Experimental Hyperosmolar Diabetic Syndrome

Ketogenic Response to Medium-chain Triglycerides

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SUMMARY

The clinical features of the experimental hyperosmolar diabetic (EHD) rat model resemble those seen in the human syndrome —extreme hyperglycemia without ketoacidosis is common to both. The absence of ketoacidosis in the syndrome has been ascribed to both substrate (free fatty acid) deficiency and to interference with hepatic ketone body synthesis. The potential for hepatic ketone body synthesis in the experimental model has been directly assessed by challenging the EHD animals with medium-chain triglycerides (MCT) administered intragastrically. This neutral lipid, largely consisting of C_8 and C_{10} fatty acids, leads to a dose- and time-related increase in the plasma concentration of acetoacetate and β-hydroxybutyrate. The EHD rats respond to MCT with an increase in plasma ketone bodies that rises to levels that are twice as high as those observed in normal rats receiving MCT and are equivalent to the levels seen in untreated ketoacidotic animals. These data indicate that hepatic medium-chain fatty acid oxidation and ketogenesis are unimpaired in the EHD animal. An analysis of the factors responsible for the greater ketogenic response in the EHD rat reveals that moderate diabetes and dehydration enhance MCT-induced ketone body accumulation, while cortisol is without effect. The plasma free fatty acid concentration in EHD animals does not differ from normal rats, but is significantly lower than that seen in diabetic ketoacidosis. These data support the concept that a principal reason for the absence of ketoacidosis in the EHD syndrome is the limitation in availability of substrate, free fatty acids, for ketone body synthesis. DIABETES *24*:301-06, March, 1975.

The hyperglycemic, hyperosmolar syndrome has been described in diabetics not requiring exogenous insulin, and less frequently in insulin-dependent diabetics and in nondiabetic individuals. During the acute episode, the peripheral plasma immunoreactive insulin (IRI) levels are inappropriately low for the hyperglycemia in all patients. Despite low plasma insulin concentrations, which do not differ appreciably from those seen in diabetic ketoacidosis, accumulation of plasma ketones and acidosis are absent.[1] Conflicting hypotheses have been offered to explain this paradox. Joffe and associates[2,3] have advanced the view that hepatic ketogenesis is restricted, while others[1,4] ascribe the absence of ketosis to a limited supply of free fatty acids (FFA) for ketone body synthesis. The present study has been designed to examine these two possibilities in the experimental hyperosmolar diabetic (EHD) rat model.[5] Our data point to a restriction in the availability of precursors, the FFA, as the prime determinant for the lack of the development of ketoacidosis, and are consistent with the hypothesis that dehydration is importantly involved.

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METHODS

Male Sprague-Dawley rats weighing 200 to 300 gm. were used in all experiments. Rats were made moderately diabetic by the intravenous administration of 40 to 50 mg. streptozotocin per kilogram body weight after an eighteen to twenty-four hour fast. These animals were not used until at least two weeks after production of diabetes. Rats that lost appreciable amounts of weight during this period were discarded. Ketoacidosis was induced by intravenous administration of 65 mg. alloxan per kilogram of body weight after an overnight fast; these rats were used three days after injection. All diabetic rats were maintained without exogenous insulin. Adrenalectomized animals were given 0.9 per cent NaCl in their drinking water and were used approximately one week after surgery; cortisol-treated rats received 5 mg. cortisol intramuscularly for six days. Control animals in this series of experiments were subjected to a sham operative procedure.

Production of the EHD syndrome was accomplished as described earlier by treating moderately diabetic rats with 5 mg. cortisol intramuscularly followed by water deprivation starting on the third day of cortisol administration.[5] In recent experiments,

more consistent production of the EHD syndrome was induced by withdrawing water on the first day of cortisol administration. Figure 1 shows the plasma and urine glucose values during development of the syndrome using this protocol. Plasma glucose concentrations in the 1,000 mg. per 100 ml. range were generally observed between forty and sixty hours after initiation of cortisol treatment and dehydration. Urine glucose in the twelve hours before dehydration and cortisol administration was 6.38 ± 0.05 gm.; diminution of glucosuria occurred concomitantly with a marked decrease in urine volume. Medium-chain triglyceride (MCT) in undiluted form was given by gastric instillation with a metal cannula. The fatty acids of MCT are principally of C_8 and C_{10} chain length (>94 per cent) and less than 6 per cent fatty acids are of chain length C_6 and C_{12}.

