In Vivo Effects of Lipopolysaccharide on Hepatic Free-NAD(P)$^+$ -Linked Redox States and Cytosolic Phosphorylation Potential in 48-Hour–Fasted Rats

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This study was performed to determine the magnitude and time of onset of in vivo changes in hepatic bioenergetics in response to a sublethal dose of lipopolysaccharide (LPS), a bacterial endotoxin. Male rats (48-hour–fasted) were administered an intraperitoneal injection of LPS (5 mg/kg body weight) or vehicle alone, and the livers were freeze-clamped 5, 30, or 180 minutes or 24 hours later. Liver tissue was extracted with perchloric acid, and the metabolites necessary to calculate NAD$^+$- and NADP$^+$-linked redox states and the cytosolic phosphorylation potential were measured. There was no significant difference in hepatic cytosolic phosphorylation potential between LPS and control groups at any of the times investigated. This indicated that the ability of the liver to synthesize adenosine triphosphate (ATP) was not compromised under the conditions of the study. No changes in hepatic redox states were observed 5 or 30 minutes after LPS treatment. Three hours after LPS treatment, hepatic cytosolic and mitochondrial free-[NAD$^+$]/[NADH] redox states and the cytosolic free-[NADP$^+$]/[NADPH] redox state were more oxidized. By 24 hours, only NAD$^+$-linked redox states were more oxidized than the time-matched controls. Hepatic urea content was elevated at both 3 and 24 hours, compatible with an increased rate of urea synthesis as a consequence of increased amino acid metabolism, whereas hepatic β-hydroxybutyrate and total ketone bodies were decreased 24 hours after LPS treatment, indicating decreased hepatic ketogenesis. The oxidation of hepatic NAD$^+$ redox states in response to LPS appears to be due to a change in the metabolic fuels available to the liver; however, a partial uncoupling of oxidative phosphorylation cannot be ruled out.

SEPSIS CAUSES a systemic derangement of host metabolism.[1-3] The integral role of the liver in the regulation of systemic metabolism has resulted in investigations focusing on liver function during sepsis.[4-7] Many of the metabolic changes observed during sepsis can be duplicated by in vivo treatment of animals with lipopolysaccharide (LPS).[8] Changes in hepatic metabolism due to sepsis and LPS treatment have been attributed to altered regulation of hepatic intermediary metabolism.[9-11] One proposed mechanism by which these changes could occur is a disruption of cellular energetics. This would explain the observed decreases in hepatic adenosine triphosphate (ATP) content,[7,12] uncoupling of oxidative phosphorylation,[13] and decreases in mitochondrial membrane potential[14] reported after LPS treatment.

Bioenergetic reactions of the cell are linked by a network of near-equilibrium reactions that couple the cytosolic phosphorylation potential with the redox states of pyridine nucleotides.[15] Within the liver cell, these near-equilibrium reactions are considered fundamental to the coordination of carbohydrate flux through glycolysis and gluconeogenesis, reductive syntheses, and energy transduction.[15] The methodology for evaluating these bioenergetic functions from measured concentrations of metabolites in freeze-clamped samples has been well established.[15-18] Analyses of this type yield important information on cellular bioenergetics within different compartments, and have been shown to be sensitive indicators of changes in cellular bioenergetics within both the cytosol and mitochondria in response to variations in nutritional, disease, and hormonal status.[15-17] To determine if the disruption of intermediary metabolism caused by LPS could be attributed to changes in hepatic bioenergetics, the methodology reported by Williamson et al[16] and Veech et al[17,18] for the assessment of hepatic bioenergetics was used to characterize the extent and time dependence of in vivo changes in these parameters caused by a sublethal dose of LPS.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150 to 240 g body weight; Sasco, Houston, TX) were maintained on standard laboratory chow and water ad libitum before experiments began. Rats were fasted for 48 hours and then under light ether anesthesia received an intraperitoneal injection of *Salmonella typhosa* LPS (WS Typhosa 09011; Difco Laboratories, Detroit, MI) 0.5 mg/mL in 0.9% NaCl (1.0 mL/100 g body weight, 5 mg/kg body weight, a dosage of LPS that resulted in no deaths of the treated animals) or 0.9% NaCl alone in time-, age-, and weight-matched controls.[19] Starvation was maintained throughout the study. At the times indicated, animals were anesthetized with ether, and as soon as the eye reflex was lost, the abdominal cavity was opened and the livers were rapidly removed (<2 seconds) and freeze-clamped to a thickness of approximately 1 mm using aluminum tongs prechilled in liquid nitrogen.[20] For the 5-minute treatment, rats were maintained under ether anesthesia for the entire period between injection and freeze-clamping of the liver. To control for the possible metabolic effects of ether anesthesia, all statistical analyses of the LPS-treated group were performed using matched controls as described earlier. After freeze-clamping, livers were pulverized in a mortar and pestle at liquid-nitrogen temperature and stored at −80°C. Perchloric acid extracts were prepared using weighed portions of powdered liver.[21] Gross examination of the liver just before freeze-clamping indicated no observable histologic damage to the liver at any of the time points studied.

