JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 461–465

Received October 14, 2007
Accepted January 7, 2008
DOI 10.1211/jpp.60.4.0008
ISSN 0022-3573

# Soybean fat supplementation controls insulin resistance caused by fat-free total parenteral nutrition

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## Abstract

Conscious young adult male rats were given total parenteral nutrition (TPN) with or without soybean fat for 4 days. Those given fat-free TPN developed severe fatty liver, with hyperglycaemia, hyperinsulinaemia, and hypotriglyceridaemia. These disorders were clearly improved by supplementing TPN with soybean fat, in an amount equivalent to 20% of total calories, and correspondingly reducing glucose. Insulin resistance also developed over a 4-day infusion of fat-free TPN in mature rats. Even after overnight fasting after stopping the TPN infusion, the levels of serum glucose and insulin were higher in the fat-free TPN group than in the control group, and intravenous glucose tolerance test results indicated insulin resistance in the fat-free TPN group. The HOMA-IR index of insulin resistance was significantly improved by supplementation with soybean fat. In conclusion, fat-free TPN infusion induced hyperglycaemia and hyperinsulinaemia, leading to fatty liver and insulin resistance. TPN with glucose should be supplemented with soybean fat emulsion as replacement for part of the glucose calories.

## Introduction

The American Society for Parenteral and Enteral Nutrition guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients state that, when initiating parenteral nutrition, insulin therapy should be continued in patients with type 1 diabetes and in patients with type 2 diabetes who previously required insulin (August et al 2002). The rate at which glucose is administered is important in preventing hyperglycaemia, and it should be given at a rate less than 4–5 $mg\,kg^{-1}min^{-1}$, even in non-diabetic patients (Rosmarin et al 1996; McMahon 1997). An investigation of US medical centres revealed that 16% of patients received greater than 4 $mg\,kg^{-1}min^{-1}$ glucose during total parenteral nutrition (TPN), suggesting a risk for induction of not only hyperglycaemia and hyperinsulinaemia but also hepatic steatosis (Schloerb 2004). On the other hand, in spite of this evidence, there is a tendency to avoid addition of fat in TPN in Japan, because of unease about the risk of thrombosis, and increased liver burden etc. The purpose of this study is to indicate that addition of an appropriate amount of fat emulsion is important to TPN therapy.

The energy content of lipids, at 9 $kcal\,g^{-1}$, is more than double that of glucose, at 4 $kcal\,g^{-1}$. Thus, it is possible to prevent excessive administration of glucose during TPN by replacing part of the glucose with a fat emulsion. Oshita et al (2004) and Nishimura et al (2006) have indicated that metabolic complications in the liver occurred as a result of excessive administration of fat-free TPN in infant rats and could be prevented by supplying 20% of total calories from soybean oil.

In this study, using conscious young adult rats, the infusion of fat-free TPN for 4 days caused severe hepatic steatosis associated with insulin resistance. These symptoms were improved by replacing 20% of the total glucose calories with fat.

## Materials and Methods

### Animals

Male Sprague–Dawley rats, 6 weeks of age, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The rats were housed in a light-controlled room (light from 09:00 to 21:00) at a

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temperature of 25 ± 2°C and a humidity of 60 ± 10%. The rats were allowed free access to water and standard rat diet, which contained, per 100 g, 54.7 g starch, 18.8 g protein, 3.9 g fat, 6.6 g fibre, and 6.9 g mineral and vitamin mixture (Labo MR stock, Nihon Nosan Kogyo Ltd., Yokohama, Japan). All animal procedures were in accordance with the standards set forth in the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi campus of Kanazawa University, Japan.

