Nutritional and metabolic effects of the endotoxin bacterial lipopolysaccharide in orally and parenterally fed rats[1–3]

Nilima Raina, Junichi Matsui, and Khursheed N Jeejeebhoy

ABSTRACT

Background: Animals treated with tumor necrosis factor α (TNF-α) developed severe metabolic abnormalities despite receiving sufficient protein and energy by total parenteral nutrition (TPN).

Objective: We sought to investigate the nutritional and metabolic effects of bacterial lipopolysaccharide (LPS) in rats.

Design: Rats were randomly allocated to 5 groups: oral nutrition (ON control; n = 7), TPN control (n = 7), ON+LPS (n = 6), TPN+LPS (n = 9), and pair fed (PF) in relation to ON+LPS (n = 6).

Results: Body weight decreased significantly as diet consumption decreased in the ON+LPS and PF groups compared with the ON control group. Relative carcass weights were significantly lower in the TPN+LPS and ON+LPS groups than in their respective control groups. Diaphragm and extensor digitorum longus weights were significantly lower in the ON+LPS and PF rats, but not in the TPN+LPS rats, compared with their respective controls. Biochemical abnormalities and plasma corticosterone concentrations were greater in the TPN+LPS group than in the other groups.

Conclusions: These data suggest that provision of sufficient protein and energy by TPN does not prevent general carcass wasting induced by LPS but may protect individual muscles. However, compared with an oral ad libitum diet, TPN providing sufficient protein and energy worsens the biochemical abnormalities induced by LPS. More rapid clearance of TNF-α and low corticosterone concentrations in weight-losing animals may help reduce the severity of the metabolic effects of LPS. *Am J Clin Nutr* 2000;71:835–43.

KEY WORDS Oral nutrition, total parenteral nutrition, lipopolysaccharide, central vein catheter, continuous infusion, metabolic effects, body composition, corticosterone, rats, endotoxin, tumor necrosis factor

INTRODUCTION

Bacterial lipopolysaccharide (LPS), an integral glycolipid component of the outer membrane of gram-negative bacteria, is the most potent endotoxin that induces production of tumor necrosis factor α (TNF-α) by macrophages (1). TNF-α can be detected in peripheral blood immediately after LPS injection, reaches a maximum concentration by 1.5–2 h (2), and is then cleared from the plasma with a half-life of between 6.5 (3) and 10.5 (4, 5) min.

TNF-α, a mediator of shock and cachexia (general wasting) due to sepsis, is a 17-kD peptide produced by macrophages in response to endotoxin exposure (6). The known effects of bacterial endotoxins, such as fever, hypermetabolism, anorexia, protein catabolism, and cachexia, are mediated by TNF-α (7). These effects occur via direct interaction with tissues and also indirectly via stimulation of the endocrine and central nervous systems (8).

In a previous study (9), we showed that a continuous infusion of TNF-α in orally fed rats resulted in anorexia, weight loss, hypermetabolism, loss of protein and muscle mass, and increase in weight and total protein content of the viscera. However, these wasted animals did not develop severe hyperglycemia, azotemia, or hepatic failure. In another study (10), to overcome the nutritional consequences of anorexia in the TNF-α–treated animals, we administered total parenteral nutrition (TPN) to them in amounts that were sufficient to promote normal growth. The animals developed hyperglycemia, hyperosmolality, diuresis, dehydration, and renal and hepatic dysfunction that were comparable with the complications seen in clinical sepsis. Because these observations suggested that nutritional intake sufficient to maintain normal growth in rats intensified the undesirable metabolic effects of TNF-α, it was important to determine whether similar effects would occur with LPS administration.

Therefore, in this study we compared the nutritional and metabolic effects of LPS in rats that were receiving parenteral or oral nutrition and were subjected to a continuous LPS infusion at a rate of 800 $\mu g \cdot kg^{-1} \cdot d^{-1}$ for 10 d.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River Canada Inc, St-Constant, Canada) that weighed 200–220 g were housed individually in metabolic cages (Nalgene, Sevenoaks, Kent, United Kingdom). The animals were kept at an environmental temperature of 22 °C in a light-controlled room that was light for 12 h (0800–2000) and dark for 12 h (2000–0800). The rats were fed a nonpurified

[1] From the Departments of Medicine and Nutritional Sciences, University of Toronto.

[2] Supported by the Medical Research Council of Canada (no. MT 12238) and by Mead Johnson (Bristol-Myers Squibb Company), Evansville, IN.

[3] Address reprint requests to N Raina, Room 6352, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada, M5S 1A8. E-mail: nilima42@hotmail.com.

Received February 10, 1999.

Accepted for publication August 20, 1999.

diet (Purina rodent chow 5001; Ralston Purina Corp, Strathroy, Canada) ad libitum for 1 wk. Under general anesthesia (50 μg sodium pentobarbital/g given intraperitoneally), a silicone catheter was advanced through the internal jugular vein and positioned in the superior vena cava. The catheter was tunneled through the back of the rat and was circled by a protective wire spring secured to the rat by a stainless steel button (Instec Labs, Horsham, PA), as described previously (11, 12). Both the catheter and spring were connected to a swivel device that allowed movement of the animal about the cage.

During the postoperative period, the rats were housed individually for 6 d and were given the same nonpurified diet and water ad libitum to allow them to recover from surgery. The catheters were kept patent by injecting 0.25 mL saline containing heparin (10 000 U heparin sodium/L) daily. The protocol was approved by the University of Toronto Animal Care Committee.