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Metabolite Assays and Calculated Values

Levels of the following metabolites were measured in neutralized perchloric acid extracts of freeze-clamped liver samples using standard methods: lactate, ATP, and isocitrate,[22] β-hydroxybutyrate and acetoacetate,[23] and all others.[24,25] The cytosolic free-[NAD^+]/[NADH] redox state was calculated using the measured hepatic content of lactate and pyruvate and the equilibrium constant (K_{eq}) for the lactate dehydrogenase (EC 1.1.1.27)–catalyzed reaction at pH 7.2[16]:

$$K_{eq} = [\text{pyruvate}][\text{NADH}][H^+]/([\text{lactate}][NAD^+])$$

$$= 1.11 \times 10^{-11}\ \text{mol/L}. \quad (1)$$

The cytosolic free-[$NADP^+$]/[NADPH] redox state was calculated from the measured hepatic content of isocitrate and α-ketoglutarate and the equilibrium constant for the isocitrate dehydrogenase (EC 1.1.1.42)–catalyzed reaction at pH 7.2 and [CO_2] of 1.63 mmol/L[17]:

$$K_{eq} = [\alpha\text{-ketoglutarate}][CO_2][\text{NADPH}]/([\text{isocitrate}][NADP^+])$$

$$= 1.17\ \text{mol/L}. \quad (2)$$

The mitochondrial free-[NAD^+]/[NADH] redox state was calculated from the measured hepatic content of acetoacetate and β-hydroxybutyrate and the equilibrium constant for the β-hydroxybutyrate dehydrogenase (EC 1.1.1.30)–catalyzed reaction at pH 7.4[16]:

$$K_{eq} = [\text{acetoacetate}][\text{NADH}][H^+]/([\beta\text{-hydroxybutyrate}][NAD^+])$$

$$= 4.93 \times 10^{-9}\ \text{mol/L}. \quad (3)$$

The free cytosolic phosphorylation potential, [ΣATP/([ΣADP] × [ΣP_i]), was calculated from the measured hepatic content of lactate, pyruvate, dihydroxyacetone phosphate, and 3-phosphoglycerate and the equilibrium constants for lactate dehydrogenase (EC 1.1.1.27)–, 3-phosphoglycerate kinase (EC 2.7.2.3)–, triosephosphate isomerase (EC 5.3.1.1)–, and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12)–catalyzed reactions at a free [Mg^{2+}] of 1 mmol/L and pH 7.2[18]:

$$K_{eq} = \frac{[\text{3-phosphoglycerate}][\Sigma\text{ATP}][\text{lactate}]}{([\text{DHAP}]/22)[\Sigma\text{ADP}][\Sigma\text{Pi}][\text{pyruvate}]}$$

$$= 1.65 \times 10^{7}\ \text{L/mol}, \quad (4)$$

where ADP is adenosine diphosphate, DHAP is dihydroxyacetone phosphate, and Pi is inorganic phosphate.

