CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis

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The striking and unexpected relative risk reductions in cardiovascular (CV) mortality (38%), hospitalization for heart failure (35%), and death from any cause (32%) observed in the EMPA-REG OUTCOME trial using an inhibitor of sodium–glucose cotransporter 2 (SGLT2) in patients with type 2 diabetes and high CV risk have raised the possibility that mechanisms other than those observed in the trial—modest improvement in glycemic control, small decrease in body weight, and persistent reductions in blood pressure and uric acid level—may be at play. We hypothesize that under conditions of mild, persistent hyperketonemia, such as those that prevail during treatment with SGLT2 inhibitors, β-hydroxybutyrate is freely taken up by the heart (among other organs) and oxidized in preference to fatty acids. This fuel selection improves the transduction of oxygen consumption into work efficiency at the mitochondrial level. In addition, the hemoconcentration that typically follows SGLT2 inhibition enhances oxygen release to the tissues, thereby establishing a powerful synergy with the metabolic substrate shift. These mechanisms would cooperate with other SGLT2 inhibition–induced changes (chiefly, enhanced diuresis and reduced blood pressure) to achieve the degree of cardioprotection revealed in the EMPA-REG OUTCOME trial. This hypothesis opens up new lines of investigation into the pathogenesis and treatment of diabetic and nondiabetic heart disease.

First among cardiovascular (CV) end point trials of glucose-lowering agents (1), the EMPA-REG OUTCOME trial—using 10 or 25 mg/day sodium–glucose cotransporter 2 (SGLT2) inhibitor empagliflozin against placebo in 7,020 patients with type 2 diabetes (T2D) who were at increased CV risk—reported a 14% reduction in major CV events and marked relative risk reductions in CV mortality (38%), hospitalization for heart failure (35%), and death from any cause (32%) over a median time period of 2.6 years (2). Of note, all the pathologic categories of CV death (ischemic, pump failure, arrhythmic, embolic) contributed to the overall reduction in CV death in a patient cohort well treated with the use of renin-angiotensin-aldosterone inhibitors, statins, and acetylsalicylic acid. Furthermore, separation of the cumulative incidence functions between pooled-dose groups and placebo was already evident months after randomization. This unusual time course and the discrepancy between the relative risk reduction of the primary end point (nonfatal myocardial infarction, stroke, and CV mortality) and CV mortality itself suggests that active treatment affected case fatality rates more than event rates. In other words, empagliflozin treatment appeared mostly to rescue patients from impending cardiac decompensation. This interpretation is supported by the recent post hoc analyses of heart failure, documenting a large benefit in first and recurrent heart failure hospitalization across virtually every patient subgroup (3). Despite the fact that the diagnosis of heart failure was based on investigator reporting, this outcome of the EMPA-REG

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OUTCOME trial stands out against the recognized lack of evidence on the safety or efficacy of glucose-lowering drugs in patients with heart failure (4,5); indeed, a risk signal of incident heart failure after treatment with saxagliptin emerged from the SAVOR-TIMI trial (1).

