# Effect of Inflammatory and Noninflammatory Stress on β-Hydroxybutyrate and Free Fatty Acids in Rat Blood

Harold A. Neufeld, Mitchell V. Kaminski, Jr., and Judith A. Pace

Running head: Ketone Body Concentrations Under Differing Stresses

From the United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

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β-Hydroxybutyrate and free fatty acid concentrations in rat blood were measured during a variety of inflammatory and noninflammatory stresses. Inflammatory stresses included infection with Streptococcus pneumoniae, endotoxemia and induced turpentine abscess. Noninflammatory stresses included fasting plus screen-restraint and fasting plus femoral fracture. Inflammatory stresses caused a marked inhibition of the normal fasting-induced ketosis and a reduction in the level of circulating free fatty acids. Noninflammatory stresses caused no inhibition of the →

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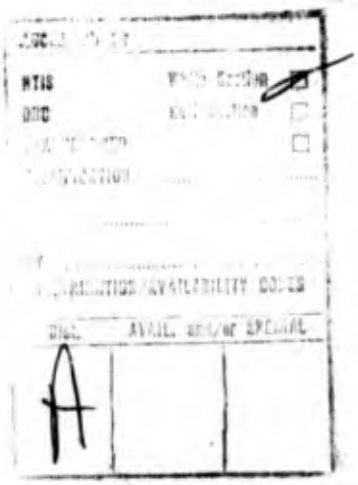
normal fasting-induced ketosis but did cause a reduction in the level of circulating free fatty acids.



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β-Hydroxybutyrate and free fatty acid concentrations in rat blood were measured during a variety of inflammatory and noninflammatory stresses. Inflammatory stresses included infection with Streptococcus pneumoniae, endotoxemia and induced turpentine abscess. Noninflammatory stresses included fasting plus screen-restraint and fasting plus femoral fracture. Inflammatory stresses caused a marked inhibition of the normal fasting-induced ketosis and a reduction in the level of circulating free fatty acids. Noninflammatory stresses caused no inhibition of the normal fasting-induced ketosis but did cause a reduction in the level of circulating free fatty acids.

Recently it has been suggested that metabolic response to stress is dependent on the type of stress imposed upon the subject [1]. Starvation, uncomplicated by trauma or sepsis, results in a high level of circulating free fatty acids and a corresponding rise in the level of circulating ketone bodies (ketosis). The stress of prolonged or severe exercise has been shown to cause marked ketosis [2-8]. Blackburn et al. [9] and Ryan et al. [10] have shown that sepsis causes a marked reduction in the level of circulating ketone bodies and free fatty acids despite concurrent starvation. Neufeld et al. [11] have shown in the rat model that sepsis causes a depression in both circulating and hepatic ketone bodies and a lowering of circulating free fatty acids. The purpose of this investigation was to examine further the effect of inflammatory and noninflammatory stress on the level of β-hydroxybutyrate and free fatty acids in the blood using the rat model. Inflammatory related stresses were produced by turpentine abscess, endotoxemia and Streptococcus pneumoniae infection. Noninflammatory stresses were produced by starvation plus screen-restraint or noninvasive femoral fracture.

## Materials and Methods

Male, Fisher-Dunning rats, F-344/Mai-F were obtained from Microbiological Associates, Walkersville, MD.

Two noninflammatory stresses were produced according to the following methods. General anesthesia using halothane was induced prior to manipulation. The first method utilized wire screen-restraint as described by Rossi et al. [12] and Brodie et al. [13]. A small hole was cut in the screen to allow placement of a water spigot within the compartment providing water ad libitum. In the second method, femoral

fracture was produced by digital noninvasive technique. The pelvis, acetabulum and proximal femur were grasped firmly between the index and forefinger of the right hand. The distal femur and knee joint were similarly grasped by the left hand. The index fingers were held against a table. A fracture, consistently located between the proximal third and distal two-thirds of the femur, was produced by controlled, downward thumb pressure and signaled by an audible snap.