### Surgery and diet

The rats, weighing 180–200 g each, were randomly allocated to four groups (n = 5 in each group): the untreated control group (control), the oral diet group (OD), the TPN group without fat (fat-free), and the TPN group with 20% of experimental calories from a fat emulsion of soybean oil and egg lecithin group (20% fat). After rats in the OD and TPN groups were fasted overnight, a polyethylene catheter (0.58 mm i.d., 0.96 mm o.d., Biotechnology Research Center, Nagoya, Japan) was implanted, under general anaesthesia with pentobarbital (50 mg/100 g, intraperitoneally). The catheter was surgically inserted via the right jugular vein into the superior vena cava and was routed to the back of the neck, to exit through a coil spring attached to a swivel, allowing free mobility of the animals within their individual metabolic cages. All TPN regimens were isocaloric (1000 kcal $L^{-1}$) and isonitrogenic. The compositions of the fat-free TPN and 20% fat TPN are shown in Table 1. The fat-free TPN group received only basal TPN solution (Amiparen, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan), and the 20% fat TPN group received basal solution supplemented with soybean fat equivalent to 20% of total calories (Intralipos, Otsuka Pharmaceutical), with a corresponding decrease in glucose. Vitamedin IV (Daiichi-Sankyo Co. Ltd., Tokyo, Japan) was used to supply vitamins. The TPN groups continuously received an increasing amount of TPN, in a stepwise manner, using an infusion pump (BRIFSYS-R1, Bioresearch Center Co., Tokyo, Japan). Immediately after cannulation, the infusion was started at 5 mL $kg^{-1}$ $h^{-1}$ on the first day. On the second day, the infusion rate was increased to 10 mL $kg^{-1}$ $h^{-1}$, and on the third day, to 15 mL $kg^{-1}$ $h^{-1}$. The total experimental period was 4 days, and the catheter was washed out once a day with heparin.

**Table 1** Formulation of TPN solutions

| | Fat-free | 20% Fat |
|---|---|---|
| Glucose (g) | 184 | 140 |
| Fat (g) | 0 | 19.5 |
| Amino acid (g) | 36 | 36 |
| Na (mEq) | 35 | 35 |
| K (mEq) | 27 | 27 |
| Ca (mEq) | 7.8 | 7.8 |
| Cl (mEq) | 54 | 54 |
| Mg (mEq) | 5 | 5 |
| P (mmol) | 6.5 | 7.8 |
| Zn (μmol) | 20 | 20 |

TPN solution contains vitamin B. TPN calories per unit volume: 880 kcal/880 mL. Caloric ratio of nonprotein calories to nitrogen (NPC/N): 130.

### Sample collection

Blood samples were collected from the left jugular vein at the end of the experiment (without fasting) and were centrifuged (3500 rev $min^{-1}$, 10 min). Serum samples were stored at −80°C. After weighing the liver, part of it was fixed in 10% buffered formalin and stained with Oil red O and haematoxylin and eosin (H&E) according to standard methods; the rest of the tissue was used for biochemical analysis.

### Biochemical analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and phospholipids (PL) were measured by SRL Co., Ltd. (Tokyo, Japan). Triglyceride (TG), total cholesterol (TC), and free fatty acid (FFA) were measured in our laboratory, using commercially available assay kits (Wako Pure Chemical Co., Osaka, Japan). Serum glucose was measured with a blood glucose monitoring system (Glucocard, Arkray Inc., Kyoto, Japan). Serum insulin was measured using an ELISA kit for rat insulin (ARKIN-010, Shibayagi Co., Gunma, Japan).

### Intravenous glucose tolerance tests (IGTT)

After 4 days of TPN infusion, glucose (4.4 mmol $kg^{-1}$) was intravenously injected into rats that had been fasted overnight. Blood samples were collected from the left jugular vein just before and at 2, 6, 15, 30, and 60 min after starting the IGTT. The blood samples were centrifuged (3500 rev $min^{-1}$, 10 min), and serum glucose and insulin were measured.

### Index of insulin sensitivity

The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using glucose (FSG) and insulin (FSI) values determined in the early morning, after overnight fasting (Matthews et al 1985; Cutfield et al 1999), as follows: HOMA-IR = FSG × FSI / 405.

### Statistical analysis

Data are expressed as means ± s.d. The statistical significance of differences between four groups was analysed by the parametric analysis of variance (ANOVA). *P* values of less than 0.05 or 0.01 were considered statistically significant.