RESULTS

Measured hepatic contents of intermediary metabolites at 5, 30, or 180 minutes or 24 hours after in vivo LPS or normal saline administration are listed in Table 1. Hepatic redox states calculated from these measured metabolites were not significantly different between LPS-treated and time-matched control groups at 5 or 30 minutes after treatment (Table 2). However, by 3 hours, the mitochondrial free-[NAD^+]/[NADH] redox ratio (Eq 3) was more oxidized (Table 2), reflecting an increased hepatic acetoacetate content (Table 1). The cytosolic free-[NAD^+]/[NADH] redox ratio (Eq 1) was more oxidized (Table 2), reflecting an increased hepatic pyruvate content (Table 1), and the cytosolic free-[$NADP^+$]/[NADPH] redox ratio (Eq 2) was also more oxidized (Table 2), reflecting an increased α-ketoglutarate content (Table 1). By 24 hours, the cytosolic free-[$NADP^+$]/[NADPH] redox ratio had returned to control values, but the cytosolic free-[NAD^+]/[NADH] redox ratio was even more oxidized (Table 2), reflected in a further increase in pyruvate content (Table 1). Although acetoacetate returned to control values 24 hours after LPS treatment (Table 1), the mitochondrial free-[NAD^+]/[NADH] redox ratio remained oxidized (Table 2), and this was reflected in the significant decrease in β-hydroxybutyrate (Table 1). Total ketone bodies (acetoacetate + β-hydroxybutyrate) were significantly decreased at 24 hours (control 1.73 ± 0.19 [n = 5] *v* LPS 0.698 ± 0.075 [n = 7], $P < .001$), whereas the hepatic urea content was increased at 3 hours (control 5.31 ± 0.60 [n = 13] *v* LPS 7.63 ± 0.72 [n = 13], $P = .02$) and 24 hours (control 5.24 ± 0.17 [n = 5] *v* LPS 7.78 ± 0.89 [n = 7], $P < .01$) after LPS treatment. There was no significant difference in hepatic ketone body or urea content between the groups at the 5- and 30-minute time points. (All values are expressed as micromoles per gram fresh weight of freeze-clamped liver and are the mean ± SEM from n different livers.)

The free cytosolic phosphorylation potential was not significantly different between LPS-treated and control groups at any of the times studied (Table 2; Eq 4), although both 3-phosphoglycerate and dihydroxyacetone phosphate

Table 1. Metabolite Content of Freeze-Clamped Rat Livers as a Function of Time After LPS Administration to Fasted Rats

	5 Minutes		30 Minutes		180 Minutes		24 Hours	
	Control (n = 6)	LPS (n = 6)	Control (n = 6)	LPS (n = 6)	Control (n = 13)	LPS (n = 13)	Control (n = 5)	LPS (n = 7)
ATP	2.51 ± 0.14	2.42 ± 0.16	2.73 ± 0.01	2.57 ± 0.05*	2.50 ± 0.09	2.38 ± 0.04	2.74 ± 0.07	2.49 ± 0.07
DHAP	0.0188 ± 0.0023	0.0202 ± 0.0016	0.0174 ± 0.0008	0.0186 ± 0.0005	0.0191 ± 0.0013	0.0260 ± 0.0020*	0.0202 ± 0.0014	0.0252 ± 0.0014*
3-Phosphoglycerate	0.128 ± 0.056	0.131 ± 0.049	0.179 ± 0.013	0.158 ± 0.015	0.134 ± 0.021	0.320 ± 0.048†	0.155 ± 0.014	0.539 ± 0.077†
Pyruvate	0.0202 ± 0.0044	0.0199 ± 0.0032	0.0210 ± 0.0017	0.0204 ± 0.0014	0.0141 ± 0.0013	0.0256 ± 0.0036†	0.0157 ± 0.0016	0.0345 ± 0.0047†
Lactate	0.365 ± 0.042	0.367 ± 0.053	0.331 ± 0.020	0.366 ± 0.022	0.361 ± 0.035	0.465 ± 0.067	0.432 ± 0.076	0.445 ± 0.062
Acetoacetate	0.303 ± 0.037	0.319 ± 0.047	0.280 ± 0.014	0.290 ± 0.035	0.283 ± 0.021	0.399 ± 0.028†	0.234 ± 0.031	0.249 ± 0.025
β-Hydroxybutyrate	1.95 ± 0.24	2.10 ± 0.31	1.82 ± 0.20	2.05 ± 0.17	1.92 ± 0.21	1.70 ± 0.35	1.50 ± 0.17	0.449 ± 0.057†
Isocitrate	0.0146 ± 0.0023	0.0161 ± 0.0032	0.0103 ± 0.0007	0.0097 ± 0.0010	0.0074 ± 0.0007	0.0097 ± 0.0008*	0.0071 ± 0.0012	0.0090 ± 0.0010
α-Ketoglutarate	0.076 ± 0.013	0.071 ± 0.017	0.0647 ± 0.0075	0.0610 ± 0.0117	0.0517 ± 0.0053	0.113 ± 0.015†	0.0482 ± 0.0020	0.0734 ± 0.0118