As expected from previous clinical studies, in the EMPA-REG OUTCOME trial the difference in glycemic control between the treatment and placebo arms (with HbA_{1c} averaging 0.54–0.60% at 12 weeks and 0.24–0.36% at study end) was accompanied by a small decrease in body weight and persistent reductions in blood pressure and uric acid level. Ahead of the study end, the EMPA-REG OUTCOME trial investigators themselves had outlined additional risk factors that the use of SGLT2 inhibitors influences positively: visceral adiposity, hyperinsulinemia, arterial stiffness, albuminuria, and oxidative stress (6). However, isolated reductions in HbA_{1c} level, body weight, or uricemia of the degree seen in the trial have been generally reputed to be insufficient to explain the outcome (7–11). For example, a meta-analysis of macrovascular outcomes in intensive glucose control trials (12) reported a 9% reduction in major CV events for an average HbA_{1c} reduction of 0.7%, but no significant reduction in CV or all-cause mortality. By their mode of action, SGLT2 inhibitors induce natriuresis and osmotic diuresis (13,14) and a drop in systolic and diastolic blood pressure levels (2); indeed, these effects have been hypothesized to play a pivotal role in the CV benefit shown by the EMPA-REG OUTCOME trial (10,11), especially during the early weeks of treatment when the systolic blood pressure gradient between the arms was largest (5 mmHg) (10). Clearly, such hemodynamic changes can be beneficial, particularly in patients with high CV risk, insofar as a reduced blood volume lowers the preload and a reduced blood pressure lowers the postload burden to the heart. There remain, however, inconsistencies between EMPA-REG OUTCOME trial outcomes and the CV benefit profile of antihypertensive and diuretic therapy. For example, a recent systematic review of blood pressure lowering in T2D patients (15) reported significant reductions in major CV events (including stroke), but smaller reductions in heart failure and mortality associated with a 10 mmHg decrease in systolic blood pressure. Likewise, in systematic meta-analyses (16,17), diuretic agents appear to be associated with a smaller (~20%) reduction in CV mortality than that seen in the EMPA-REG OUTCOME trial and a clear risk reduction in stroke, which was not observed in the EMPA-REG OUTCOME trial. In a recent trial (18), the use of the mineralocorticoid receptor antagonist eplerenone in patients with systolic heart failure resulted in a reduction in hospitalization for heart failure similar in size and time course to the corresponding outcome in the EMPA-REG OUTCOME trial. The eplerenone-treated patients, however, did not have diabetes, were selected on the basis of high levels of B-type natriuretic peptide (BNP) and N-terminal pro-BNP, and the trial outcome showed no protection against nonfatal myocardial infarction or stroke. The EMPA-REG OUTCOME trial cohort (2) was composed of older patients with long-standing T2D whose heart failure, when reported, was not characterized either for ejection fraction or levels of natriuretic peptides. Also, most patients in the EMPA-REG OUTCOME trial were being treated with antihypertensive drugs, including diuretic agents, so SGLT2 inhibition conferred a benefit for heart failure above and beyond antihypertensive treatment. Interestingly, in a small, short-term (12-week) mechanistic study in T2D patients (19), treatment with dapagliflozin (another selective SGLT2 inhibitor) resulted in a smaller reduction in blood pressure than did treatment with a comparator diuretic (25 mg hydrochlorothiazide), but also in a 7% decrease in plasma volume (as directly measured with the use of ^{125}I-labeled albumin) and some increment in N-terminal pro-BNP levels, neither of which were observed with the treatment with the diuretic agent. Thiazide diuretic agents decrease plasma volume only transiently, whereas in the EMPA-REG OUTCOME trial the observed increase in hematocrit persisted for the entire duration of the trial (20).

Collectively, these considerations suggest that other mechanisms triggered by SGLT2 inhibition may contribute to the CV outcomes of the EMPA-REG OUTCOME trial.

HYPOTHESIS

Raised circulating levels of β-hydroxybutyrate offered significant cardioprotection to the high-risk diabetes patients in the trial.

RATIONALE

In diet-induced obese rats treated with dapagliflozin (21), ipragliflozin (22), or tofogliflozin (23), lipolysis is accelerated and circulating ketone body levels are increased, especially in the fasting state or when animals are fed in pairs. In patients with T2D, empagliflozin-induced glycosuria lowers plasma glucose and insulin levels and raises fasting and postmeal glucagon concentrations. The subtraction of large amounts of glucose from the glucose pool, coupled with the dual hormonal changes, results in a 25% restriction of glucose utilization, and a concomitant increase in lipid mobilization and usage for energy production (24). Under conditions of reduced portal insulin-to-glucagon ratio, the increased delivery of free fatty acids (FFAs) to the liver stimulates ketogenesis (25), resulting in a metabolic condition resembling a prolonged fast (26). In well-controlled Caucasian patients with T2D who were receiving stable doses of metformin or were drug-naive (27), a 4-week course of treatment with 25 mg empagliflozin was associated with raised fasting and postmeal circulating FFA and glycerol levels, indicating enhanced lipolysis. Concomitantly, both fasting and postmeal plasma β-hydroxybutyrate concentrations were increased twofold to threefold; these changes were similar in time course, though attenuated in extent, in a group of nondiabetic volunteers receiving the drug. Similar results have been reported in Japanese patients with T2D with the use of empagliflozin, tofogliflozin, luseogliflozin, or canagliflozin (28–32). For example, in a 24-week phase III study of drug-naive patients with T2D (32), plasma ketones rose dose dependently with the administration of 100 or 200 mg canagliflozin versus placebo throughout the study period. Of note, though mean plasma ketone levels are only modestly elevated, in a sizeable proportion of subjects they rise into the millimolar range (27–32), particularly in the more insulinopenic patients (27).

