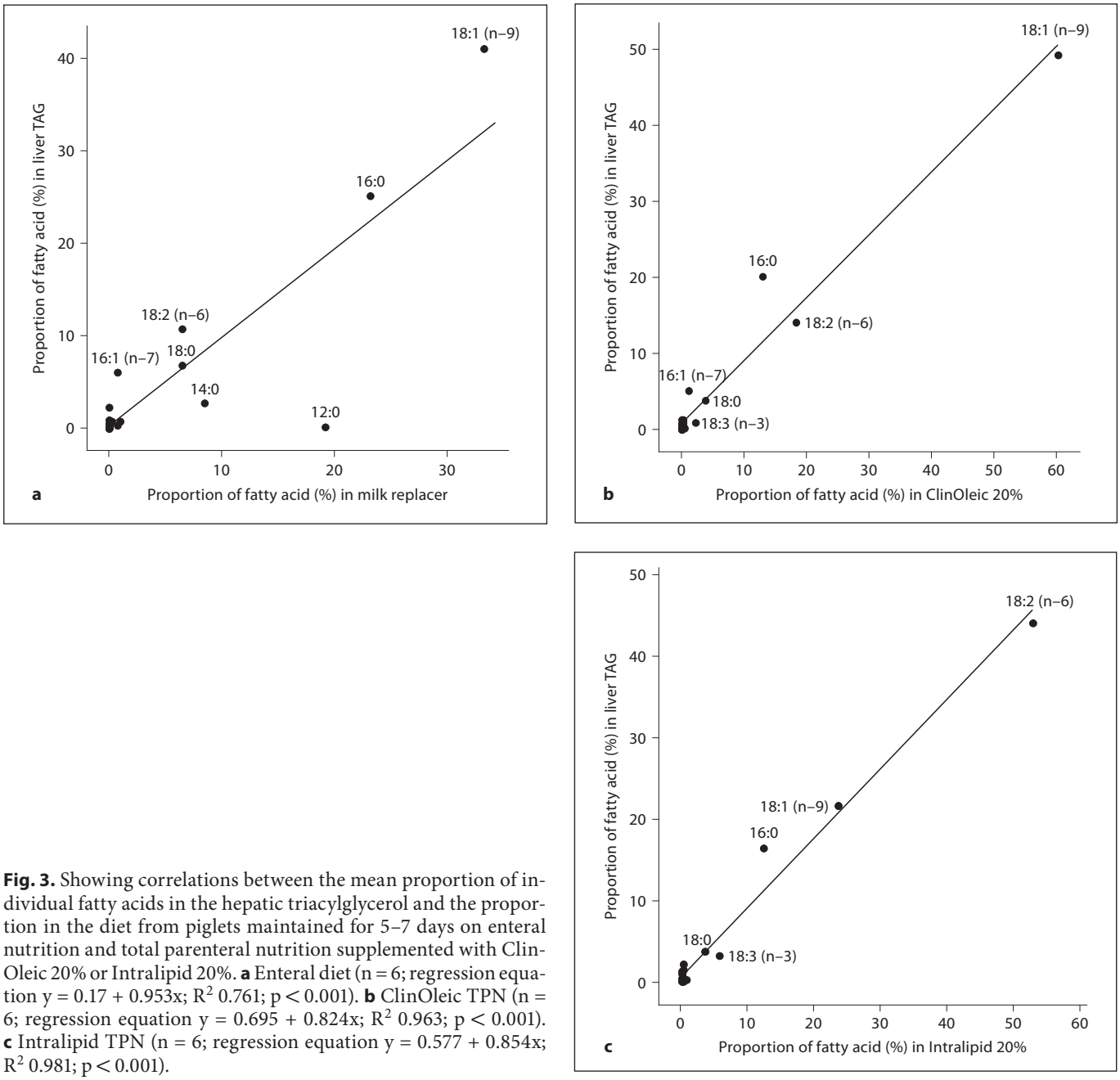


**Fig. 3.** Showing correlations between the mean proportion of individual fatty acids in the hepatic triacylglycerol and the proportion in the diet from piglets maintained for 5–7 days on enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20%. **a** Enteral diet (n = 6; regression equation y = 0.17 + 0.953x; $R^2$ 0.761; $p < 0.001$). **b** ClinOleic TPN (n = 6; regression equation y = 0.695 + 0.824x; $R^2$ 0.963; $p < 0.001$). **c** Intralipid TPN (n = 6; regression equation y = 0.577 + 0.854x; $R^2$ 0.981; $p < 0.001$).

this experiment. This finding, however, is consistent with a previous study demonstrating that the fatty acid profile of the diet had a marked effect on growth rate in neonatal rats; this was attributed to low concentrations of arachidonic acid in the plasma and liver of the rats [45]. Further research is required to explain the complex relationship between the fatty acid profile of the diet and growth rate.