Lipopolysaccharide

LPS endotoxin from *Escherichia coli* serotype 0127:B8 (Sigma Chemical Co, St Louis) was diluted in physiologic saline and stored in 2-mL aliquots, each of which contained 0.2 mg LPS. Each aliquot was thawed immediately before use and was infused intravenously over 24 h.

Experimental design

The 35 rats were randomly allocated to 5 groups. Rats in the oral nutrition (ON) group (n = 7) consumed an average of 60 mL liquid defined-formula diet and were infused with physiologic saline at a rate of 0.083 mL/h through the central venous catheter. The ON group served as a control group for the ON+LPS group. Rats in the ON+LPS group (n = 6) were offered the same diet as the ON group and were infused with LPS (800 $\mu g \cdot kg^{-1} \cdot d^{-1}$) at a rate of 0.083 mL/h through the central venous catheter. The pair-fed (PF) group (n = 6) was included as a nutritionally matched control group. Individual rats in the PF group received exactly the same volume of diet taken by their matched ON and LPS rats; PF animals were also infused with physiologic saline at a rate of 0.083 mL/h through the central venous catheter. To match the food intake of these 2 groups for each study day, the PF rats were given intakes equal to those consumed by the ON+LPS rats on the previous day. Rats in the TPN (n = 7) and TPN+LPS (n = 9) groups were infused with 40 mL TPN on day 1 and the rate was increased to 60 mL/d (2.5 mL/h given continuously) over the next 2 d; this rate was then continued until the end of the experiment. The TPN group served as a control group for the TPN+LPS group. Rats in the TPN+LPS group received TPN at the same rate as that of the TPN group but in addition received a continuous infusion of LPS (800 $\mu g \cdot kg^{-1} \cdot d^{-1}$).

All the animals were observed carefully, weighed daily, and allowed to drink water ad libitum throughout the experiment. The TPN solution was mixed aseptically each day in a laminar air flow hood and was administered continuously for 10 d at a constant rate with a Harvard infusion pump (pump 22; Harvard Apparatus, Wellesley, MA). LPS and saline were also administered with the infusion pump.

Study diet

For 6 d after the catheters were inserted, rats in all groups (those fed orally and parenterally) were given a liquid-formula diet in which carbohydrate provided 84.33% and lipid provided 14.16% of the total energy requirement (**Table 1**). The liquid diet also met the recommended nitrogen requirement for rats, which is 2.0 g/kg body wt. For rats, choline can be replaced by methionine in diets formulated from amino acids (13). The amino acid mixture used in the present study (Travasol 10%; Baxter Corporation, Toronto) consisted of all the essential amino acids. This liquid diet allowed the control rats to grow at the same rate as animals fed a nonpurified diet ad libitum in a previous study (9).

Blood, organ, and muscle analysis

On day 10, the rats were anesthetized with pentobarbital and exsanguinated by cardiac puncture. The plasma was separated, stored at −70 °C, and later analyzed for creatinine, blood urea nitrogen (BUN), bilirubin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, sodium, chloride, potassium, transferrin, triacylglycerol, and glucose. The liver, heart, lungs, kidneys, diaphragm, soleus, and extensor digitorum longus (EDL) were removed and weighed. Aliquots of the muscles and organs were freeze-dried and the dry weights were recorded. The remaining tissues were frozen at −70 °C and stored for further analysis. The total protein and DNA contents of tissues were measured with methods that have been used in our laboratory and described previously (14).

Plasma corticosterone assay

Plasma corticosterone concentrations were measured in duplicate (15) with a radioimmunoassay kit (Diagnostics Products Cor-

TABLE 1
Composition of liquid defined formula diet (per liter)

Dietary component	Amount
Dextrose (g)[1]	279
Amino acids (g)[2]	39.7
Fat (g)[3]	20.8
Energy (MJ)	5.3
Total nitrogen (g)	6.7
Sodium (mmol)	60.7
Potassium (mmol)	81.2
Chloride (mmol)	145.2
Calcium (mmol)	6.3
Magnesium (mmol)	5.3
Zinc (mmol)	0.008
Manganese (mmol)	0.01
Copper (mmol)	0.004
Iron (mmol)	0.013
Selenium (mmol)	0.0003
Chromium (mmol)	0.00006
Acetate (mmol)	61.9
Phosphate (mmol)	7.0
Iodide (mmol)	0.0008
Thiamine (mg)	62.5
Riboflavin (mg)	13.8
Niacinamide (mg)	138.8
Pyridoxine (mg)	16.7
Pantothenate (mg)	36.17
Vitamin A palmitate (RE)	4170.66
Vitamin D_3 (mg)	0.035
D-α-Tocopherol (mg α-TE)	9.27
Ascorbic acid (mg)	1388.3

[1] Dextrose 50% (Baxter Corporation, Toronto).
[2] Travasol 10% (Baxter Corporation).
[3] Intralipid 20% (Clintec, Mississauga, Canada).