Inflammatory stress was produced either by a sc inoculation into the nape of the neck of $10^4$ S. pneumoniae, Ia5, or 1 ml of pure spirits of gum turpentine manufactured by Warren-Graham Company or by the ip injection of 1 ml of endotoxin (1 mg/rat, lipopolysaccharide W, E. coli 01271B8 supplied by Difco Laboratories). Controls for the rats receiving viable organisms was an equal quantity of heat-killed organisms. Controls for rats injected with turpentine or lipopolysaccharide were equal volumes of sterile pyrogen-free saline. Blood was obtained as described by Neufeld et al. [11] at 24 and 48 hr postinoculation. β-Hydroxybutyrate was determined according to the method of McGarry et al. [14]. Serum free fatty acids were determined by the method of Dalton et al. [15].

## Results

A comparison of the effect of inflammatory and noninflammatory stress on circulating β-hydroxybutyrate acid is shown in figure 1. Of particular interest is that the inhibition of the normal fasting-induced ketosis by infection previously reported by Neufeld et al. [11] can also be caused by the administration of endotoxin or by the induction of a turpentine abscess. These inflammatory stresses are also accompanied by anorexia and elevation in body temperature. Further, these stresses

result in an increase in circulating leukocytes. Inflammatory stresses can be distinguished from noninflammatory in that the latter do not inhibit fasting-induced ketosis.

Neufeld et al. [11] showed that the inhibition of fasting-induced ketosis by infection was accompanied by a decrease in the level of circulating free fatty acids. Our data further demonstrate that the difference between the effect of noninflammatory and inflammatory stress is on serum β-hydroxybutyrate only, not serum free fatty acids. Figure 2 shows that both inflammatory and noninflammatory stresses caused a reduction in serum free fatty acids.

## Discussion

Neufeld et al. [11] reported that the infection-induced inhibition of ketosis was accompanied by a concurrent lowering of the level of circulating free fatty acids. It was proposed by them that this was probably the result of inhibition of lipolysis. The data presented in this paper suggest that the effect is a good deal more complicated since all stresses tested, inflammatory or noninflammatory, caused a lowering of circulating free fatty acids while only the inflammatory stresses caused the inhibition of fasting-induced ketosis. The data presented in this paper suggest that the levels of β-hydroxybutyrate acid and free fatty acids are influenced by the type of stress imposed upon the animal. It might be hypothesized from these data that at least three types of stress can be described by the metabolic response in terms of serum ketone body and free fatty acid concentrations.

The first may be described as a physical stress induced by starvation and exercise which impose severe demands on the body for

energy reserves. This results in a rapid release of fatty acids from fat stores causing a high level of circulating free fatty acids followed by an increase in ketone bodies which can then be used as fuel by the peripheral tissue. The second can, perhaps, be described as a noninflammatory stress illustrated here by the screening-restraint technique and aseptic femoral fracture. Both of these are manifested by a normal production of β-hydroxybutyrate acid during the fasting state but a reduction in the level of free fatty acids. The third can be described as a stress of inflammation and is illustrated by infection, administration of endotoxin, and the creation of a turpentine abscess. This results in a marked inhibition of fasting-induced ketosis and a concomitant decrease in circulating free fatty acids.

It is important, therefore, that differing forms of stress not be indiscriminantly considered together. As shown here, the difference in response with regard to serum β-hydroxybutyrate and free fatty acids was noted only when the stress was inflammatory in nature. This implies a need for specific forms of metabolic support in human patients suffering trauma, infection, or trauma and infection.