NOTE. Values are micromoles per gram fresh weight and are the mean ± SEM from (n) different liver samples.
Abbreviation: DHAP, dihydroxyacetone phosphate.
*$P < .05$, †$P < .01$, Significant difference between time-matched LPS and control groups evaluated using a two-tailed unpaired Student's *t* test.

Table 2. Calculated Values of Hepatic Cytosolic and Mitochondrial Free-[NAD$^+$]/[NADH] Ratio, Cytosolic Free-[NADP$^+$]/[NADPH] Ratio, and Cytosolic Free Phosphorylation Potential in Freeze-Clamped Livers as a Function of Time After Administration of LPS to Fasted Rats

	5 Minutes		30 Minutes		180 Minutes		24 Hours	
	Control (n = 6)	LPS (n = 6)	Control (n = 6)	LPS (n = 6)	Control (n = 13)	LPS (n = 13)	Control (n = 5)	LPS (n = 7)
Cytosolic free-[NAD$^+$]/[NADH]	311 ± 64	364 ± 95	364 ± 30	322 ± 29	237 ± 22	327 ± 25*	219 ± 22	443 ± 21†
Mitochondrial free-[NAD$^+$]/[NADH]	1.28 ± 0.10	1.30 ± 0.18	1.30 ± 0.14	1.15 ± 0.12	1.41 ± 0.21	2.56 ± 0.35†	1.28 ± 0.13	4.65 ± 0.50†
Cytosolic free-[NADP$^+$]/[NADPH]	0.0078 ± 0.0014	0.0065 ± 0.0009	0.0088 ± 0.0008	0.0088 ± 0.0015	0.0110 ± 0.0016	0.0171 ± 0.0019*	0.0104 ± 0.0017	0.0115 ± 0.0014
Cytosolic free-[ΣATP]/([ΣADP] × [ΣP$_i$]) L/mol	10,200 ± 3,600	12,800 ± 5,500	4,680 ± 350	5,070 ± 330	5,580 ± 860	4,390 ± 670	3,910 ± 640	3,020 ± 350

NOTE. Values were calculated from data in Table 1 and are the mean ± SEM from (n) different liver samples. The pH was assumed to be 7.2 for cytosol and 7.4 for mitochondria, and [CO_2] = 1.63 mmol/L.

Abbreviations: ADP, adenosine diphosphate; P_i, inorganic phosphate.

*P < .05, †P < .01: Significant difference between time-matched LPS and control groups evaluated using a two-tailed unpaired Student's t test.

contents were significantly elevated 3 and 24 hours after LPS treatment (Table 1). The total hepatic content of ATP measured in freeze-clamped livers did not change in any of the treated groups, except for a slight decrease after 30 minutes (Table 1).

DISCUSSION

In vivo LPS treatment has been shown to change hepatic substrate supply[26,27] and circulating hormone concentrations.[28,29] For these reasons, we specifically did not want to study isolated cell or organ systems, since by virtue of the very act of isolation the liver tissue would no longer be in the milieu of hormones and substrates that affect its biochemical functions in vivo. Only by studying the intact animal can one determine the in vivo metabolic and bioenergetic status of the liver.

The cytosolic phosphorylation potential after LPS treatment was not different from control values at any of the time points examined (Table 2). This demonstrates that hepatic phosphoenergetics were not compromised after a sublethal dose of LPS. Unlike the phosphorylation potential, cellular NAD(P)$^+$ redox states (Table 2) were oxidized 3 and 24 hours after treatment with LPS. Changes in cellular redox states can be caused by a number of different mechanisms, two of which are the uncoupling of oxidative phosphorylation[30,31] and changes in the hepatic fuel source. For example, when changing from fatty acids to carbohydrates or amino acids as substrate, hepatic NAD(P)$^+$ redox states become more oxidized.[15-17]