Periodic plasma glucose concentrations were done at indicated times on tail vein blood. Plasma and urine glucose were assayed either with the Beckman glucose analyzer or with Glucostat reagent. At the end of each experiment, heparinized blood was obtained either by cardiac puncture of the anesthetized animal (ether) or following decapitation from the carotid and jugular vessels. The formed elements of the blood were immediately separated by centrifugation. A protein-free filtrate of the plasma was prepared without delay by addition of cold perchloric acid. The filtrate was then titrated with KOH to pH 7.0 with an automatic burette. Acetoacetate and β-hydroxybutyrate were determined on the neutralized filtrate using an enzymatic-spectrophotometric technic.[6,7] Plasma free fatty acids (FFA) were assayed by the colorimetric method of Lauwerys.[8]

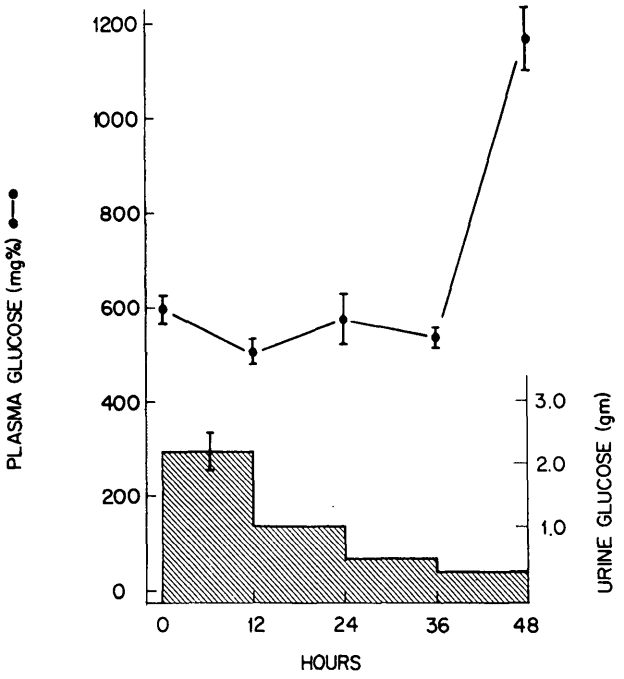

FIG. 1. Plasma and urine glucose concentrations in water-deprived, diabetic rats receiving cortisol. Each value represents the mean ± S.E. of determinations in at least four animals. The forty-eight hour value actually represents plasma glucose concentrations obtained at the time of sacrifice; the mean time of sacrifice after initiation of cortisol administration and water deprivation was forty-nine hours. The standard error for urine glucose was less than ± 0.1 gm. per twelve hours unless indicated.

The MCT oil was kindly supplied by Mead Johnson Laboratories, Evansville, Indiana, and streptozotocin from the Upjohn Company, Kalamazoo, Michigan. Glucostat was obtained from Worthington Biochemical Corporation. β-hydroxybutyrate dehydrogenase was purchased from Boehringer Mannheim Corporation and pyridine nucleotide from Sigma Chemical Company, St. Louis, Missouri.

RESULTS

Ketogenic effect of medium-chain triglyceride (MCT). Medium-chain fatty acids (MCFA) are more effective precursors for ketone body synthesis in liver than long-chain fatty acids.[9] Although parenteral administration of MCFA has been shown to result in a modest elevation of plasma ketones in rats,[10] these fatty acids are not well tolerated. However, the MCFA esterified with glycerol and administered intragastrically as neutral lipid (MCT) are well tolerated and result in substantial production of ketone bodies, as manifested by an increase in the plasma ketone body concentration (figure 2). The levels of plasma ketone bodies attained is dependent upon the amount of MCT administered. After 2.5 ml. of MCT, a peak concentration of approximately 3 mEq. of ketone bodies (sum of acetoacetate and β-hydroxybutyrate) per liter is reached after ninety minutes, and then the level falls. Doubling the administered dose to 5.0 ml. results in approximately twice as high plasma ketone body levels at ninety minutes with a plateauing effect thereafter. In all subsequent experiments 5.0 ml. of MCT has been given intragastrically with plasma sampling at ninety minutes after administration.