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## References


1. Neufeld, H. A., Kaminski, M. V., Pace, J. A., Stuhl, H. A. The effect of inflammatory and noninflammatory stress on ketosis in the rat. Fed. Proc. 35:1502, 1976.
2. Askew, E. W., Dohm, G. L., Huston, R. L. Fatty acid and ketone body metabolism in the rat: response to diet and exercise. J. Nutr. 105:1422-1432, 1975.
3. Askew, E. W., Huston, R. L., Plopper, C. G., Hecker, A. L. Adipose tissue cellularity and lipolysis. Response to exercise and cortisol treatment. J. Clin. Invest. 56:521-529, 1975.
4. Johnson, R. H., Walton, J. L. Fitness, fatness, and post-exercise ketosis. Lancet 566-568, 1971.
5. Johnson, R. H., Walton, J. L., Krebs, H. A., Williamson, D. H. Metabolic fuels during and after severe exercise in athletes and nonathletes. Lancet 452-455, 1969.
6. Johnson, R. H., Walton, J. L., Krebs, H. A., Williamson, D. H. Post-exercise ketosis. Lancet 1383-1385, 1969.
7. Molé, P. A., Oscai, L. B., Holloszy, J. O. Adaptation of muscle to exercise. Increase in levels of palmityl CoA synthetase, carnitine palmityltransferase, and palmityl CoA dehydrogenase, and in the capacity to oxidize fatty acids. J. Clin. Invest. 50:2323-2338, 1971.
8. Winder, W. W., Baldwin, K. M., Holloszy, J. O. Exercise-induced adaptive increase in rate of oxidation of β-hydroxybutyrate by skeletal muscle. Proc. Soc. Exp. Biol. Med. 143:753-755, 1973.
9. Blackburn, G. L., Flatt, J. P., Clowes, G. H. A., O'Donnell, T. E., Hensle, T. E. Protein sparing therapy during periods of starvation with sepsis and trauma. Ann. Surg. 177:588-594, 1973.

10. Ryan, N. T., Blackburn, G. L., Clowes, Jr., G. H. A. Differential tissue sensitivity to elevated endogenous insulin levels during experimental peritonitis in rats. Metabolism 23:1081-1089, 1974.

11. Neufeld, H. A., Pace, J. A., White, F. E. The effect of bacterial infections on ketone concentrations in rat liver and blood and free fatty acid concentrations in rat blood. Metabolism 25: in press.

12. Rossi, G., Bonfils, S., Lieffogh, F., Lambling, A. Technique nouvelle pour produire des ulcérations gastriques chex le rat blanc: l'ulcère de contrainte. C. R. Soc. Biol. (Paris) 150:2124-2126, 1956.

13. Brodie, D. A., Hanson, H. M. A study of the factors involved in the production of gastric ulcers by the restraint technique. Gastroenterology 38:353-360, 1960.

14. McGarry, J. D., Guest, M. J., Foster, D. W. Ketone body metabolism in the ketosis of starvation and alloxan diabetes. J. Biol. Chem. 245:4382-4390, 1970.