Although it has been previously reported that sepsis causes uncoupling of oxidative phosphorylation,[13] this does not appear to be the mechanism operating in our system, since uncoupling of oxidative phosphorylation with carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) has been shown to cause a discordant change in cellular NAD$^+$ redox states. In isolated perfused livers the mitochondrial free-[NAD$^+$]/[NADH] redox ratio became more oxidized, whereas in the intact animal there was no change in this ratio after FCCP treatment.[30,31] The cytosolic free-[NAD$^+$]/[NADH] redox ratio, on the other hand, became more reduced in both the perfused liver and the intact animal treated with FCCP.[30,31] However, these studies were performed using a lethal dose of uncoupler (in the intact studies, the animals died within 5 minutes[31]), and the possibility that a partial uncoupling of oxidative phosphorylation could cause the oxidation of both the cytosolic and mitochondrial redox states cannot be ruled out.

In this study, fasting of the animals 48 hours before treatment should result in fatty acids being the primary hepatic substrate before LPS administration. Thus, a shift to a more oxidized fuel source such as amino acids derived from peripheral-tissue protein breakdown, which has been shown to increase after LPS treatment,[4,26,32-34] would be a possible mechanism for the oxidation of cellular NAD$^+$ redox states that were observed 3 and 24 hours after LPS treatment (Table 2). The oxidation of hepatic redox states after in vivo administration of LPS to fasted rats occurred after a lag phase with no significant changes until 3 hours. This time course is distinct from the rapid LPS activation of glycogen phosphorylase and glycogenolysis that occurs within seconds of LPS addition to perfused rat livers,[35] and is consistent with the time course of a LPS-induced alteration in substrate availability.

The hepatic urea content in LPS-treated groups was elevated at 3 and 24 hours after LPS treatment, which is consistent with an increased hepatic catabolism of amino acids and an obligatory increase in ammonia detoxification. The hepatic content of β-hydroxybutyrate (Table 1) and total ketone bodies was not decreased 3 hours after LPS treatment, which would indicate that fatty acids were still being actively metabolized.[36] However, the hepatic content of acetoacetate was significantly greater 3 hours after LPS treatment (Table 1), as has been reported by others.[37] This was a consequence of the near-equilibrium reaction catalyzed by β-hydroxybutyrate dehydrogenase in response to the oxidation of the mitochondrial [NAD$^+$]/[NADH] redox state.[16] In addition, the increase in hepatic acetoacetate indicates that the plasma acetoacetate concentration also increased, since it has been shown that the liver actively maintains a gradient of acetoacetate approximately twofold greater in the plasma relative to the liver.[38] Total hepatic ketone body content was not decreased until 24 hours after LPS treatment. It has been shown that the rate of ketone body utilization in extrahepatic tissue is concentration-dependent[36]; therefore, the rate of ketogenesis also would not be expected to decrease until 24 hours after LPS

treatment, since the rate of formation must equal the rate of utilization to maintain steady-state concentrations.

The oxidation of hepatic redox states in response to in vivo administration of LPS can best be explained by the following hypothesis based on the data presented in this report and previous studies by others under similar in vivo conditions. LPS stimulates peripheral protein degradation, which results in an increased hepatic uptake and catabolism of amino acids (mainly glutamine and alanine).[4,26,33] The increased amino acid utilization, as reflected in the increase of hepatic urea content, leads to an increase in the mitochondrial free-[NAD^+]/[NADH] ratio due to a shift to a more oxidized fuel source (ie, amino acids) from free fatty acids and triglycerides.[15,17] The oxidation of mitochondrial free-[NAD^+]/[NADH] caused an increase in the acetoacetate to β-hydroxybutyrate ratio (Eq 3) and hepatic and plasma acetoacetate contents (Table 1). Increased circulating acetoacetate has been shown to stimulate insulin secretion,[39] which then would be expected to contribute to decreased circulating fatty acid levels.[28] Decreased free fatty acid levels then lead to the decreases in hepatic ketone body concentrations that were observed at 24 hours as a result of a decreased rate of ketogenesis. The decrease in fatty acid utilization could also be due to LPS-stimulated release of tumor necrosis factor, which has been reported to suppress fatty acid metabolism,[40] or could even be a direct effect of the elevated insulin levels, as has been reported in man.[41]

