

1 **Effects on Bacterial Translocation of High-Fat Enteral Nutrition in Bile Duct Ligated**

2 **Rats**

3

4 **ABSTRACT**

5 **Objective:** Bacterial Translocation (BT) from gastrointestinal system is in the center of
6 current sepsis theories. In the patients with obstructive jaundice the absence of intraluminal
7 bile flow causes some alterations and mucosal damage in gut. In the present study, it was
8 aimed to investigate the effects on BT of high-fat enteral nutrition in bile duct ligated rats.

9 **Material and Methods:** In this study, a total of 28 healthy Spraque-Dawley rats, weighing
10 230 – 300 gr, were grouped into four as sham group, control group, high-fat enteral nutrition
11 group and low-fat enteral nutrition group. The rats in the all groups were sacrificed on seventh
12 day of postoperatively. The values of aspartate aminotransferase (AST), alanine
13 aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT),
14 total and direct bilirubine were measured for biochemical evaluation. Also, the samples were
15 taken from blood, lung, liver, spleen and mesenteric lymph nodes for microbiological
16 evaluation. The results were calculated as CFU/gr and evaluated statistically.

17 **Results:** In all bile duct ligated rats, all findings of obstructive jaundice were observed as
18 clinically (in postoperatively third day) and in laboratory. It was determined that the
19 lymphatic system is essential way for BT likewise reported by similar studies. But it was
20 observed in this study that the high-fat enteral nutrition may be not very affected on reducing
21 of BT in bile duct ligated rats. The results were supported by statistical analyses.

22 **Conclusion:** It was observed that high-fat enteral nutrition has not meaningful effects on
23 reducing BT in bile duct ligated rats.

24

25 **Keywords:** Obstructive jaundice, high-fat enteral nutrition, bacterial translocation.

26

27 INTRODUCTION

28 In physiopathology of obstructive jaundice, the role of endotoxemia attracts attention
29 and it is observed that both portal and systemic endotoxins increase in bile duct obstruction
30 (1-6). It is believed that endotoxin in portal venous blood comes from lumen of
31 gastrointestinal tract by translocation (3-6).

32 The pathogenesis of bacterial translocation (BT) is not adequately known. However,
33 homeostasis defects among defence mechanisms such as intestinal microflora and mucosal
34 barrier, gastric acidity and gastrointestinal motility may cause BT. When gastrointestinal
35 barrier has damaged as functionally or physically, some bacteria may be potential pathogen or
36 may be sources of sepsis (7). It is supposed that, in mechanical biliary obstruction, the
37 absence biliary salts in gut indirectly increases endotoxin translocation into portal vein blood
38 because of the biliary salts holds and inactivates endotoxin (8).

39 Enteral nutrition prevents mucosal atrophy in gastrointestinal system and provides
40 completeness of immune system by the protecting normal gut flora (9). High-fat enteral
41 nutrition protects the gut barrier function and reduces endotoxemia (10,11). The fat in diet
42 strongly increases the secretion of biliary salts, which is a potential inhibitor of endotoxin.
43 Healthy secretion of bile is essential for formation of chylomicrons after high-fat enteral
44 nutrition. These lipoproteins rich in triacylglycerol effectively neutralise the endotoxin and
45 protect the organisms against to mortality caused by endotoxin (12).

46 The most important target of treatment on bile duct ligation is endotoxin and
47 inflammatory reply depending on endotoxin. Despite a lot of different experimental studies,
48 no clinical treatment reducing the high complication values of the patients having obstructive
49 jaundice after major surgical operation has been yet reported (13,14).

50 Up to now, some studies which investigate the BT in obstructive jaundice models were
51 done (15-22). In some of them, different materials were given to the models which bile duct

52 ligated to observe the effects of them on BT (16-22). Erbil et al. (16) have studied the effects
53 of deoxycholate, lactulose and glutamine on BT included by obstructive jaundice. Aldemir et
54 al. (17) have searched the effects of ursodeoxycholic acid, glutamine and polyclonal
55 immunoglobuline to BT in bile duct ligated rats. Luyer et al. (18) have investigated the levels
56 of plasma endotoxin and TNF- α on the models which have bile duct ligated and fed by high-
57 fat nutrition during seven day after hemorrhagic shock procedure. Geyik et al. (21) have
58 studied the effects of a yeast on BT in the models with obstructive jaundice. Gencay et al. (22)
59 have reported the effect of honey on BT in obstructive jaundice. The effects of ciprofloxacin
60 and ursodeoxycholic acid on BT in obstructive jaundice were investigated by Kaya et al. (20).
61 The effects of glutamine and curcumin on BT in the rat models with obstructive jaundice were
62 searched by Karatepe et al. (19).