Circulating β-hydroxybutyrate is taken up (through the monocarboxylate transporter, which also transports pyruvate) in proportion to its plasma concentration into most organs, including heart, brain, and kidney, by a saturable transport mechanism (33). In humans, β-hydroxybutyrate

uptake by forearm tissues is not influenced by local hyperinsulinemia (34) (Fig. 1), and exogenous β-hydroxybutyrate infusion does not interfere with insulin-mediated glucose utilization (35). Thus, β-hydroxybutyrate transport is insulin independent. In the fasting state, β-hydroxybutyrate is taken up by the human heart, along with glucose, lactate, pyruvate, glycerol, and FFA, with the highest avidity per unit mass among body tissues and with a fractional extraction (~40%) comparable to that of pyruvate and far higher than that of glucose (~2%) or FFA (15–20%) (36) (Fig. 2). By combining mass uptake (~10 μmol/min) with the heat of combustion (Table 1), it can be calculated that in the overnight fasted state β-hydroxybutyrate contributes 15% of resting cardiac energy expenditure compared with 45% of FFAs and 8% of glucose/lactate/pyruvate. When the rate pressure product is increased by incremental atrial pacing (Fig. 3), the fractional extraction of β-hydroxybutyrate remains high at ~40%, thereby continuing to support external cardiac work (37).

In perfused working rat hearts, β-hydroxybutyrate supplementation inhibits pyruvate oxidation by deactivating pyruvate dehydrogenase (38), mimics insulin action (39), and competes with oxidation of FFAs (possibly also by impeding their transport into cells [40]). Within the cell, after conversion to acetoacetate (catalyzed by the mitochondrial isoform of 3-hydroxy-3-methylglutaryl-CoA synthase) and breakdown to acetyl-CoA, β-hydroxybutyrate enters the tricarboxylic acid cycle (TCA) to be oxidized. By expanding the mitochondrial acetyl-CoA pool, β-hydroxybutyrate competes for entry into the TCA cycle with the acetyl-CoA originating from FFA oxidation and glucose-derived pyruvate (Fig. 4) (39,40). Thus, during fasting and starvation, β-hydroxybutyrate partially replaces glucose as a fuel, which is critical for the brain in circumstances of low glucose availability (26). Importantly, the energetics of mitochondrial β-hydroxybutyrate oxidation compares favorably with the oxidation of pyruvate (Table 1); in fact, when β-hydroxybutyrate is added to the perfusion medium of working rat hearts, the heat of combustion per unit of carbon has been calculated to be 31% increased and the oxygen cost of this energy output to be 27% decreased (41). This advantage is granted by a more efficient oxidation of the mitochondrial coenzyme Q couple and an increase in the free energy of cytosolic ATP hydrolysis. As a consequence, in the isolated working heart β-hydroxybutyrate increases external cardiac work at the same time as it reduces oxygen consumption, thereby improving cardiac efficiency by 24%. Furthermore, a persistently elevated rate of FFA oxidation generates reactive oxygen species in excess of scavenging capacity (42); the resulting oxidative stress contributes to mitochondrial damage in a range of pathologies (43). In in vitro systems, β-hydroxybutyrate has been shown to suppress oxidative stress (44) by inhibiting histone deacetylases. Finally, ketone bodies have been shown to upregulate mitochondrial biogenesis and, by stabilizing cell membrane potential, to exhibit antiarrhythmic potential (45).

In the fed state, as insulin restrains lipolysis and stimulates glucose oxidation, the circulating concentrations of β-hydroxybutyrate decline along with FFA flux and oxidation. In the normal human heart, systemic insulin administration at physiologic concentrations stimulates myocardial glucose uptake, both directly and by suppressing plasma FFA concentrations, without altering coronary blood flow; the uptake of β-hydroxybutyrate and its contribution to cardiac energy expenditure drop to nil (Fig. 2) (36). However, in patients with T2D (27,46) as well as in patients without diabetes with coronary artery disease (47) or stress-induced ischemia (48), whole-body and myocardial insulin-mediated glucose utilization are impaired (i.e., insulin resistance), and a larger (>80%) than normal (50–70%) proportion of energy is derived from the oxidation of fatty substrates (49). FFAs require ~8% more oxygen than glucose to produce the same number of calories, and the external power of the left ventricle for a given oxygen consumption is higher when rates of fatty acid β-oxidation are low relative to glucose and lactate oxidation (50). This coupling between substrate selection and mechanical efficiency is especially relevant to heart failure, regardless of its nature (ischemic or nonischemic) and functional manifestation (reduced or preserved ejection fraction) (51). Whether primarily or secondary to systemic changes induced by cardiac failure itself (e.g., adrenergic activation [47]), the failing heart is an "engine out of fuel" (52), especially when the energy demand increases, such as it does during exercise. Indeed, most kinds of cardiac injury—ischemic, myopathic, and reperfusion injury (53)—converge on insufficient mitochondrial energy output and contractile failure as the basic mechanism. Enhancing oxygen use and mechanical efficiency through the long-term