In summary, the changes in hepatic bioenergetics in response to LPS reported here apparently result from extrahepatic events to which the liver has responded. The oxidation of hepatic NAD^+ redox states was consistent with an LPS-induced change in metabolic fuels available to the liver, as would be expected from the previously reported data regarding increased muscle protein degradation[4,26,32-34] and decreased fatty acid mobilization.[42-44]

REFERENCES

1. Berry LJ: Metabolic effects of bacterial endotoxins, in Kadis S, Weinbaum G, Ajl SJ (eds): Microbial Toxins, vol 5. New York, NY, Academic, 1971, pp 165-208
2. Morrison DC, Ulevitch RJ: The effects of bacterial endotoxins on host mediation systems. Am J Pathol 93:526-617, 1978
3. Siegel JH, Cerra FB, Coleman B, et al: Physiological and metabolic correlations in human sepsis. Surgery 86:163-193, 1979
4. Beisel WR, Wannemacher RW Jr: Gluconeogenesis, ureagenesis, and ketogenesis during sepsis. JPEN 4:277-285, 1980
5. Takeyama N, Itoh Y, Kitazawa Y, et al: Altered hepatic mitochondrial fatty acid oxidation and ketogenesis in endotoxic rats. Am J Physiol 259:E498-E505, 1990
6. Ardawi MSM, Majzoub MF, Kateilah SM, et al: Maximal activity of phosphate-dependent glutaminase and glutamine metabolism in septic rats. J Lab Clin Med 118:26-32, 1991
7. Pelias ME, Townsend MC: In vivo [^{31}P]NMR assessment of early hepatocellular dysfunction during endotoxemia. J Surg Res 52:505-509, 1992
8. McCabe WR: Gram-negative bacteremia. Adv Intern Med 19:135-158, 1974
9. Filkins JP, Cornell RP: Depression of hepatic gluconeogenesis and the hypoglycemia of endotoxin shock. Am J Physiol 227:778-781, 1974
10. Clemens MG, Chaudry IH, McDermott PH, et al: Regulation of glucose production from lactate in experimental sepsis. Am J Physiol 244:R794-R800, 1983
11. Ohtake Y, Clemens MG: Interrelationship between hepatic ureagenesis and gluconeogenesis in early sepsis. Am J Physiol 260:E453-E458, 1991
12. Kopprasch S, Hörkner U, Orlik H, et al: Energy state, glycolytic intermediates and mitochondrial function in the liver during reversible and irreversible endotoxin shock. Biomed Biochim Acta 48:653-659, 1989
13. Schumer W, Das Gupta TK, Moss GS, et al: Effect of endotoxemia on liver cell mitochondria in man. Ann Surg 171:875-882, 1970
14. Kurose I, Kato S, Ishii H, et al: Nitric oxide mediates lipopolysaccharide-induced alteration of mitochondrial function in cultured hepatocytes and isolated perfused liver. Hepatology 18:380-388, 1993
15. Krebs HA, Veech RL: Pyridine nucleotide interrelations, in Papa S, Tager JM, Quagliariello E, et al (eds): The Energy Level and Metabolic Control in Mitochondria. Bari, Italy, Adriatica Editrice, 1969, pp 329-382
16. Williamson DH, Lund P, Krebs HA: The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem J 82:514-527, 1967
17. Veech RL, Eggleston LV, Krebs HA: The redox state of free nicotinamide adenine dinucleotide phosphate in the cytoplasm of rat liver. Biochem J 115:609-619, 1969
18. Veech RL, Lawson JWR, Cornell NW, et al: Cytosolic phosphorylation potential. J Biol Chem 254:6538-6547, 1979
19. Miller BC, Ishikawa E, Uyeda K, et al: Endotoxin increases the liver fructose 2,6-bisphosphate concentration in fasted rats. Biochem Biophys Res Commun 165:1072-1078, 1989
20. Wollenberger A, Ristan O, Schoffa G: Eine einfache technik der extrem schnellen abkühling größerer gewebestücke. Pflugers Arch Gesamte Physiol Menschen Tiere 270:399-412, 1960
21. Kim Y-A, King MT, Teague WE Jr, et al: Regulation of the purine salvage pathway in rat liver. Am J Physiol 262:E344-E352, 1992
22. Lowry OH, Passonneau JV: A Flexible System of Enzymatic Analysis. New York, NY, Academic, 1972
23. Williamson DH, Mellanby J, Krebs HA: Enzymic determination of D(−)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J 82:90-96, 1962
24. Veech RL, Guynn R, Veloso D: The time-course of the effects of ethanol on the redox and phosphorylation states of rat liver. Biochem J 127:387-397, 1972
25. Veloso D, Passonneau JV, Veech RL: The effects of intoxicating doses of ethanol upon intermediary metabolism in rat brain. J Neurochem 19:2679-2686, 1972
26. Austgen TB, Chen MK, Flynn TC, et al: The effects of endotoxin on the splanchnic metabolism of glutamine and related substrates. J Trauma 31:742-752, 1991
27. Pacitti A, Austgen T, Souba W: Adaptive regulation of alanine transport in hepatic plasma membrane vesicles from the endotoxin-treated rat. J Surg Res 51:46-53, 1991
28. Neufeld HA, Pace JG, Kaminski MV, et al: A probable endocrine basis for the depression of ketone bodies during infectious or inflammatory state in rats. Endocrinology 107:596-601, 1980
29. Kelleher DL, Fong BC, Bagby GJ, et al: Metabolic and hormonal changes following endotoxin administration to diabetic rats. Am J Physiol 243:R77-R81, 1982