Lipid metabolism in the EHD rat. The plasma FFA, the immediate precursors of hepatic ketone body production, are significantly lower ($p<0.001$) in the EHD than in the ketoacidotic animal; the mean plasma FFA concentration in EHD rats is identical to that found in control, nondiabetic rats (figure 3).

After MCT is administered to EHD rats, an enor-

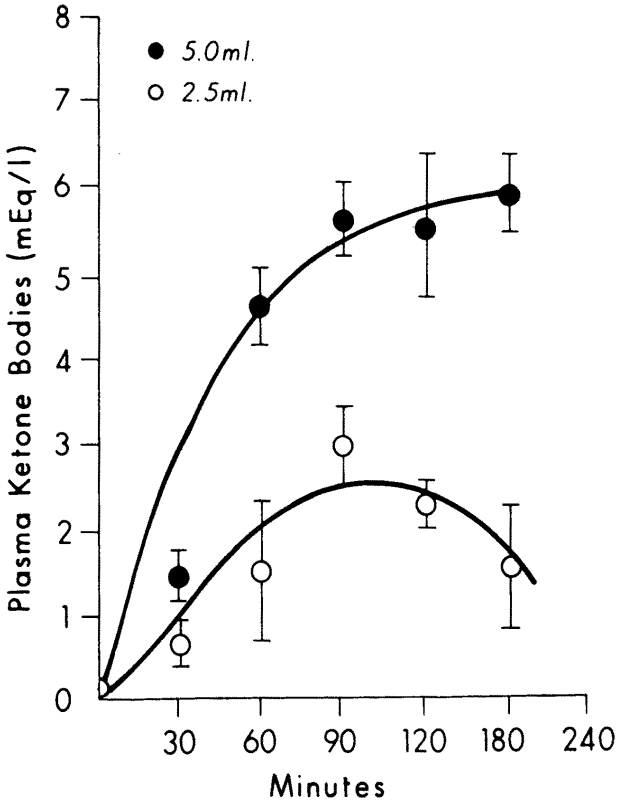

FIG. 2. The effect of medium chain triglyceride (MCT) administered intragastrically on the sum of plasma acetoacetate and β-hydroxybutyrate in normal rats. Each value represents the mean ± S.E. response of at least five rats.

mous increase in plasma ketone bodies is observed (figure 4). The plasma ketones of EHD rats so treated reached levels that are approximately twice those ob-

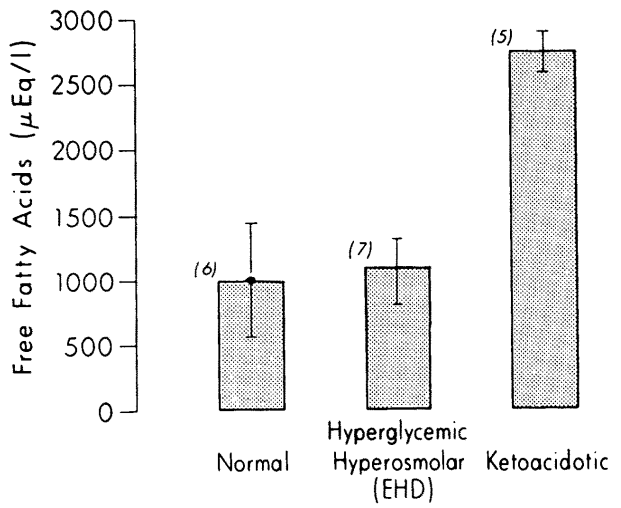

FIG. 3. The plasma free fatty acids (FFA) in normal, EHD and ketoacidotic rats. The height of the bars represents the mean values ± S.E. of the number of animals in each group indicated in parentheses.