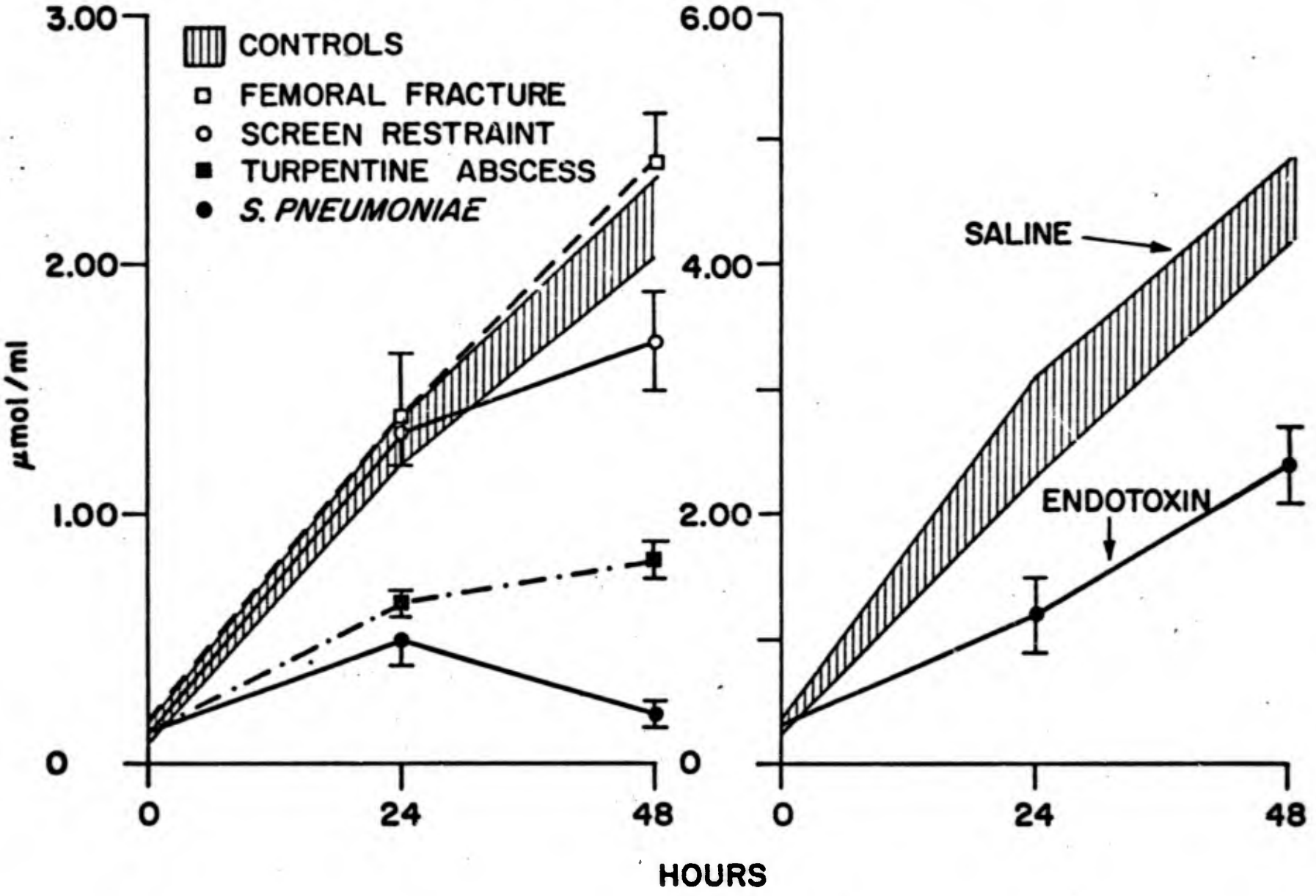
15. Dalton, C., Kowalski, C. Automated colorimetric determination of free fatty acids in biologic fluids. Clin. Chem. 13:744-751, 1967.

**Legends for Figures**

**Figure 1.** Effect of inflammatory and noninflammatory stress on serum β-hydroxybutyrate. (band) starvation-induced elevation of serum β-hydroxybutyrate, (□) femoral fracture, (○) screen-restraint, (■) turpentine abscess, (●) S. pneumoniae infection. The data for endotoxin are presented separately since it has been observed that the ip injection of sterile pyrogen-free saline causes an approximate doubling of the normal level of β-hydroxybutyric acid. A minimum of six rats was used for each data point.

**Figure 2.** Effect of inflammatory and noninflammatory stress on serum free fatty acids. (band) fasted controls, (□) femoral fracture, (○) screen-restraint, (■) turpentine abscess, (●) infection with S. pneumoniae, (▲) endotoxin.

EFFECT OF INFLAMMATORY AND NONINFLAMMATORY STRESS ON SERUM β-HYDROXYBUTYRATE



# EFFECT OF INFLAMMATORY AND NONINFLAMMATORY STRESS ON SERUM FREE FATTY ACIDS