30. Stubbs M: Relationships between the redox state of the pyridine nucleotides, the phosphorylation state of the adenine nucleotides and substrate utilization in animal tissues. BSc thesis, University of Oxford, Oxford, UK, 1971

31. Stubbs M, Veech RL, Krebs HA: Control of the redox state of the nicotinamide couple in rat liver cytoplasm. Biochem J 126:59-65, 1972

32. Imamura M, Clowes GH Jr, Blackburn GL, et al: Liver metabolism and glucogenesis in trauma and sepsis. Surgery 77:868-880, 1975

33. O'Donnell TF Jr, Clowes GHA Jr, Blackburn GL: Proteolysis associated with a deficit of peripheral energy fuel substrates in septic man. Surgery 80:192-200, 1976

34. Hasselgren PO, Talamini M, James JH, et al: Protein metabolism in different types of skeletal muscle during early and late sepsis in rats. Arch Surg 121:918-923, 1986

35. Casteleijn E, Kuiper J, Van Rooij HJC, et al: Endotoxin stimulates glycogenolysis in the liver by means of intercellular communication. J Biol Chem 263:6953-6955, 1988

36. Williamson DH, Bates MW, Page MA, et al: Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. Biochem J 121:41-47, 1971

37. Takeyama N, Takagi D, Matsuo N, et al: Altered hepatic fatty acid metabolism in endotoxicosis: Effect of L-carnitine on survival. Am J Physiol 256:E31-E38, 1989

38. Fafournoux P, Demigné C, Rémésy C: Mechanisms involved in ketone body release by rat liver cells: Influence of pH and bicarbonate. Am J Physiol 252:G200-G208, 1987

39. Hawkins RA, Alberti KGMM, Houghton CRS, et al: The effect of acetoacetate on plasma insulin concentration. Biochem J 125:541-544, 1971

40. Tracey KJ, Cerami A: Tumor necrosis factor, other cytokines and disease. Annu Rev Cell Biol 9:317-343, 1993

41. Keller U, Gerber PG, Stauffacher W: Fatty acid–independent inhibition of hepatic ketone body production by insulin in humans. Am J Physiol 254:E694-E699, 1988

42. Neufeld HA, Pace JA, White FE: The effect of bacterial infections on ketone concentrations in rat liver and blood and on free fatty acid concentrations in rat blood. Metabolism 25:877-884, 1976

43. Kaminski MV Jr, Neufeld HA, Pace JG: Effect of inflammatory and noninflammatory stress on plasma ketone bodies and free fatty acids and on glucagon and insulin in peripheral and portal blood. Inflammation 3:289-294, 1979

44. Wannemacher RW Jr, Pace JG, Beall FA, et al: Role of the liver in regulation of ketone body production during sepsis. J Clin Invest 64:1565-1572, 1979