Lack of effects of hyperglycemia on the disposal of 3-hydroxybutyrate in insulin-dependent diabetic patients

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Abstract. There is evidence that hyperketonemia in insulin-dependent diabetes may be aggravated by a decreased disposal rate for ketone bodies. To test the hypothesis that this decrease may be induced by concomitant hyperglycemia through substrate competition at the acetyl-CoA level, 5 young insulin-dependent diabetic subjects received at 2-h iv infusion of 0.9 mmol 3-hydroxybutyrate · kg⁻¹ · h⁻¹ at clamped 1. euglycemia (5 mmol/l) and 2. hyperglycemia (11 mmol/l) on separate occasions. To ensure similar metabolic conditions, a low-dose hyperinsulinemic euglycemic clamp was performed during the 5 h preceding the actual studies. Substrate fluxes in muscle were assessed through the forearm technique. The glucose infusion rate was 4.9 and 2.9 mg · kg⁻¹ · min⁻¹, and the forearm arteriovenous difference for glucose was 0.72 during hyperglycemia and 0.39 mmol/l (p<0.05) during euglycemia. Hyperglycemia did not affect circulating levels of free insulin, glucagon, non-esterified fatty acids, 3-hydroxybutyrate (hyperglycemia: 665, euglycemia: 770 µmol/l, p>0.05) or acetocacetate, nor forearm uptake of 3-hydroxybutyrat (hyperglycemia, 152, euglycemia: 168 µmol/l, p>0.05). In conclusion, our results do not suggest any inhibitory role for hyperglycemia in the disposal of ketone bodies. In as much as extrapolation from the present well insulinized state is appropriate, the data indicate that alternative mechanisms may be involved in the observed impairment of ketone body clearance in hyperketonemic insulin-dependent diabetic patients.

Recently much interest have focused on the potential actions of lipid fuel substrates to inhibit glucose disposal, and there is evidence that free fatty acids (FFA) may impair insulin sensitivity in vivo (1).

During relative hypoinsulinemia patients with insulin-dependent diabetes are characterized by hyperglycemia accompanied, and perhaps further aggravated, by increased circulating levels of FFA and ketone bodies. The hyperketonemia is primarily due to increased ketogenesis, but may also be substantially worsened by a decreased disposal rate (2-7). A decrease in ketone body disposal could theoretically be induced by hyperglycemia through mass action and subsequent substrate competition at the acetyl-CoA level leading to decreased ketone oxidation. We are, however, not aware of any in vivo studies that have addressed this question.

The current study was initiated to establish the possible effects of isolated hyperglycemia on serum concentrations and forearm uptake of 3-hydroxybutyrate (3-OHB) during moderate hyperinsulinemia in insulin-dependent diabetic patients.

Subjects and Methods

Five lean (BMI: 23 (20-25) kg/m²), young (aged 27 (20-33) years), C-peptide negative male patients with insulin-dependent diabetes for 8 (4-15) years gave informed consent to participate in the study, which had been approved by the local Ethical Committee. HbA1c of the patients was 8.6 (7.2-9.4)%. They were all treated with multiple daily insulin injections and they all received soluble insulin.
(Actrapid®, Novo, Bagsvaerd, DK) exclusively for 36 h prior to the study. The participants attended hospital the evening before the study and were given sc insulin at 23.00 and 03.00 h depending on actual blood glucose values. After a 10-h fast subjects were studied for 7 h from 08.00 h and onwards. All were studied twice on separate occasions and in random order, i.e. during 1. euglycemia and 2. hyperglycemia.

Protocol
At 07.30 h one catheter was placed retrogradely into a deep antecubital vein and one catheter was inserted retrogradely into a contralateral heated dorsal hand vein to allow for measurement of forearm substrate exchange as described (8). A third catheter was placed into a deep antecubital vein of the heated arm for infusions. Total ipsilateral blood flow was determined by venous occlusion plethysmography preceding each deep venous blood sample (9). Hand blood flow was interrupted by inflation of a wrist cuff to a pressure of 250 mmHg before each blood flow determination and 1 min prior to all deep venous samples. At 08.00 h a continuous iv infusion of insulin (Actrapid, Novo) was commenced at rates of 1 mU/kg·min⁻¹ for 1 h, 0.5 mU kg⁻¹·min⁻¹ for the following 1 h and 0.3 mU kg⁻¹·min⁻¹ for the remaining 5 h. During insulin infusion plasma glucose was clamped at 5 mmol/l on the basis of plasma glucose measurements every 5-10 min and appropriate adjustment of iv infused 20% glucose. From 13.00 to 15.00 h sodium-hydroxybutyrate/HCl was infused at a rate of 1.8 mmol kg⁻¹·h⁻¹ for 20 min and 0.9 mmol kg⁻¹·h⁻¹ for the remaining 100 min on both occasions. To minimize the alkalinizing effect of sodium-hydroxybutyrate, HCl was added to the infusate to obtain a pH of 6.5. On one occasion the preset plasma glucose of 5 mmol/l was acutely increased to and maintained at 11.2 mmol/l by infusion of additional 50% glucose synchronously with initiation of the sodium-hydroxybutyrate infusion.

Plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA). Glycerol and 3-hydroxybutyrate (3-OHB) were determined by automated fluorimetric methods (10). Serum non-esterified fatty acids (NEFA) were assayed radiochemically (11). Plasma acetacetate was determined by an enzymatic micro-method (12). Radioimmunoassays were employed for measurements of plasma glucagon and serum free insulin (13,14). Standard bicarbonate, pH, oxygen saturation, and HbA1c were analysed by routine laboratory methods. Forearm arteriovenous substrate balances are given as simple differences. Results are given as means and range in the text and means ± SEM in the figures. Statistical significance was assessed by ANOVA for two repeated measures (time and treatment). A significance level of 0.05 (two-tailed) was employed.

Unless specified otherwise concentrations referred to below are obtained with arterialized blood.

Results

Circulating hormones
Introduction of 3-OHB and euglycemia or hyperglycemia did not affect circulating concentrations of free insulin or glucagon, which remained at pre-levels of 33 (range: 27-45) mU/l and 15 (8-33) ng/l in both situations.

Glucose metabolism
Plasma glucose levels were clamped at 5.0 (4.8-5.2) and 11.2 (10.4-12.0) mmol/l (p<0.05) (Fig. 1). During euglycemia 2.9 (0.3-4.6) mg · kg⁻¹ · min⁻¹

![Graph showing 3-OH butyrate levels](https://example.com/graph)

**Fig. 1.** Plasma glucose, exogenously administered glucose and glucose arteriovenous differences during a 2-h infusion of 3-hydroxybutyrate (3-OH butyrate), 0.9 mmol · kg⁻¹ · h⁻¹ at hyperglycemia (△-△) and euglycemia (○-○).
exogenously administered glucose was required and during hyperglycemia 4.9 (1.0-8.0) mg·kg\(^{-1}\)·min\(^{-1}\) (p<0.05). The increased requirement for exogenous glucose was reflected in the forearm arteriovenous differences for glucose (euglycemia: 0.39 (0.06-0.95) and hyperglycemia: 0.72 (0.39-1.28) mmol/l at 120 min, p<0.05).

**Lipid substrates**

During infusion, blood concentrations of 3-OHB increased gradually from pre-levels of 127 (30-435) (euglycemia) and 134 (20-350) (hyperglycemia) µmol/l to comparable levels of 770 (520-955) and 665 (450-910) µmol/l, respectively (Fig. 2). Pre-infusion (euglycemia: 26 (5-50), hyperglycemia: 16 (5-45) µmol/l) and hyperketonemic (euglycemia: 168 (70-350), hyperglycemia: 152 (80-365) µmol/l) arteriovenous differences of 3-OHB did not differ between the two experimental settings. Plasma acetacetate measured in 3 subjects rose from 40 (20-75) µmol/l to levels of 340 (225-480) µmol/l (arterialized) and 266 (195-425) (venous) µmol/l in both situations.

Under both circumstances 3-OHB infusion was followed by a steady decrease (p<0.05) in circulating NEFA levels (from 383 (287-685) and 436 (247-760) to 212 (175-251) and 198 (119-245) µmol/l, respectively). Forearm clearance of NEFA was not affected by infusion of 3-OHB in any of the experiments.

Total forearm blood flow remained at a baseline level of 3.0 (2.1-3.8) ml·100 ml\(^{-1}\)·min\(^{-1}\) on both occasions. Oxygen saturations and pH in arterialized and deep venous blood did not change with time or between treatments (data not shown).

**Discussion**

The present study was designed to establish whether an increased glucose flux per se influences circulating concentrations and disposal of ketone bodies in insulin-dependent diabetic patients. To ensure comparable metabolic conditions a glucose clamp with a low insulin infusion rate was employed. Despite a 70% increase in the exogenously administered glucose and a 90% increase in forearm glucose uptake during hyperglycemia no difference in blood concentration or forearm clearance of 3-OHB was recorded, clearly indicating that hyperglycemia/increased glucose disposal does not affect overall utilisation of ketone bodies. When interpreting these results it should, however, be borne in mind that they have been obtained during relative hyperinsulinaemia and that it cannot be excluded that the effects of hyperglycemia may be different under hypoinsulinemic and preketotic circumstances.

There are several alternative mechanisms which could explain the observed impairment of the disposal of ketone bodies in poorly controlled hyperketonemic insulin-dependent diabetes: First, ketone body utilisation is linearly related to circulating concentrations only at low concentrations and decreases relatively at high concentrations (5,15). Second, there is evidence that insulin may
increase ketone body clearance (7); it should, however, be mentioned that in a very recent study there was no acute effect of insulin on forearm ketone body utilisation (16). Third, a concomitant increase in other lipid substrates could inhibit ketone disposal. One study (17) has indicated this, though the observed decrease of disposal could also be secondary to an increase in ketone body concentration per se. Fourth, extraction of ketones could theoretically be affected by increased levels of counterregulatory hormones; we are not aware of any studies in this field. Finally, it should be emphasized that the validity of many of the isotope dilution techniques employed in the above studies is still a subject of controversy and that it has been suggested that net production of ketones may take place in muscle in ketotic diabetic subjects (6).

In summary, we find no evidence for any inhibition of ketone body disposal by hyperglycemia-induced increases in glucose fluxes and suggest that alternative mechanisms behind the reported impairment of ketone body clearance in ketotic insulin-dependent diabetic patients should be sought.

Acknowledgments

This work was supported by Michaelsen Fonden and Diabetesforeningen. The expert technical assistance of Anette Mengel is gratefully acknowledged.

References