Ketone Body Infusion Increases Circulating Erythropoietin and Bone Marrow Glucose Uptake

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Sodium–glucose cotransporter 2 (SGLT2) inhibition was originally developed as a facile way of lowering blood glucose through increased renal elimination. However, clinical trials have demonstrated that SGLT2 inhibition not only improves glycemic control but also increases circulating ketone bodies and hematocrit (1,2). Recently, SGLT2 inhibitor–driven modest hyperketonemia has attracted much focus as a possible explanation of the improved cardiac outcome in patients treated with SGLT2 inhibitors (3), whereas the increased hematocrit has mostly been ascribed to hemoconcentration due to increased osmotic diuresis. In this Observation, we present data indicating that hyperketonemia directly stimulates circulating erythropoietin concentrations and is associated with increased bone marrow activity assessed by ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT).

Seventeen healthy subjects undergoing either cardiac (study A) or brain (study B) PET/CT as part of protocols involving experimental hyperketonemia were investigated. Study A (4) consisted of 8 subjects (3 women) aged mean 60 (range 50–68) years with BMI mean 26 (range 22–35) kg/m^2, and study B (5) of 9 subjects (4 women) aged 62 (56–69) years with BMI 24 (21–27) kg/m^2. Both studies were randomized, single-blinded crossover trials in which participants were infused with either 7.5% Na-3-β-hydroxybutyrate (3-OHB) (infusion rate 0.18 g/kg/h [study A] and 0.22 g/kg/h [study B]) or saline (0.9%). In study A, bone marrow palmitate and glucose uptake was measured with dynamic ^{11}C-palmitate and ^{18}F-FDG PET ($n = 8$). Bone marrow time-radioactivity curves were measured in two vertebrae in the spine, and the input function was derived from either arterial samples (^{11}C-palmitate) or from a volume of interest drawn in the aorta (^{18}F-FDG). Uptake rates were obtained using linear graphical analysis (Logan plot for ^{18}F-FDG and Patlak analysis for ^{11}C-palmitate).

A serum sample from the end of each study day (study A: $t = 390$ min, study B: $t = 240$ min) was analyzed for erythropoietin by a chemiluminescent immunoassay on Immulite 2000 (Siemens Healthineers, Erlangen, Germany).

By design, 3-OHB concentrations increased rapidly during ketone body infusions (Fig. 1*A*), reaching mean ± SEM 3.8 ± 0.2 mmol/L (study A) and 5.5 ± 0.4 mmol/L (study B). Ketone body levels were undetectable on saline study days.

As seen in Fig. 1*C*, erythropoietin concentrations were significantly greater during hyperketonemia than after saline infusions (mean ± SD 9.9 ± 1.1 vs. 7.6 ± 1.0 IU/L, $P = 0.01$). Hyperketonemia was also associated with 25% increased bone marrow ^{18}F-FDG uptake compared with the saline study day (mean ± SD 0.64 ± 0.08 vs. 0.51 ± 0.12 mL plasma/mL tissue, $P = 0.04$) (Fig. 1*D*) but did not affect bone marrow ^{11}C-palmitate uptake (1.7 ± 0.4 vs. 1.9 ± 0.6 μmol/g/min, $P = 0.62$). No correlation was observed between the increase in erythropoietin concentration and increase in bone marrow glucose uptake ($r = -0.09$, $P = 0.84$).

Some data supporting a connection between ketone bodies and erythropoietin exist. Thus, it has been shown that the hematocrit of infants born to mothers with diabetes is correlated with maternal 3-OHB concentrations and also that 4 weeks of SGLT2 inhibition by empagliflozin results in both increased levels of ketone bodies and a most-likely-transient increase in erythropoietin (2). In addition, dapagliflozin treatment causes not only hemoconcentration but also increased total red cell mass and transient increases in reticulocytes and erythropoietin.

Although the levels of hyperketonemia obtained in our experimental protocols were markedly greater than what has