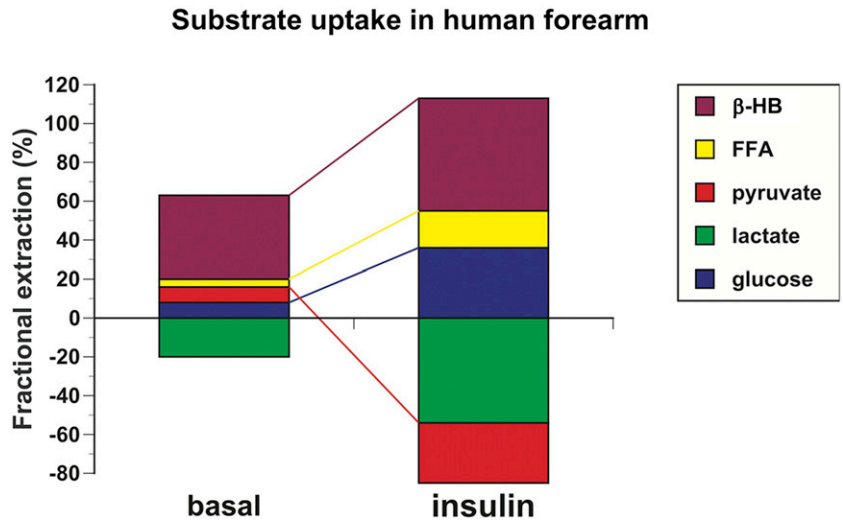

Figure 1—Fractional exchange of substrates by the human forearm under basal conditions and during local physiological hyperinsulinemia. Note the insulin-induced increased net release of lactate and the switch from net uptake to net release of pyruvate in the face of a constant extraction of β-hydroxybutyrate. Data were recalculated from the study by Natali et al. (34).

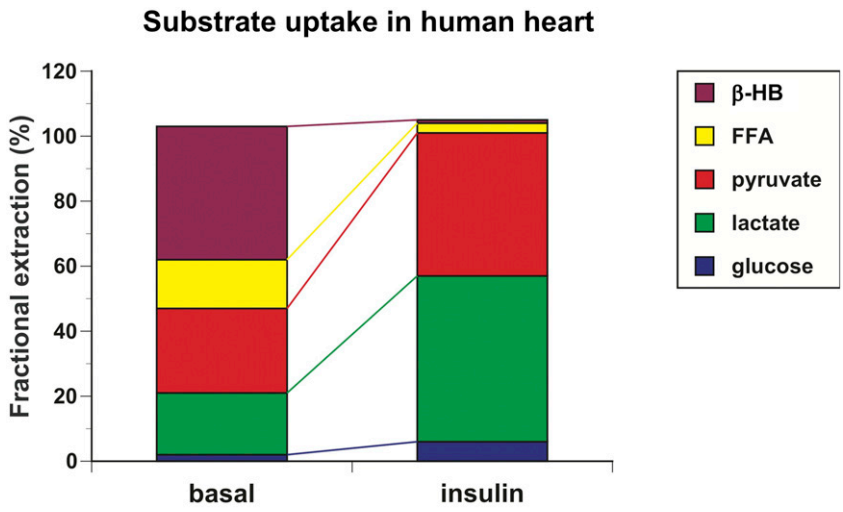

Figure 2—Fractional extraction of substrates by the human heart under basal (overnight fast) and systemic hyperinsulinemia (euglycemic-hyperinsulinemic clamp). Note the increased net extraction of glucose, lactate, and pyruvate in the face of a marked reduction in the net extraction of FFA and β-hydroxybutyrate (due to insulin-induced suppression of whole-body lipolysis). Data were recalculated from the study by Ferrannini et al. (36).