63 In this study, the effects of high-fat enteral nutrition on barrier of gut mucosa were
64 investigated using the bile duct ligated rat models. For this aim, BT in the samples from blood
65 and some tissues likewise lung, liver, spleen, mesenteric lymph nodes in the rats which have
66 obstructive jaundice was investigated. Thus, our findings on the effects of high-fat enteral
67 nutrition on BT in obstructive jaundice were discussed with literatures.

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69

70 **MATERIAL and METHODS**

71 The present study was performed on the rats from the Unit of Experimental Medicine
72 Researches and Application of Kocaeli University and approved by the Ethical Committee.

73 A total of 28 healthy Sprague-Dawley rats (without separating sex), weighing 230-300
74 gr, were used in the study. The rats were sorted into four different groups. Every group had 7
75 rats which has been hold in plastic cages in stabile environmental conditions. Experimental
76 groups consisted of:

77 **Group A:** Sham group; bile duct dissection was done by laparotomy. And then, they were
78 fed by standard rodent chow during seven days and had free access to water. The
79 experimental processes were used after sacrifice.

80 **Group B:** Control group; bile duct ligation was done by laparotomy. And then, they were
81 fed by standard rodent chow during seven days and had free access to water. The
82 experimental processes were used after sacrifice.

83 **Group C:** High-fat enteral nutrition group; bile duct ligation was done by laparotomy.
84 And then, they were fed by high-fat nutrition during seven days and had free access to
85 water. The experimental processes were used after sacrifice.

86 **Group D:** Low-fat enteral nutrition group; bile duct ligation was done by laparotomy.
87 And then, they were fed by low-fat nutrition during seven days and had free access to
88 water. The experimental processes were used after sacrifice

89

90 **Surgical Procedures**

91 The rats were operated with inhalation anaesthesia by ether. Midline of the rats was
92 cleaned for operation using povidon-iodine (Betadin[®]) as sterilizer. About 4 cm midline
93 incision was explored under from xiphoid with passing skin, under of skin and fascia. In
94 Sham Group, only common bile duct was dissected after laparotomy. In the other groups,
95 common bile duct was made free after dissection. It was cut after twice binding with silk 4/0.
96 Abdominal strata were closed as continued sutures with 4/0 polipropylene after providing
97 liquid resuscitation by using 5 cc of 0.9 % NaCl into abdominal cavity. In postoperatively
98 period, while the rats in first and second groups were fed by standard rodent chow, the rats in
99 third group were fed by high-fat nutrition (Glucerna[®]) and the rats in fourth group were fed by
100 low-fat nutrition (Biosorb fibre[®]) during seven days.

101

102 **Biochemical Analyses:**

103 Before the sacrifice, about 2 cc bloods were taken intracardiacly from subxiphoid
104 area. The blood samples were centrifuged and then 1 cc serum was separated. The values of
105 total bilirubine, direct bilirubine, aspartate aminotransferase (AST), alanine aminotransferase
106 (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were measured
107 by using Abbot Aeroset.

108 In the all operated bile duct ligated rats, all findings of obstructive jaundice were
109 observed clinically (in third day after operation) and in laboratory.

110

111 **Microbiological Analyses:**

112 At the end of the feeding days, all rats in all groups were sacrificed by cervical
113 dislocation and then thoraco-laparotomy was done. The blood samples taken from right
114 atrium of the rats were put into sterile tubes including 1 cc liquid culture. To investigate BT,
115 tissue samples from lung, liver, spleen, mesenteric lymph nodes were put into sterile tubes
116 including 1 cc 0.9 % NaCl and they were weighed. The tissue samples were put into sterile
117 plastic bags and they were homogenised. These homogenised sterile blood and tissue samples
118 were planted into Petri dish as zigzag by using line sowing methods (shallow planting) under
119 Bunzen burner flame conditions by the sterile loop. The blood samples and homogenised
120 tissue samples were planted into EMB nutrition as 10 μ L and 50 μ L. The incubation was
121 performed during 24-48 hours at 37°C. The results were evaluated as CFU (Colony Forming
122 Unit) in per gram, quantitatively.

123

124 **Statistical Analyses:**

125 The average and standard deviation values of all groups were compared statistically
126 with each other by using SPSS (Statistical Package for the Social Sciences) programme for

127 windows 10.0. Statistical evaluation was also made by using Kruskal-Wallis variance
128 analyses. The results were reported to be statistically meaningful if *p* value was less than 0.05.

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131 **RESULTS**

132 The results belonging to total bilirubine, direct bilirubine, AST, ALT, ALP, GGT from
133 blood samples of the rats were shown in Figure1 and Table 1.