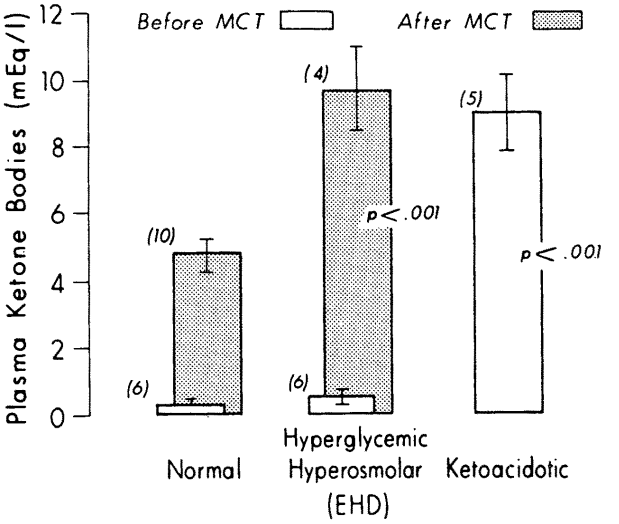

FIG. 4. The basal plasma ketone body concentration (sum of acetoacetate and β-hydroxybutyrate) in normal, EHD and ketoacidotic rats and the ketone body response to MCT (5 ml. intragastrically) in normal and EHD rats. The values represent the means ± S.E.; the number of rats in each group is indicated in parentheses. The p values indicate statistically significant differences between experimental and normal animals.

served in normal rats receiving MCT and are equivalent to the plasma concentration seen in untreated ketoacidotic rats. These data clearly demonstrate that the EHD rats have the potential for a high rate of ketone body synthesis.

Effects of diabetes, dehydration, and cortisol on MCT-induced ketosis. Development of the experimental EHD syndrome is accomplished by dehydrating the cortisol-treated, moderately diabetic rat. For this reason, the influence of each of these states on MCT-induced ketosis has been examined (figure 5). MCT administration to moderately diabetic rats and to nondiabetic rats that have been water deprived for three days results in a significantly higher level of ketone bodies than in the plasma of normal rats. In contrast, nondiabetic, cortisol-treated rats respond to MCT administration with a rise in plasma ketone body levels to the same extent as control animals. Additional support for an absence of a cortisol effect of MCT-induced ketosis is derived from experiments with adrenalectomized rats. Neither the acetoacetate levels nor the β-hydroxybutyrate levels are significantly altered by cortisol deficiency or by cortisol excess after MCT administration (figure 6).

DISCUSSION

The extreme hyperglycemia and the absence of ketoacidosis in the EHD syndrome suggest a profound

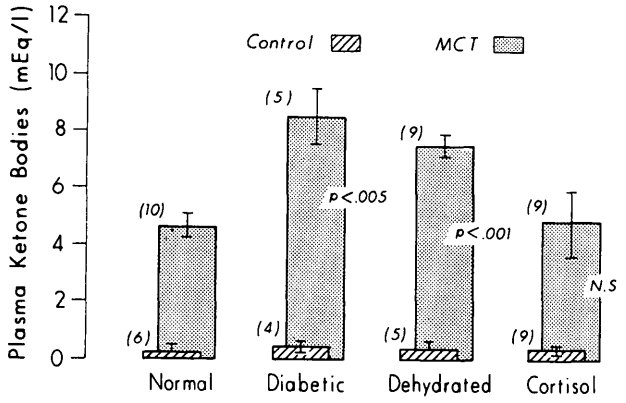

FIG. 5. The effect of MCT on the plasma concentration of ketone bodies (sum of acetoacetate and β-hydroxybutyrate) in normal, moderately diabetic, water-deprived (three days) normal, and cortisol-treated (5 mg. intramuscularly x 3 days) rats. The number of animals in each group is indicated in parentheses. The values for total plasma ketone bodies did not differ significantly between any of the groups prior to MCT administration. The levels of significance between normal and other groups after MCT administration are indicated by the "p" values.

alteration of carbohydrate metabolism without a concomitant, clinically apparent, disturbance in the handling of lipids. Since adipose tissue is known to be extremely responsive to small amounts of insulin, it has been suggested that there is adequate peripheral insulin to inhibit the release of FFA from adipose tissue.[4,11] However, carefully controlled studies in human subjects[1] and in a hyperosmolar rat model[3] have failed to demonstrate a difference in the concentration of plasma IRI between the ketoacidotic or-