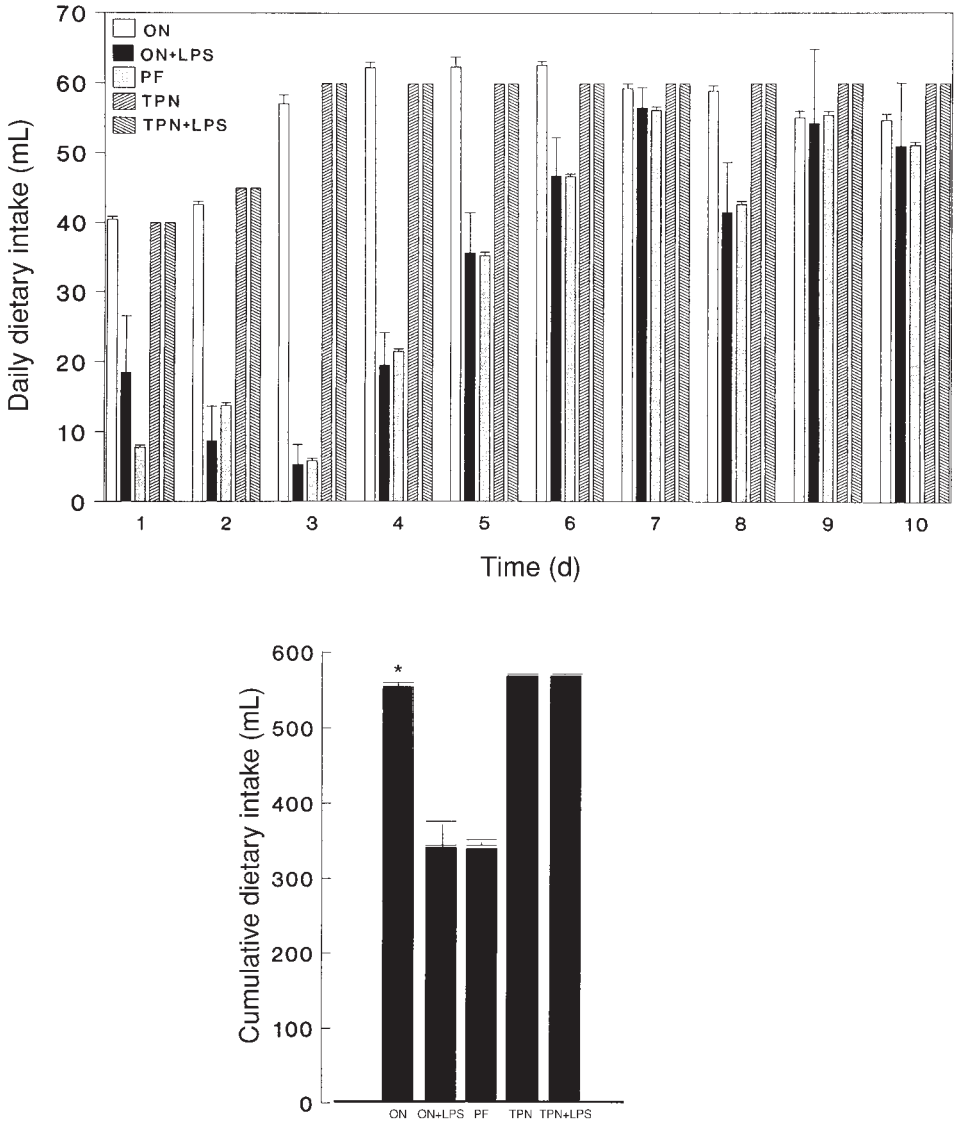


FIGURE 1. Mean (±SEM) daily and cumulative (0–10 d) dietary intakes in 35 rats weighing 200–220 g initially and randomly allocated to the following groups: oral nutrition (ON; n = 7), total parenteral nutrition (TPN; n = 7), and pair-fed (PF; n = 6) control groups, which were infused with physiologic saline at a rate of 0.083 mL/h, and ON plus bacterial lipopolysaccharide (ON+LPS; n = 6) and TPN+LPS (n = 9) groups, which were infused with LPS (800 $\mu g \cdot kg^{-1} \cdot d^{-1}$) at a rate of 0.083 mL/h. *Significantly different from the ON+LPS and PF groups, $P < 0.01$ (one-way ANOVA followed by Duncan's new multiple-range test).

poration, Los Angeles). Fluctuations in corticosterone concentrations in TNF-α–infused rats have been shown to be significantly smaller than those in normal rats not given TNF-α infusions (N Raina and KN Jeejeebhoy, unpublished observations, 1993); therefore, one time point under steady state conditions was adequate to characterize corticosterone concentrations.