## Results

After young adult rats had received TPN with or without fat for 4 days, the body weight did not differ among the four groups, but the livers of the group given fat-free TPN were enlarged to double the weight of the livers of the control groups (Table 2). The liver weight of the 20% fat TPN group was intermediate between those of the control and fat-free TPN groups.

**Table 2** Body, liver weight and biochemical data

| | Control | OD | Fat-free TPN | 20% fat TPN |
|---|---|---|---|---|
| Body weight (g) | 201 ± 1.4 | 202 ± 1.6 | 195 ± 6 | 193 ± 1.9 |
| Liver weight (g) | 6.6 ± 0.0$^{\#\#}$ | 8.0 ± 0.9$^{\#\#}$ | 16.7 ± 1.7** | 12.2 ± 2.0**,$^{\#\#}$ |
| Serum | | | | |
| AST ($IUL^{-1}$) | 91 ± 12$^{\#}$ | 89 ± 6$^{\#}$ | 204 ± 67* | 98 ± 7$^{\#}$ |
| ALT ($IUL^{-1}$) | 55 ± 3$^{\#}$ | 53 ± 5$^{\#}$ | 96 ± 21* | 39 ± 14$^{\#\#}$ |
| ALP ($IUL^{-1}$) | 1148 ± 115 | 1122 ± 223 | 1274 ± 241 | 1175 ± 220 |
| γ-GTP ($IUL^{-1}$) | <2 | <2 | 4 ± 2 | 3 ± 1 |
| TG ($mgdL^{-1}$) | 48 ± 9$^{\#\#}$ | 60 ± 10$^{\#\#}$ | 7.7 ± 2.0** | 21 ± 5**,$^{\#\#}$ |
| TC ($mgdL^{-1}$) | 92 ± 5$^{\#\#}$ | 87 ± 15$^{\#\#}$ | 36 ± 13** | 74 ± 10$^{\#\#}$ |
| FFA ($\mu EqL^{-1}$) | 281 ± 96$^{\#}$ | 294 ± 73$^{\#}$ | 130 ± 42* | 246 ± 56$^{\#}$ |
| PL ($mgdL^{-1}$) | 128 ± 19 | 111 ± 7 | 107 ± 24 | 113 ± 20 |
| Glucose ($mgdL^{-1}$) | 103 ± 15$^{\#\#}$ | 117 ± 4$^{\#\#}$ | 304 ± 83** | 147 ± 57$^{\#\#}$ |
| Insulin ($\mu UmL^{-1}$) | 63 ± 12$^{\#\#}$ | 73 ± 21$^{\#\#}$ | 248 ± 16** | 142 ± 14**,$^{\#\#}$ |

After 4 days of oral feeding of standard diet (control group, OD group) or TPN infusion (fat-free TPN group, 20% fat TPN group), samples were collected from rats without fasting. Data represent the means ± s.d. of 5 rats. *, **Significantly different from the OD group at $P < 0.05$ and 0.01, respectively. $^{\#}$, $^{\#\#}$Significantly different from the fat-free TPN group at $P < 0.05$ and 0.01, respectively.

The pathological findings included diffuse fatty degeneration in the liver of rats receiving fat-free TPN, whereas the liver of rats receiving fat supplementation showed decreased fatty degeneration (Figure 1). Among hepatic biomarkers, serum AST and ALT were significantly increased after fat-free TPN and were improved by fat supplementation (Table 2). Infusion of fat-free TPN significantly decreased the serum concentrations of TG, TC, and FFA, and fat supplementation of TPN almost completely blocked the decrease in lipid concentrations, except for TG (Table 2). Serum glucose and insulin levels were significantly increased with fat-free TPN and returned to normal with 20% fat TPN (Table 2). In all these experiments, the surgical operation of catheter implantation had hardly any influence on the biomarkers upto 4 days after the operation.