Despite the differences in growth rate observed between E and TPN-fed piglets, crown-to-rump length, femur length and head circumference were similar between groups. In contrast, abdominal circumference and ponderal index were reduced following TPN, suggesting that these animals were exhibiting signs of disproportionate body growth, akin to that observed in intra-uterine growth restricted human pregnancies [56].

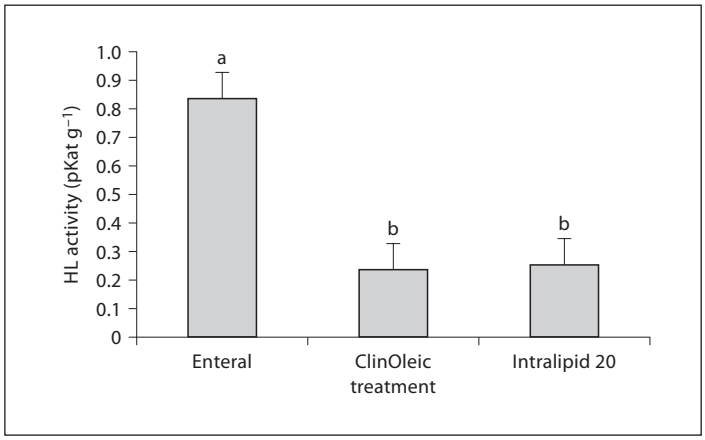


**Fig. 4.** The effect of enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20% on hepatic lipase activity. For all groups n = 6. Values are least-squares means, error bars show SEM. Bars with a different letter are significantly different $p < 0.001$.

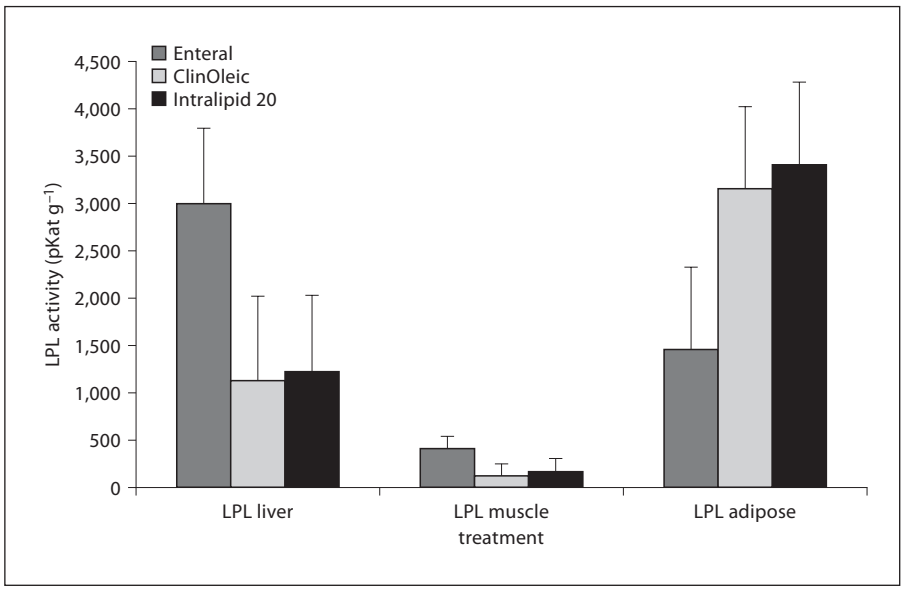


**Fig. 5.** The effect of enteral nutrition and total parenteral nutrition supplemented with ClinOleic 20% or Intralipid 20% on lipoprotein lipase activity in the liver, skeletal muscle and adipose tissue. For all groups n = 6. Values are least-squares means. Error bars show SEM.

The differences seen in organ weight are typical of those previously reported in piglets on TPN [38, 39]. Reduced gut weight results from the absence of nutritional stimulus [40], whilst the increase in liver weight may be partially attributed to the increase in lipid content and in some cases inflammation of the liver. During TPN some piglets showed transient jaundice, although there was no significant increase in plasma bilirubin concentration during TPN. Without the histological findings, the jaundice could have been attributed to cholestasis rather than fatty liver.