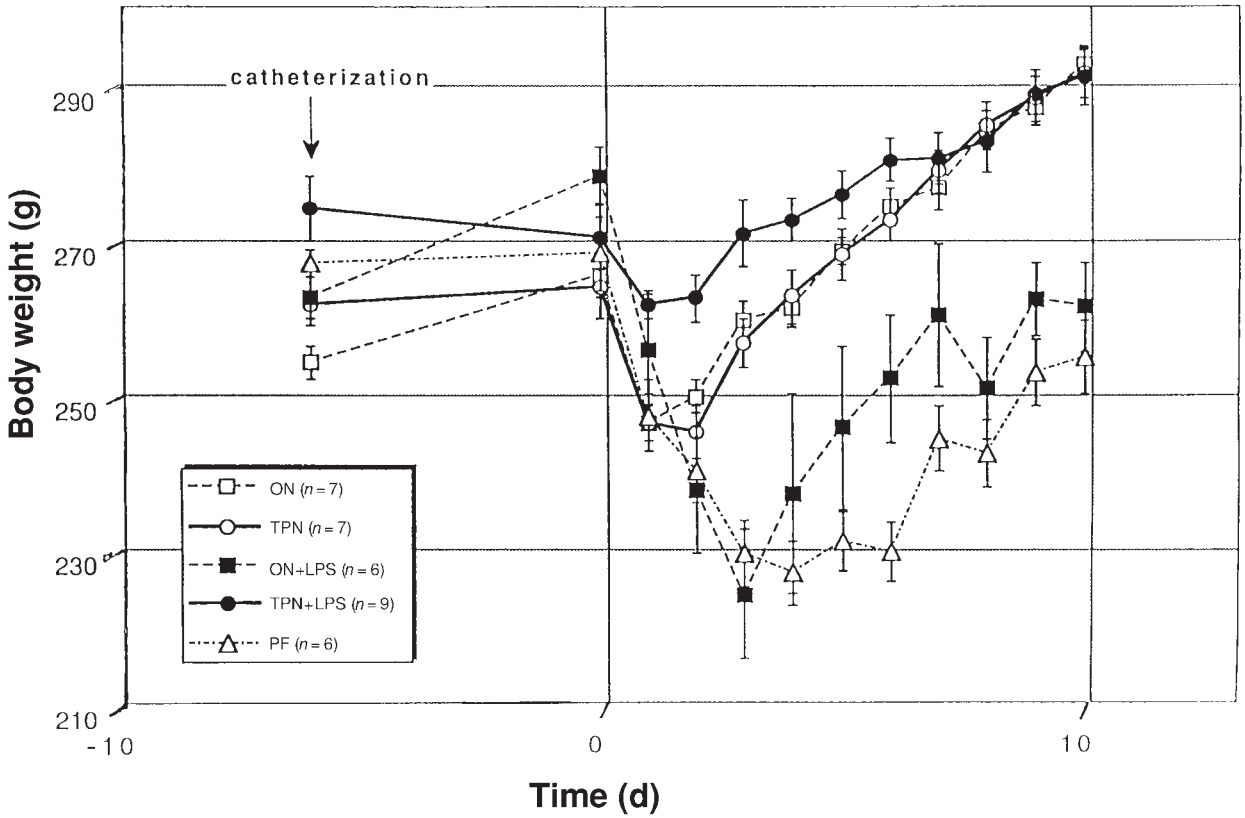
Statistical analysis

Data were expressed as means ± SEMs. Differences among groups were analyzed by one-way analysis of variance (ANOVA) and were considered statistically significant at $P < 0.05$. This was followed by Duncan's new multiple-range test (16). Paired differences in weight and cumulative dietary intake between days 0 and 10 were also analyzed by ANOVA followed by Duncan's new multiple-range test to determine whether the groups differed significantly.

RESULTS

Dietary intake and body weight

Oral dietary intake was markedly reduced in the ON+LPS group for the first 3 d and then gradually increased to the intake of control (ON) rats by about the ninth day (**Figure 1**). The body weights of the ON+LPS rats were lower than those of the ON rats until the end of the experiment (**Figure 2**). The loss of body weight was entirely due to reduced dietary intake because the body weight changes in the ON+LPS rats were comparable with



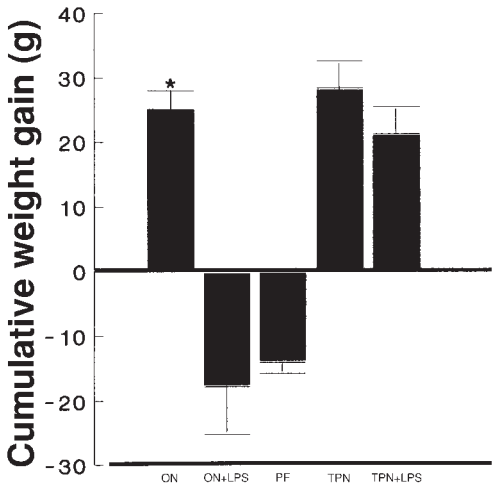


FIGURE 2. Mean (±SEM) daily body weights and cumulative weight gains in 35 rats weighing 200–220 g initially and randomly allocated to the following groups: oral nutrition (ON; n = 7), total parenteral nutrition (TPN; n = 7), and pair-fed (PF; n = 6) control groups, which were infused with physiologic saline at a rate of 0.083 mL/h, and ON plus bacterial lipopolysaccharide (ON+LPS; n = 6) and TPN+LPS (n = 9) groups, which were infused with LPS (800 $\mu g \cdot kg^{-1} \cdot d^{-1}$) at a rate of 0.083 mL/h. *Significantly different from the ON+LPS and PF groups, $P < 0.01$ (one-way ANOVA followed by Duncan's new multiple-range test).

those of their respective PF controls. Cumulative dietary intake and weight gain (Figures 1 and 2, respectively) were significantly lower in the ON+LPS and PF groups than in the ON group. Rats in the TPN and TPN+LPS groups lost weight initially, after which time they gained weight at the same rate as did the ON group (Figure 2). Cumulative dietary intake and weight gain (Figures 1 and 2, respectively) did not differ significantly between the TPN and TPN+LPS groups.