As these data suggested impairment of insulin sensitivity in rats receiving fat-free TPN, an IGTT was conducted after overnight fasting (Figure 2). Fasting serum glucose concentrations were 85.3 ± 13.4 in the OD control group, 122.4 ± 7.6

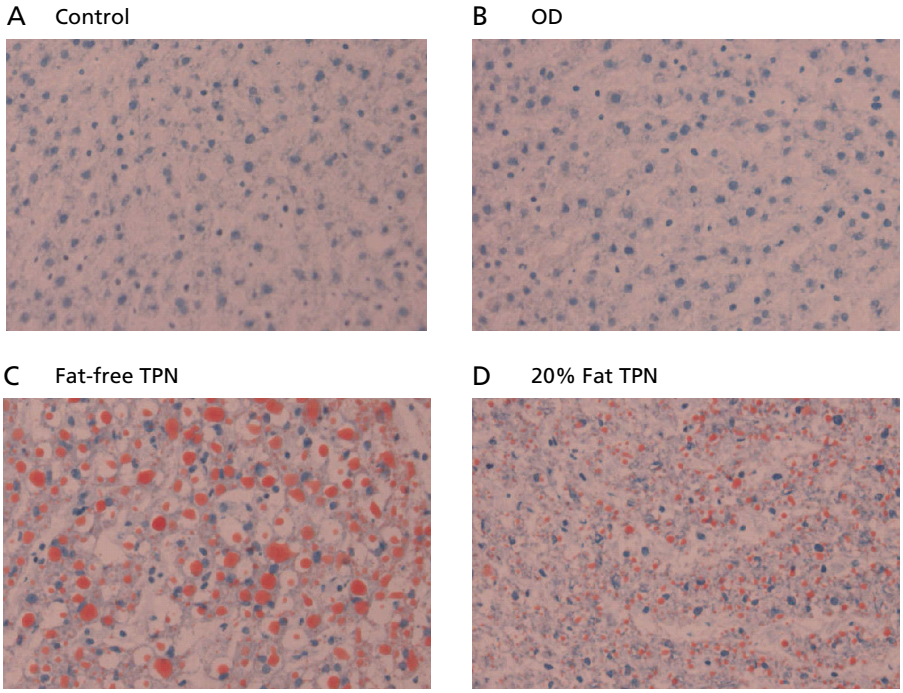


**Figure 1** Light micrographs of the livers of rats receiving TPN with or without fat for 4 days (haematoxylin & eosin and Oil red O staining).

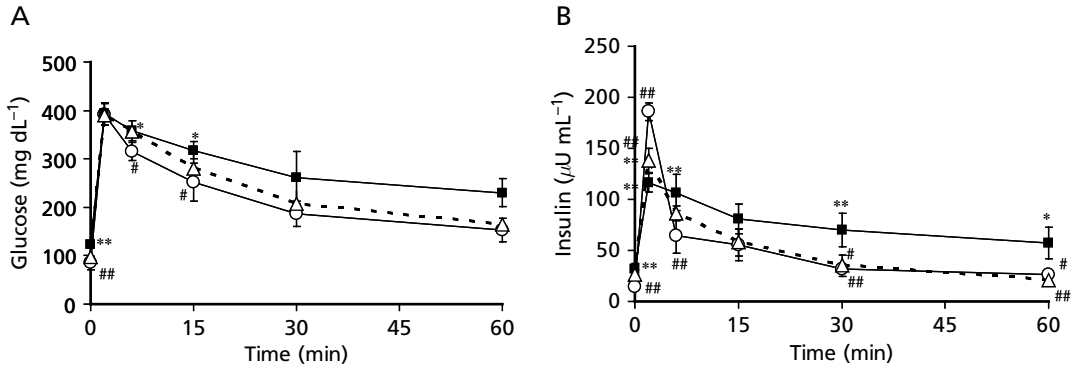


**Figure 2** Time courses of serum concentrations of glucose(A) and insulin(B) following the IGTT in rats. Rats were used for IGTT after overnight fasting. Glucose (4.4 mmol kg$^{-1}$) was intravenously administered and samples were collected at 0, 6, 15, 30 and 60 min. O, OD group; ■, fat-free TPN group; △, 20% fat TPN group. Each symbol and bar represents the mean ± s.d. of 5 rats. *, ** Significantly different from the oral diet control group at $P < 0.05$ and 0.01, respectively. #, ## Significantly different from the fat-free TPN group at $P < 0.05$ and 0.01, respectively.