provision of an energetically thrifty substrate should therefore benefit most conditions of extensive CV damage in a relatively short time frame, precisely as was the case in the EMPA-REG OUTCOME trial. In this context, β-hydroxybutyrate can be viewed as a constitutive mitochondrial helper, just as is its closest kin of energetics, pyruvate (54,55) (Table 1). Of note is that, in a mouse model of heart failure, metabolite signatures of fatty acid oxidation are reduced, whereas signatures of the oxidation of ketone bodies (e.g., β-hydroxybutyrate dehydrogenase) are increased (56). Furthermore, in patients with heart failure, the use of circulating ketones is impaired by >50% in skeletal muscle but is preserved in myocardial tissues (57). Finally, in a study of myocardial tissue obtained from patients with advanced heart failure at the time of cardiac transplantation (58), levels of acyl-CoAs were reduced and those of acetyl-CoA (and succinyl-CoA:3-oxoacid CoA transferase expression) were increased, as predicted by the metabolic sequence sketched in Fig. 4. Thus, the failing heart attempts to cope with the increased energy expenditure (59) by turning to ketone use at the expense of fatty acid use (60).

A contributing factor to the CV outcomes of the EMPA-REG OUTCOME trial could be represented by an increased delivery of oxygen to tissues. In fact, SGLT2 inhibitors cause an increase in hematocrit (61), which in the EMPA-REG OUTCOME trial averaged 5% in absolute values, and 11% in percentage points (2). This change likely reflects the hemoconcentration associated with the diuretic effect. However, preliminary results in patients with T2D who were receiving treatment with dapagliflozin (19) have shown that red blood cell mass (as measured by ^{51}Cr-labeled erythrocytes) was expanded by ~6%, a change that was preceded by a transient increase in erythropoietin concentrations and reticulocyte count. Thus, the observed increase in hematocrit might result in part from the stimulation of erythropoiesis, an intriguing possibility that awaits confirmation. Interestingly, the blood volume contraction was not associated with a significant increase in heart rate (2), suggesting that cardiac output was maintained at least at pretreatment levels. A higher hematocrit for the same blood flow is expected to deliver more oxygen to tissues (62). In quantitative terms, experiments in hamsters transfused with packed red blood cells (63) demonstrate that short-term increments in the hematocrit (up to ~10%) induce a 20% rise in oxygen delivery to tissues, coupled with a 10% drop in arterial blood pressure and an increase in cardiac output. In patients (including patients with diabetes) with decompensated heart failure and renal dysfunction, hemoconcentration induced with very high doses of a loop diuretic has been associated with a substantially improved survival time despite the deterioration of renal function (64–67).

Table 1—Comparative mitochondrial energetics of β-hydroxybutyrate oxidation

	Glucose	Palmitate	Pyruvate	β-HB
O_2 used (L/mol)	134	515	56	101
Heat of combustion (kcal/mol)	670	2,385	279	487
O_2 cost of calorie (mL/kcal)	200	216	201	207
Heat of combustion per C_2 (kcal/mol)¶	224	298	186	244

In terms of oxygen (O_2) cost, the energy yield of β-HB oxidation is comparable to those of glucose and pyruvate, and lower than that of palmitate. If the energy yield is calculated per C_2 unit, β-HB oxidation is superior to glucose and far better than palmitate. β-HB, β-hydroxybutyrate. ¶According to Sato et al. (41).

CAVEATS

The metabolic mechanism laid out here is basic, and as such it should apply across organs (foremost, the kidney and the brain) and pathologies (ischemic and nonischemic). However, at present crucial information is missing. Thus, we do not know *1*) the dose-response relationship between raised ketone bodies and cardiac function in humans, *2*) the time course of hyperketonemia during SGLT2 inhibitor treatment, and *3*) the impact of other medications. For instance, the combination of an SGLT2 inhibitor with glucose-lowering agents that stimulate insulin secretion (sulfonylureas, dipeptidyl peptidase-4 inhibitors, and glucagon-like peptide 1 receptor agonists) or with exogenous insulin itself may quench or obliterate the glucagon and/or the ketone response. Furthermore, our hypothesis posits that the combination of improved oxygen use/supply with sodium/volume reduction should work best toward heart failure and organ ischemia (as well as arrhythmias), but in the EMPA-REG OUTCOME trial these end points were affected differentially in size and, possibly, in time course (2). Thus, the incidence of stroke was, if anything, numerically