134 It was observed that the Group C had the highest values of total bilirubine
135 (15.95±3.79) and direct bilirubine (11.37±2.54). Also, it was observed that the Group B had
136 the highest values of AST (1558.71±837.2) and ALT (513.0±255.30), GGT (67.85±35.80)
137 and ALP (727.0±651.23).

138 The average values of CFU and standard deviation were shown in Table 2 and Figures
139 2-3. When the all groups compared with each other for the samples of 10µL and 50 µL,
140 bacterial growth was observed in mesenteric lymph nodes in all groups. While the highest
141 bacterial growth in mesenteric lymph nodes was observed at Group D for the samples of
142 10µL, for 50 µL was observed at the Group C (Table 2).

143 The *p* results from Kruskal-Wallis variance analyse were shown in Table 3. As the
144 values were higher from 0.05, the differences among the groups were not found statistically
145 meaningful.

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148 **DISCUSSION**

149 Despite the improvements in the antibacterial treatments, sepsis is declared as the
150 major reason for mortality in the patients which have trauma or major surgical operation. The
151 reducing neutrophil chemotaxis and bactericidal activity after trauma, and the reducing in the

152 colony stimulating factors facilitate the sepsis (23).

153 Wells et al. (24) reported that the translocation of intestinal bacteria to the other organs
154 is by the macrophages. The macrophages increase on gut wall phagocyte the microorganisms,
155 however these live microorganisms in the macrophages are set free due to the death of
156 macrophages carried to other organs, which develops BT (24).

157 It was reported by many studies that BT is provided by the lymphatic system (25,26).
158 Reporting no growth in the blood samples from operated rats, but the first and the most
159 growth in mesenteric lymph nodes, the present study similarly supports the view that
160 lymphatic system is essential way for BT.

161 If bacteria which is translocated to mesenteric lymph nodes have high virulence or the
162 organism has inadequate immune system, bacteria can be spread to organs in far away like
163 liver and spleen. In the last step, bacteria pass to systemic circulation (27,28).

164 The advantage of early oral nutrition is that intestinal blood circulation increases,
165 which decreases the hypovolemia and ischemia in gut having important role in translocation
166 (29).

167 Although the BT in bile duct ligated models was investigated before (15-22), in a lot
168 of them (15-17, 19-22) it was studied the effects of different materials on BT, except food
169 with high-fat. In previous studies, the effects of high-fat enteral nutrition on BT were studied
170 by Luyer et al. (10, 11, 18). Although in all of these studies the hemorrhagic shock were used
171 to the models, in only one of them obstructive jaundice was formed. In our study, the bile duct
172 ligated models without hemorrhagic shock were used to feed by high-fat nutrition. Luyer et al.
173 (18) have investigated the levels of plasma endotoxin and tumor necrosis factor-alpha (TNF-
174 α) after hemorrhagic shock procedure in bile duct ligated rats fed by high-fat nutrition during
175 seven days. It has also been observed that the levels of plasma endotoxin highly decreased
176 after hemorrhagic shock in the bile duct ligated rats fed by high-fat nutrition. Interestingly, it

177 has been observed that the decrease in the levels of plasma endotoxin is related to intraluminal
178 bile salts and it is not directly affected by triacylglycerol malabsorption (18). It was also
179 observed that the levels of TNF- α decreased significantly in the rats fed by high-fat nutrition
180 when compared with rats fed by low-fat nutrition (18). In our study, only BT was investigated
181 in the bile duct ligated models that were fed by high-fat nutrition and without using
182 hemorrhagic shock.

183 When our study was compared with previous studies which have been performed on
184 the models without using hemorrhagic shock, some results were evaluated that, Erbil et al.
185 (16) have studied the effects of deoxycholate, lactulose and glutamine, and they have reported
186 that all of products cause decrease in BT, however, glutamine has the most decreasing power.
187 Aldemir et al. (17) have searched the effects of ursodeoxycholic acid, glutamine and
188 polyclonal immunoglobuline, and they have found that all of the products decreases BT.
189 Geyik et al. (21) have studied the effects of a yeast and they have recorded the *Saccharomyces*
190 *boulardii* decreases BT in the models with obstructive jaundice likewise the findings of
191 Gencay et al. (22). They have reported that honey decreases the BT (22). It was also reported
192 by Kaya et al. (20) that the ciprofloxacin and ursodeoxycholic acid decrease BT. Furthermore,
193 Karatepe et al. (19) have recorded the glutamine and curcumin decrease BT in the rat models
194 with obstructive jaundice.