in the fat-free group ($P < 0.01$, vs. OD group), and 97.3 ± 11.5 mg dL$^{-1}$ in the 20% fat group ($P < 0.01$, vs. fat-free group); fasting serum insulin concentrations were 14.8 ± 1.6 in the control group, 34.0 ± 4.3 in the fat-free group ($P < 0.01$, vs. OD group), and 26.4 ± 4.3 U mL$^{-1}$ in the 20% fat group ($P < 0.01$, vs. OD group; $P < 0.05$, vs. fat-free group). After the injection of glucose, although the serum insulin level was higher in the fat-free TPN group than in the OD group, glucose tolerance was decreased in the fat-free TPN group, suggesting insulin resistance. Glucose tolerance and hyperinsulinaemia clearly improved in the 20% fat TPN group.

Insulin sensitivity can be evaluated using the HOMA-IR, as derived from glucose and insulin levels after overnight fasting. The index values were 3.3 ± 0.9 in the OD group, 10.4 ± 2.0 in the fat-free TPN group ($P < 0.01$, vs. OD group), and 6.3 ± 0.5 in the 20% fat TPN group ($P < 0.01$, vs. OD group; $P < 0.05$, vs. fat-free group).

## Discussion

This study confirmed that fat-free TPN, after only 4 days, induced severe fatty liver with hyperglycaemia and hyperinsulinaemia in young adult rats. The conditions were clearly improved by TPN with 20% of calories from a fat emulsion. In general, about 12.5 kcal kg$^{-1}$ h$^{-1}$ are required to maintain body weight in normal adult rats (Shirotani & Numata 2006). In this study, rats were administered a maximum of 15 kcal kg$^{-1}$ h$^{-1}$ through TPN, without any acute toxic events such as dyspnoea, convulsions, hypothermia, pulmonary bleeding, congestion or ascites. However, forced administration of high glucose, as in fat-free TPN, caused fatty liver, as previously reported by Oshita et al (2004). Further, Nishimura et al (2006) recently reported that high serum glucose during the administration of fat-free TPN in infant rats decreased gluconeogenesis- and lipolysis-related gene expression in the liver but increased the expression of acetyl-coenzyme A carboxylase (ACC), a key enzyme in lipogenesis, resulting in the accumulation of lipids in the liver. On the other hand, despite the fatty liver, serum lipid concentrations were significantly lower in the fat-free TPN group. This may be attributable to fatless nutrition and hyperinsulinaemia. It has been reported that acute hyperinsulinaemia suppresses the hepatic secretion of very low density lipoprotein into the bloodstream (Lewis et al 1995; Lewis & Steiner 1996). These disorders were clearly improved by supplementation with 20% fat in the TPN solution, as has been reported elsewhere (Oshita et al 2004; Nishimura et al 2006).

Surprisingly, systemic insulin resistance developed over the short term: here, a 4-day TPN infusion in mature rats. Even after overnight fasting after stopping TPN infusion, the levels of serum glucose and insulin were far higher in the fat-free TPN group than in the oral diet control group, and the results of the IGTT indicated insulin resistance in the fat-free TPN group. The HOMA-IR index of insulin sensitivity was significantly improved by supplementation with fat. Although the mechanism for insulin resistance should be further studied, it seems that hyperinsulinaemia is an important factor in hepatic lipid accumulation. It is known that excess insulin activates hepatic sterol regulatory element binding protein-1 (SREBP-1), followed by gene expression of lipid synthesis-related enzymes such as ACC and fatty acid synthase (Liang et al 2002). The mRNA expression of SREBP-1c, ACC and fatty acid synthase were significantly increased in the fat-free TPN group, and this was reversed by supplementation with soybean fat (unpublished data).

In a randomized clinical trial, the use of a balanced energy-source TPN solution prevented liver dysfunction, indicating that a TPN solution containing 40% of total calories from lipids was more effective than one containing 8.5% of total calories from lipids (Buchmiller et al 1993). Oshita et al (2004) suggested that fatty changes in the liver of infant rats could be prevented by supplying 20% or 40% of calories from a fat emulsion based on soybean oil. In contrast, there are some reports that liver dysfunction and fatty changes were not improved by replacement of glucose with a fat emulsion in patients (Wagner et al 1983; Tayek et al 1990) or in rats (La Scala et al 1993). This study suggested the importance of adding an appropriate amount (calories) of fat emulsion in TPN.