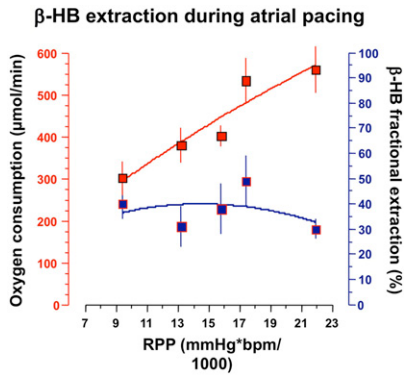

Figure 3—β-hydroxybutyrate (β-HB) extraction by the normal human heart during graded atrial pacing. RPP, rate pressure product. Data were recalculated from the study by Camici et al. (37).

higher in the treatment arm than in the placebo arm (hazard ratio 1.24 [95% CI 0.92–1.67], P = 0.16), although death from stroke was recorded in 0.34% of treated patients versus 0.47% of placebo patients, and transient ischemic attacks were recorded in 0.8% of treated patients versus 1.0% of placebo participants. Given the small number of these latter events, a satisfactory explanation is not at hand, but it is theoretically possible that in vulnerable patients the increased blood viscosity associated with hemoconcentration may play a role. More adjudicated events and/or a longer trial duration and better definition of end points (e.g., type of heart failure), as well as measures of circulating substrates (e.g., β-hydroxybutyrate) and biomarkers (e.g., natriuretic peptides), would be needed to refine the quantitative aspects of any hypothesis. We hope that ongoing CV outcomes trials with other SGLT2 inhibitors (1) will provide additional information on these important issues.

Starting from the evolutionary role of ketosis (26), other biology of ketone bodies (e.g., their effects on cardiac remodeling, oxidative stress, and mitochondrial biogenesis [42–45]) has generated some enthusiasm for their therapeutic exploitation (68–70). Low-carbohydrate ketogenic diets are still widely used, mainly for weight loss, but their long-term impact on CV function is still uncertain and controversial (71). In patients with type 1 diabetes or insulin-treated patients with T2D who are receiving therapy with SGLT2 inhibitors, inappropriate reductions in exogenous insulin administration or intercurrent illness may precipitate episodes of nonhyperglycemic ketoacidosis (72). It is conceivable that the overall physiological impact of ketone bodies follows an inverted U-shaped curve, whereby mild elevations are beneficial, but higher levels may be harmful. According to the present evidence, treatment with SGLT2 inhibitors appears to superimpose intermittent mild hyperketonemia on an otherwise normal daily pattern of fast/feeding, with consistent but limited restriction of carbohydrate usage (24). More drastic changes in circulating ketone levels, carbohydrate availability, and fat composition may be counterproductive. Above all, it should not be forgotten that the cardioprotection of the EMPA-REG OUTCOME trial was seen in a cohort of patients with T2D who were at high CV risk.