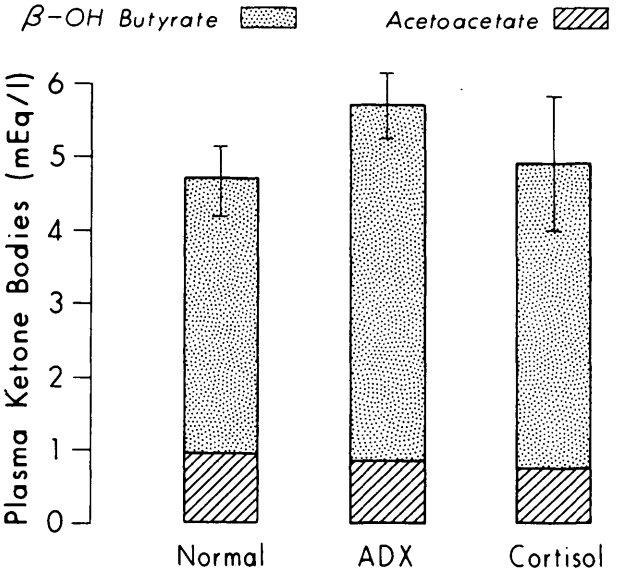

FIG. 6. Cortisol and plasma ketone body values after MCT administration. The cortisol treated animals received 5 mg. cortisol intramuscularly for three days. The values represent the mean ± S.E. of at least six rats in each group.

ganism where there is a very high concentration of FFA and the hyperglycemic, hyperosmolar state where the FFA concentration is normal (figure 3). Elevated FFA concentrations have been reported in two clinical studies.[2,12] In some cases, the data suggest that the syndrome might not have been "pure," but rather a combination of hyperglycemic hyperosmolar and diabetic ketoacidosis;[2] the calculated anion gap might be explained by elevated concentrations of plasma ketone bodies. These data point to a factor(s) other than insulin which restricts the release of FFA from adipose tissue in the hyperglycemic, hyperosmolar state.

On the basis of earlier experiments with an experimental rat model, we concluded that dehydration is an important etiologic factor for the development of the hyperglycemic, hyperosmolar syndrome.[5] In the diabetic rat, fluid restriction is required for the development of the syndrome. In man, fluid deprivation or excessive fluid loss is often a prominent feature of the history. The possibility that severe dehydration in and of itself could restrict ketone body formation by limiting substrate availability was introduced by Gerich et al.[13] They found that restriction of water blunted the elevation of plasma FFA and ketone bodies induced by fasting normal rats. Moreover, the plasma FFA response to epinephrine was markedly attenuated when rats were dehydrated. In other experiments, it was shown that adipose tissue from normal rats incubated in hypertonic media exhibited a depressed release of free fatty acids. Kuzuya et al.[14] have also observed similarities between insulin action and hypertonicity on certain metabolic activities. In normal human subjects, a ketogenic diet results in lower levels of serum ketones and decreased urinary ketone excretion when the subject's fluid intake is restricted.[15] These findings are consistent with the interpretation that the limited release of FFA in the hyperglycemic, hyperosmolar organism is due at least in part to dehydration and could be a determinant explaining the lack of ketosis. Emphasis should be given to the normal level of plasma FFA observed in the EHD syndrome since this indicates that adipose tissue has not been lipid depleted despite appreciable weight loss during development of the syndrome.

The absence of ketosis in the hyperosmolar state could be due to limited hepatic synthesis of ketone bodies. On the basis of indirect evidence, Joffe et al.[3] have reasoned along these lines. They observed equivalent levels of portal venous IRI levels in normoglycemic rats and in extremely hyperglycemic animals

together with a twofold increase in liver glycogen in the latter group. A direct assessment of ketone body synthesis in the hyperosmolar model has led us to conclude that ketogenesis proceeds at an enhanced rate when appropriate substrate is present.