In conclusion, excessive administration of glucose via fat-free TPN infusion induced hyperglycaemia and hyperinsulinaemia, which caused fatty liver and systemic insulin resistance, but these conditions were clearly less severe when TPN supplemented with soybean fat, equivalent to 20% of total calories, was used. Further study is necessary to determine the optimum lipid ratio in TPN solutions.

## References


August, D., Teitelbaum, D., Albina, J., Bothe, A., Guenter, P., Heitkemper, M., Ireton-Jones, C., Mirtallo, J.M., Seidner, D., Winkler, M. (2002) Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. *J. Parenter. Enter. Nutr*. **26** (Suppl): Jan–Feb

Buchmiller, C. E., Kleiman-Wexler, R. L., Ephgrave, K. S., Booth, B., Hensley, C. E. 2nd (1993) Liver dysfunction and energy source: results of a randomized clinical trial. *J. Parenter. Enter. Nutr*. **17**: 301–306

Cutfield, W. S., Jefferies, C. A., Jackson, W. E., Robinson, E. M., Hofman, P. L. (1999) Evaluation of HOMA and QUICKI as measures of insulin sensitivity in prepubertal children. *Pediatr. Diabetes* **4**: 119–125

La Scala, G. C., Le Coultre, C., Roche, B. G., Bugmann, P., Belli, D. C. (1993) The addition of lipids increases the total parenteral nutrition-associated cholestasis in the rat. *Eur. J. Pediatr. Surg*. **3**: 224–227

Lewis, G. F., Steiner, G. (1996) Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care* **19**: 390–393

Lewis, G. F., Uffelman, K. D., Szeto, L. W., Weller, B., Steiner, G. (1995) Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest*. **95**: 158–166

Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., Brown, M. S. (2002) Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J. Biol. Chem*. **277**: 9520–9528

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., Turner, R. C. (1985) Homeostasis model assessment: insulin resistance and $\beta$-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**: 412–419

McMahon, N. M. (1997) Management of hyperglycemia in hospitalized patients receiving parenteral nutrition. *Nutr. Clin. Pract*. **12**: 35–38

Nishimura, M., Yamaguchi, M., Naito, S., Yamauchi, A. (2006) Soybean oil fat emulsion to prevent TPN-induced liver damage: Possible molecular mechanisms and clinical implications. *Biol. Pharm. Bull*. **29**: 855–862

Oshita, M., Takehara, H., Yamaguchi, M., Doi, K., Ueda, N., Naito, S., Hiraoka, I., Tashiro, S. (2004) Significance of administration of fat emulsion: hepatic changes in infant rats receiving total parenteral nutrition with and without fat. *Clin. Nutr*. **23**: 1060–1068

Rosmarin, D. K., Wardlaw, G. M., Mirtallo, J. (1996) Hyperglycemia associated with high, continuous infusion of total parenteral nutrition dextrose. *Nutr. Clin. Pract*. **11**: 151–156

Schloerb, P. R. (2004) Glucose in parenteral nutrition: a survey of U.S. medical centers. *J. Parenter. Enter. Nutr*. **28**: 447–452

Shirotani, N., Numata, K. (2006) Options available for the infusion of lipid emulsion in home parenteral nutrition (HPN): a questionnaire survey for hospitals in Japan where HPN is practiced. *Nutrition* **22**: 361–366

Tayek, J. A., Bistrian, B., Sheard, N. F., Zeisel, S. H., Blackburn, G. L. (1990) Abnormal liver function in malnourished patients receiving total parenteral nutrition: a prospective randomized study. *J. Am. Coll. Nutr*. **9**: 76–83

Wagner, W. H., Lowry, A. C., Silberman, H. (1983) Similar liver function abnormalities occur in patients receiving glucose-based and lipid-based parenteral nutrition. *Am. J. Gastroenterol*. **78**: 199–202