Wet weight of carcass and muscles

Relative carcass weight (percentage of total body weight) was significantly lower in the ON+LPS rats than in their

TABLE 2
Fresh weights of carcass and muscles in orally and parenterally fed rats infused with lipopolysaccharide (LPS) and in orally fed, parenterally fed, and pair-fed controls infused with physiologic saline[1]

Group	Carcass	Diaphragm	Soleus	EDL
	% of total body wt	*g*	*g*	*g*
ON (n = 7)	78.21 ± 0.42	0.80 ± 0.03	0.12 ± 0.003	0.14 ± 0.003
ON+LPS (n = 6)	74.55 ± 0.78[2]	0.69 ± 0.02[3]	0.11 ± 0.007	0.12 ± 0.004[3]
PF (n = 6)	77.52 ± 0.23	0.70 ± 0.02[3]	0.11 ± 0.004	0.11 ± 0.005[3]
TPN (n = 7)	78.89 ± 0.95	0.78 ± 0.02	0.12 ± 0.004	0.13 ± 0.004
TPN+LPS (n = 9)	75.03 ± 0.95[4]	0.80 ± 0.03	0.11 ± 0.004	0.13 ± 0.007

[1] $\bar{x}$ ± SEM. ON, oral nutrition; TPN, total parenteral nutrition; PF, pair fed; EDL, extensor digitorum longus. All statistical analyses were by ANOVA followed by Duncan's new multiple-range test.
[2] Significantly different from ON and PF, $P < 0.01$.
[3] Significantly different from ON, $P < 0.05$.
[4] Significantly different from TPN, $P < 0.05$.

respective ON and PF controls. The TPN+LPS group also had a significantly lower relative carcass weight than the TPN control group (**Table 2**). In contrast, diaphragm and EDL weights were equally and significantly lower in both the ON+LPS and PF groups than in the ON control group (Table 2). No significant differences were observed in diaphragm, soleus, and EDL muscle weights between the TPN and TPN+LPS groups.

Wet weight of organs

Significant differences in organ weights between animals that received LPS and those that did not were observed. The weights of the liver and spleen in the ON+LPS and TPN+LPS rats were significantly higher than those of their respective controls (**Table 3**). In addition, the splenic and liver weights were significantly higher in the TPN+LPS group than in the 3 other groups. The weight of the heart in the ON+LPS rats was significantly lower than that in the ON controls and was significantly higher than that in the PF controls. In addition, lung weight was significantly higher in the ON+LPS rats than in their nutritionally matched (PF) rats, but not when compared with ON control rats. The differences referred to above were also observed when organ weights were expressed as a percentage of body weight (data not shown), which suggests that the differences were not simply due to weight loss in the orally fed animals.

Total protein and DNA contents of muscles

The protein contents of the diaphragm and EDL muscles were significantly lower in the ON+LPS and PF rats than in the ON control rats. No significant differences in the protein contents of the soleus, diaphragm, and EDL were found between the ON+LPS and PF groups (**Table 4**). The DNA contents of the soleus and EDL were also significantly lower in the ON+LPS rats than in their respective ON and PF controls. Similar trends were seen in the protein-DNA ratio of the diaphragm, which was significantly lower in the ON+LPS and PF rats than in the ON control rats. However, in the EDL, the protein-DNA ratio was significantly lower in the PF rats than in the ON and ON+LPS rats. No significant differences were found between the TPN rats and the TPN+LPS rats in the protein and DNA contents or in the protein-DNA ratio of the soleus, EDL, and diaphragm muscles.

Total protein and DNA contents of organs

ON+LPS rats, which were nutritionally comparable with PF rats, had significantly higher amounts of liver protein than their respective PF controls but not their ON controls (**Table 5**). The DNA contents of the liver and heart were significantly lower in the ON+LPS rats than in their respective ON controls. In contrast, the DNA content of the kidneys was higher in the ON+LPS rats than in their respective ON and PF controls. The protein-DNA ratio of the heart was significantly higher in the ON+LPS rats than in their respective ON and PF controls, whereas the protein-DNA ratio of the kidneys was significantly lower in the ON+LPS rats than in their respective ON controls. The protein contents of the liver and heart of the TPN+LPS rats were significantly higher than those of their respective TPN controls. The

TABLE 3
Fresh weights of organs in orally and parenterally fed rats infused with lipopolysaccharide (LPS) and in orally fed, parenterally fed, and pair-fed (PF) controls infused with physiologic saline[1]

Group	Liver	Heart	Lungs	Kidneys	Spleen
			g		
ON (n = 7)	9.9 ± 0.2	1.0 ± 0.04	1.4 ± 0.07	2.2 ± 0.09	0.89 ± 0.04
ON+LPS (n = 6)	11.6 ± 0.3[2]	0.9 ± 0.05[2]	1.6 ± 0.05[3]	2.1 ± 0.05	1.90 ± 0.09[2]
PF (n = 6)	8.5 ± 0.5	0.8 ± 0.02	1.3 ± 0.05	2.0 ± 0.06	0.91 ± 0.05
TPN (n = 7)	11.8 ± 0.6	0.9 ± 0.05	1.5 ± 0.06	2.3 ± 0.07	1.38 ± 0.15
TPN+LPS (n = 9)	16.4 ± 0.9[4]	1.0 ± 0.04	1.8 ± 0.26	2.2 ± 0.05	3.30 ± 0.60[4]

[1] $\bar{x}$ ± SEM. ON, oral nutrition; TPN, total parenteral nutrition. All statistical analyses were by ANOVA followed by Duncan's new multiple-range test.
[2] Significantly different from ON and PF, $P < 0.05$.
[3] Significantly different from PF, $P < 0.05$.
[4] Significantly different from TPN, $P < 0.05$.