MCFA are preferentially converted to ketone bodies rather than oxidized to CO_2 by isolated hepatic mitochondria.[16] The ketogenic potential of MCFA in intact rats[10] and in man[17] has also been observed. A common mitochondrial oxidative pathway is shared by both long-chain fatty acids (LCFA) and MCFA. More recent studies suggest a mechanism for the differential handling of LCFA and MCFA by the liver. Apparently the rate of LCFA oxidation is restricted by a complex mitochondrial transport mechanism involving the formation of an acyl carnitine derivative, while MCFA readily penetrate the mitochondrial membrane without undergoing intervening rate-limiting steps.[18] Moreover, an alternate pathway for LCFA, hepatic esterification with glycerol, is not open to MCFA. Triglycerides of MCFA composition lead to a modest elevation of plasma ketones in normal man and an even greater increase in noninsulin-dependent diabetics; the triglycerides composed of LCFA are without effect on plasma ketones.[19,20] When an MCT load is presented to the gastrointestinal tract of rats, appreciable levels of ketone bodies appear in the plasma, and the response is dependent upon the amount administered (figure 2). Our data clearly show that the hyperosmolar rats are capable of synthesizing massive amounts of ketone bodies. The plasma levels are about twice those observed in controls. The increment of plasma ketone body concentration above control may be related to the state of dehydration or to lack of the well known carbohydrate-sparing effect of LCFA oxidation[21] as well as to the diabetic state itself. Conceivably, the lower ketone body response to MCT in control rats could be due to inhibition of ketogenesis consequent to MCT-induced insulin release.[9] Blunting of the insulin response might be anticipated in the diabetic rats and especially under conditions of hypovolemia.[22]

The role of cortisol in ketone body metabolism is not clear. Data are available to suggest that cortisol either promotes[23,24] or inhibits[25,26] the development of ketosis. Our experiments (figure 6) do not support a role for cortisol in MCT-induced ketogenesis.

It is unlikely, although not proven, that enhanced oxidation of ketone bodies by peripheral tissues could account for the low levels of ketone bodies in the plasma of the unchallenged EHD animals. Peripheral ketone body utilization is an insulin-dependent phenomenon. Since the peripheral IRI levels are low in the EHD animal, one would anticipate a decreased rate of ketone body oxidation and an elevation of plasma ketone bodies rather than the observed low levels.

ACKNOWLEDGMENT

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BOOK REVIEW

NUTRIENTS IN PROCESSED FOODS, VOLUME II: PROTEINS, *edited by Philip L. White, Sc.D., and Dean C. Fletcher, Ph.D., American Medical Association, $16.00, 219 pages, 49 figures, 90 tables. Acton, Mass., Publishing Sciences Group, 1974.*

This is the second volume derived from a series of three symposia conducted by the Council on Food and Nutrition of the American Medical Association. It was preceded by a symposium on *Vitamins and Minerals* and is followed by one on *Fats and Carbohydrates.* Essayists in this symposium included nineteen authorities who have contributed importantly in the study of amino acid availability and protein quality in foods. In the initial section, problems dealing with the validation of concepts of protein utilization and protein quality are presented. Variations in amino acid requirements in relation to age and sex, pregnancy and lactation, and stress, infection, and physical activity are viewed in terms of nitrogen balance and protein accumulation in human and animal studies. Of interest are the discussions dealing with amino acid excess and imbalance, and observations cited concerning the effect of periodicity of feeding on the efficiency of protein utilization. The second section provides an overview of the technology of assessing protein quality and the methods employed to determine the amount of different proteins needed to meet human requirements.

Extensive investigations concerned with the influence of the processing of foods upon their nutritive qualities are delineated utilizing a variety of newer biochemical and assay methods. The final segments present an informative round-table discussion and task force reports dealing with major concerns in protein nutrition. These discussions involve the matters of protein quality analysis, amino acid availability, evaluation of human requirements, and approaches to improvement in protein quality. This volume provides the nutritionist, dietitian and physician with a sound orientation in the recent developments in the complex field of protein nutrition. C. R. SHUMAN, M.D.

Erratum

The Editors regret that a line was inadvertently dropped from "Basement Membrane Thickness in Muscle Capillaries of Normal and Spontaneously Diabetic Macaca Nigra," by Charles F. Howard, Jr., Ph.D., in DIABETES *24*:201-06, February 1975. "With the technical assistance of Audrey Griffin and Joann Wolff" should have followed the author's name.