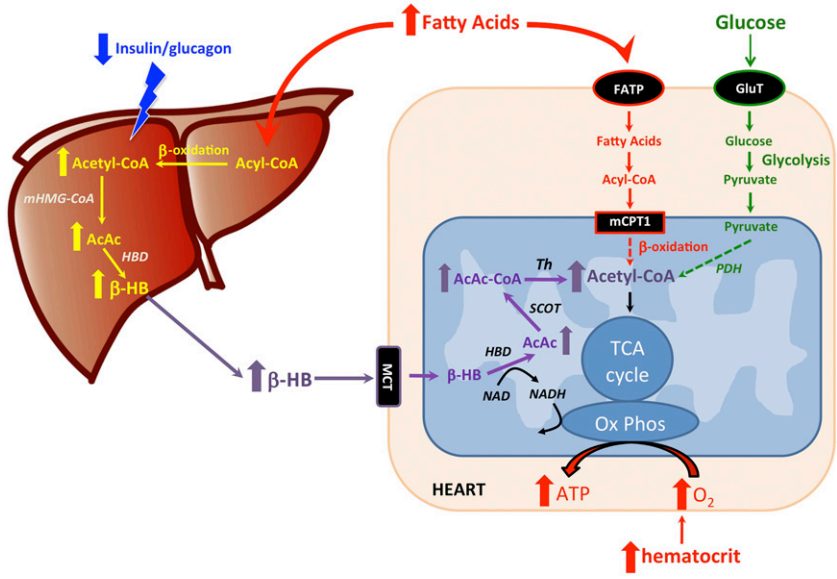

Figure 4—Raised circulating FFAs are taken up by the liver and metabolized via β-oxidation. Under circumstances of reduced insulin/glucagon ratio, in liver mitochondria the acetyl-CoA is condensed to form acetoacetate (AcAc, catalyzed by the mitochondrial isoform of 3-hydroxy-3-methylglutaryl synthase [mHMG-CoA]) and β-hydroxybutyrate (β-HB) (catalyzed by β-hydroxybutyrate dehydrogenase [HBD]). β-HB is then exported into the bloodstream, from which it is avidly taken up into the heart (through the monocarboxylate transporter [MCT]). In heart mitochondria, β-HB is converted to AcAc (by HBD), AcAc-CoA (catalyzed by succinyl-CoA: 3-oxoacid CoA transferase [SCOT], with succinyl-CoA as the CoA donor), and finally acetyl-CoA (by mitochondrial thiolase [Th]), which then enters the TCA for oxidative phosphorylation (Ox Phos). The excess acetyl-CoA restrains (dotted lines) further generation of acetyl-CoA from pyruvate (by inhibiting pyruvate dehydrogenase [PDH]) and from β-oxidation of fatty acids. Ketone body oxidation results in a more efficient oxidation of the mitochondrial coenzyme Q couple and an increase in the free energy of cytosolic ATP hydrolysis. The increase in hematocrit contributes to the improvement in cardiac efficiency by releasing more oxygen (O_2) to the muscle.

FUTURE STUDIES

A viable hypothesis should be not just plausible but verifiable. To this end, the presence, amount, and time course of SGLT2-induced ketonemia could be measured and related to cardiac outcomes in adequately powered clinical studies in patients with T2D. Short-term, low-rate β-hydroxybutyrate infusions could be used in hospital settings to test their effects on metabolic and functional parameters in patients with decompensated heart failure (59–63). The failing heart is insulin resistant (46) (Fig. 5) because of increased adrenergic tone and the release of natriuretic peptides and inflammatory molecules (73). Furthermore, there is evidence that myocardial insulin resistance is an independent risk factor for mortality in stable patients with chronic heart failure (74). Also, myocardial insulin resistance is associated with a mismatch between insulin-mediated glucose uptake and myocardial blood flow, possibly as a result of remodeling (75). However, we do not know whether reducing myocardial insulin resistance would restore contractile function and improve prognosis. Functional studies (three-dimensional

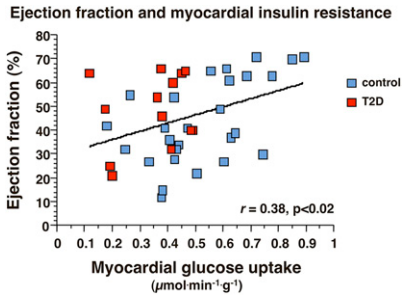

Figure 5—Direct association between insulin-induced myocardial glucose uptake and ejection fraction in control subjects and T2D patients. Redrawn with permission from Iozzo et al. (46).

echocardiography and magnetic resonance spectroscopy) and imaging studies (MRI, positron emission tomography) in patients with diabetes who have a high CV risk load could be performed before and after an SGLT2 treatment course. Finally, if the thrifty substrate paradigm held up in high-risk patients, studies in patients with lower CV risk would generate clinically useful data to inform therapeutic strategies.

SUMMARY

The hypothesis posits that, under conditions of mild but persistent hyperketonemia—such as those that prevail during treatment with SGLT2 inhibitors—β-hydroxybutyrate is freely taken up by the heart and oxidized in preference to fatty acids. This substrate selection improves the transduction of oxygen consumption into work efficiency in the endangered myocardium (and may also improve metabolic status and function of other organs, mainly the kidney). This mechanism should cooperate with other SGLT2-induced changes (reduced blood pressure [10] and enhanced diuresis [11]) to achieve the degree of cardioprotection revealed by the EMPA-REG OUTCOME trial. In addition, enhanced oxygen release to the myocardium through hemoconcentration would be in powerful synergy with the substrate shift.

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Duality of Interest. E.F. has been a speaker and consultant for Merck Sharp & Dohme, Sanofi, Eli Lilly and Company, Boehringer Ingelheim, Johnson & Johnson, and AstraZeneca. M.M. and E.M. are employees of Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. No other potential conflicts of interest relevant to this article were reported.

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