TABLE 4
Total protein and DNA contents of muscles in orally and parenterally fed rats infused with lipopolysaccharide (LPS) and in orally fed, parenterally fed, and pair-fed (PF) controls infused with physiologic saline[1]

Groups	Diaphragm	Soleus	EDL
Protein (g/muscle)			
ON (n = 7)	0.16 ± 0.01	0.03 ± 0.001	0.03 ± 0.001
ON+LPS (n = 6)	0.13 ± 0.004[2]	0.02 ± 0.002	0.02 ± 0.001[3]
PF (n = 6)	0.12 ± 0.005[2]	0.03 ± 0.002	0.02 ± 0.001[3]
TPN (n = 7)	0.13 ± 0.01	0.03 ± 0.001	0.03 ± 0.001
TPN+LPS (n = 9)	0.16 ± 0.006	0.02 ± 0.001	0.03 ± 0.001
DNA (mg/muscle)			
ON (n = 7)	0.65 ± 0.05	0.11 ± 0.007	0.06 ± 0.003
ON+LPS (n = 6)	0.70 ± 0.15	0.08 ± 0.002[4]	0.04 ± 0.003[4]
PF (n = 6)	0.76 ± 0.05	0.12 ± 0.007	0.07 ± 0.007
TPN (n = 7)	0.66 ± 0.04	0.09 ± 0.008	0.05 ± 0.006
TPN+LPS (n = 9)	0.72 ± 0.09	0.11 ± 0.011	0.05 ± 0.008
Protein:DNA (mg/mg)			
ON (n = 7)	245.1 ± 29.3	272.7 ± 14.1	505.2 ± 69.0
ON+LPS (n = 6)	185.7 ± 10.1[3]	250.5 ± 24.2	510.4 ± 34.9
PF (n = 6)	162.0 ± 11.4[3]	249.8 ± 18.3	314.5 ± 42.2[5]
TPN (n = 7)	204.8 ± 32.6	331.8 ± 20.3	608.3 ± 118.2
TPN+LPS (n = 9)	250.5 ± 16.9	223.0 ± 21.5	612.7 ± 111.1

[1] $\bar{x}$ ± SEM. ON, oral nutrition; TPN, total parenteral nutrition; EDL, extensor digitorum longus. All statistical analyses were by ANOVA followed by Duncan's new multiple-range test.
[2,3] Significantly different from ON: [2] $P < 0.05$, [3] $P < 0.01$.
[4] Significantly different from ON and PF, $P < 0.01$.
[5] Significantly different from ON and ON+LPS, $P < 0.05$.

DNA contents of the liver and heart were significantly higher, and that of the kidneys was significantly lower, in the TPN+LPS rats than in their respective TPN controls. There were no significant differences in the protein-DNA ratios of the liver, heart, and kidneys between the TPN and TPN+LPS groups.

Plasma corticosterone concentrations

Plasma corticosterone concentrations (**Figure 3**) were significantly higher in the TPN+LPS group than in the TPN control group (1257.8 ± 283.5 compared with 455.4 ± 97.3 nmol/L, respectively). In contrast, there were no significant differences in plasma corticosterone concentrations between the ON+LPS group (813.0 ± 190.2 nmol/L) and either the ON control group or the nutritionally matched PF group (793.7 ± 255.7 and 892.4 ± 165.9 nmol/L, respectively).

Plasma biochemical determinations

Plasma creatinine and BUN concentrations were significantly higher in the TPN+LPS rats than in their respective TPN controls (**Table 6**). There were no significant differences in plasma creatinine and BUN concentrations among the ON, ON+LPS, and PF groups. Plasma sodium and chloride concentrations did not differ significantly among the groups, whereas plasma potassium concentrations were significantly higher in the ON+LPS and TPN+LPS rats than in their respective controls. Lactate dehydrogenase and plasma transferrin concentrations were significantly higher in the TPN+LPS group than in the TPN control group. No significant differences were seen in aspartate aminotransferase, alanine aminotransferase, or alkaline phosphatase among the groups (data not included). The plasma total bilirubin concentration was significantly higher in the ON+LPS rats than in their respective ON and PF controls. The plasma total triacylglycerol concentration was significantly higher in the ON+LPS and TPN+LPS rats than in their respective controls. Plasma glucose did not differ significantly among the groups (data not shown).

DISCUSSION

Bacterial endotoxin is not directly toxic to most mammalian tissues (17), but it acts by stimulating the production of TNF-α (1, 18). Hence it would be expected that the interaction between nutrition and LPS would be similar to the interaction between nutrition and TNF-α. Previously, we showed that TNF-α altered the distribution of body mass between carcass and viscera, namely by causing a loss of carcass weight and a gain in visceral mass, with no change in body weight (9, 10). In addition, changes in body composition and metabolism as a result of TNF-α infusion were enhanced by administering TPN (10).

Effect of lipopolysaccharide on carcass, muscle, and organ weights

Similar to the effect of TNF-α, LPS infusion significantly reduced relative carcass weight and increased visceral mass, especially the mass of the liver and spleen, in both orally fed and parenterally fed animals (Tables 2 and 3); spleen and liver weights were markedly increased in the TPN+LPS rats. Wasting of the diaphragm and EDL in LPS-infused animals resulted from anorexia, because it occurred in both the ON+LPS and PF rats but not in the TPN+LPS rats, whose nutrient intake was maintained as a continuous infusion. In other studies, endotoxin reduced nitrogen balance, increased muscle protein

TABLE 5
Protein and DNA contents of organs in orally and parenterally fed rats infused with lipopolysaccharide (LPS) and in orally fed, parenterally fed, and pair-fed (PF) controls infused with physiologic saline[1]