The increase in lipid content of the liver falls within the biochemical definition of fatty liver in humans, the lipid content being greater than 5 g per 100 g of the wet liver weight [41, 42]. Given the similarities between the pig and humans, we speculate that the increased lipid accumulation observed in the piglets equates to the chronic lipid accumulation that occurs in human infants on TPN [1, 2]. A similar proposal was made by Wang et al. [54]. The findings of the current study showed that the weight of lipid in the liver during TPN was higher than the 1.7 g per 100 g previously reported in piglets [43], but lower than the 19 g per 100 g reported in rats [44]. The difference may have been due to differences in the TPN regime, species or the age and developmental stage of the subjects. The fatty acid profile of the TAG deposited in the liver closely mirrored the profile of the lipid emulsions used during TPN, suggesting that dietary lipid may be the source of the lipid deposition, rather than by synthesis de novo from glucose precursor supplied in the TPN solution as has previously been suggested [17]. However, the fact that the percentage of 16:0 in the liver TAG does not mirror that of

the emulsion is suggestive of some synthesis de novo. The fact that the energy intake was lower in the TPN group may have helped to reduce the lipogenic effect of excess glucose supply during TPN. It can be speculated that the supply of lipid during TPN may contribute to the incidence of fatty liver, even when supplied at concentrations which do not result in obvious hyperlipidaemia. Plasma NEFA and TAG concentrations remained stable during TPN, but fatty liver was present; therefore, measurement of plasma lipids may not be a sufficient indicator to diagnose the occurrence of fatty liver.

Although the fatty acid analysis suggests that the lipid emulsion supplied was taken up by the liver and stored, hepatic lipase activity was reduced in the TPN group, implying that uptake of lipid by the liver may be reduced. The increase in activity of LPL in the adipose tissue of piglets on TPN indicates that this is the main site of lipid deposition, but on dissection subcutaneous adipose depots were difficult to locate. This finding alongside the lack of difference in LPL activity in skeletal muscle is in agreement with work done in humans [46], but the reasons for it remain unclear. Possible explanations for the findings are (1) the fatty acids released by LPL in adipose tissue are not taken up in the adipose tissue, but instead are taken up from circulation by the liver; (2) fatty acids released by the LPL in adipose tissue are taken up by the adipose and esterified; after which they are hydrolysed and released into circulation as NEFA (via hormone sensitive lipase), which are taken up by the liver; or (3) an increase in adipose tissue LPL, after 7 days of TPN, is a later adaptation to the potentially damaging accumulation of lipid in the liver.

The decrease in liver lipases are in agreement with previous findings in rats on TPN [47, 18], which may be due to either: (1) fatty liver causing an impairment of liver function including HL and LPL activity; (2) a protective mechanism on the part of the liver to prevent fatty liver; (3) the fact that these enzymes are partially regulated by a stimulus lacking in a parenterally fed animal; or (4) hypothyroidism which can be a side effect of TPN and is known to result in pronounced reductions of HL activity in humans [48] and rats [47].

It should be noted that, although the LPL activity in the liver is decreased during TPN, it remains sufficient to make a significant contribution to the uptake of fatty acids released from TAG of circulating TAG-rich lipoproteins. As far as we are aware, this study is the first to measure LPL activity in the liver of the piglet, although it has previously been reported in the newborn rat [49], where it has been shown to be modified by nutritional manipulation [50]. The fact that newborn liver expresses LPL, suggests the liver is an importer of circulating TAG, which appears to be mediated by high expression of PPARα [51], and agrees with the correlations observed between the fatty acid profile of the diet and the liver TAG (fig. 3).

Apart from increased uptake of lipid into the liver, decreased export of lipid from the liver can also result in fat accumulation by the liver. Earlier work in humans [20, 51] has suggested that choline deficiency during TPN caused a reduction in the export of TAG as very low density lipoprotein (VLDL) from the liver. Choline deficiency was unlikely to have been a factor in this experiment because the TPN lipid emulsions contained choline in the form of phosphatidylcholine (2.25 g per 100 g). Furthermore there was no significant difference between the plasma choline concentrations. Other causes of a reduction in hepatic lipid export, e.g. apolipoprotein B degradation due to hyperinsulinaemia [53] were not studied in this experiment. Nevertheless, the fact that fat was deposited in the liver would indicate that there is either a reduction in export of lipid, or that the deposition of fat occurred at rates beyond the physiological limits of the export pathways. Further research is needed in order to elucidate this point.

In conclusion, our results suggest that the piglet is a suitable model to study the effects of TPN on hepatic function in the premature neonate. The increase in hepatic lipid content, together with the histological findings, supports the hypothesis that fatty liver does occur in the neonate given TPN, possibly more often than normally reported, as it is probably masked by symptoms of cholestasis. The mechanisms that result in increased fat storage in the liver are still points of conjecture. Three main possibilities exist: (1) increased uptake of fat; (2) decreased export of lipid from the liver and/or decreased utilisation of lipid within the liver; or (3) a combination of both increased uptake and decreased secretion of lipid.

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