195 In our study, the bacterial growth was observed in mesenteric lymph nodes in all
196 groups. For the samples of 10 μ L, the highest bacterial growth occurred in lung, liver and
197 mesenteric lymph nodes of bile duct ligated rats fed by the products of low-fat nutrition.
198 When the bacterial growth in mesenteric lymph nodes for 10 μ L samples was compared,
199 much closed values were observed in Group C (high-fat enteral nutrition group) and Group A
200 (control group) (Table 2). The bacterial growth in mesenteric lymph nodes for 10 μ L samples
201 in Group D (low-fat enteral nutrition) was observed to have to be more than Group B

202 (standard nutrition) and the others, respectively. So, in our study has indicated that the
203 bacterial growing in mesenteric lymph nodes for 10 μ L samples of the rats in high-fat enteral
204 nutrition group were found to be lower than the rats in both sham and low-fat enteral nutrition
205 groups. Therefore, it may be mentioned the high-fat enteral nutrition may cause decreasing of
206 bacterial growth in mesenteric lymph nodes. But, when the results on bacterial growth in the
207 all samples (blood and the other tissues) were evaluated totally, it is very difficult to say the
208 high-fat enteral nutrition decreases the BT. Because, the bacterial growing in the other
209 samples was not found meaningful. For example, the highest bacterial growth in mesenteric
210 lymph nodes for 50 μ L samples was observed in high-fat enteral nutrition group. When the all
211 findings from the tissues were evaluated, the results were not found to be meaningful. The
212 statistical evaluation supported the results.

213 In conclusion, BT is very important problem after surgical operations. Although it was
214 observed in this experimental study that high-fat enteral nutrition has no meaningful effect on
215 decreasing BT in bile duct ligated rats, it is need a lot of different experimental and clinical
216 studies to explain the relationships between enteral nutrition and BT.

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318 **Table 1.** The average and standard deviation values of biochemical parameters.

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	Total bilirubine (mg/dL)	Direct bilirubine (mg/dL)	Indirect bilirubine (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)
A	2.88±2.10	0.12±0.10	2.71±2.04	284.28±199.10	114.85±77.54	3.57±1.81	217.28±69.86
B	10.14±1.72	7.69±1.35	2.45±1.14	1558.71±837.22	513.00±255.30	67.85±35.80	727.00±651.23
C	15.95±3.79	11.37±2.54	4.57±2.04	763.14±846.81	181.85±135.55	50.57±21.21	599.14±110.58
D	12.04±2.40	9.19±2.08	2.84±0.41	755.71±295.94	202.00±64.84	51.85±6.03	598.28±207.27

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321 (A: Sham group, B: Control group, C: High-fat enteral nutrition group, D: Low-fat enteral nutrition group)

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Table 2. The average values of CFU and standard deviation for 10 μ L and 50 μ L of growth in groups.

Group	μ L	Lung	Liver	MLN	Spleen	Blood
A	10	-	-	7.94 \pm 15.73	4.91 \pm 12.99	-
	50	-	-	1.82 \pm 3.22	0.98 \pm 2.59	-
B	10	-	-	8.68 \pm 9.31	-	-
	50	-	-	5.09 \pm 4.66	-	-
C	10	-	0.58 \pm 1.54	7.65 \pm 20.24	-	-
	50	0.06 \pm 0.11	0.11 \pm 0.30	25.64 \pm 67.43	-	-
D	10	0.28 \pm 0.74	17.08 \pm 45.18	10.21 \pm 13.72	-	-
	50	5.49 \pm 14.11	7.76 \pm 20.53	6.83 \pm 6.13	-	-

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(A: Sham group, B: Control group, C: High-fat enteral nutrition group, D: Low-fat enteral nutrition group, MLN: Mesenteric lymph nodes)

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357 **Table 3.** Statistical results for 10 μ L and 50 μ L of growth in groups.

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		Lung	Liver	MLN	Spleen	Blood
<i>p</i> values	10 μ L	0.392	0.556	0.256	0.392	1.000
	50 μ L	0.211	0.556	0.103	0.392	1.000

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380 Legends of Figures

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382 **Figure 1.** The comparison of average values of biochemical results

383 (TB: Total Bilirubine, DB: Direct Bilirubine, IB: Indirect Bilirubine, AST: Aspartate

384 Aminotransferase, ALT: Alanine Aminotransferase, GGT: Gamma-glutamyl

385 Transferase, ALP: Alcaline Phosphatase)

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387 **Figure 2.** The comparison of average CFU values for 10 μ L of growth in groups.

388 (MLN: Mesenteric Lymph Nodes)

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390 **Figure 3.** The comparison of average CFU values for 50 μ L of growth in groups.

391 (MLN: Mesenteric Lymph Nodes)

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