Groups	Liver	Heart	Kidneys
Protein (g/organ)			
ON (n = 7)	3.08 ± 0.06	0.23 ± 0.02	0.40 ± 0.01
ON+LPS (n = 6)	3.13 ± 0.16[2]	0.20 ± 0.01	0.36 ± 0.02
PF (n = 6)	2.42 ± 0.15[3]	0.17 ± 0.01[3]	0.34 ± 0.02
TPN (n = 7)	3.05 ± 0.29	0.16 ± 0.01	0.37 ± 0.02
TPN+LPS (n = 9)	4.62 ± 0.19[4]	0.22 ± 0.01[4]	0.31 ± 0.02
DNA (mg/organ)			
ON (n = 7)	34.61 ± 5.29	1.59 ± 0.14	2.01 ± 0.25
ON+LPS (n = 6)	21.77 ± 2.50[3]	1.19 ± 0.08[5]	3.56 ± 0.33[6]
PF (n = 6)	15.34 ± 2.18[5]	1.31 ± 0.04	2.54 ± 0.22
TPN (n = 7)	22.31 ± 1.19	1.05 ± 0.10	4.03 ± 0.23
TPN+LPS (n = 9)	34.87 ± 7.29[7]	1.74 ± 0.12[4]	3.22 ± 0.16[7]
Protein:DNA (mg/mg)			
ON (n = 7)	103.5 ± 25.0	149.7 ± 10.9	219.0 ± 37.7
ON+LPS (n = 6)	152.5 ± 19.5	170.7 ± 2.9[6]	105.9 ± 12.1[5]
PF (n = 6)	172.2 ± 25.4	132.9 ± 14.4	141.0 ± 17.9
TPN (n = 7)	138.7 ± 14.8	164.7 ± 18.9	91.3 ± 4.1
TPN+LPS (n = 9)	134.6 ± 14.0	130.5 ± 11.0	101.3 ± 11.9

[1] $\bar{x}$ ± SEM. ON, oral nutrition; TPN, total parenteral nutrition. All statistical analyses were by ANOVA followed by Duncan's new multiple-range test.
[2] Significantly different from PF, $P < 0.01$.
[3,5] Significantly different from ON: [3] $P < 0.01$, [5] $P < 0.05$.
[4,7] Significantly different from TPN: [4] $P < 0.01$, [7] $P < 0.05$.
[6] Significantly different from ON and PF, $P < 0.01$.

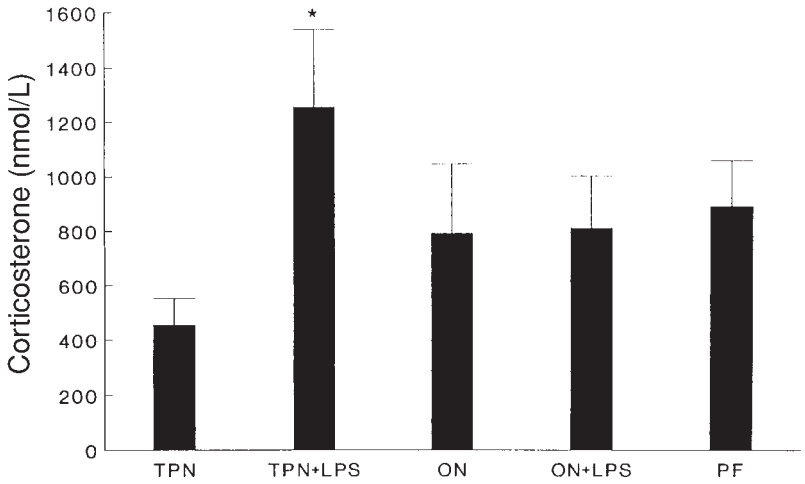


FIGURE 3. Mean (±SEM) plasma corticosterone concentrations in 35 rats randomly allocated to the following groups: oral nutrition (ON; $n = 7$), total parenteral nutrition (TPN; $n = 7$), and pair-fed (PF; $n = 6$) control groups, which were infused with physiologic saline at a rate of 0.083 mL/h, and ON plus bacterial lipopolysaccharide (ON+LPS; $n = 6$) and TPN+LPS ($n = 9$) groups, which were infused with LPS (800 $\mu g \cdot kg^{-1} \cdot d^{-1}$) at a rate of 0.083 mL/h. *Significantly different from all other groups, $P < 0.05$.

catabolism, reduced skeletal and heart muscle protein synthesis, and increased liver protein synthesis (19, 20). These studies did not measure separately the net effects of endotoxin on carcass and liver mass. The data suggest that provision of sufficient protein and energy from TPN does not prevent general carcass wasting but may protect individual muscles, such as the diaphragm and EDL.

Effect of lipopolysaccharide on serum biochemistry

Biochemical abnormalities were seen in LPS-treated rats that received either ON or TPN, but the degree of abnormality was greater in the TPN-fed rats (Table 6). In a previous study, we showed that 60% of the TNF-α–treated rats that received TPN developed hyperosmolar diuresis caused by marked hyperglycemia and glycosuria (10). In the present study, although both the ON+LPS and TPN+LPS groups had higher plasma concentrations of potassium and triacylglycerol than their respective controls, the TPN+LPS rats had markedly higher plasma concentrations of creatinine, BUN, and lactate dehydrogenase, which is indicative of renal impairment. In addition, we found higher plasma concentrations of transferrin in the TPN+LPS group and of bilirubin in the ON+LPS group than in their respective controls. In a previous study, we found that even when groups had the same protein and energy intakes, TNF-α infusion resulted in a greater degree of biochemical abnormalities in enterally fed animals than in parenterally fed animals (21). It is therefore likely that in the present study, the lower biochemical response in LPS-infused rats that received oral nutrition, as compared with TPN-fed rats, may have been related to reduced protein and energy intakes rather than to the route of feeding.