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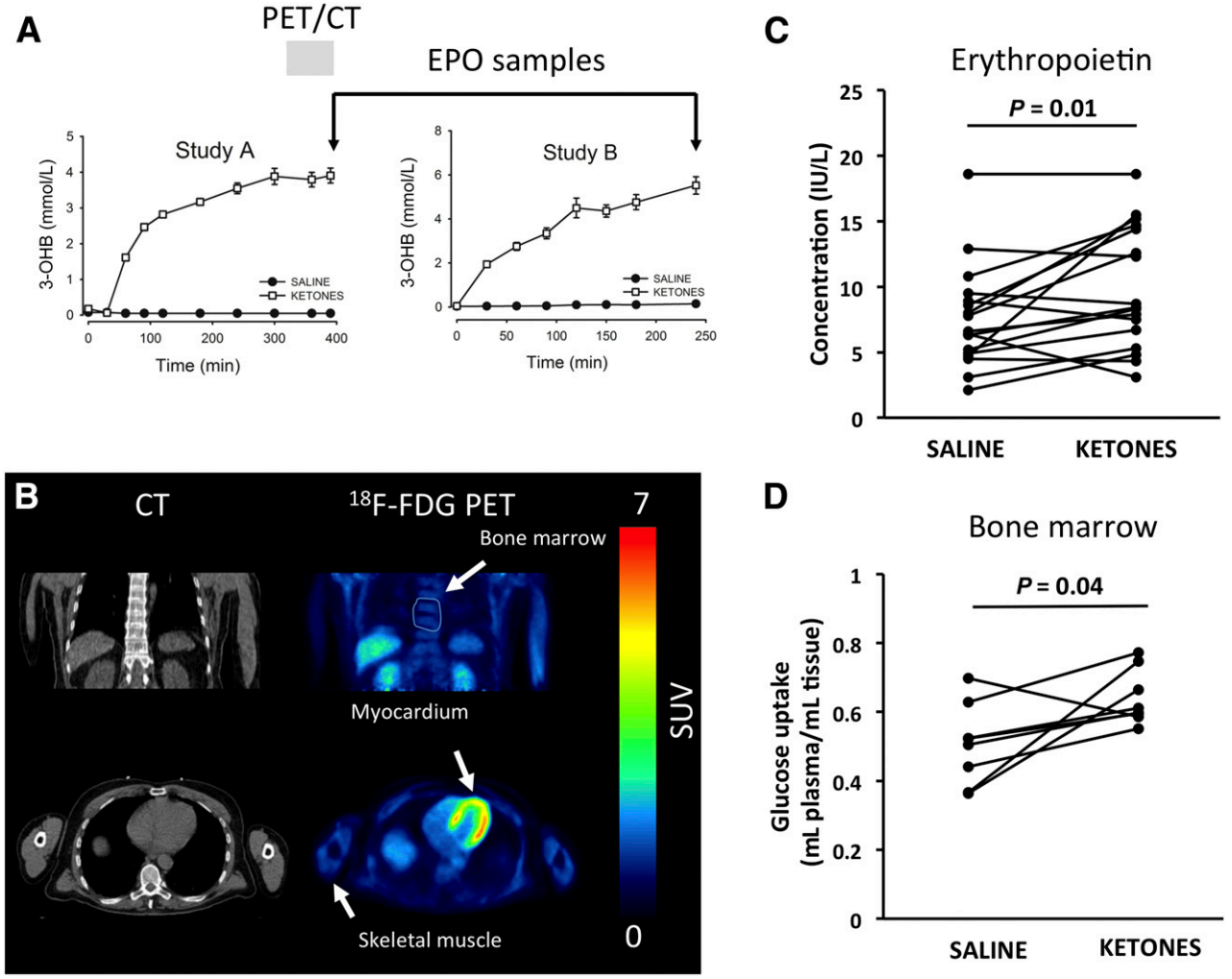

Figure 1—*A*: 3-OHB concentrations during hyperketonemia (open boxes) and saline (closed circles). Error bars are SEM. EPO, erythropoietin. *B*: Representative CT and PET images (coronal and axial projections) of subject no. 8 on the ketone body infusion study day. PET images were summed from 5–50 min and represent average standardized uptake values (SUV). As seen, the bone marrow was clearly visible. *C*: Erythropoietin levels. *D*: Bone marrow glucose uptake (volume of distribution) assessed by ^{18}F-FDG PET/CT.

been observed during SGLT2 inhibitor treatment (4–5 vs. ~0.6 mmol/L) (3), it is possible that a more modest but sustained increase in ketone bodies has a similar effect on erythropoietin levels. Erythropoietin is primarily produced in specialized renal cells, and increased secretion of the hormone in response to, e.g., altitude-induced hypoxia is not necessarily closely related to the stimulus. In other words, we may have only detected the initial increase in erythropoietin levels that the experimental hyperketonemia would eventually have produced.

Erythropoietin stimulates red blood cell maturation and release from the bone marrow, an energy-consuming process visible as avid ^{18}F-FDG uptake on PET/CT. The time to maximum effect of a single dose of human recombinant erythropoietin on reticulocyte cell count is 200–300 h, and it is therefore not surprising that we were unable to demonstrate a correlation between erythropoietin levels and bone marrow activity. Alternatively, the increase in bone marrow glucose uptake could be the result of increased leukopoiesis or thrombopoiesis or increased blood flow. We find this unlikely since *1*) ketone bodies have been reported to be anti-inflammatory, *2*) it takes several weeks of ketone body ingestion to increase thrombocytes discretely, and *3*) bone marrow ^{11}C-palmitate uptake was unaffected.

In conclusion, experimental hyperketonemia results in ~30% concomitant increase in erythropoietin levels and bone marrow glucose uptake. Although the level of hyperketonemia in our study was markedly higher than what is typically observed during SGLT2 inhibition, our data provide a possible explanation for the unknown link between SGLT2 inhibitor treatment and increased erythropoiesis.

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