It is tempting to speculate that the higher plasma concentrations of triacylglycerol observed in the LPS-infused ON and TPN rats than in their respective controls (Table 6) may have been a result

TABLE 6
Plasma indexes in orally and parenterally fed rats infused with lipopolysaccharide (LPS) and in orally fed, parenterally fed, and pair-fed (PF) controls infused with physiologic saline[1]

Index	ON ($n = 7$)	ON+LPS ($n = 6$)	PF ($n = 6$)	TPN ($n = 7$)	TPN+LPS ($n = 9$)
Creatinine (μmol/L)	55.8 ± 0.3	51.7 ± 1.3	52.7 ± 2.2	30.4 ± 4.6	54.5 ± 1.5[2]
BUN (mmol/L)	3.1 ± 0.4	3.0 ± 0.6	2.5 ± 0.3	3.4 ± 0.3	6.9 ± 1.2[3]
Bilirubin (μmol/L)	4.8 ± 0.5	8.2 ± 0.5[4]	5.0 ± 0.5	2.6 ± 1.1	5.1 ± 0.3
LDH (nkat/L)	1700 ± 463.3	2217 ± 135	1783 ± 253.3	1417 ± 238.3	1967 ± 288.3[3]
Potassium (mmol/L)	3.6 ± 0.2	4.3 ± 0.2[4]	3.7 ± 0.1	3.6 ± 0.2	4.7 ± 0.3[2]
Sodium (mmol/L)	152 ± 3.7	151 ± 1.9	143 ± 2.6	145 ± 4.6	152 ± 2.6
Chloride (mmol/L)	111 ± 2.8	107 ± 1.9	107 ± 0.9	107 ± 1.6	105 ± 1.9
Transferrin (mmol/L)	1.4 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.02	1.8 ± 0.1[3]
Triacylglycerol (mmol/L)	0.4 ± 0.1	1.0 ± 0.3[4]	0.3 ± 0.1	0.3 ± 0.04	1.0 ± 0.04[3]

[1] $\bar{x}$ ± SEM. ON, oral nutrition; TPN, total parenteral nutrition; BUN, blood urea nitrogen; LDH, lactate dehydrogenase. All statistical analyses were by ANOVA followed by Duncan's new multiple-range test.

[2,3] Significantly different from TPN: [2] $P < 0.01$, [3] $P < 0.05$.

[4] Significantly different from ON and PF, $P < 0.05$.

of reduced lipoprotein lipase activity. Rouzer and Cerami (22) noted that hypertriglyceridemia was attributable to a clearing defect caused by a systemic reduction in lipoprotein lipase activity. Kawakami and Cerami (23) showed that in endotoxin-sensitive (C^3H/HeN) mice, LPS injections resulted in systemic suppression of lipoprotein lipase activity and lipemia as compared with endotoxin-resistant (C^3H/HeJ) mice. Because these studies were done in animals, the predominant mechanism responsible for the reduction of lipoprotein lipase activity could be species dependent.

Interaction of lipopolysaccharide and corticosterone in sepsis

Corticosterone is the most abundant circulating glucocorticoid in rats (24). The results of our previous study suggest that the catabolic effect of TNF-α on a critically important muscle, the diaphragm, is potentiated by corticosterone (25). In the present study, there was some degree of muscle weight loss in the ON+LPS rats, which was not related to corticosterone concentrations because these concentrations were not significantly higher in this group than in the other groups. The muscle loss was mainly a result of deficient dietary intake caused by anorexia.

Baumann et al (26) reported that high circulating concentrations of glucocorticoids enhanced the synthesis of proteins in the liver. In the present study (Table 5), the highest liver protein and DNA contents were seen in the TPN+LPS rats. Our data also suggest that LPS induces the production of corticosterone, which has been reported by Perretti et al (27), who found that corticosterone was significantly elevated for 24 h as a result of LPS as compared with saline infusion in rats. The significantly higher corticosterone concentrations in the TPN+LPS group than in the other 4 groups (Figure 3) may have accounted for the greater degree of metabolic abnormalities in this group. Despite higher corticosterone concentrations, plasma glucose concentrations were not significantly different between any of the groups. Soto et al (28) reported that acute LPS injections (2.5, 25, or 250 g/kg) in rats raised serum corticosterone in a dose-dependent manner. However, serum glucose concentrations did not differ significantly between the control and LPS-treated rats. Insulin secretion was reduced after acute, but not during chronic, LPS administration (250 g LPS/kg for 8 d). Tolerance to chronic LPS administration seems to be related to reduced macrophage responsiveness to LPS, rather than to reduced neuroendocrine response to cytokines (29, 30). Although we did not measure insulin concentrations, it is reasonable to assume that insulin released in response to plasma glucose concentrations would have followed the same pattern.

In summary, metabolic abnormalities were most profound in the TPN+LPS rats, which had sufficient protein and energy intakes and had no weight loss. In contrast, LPS-infused ON rats with anorexia and weight loss had less severe metabolic abnormalities. The underlying reasons why the effects of LPS were masked in orally fed anorexic rats remain speculative. Keith et al (31) showed that retention of labeled TNF-α was higher in weight-gaining rats and lower in weight-losing rats. This suggests that faster clearance of TNF-α in weight-losing rats may help reduce the severity of its metabolic effects. In addition, low concentrations of corticosterone may act synergistically in preventing the catabolism caused by LPS.

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