Reinterpreting Cardiorenal Protection of Renal Sodium–Glucose Cotransporter 2 Inhibitors via Cellular Life History Programming

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Cardiovascular outcome trials have provided evidence that sodium–glucose cotransporter 2 inhibitor (SGLT2i) treatment is associated with remarkably favorable cardiovascular outcomes. Here, we offer a novel hypothesis that may encompass many of these hypothetical mechanisms, i.e., the ability of SGLT2i to modify the trajectory of cell response to a toxic environment through modifications of cellular life history programs, either the defense program or the dormancy program. The choice between these programs is mainly determined by the environment. Hyperglycemia can be considered a toxic determinant able to interfere with the basic programs of cell evolution. While the defense program is characterized by activation of the immune response and anabolic metabolism, the dormancy program is an energy-preserving state with high resistance to environmental stressors, and it has strong analogy with animal hibernation where fuel is stored, metabolic rate is suppressed, and insulin secretion is reduced. The metabolic changes that follow treatment with SGLT2i are reminiscent of the metabolic picture characteristic of the dormancy program. Therefore, we hypothesize that the beneficial cardioprotective effects of SGLT2i may be related to their ability to switch cell life programming from a defense to a dormancy state, thus lending additional benefit.

Glucose reabsorption by the renal Na^+-dependent glucose cotransporters is regarded as a mechanism to restrain energy loss. However, when maximum transport (Tm) for glucose exceeds ~380 mg/min, corresponding to a concentration of 180–200 mg/dL, glucose is excreted into the urine. Therefore, glycosuria can be considered a protective mechanism to remove glucose when it reaches concentrations that are toxic for the body. The sodium–glucose cotransporter 2 inhibitors (SGLT2i) act mainly in the kidney where they rapidly inhibit glucose reabsorption by 30–50%. The amount of glucose excreted through the urine is the product of glomerular filtration by the difference between plasma glucose concentration and tubular threshold for glucose reabsorption (1). The latter, as a consequence of SGLT2 inhibition, falls from ~200 mg/dL to less than 40 mg/dL (2). The increased loss of urinary glucose results in a reduction of the glucose pool in the body and of plasma glucose levels. Cardiovascular outcome trials have provided evidence that SGLT2i treatment is associated with remarkably positive cardiovascular outcomes (3–6). Several hypotheses have been offered to explain such beneficial effects: direct renal protection (7), ability to shift energy metabolism (8),

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increased hematocrit (9), improved sodium concentration in cardiomyocytes (10), and many more. Here, we offer a novel hypothesis that may encompass many of these hypothetical mechanisms, i.e., the ability of SGLT2i to modify the trajectory of cell response to a toxic environment though modification of cellular life history programs.

THE CONTEXT

Biological programs can be divided into growth, reproduction, and maintenance. The choice among these programs is mainly determined by the environment. A favorable environment promotes growth and reproduction, whereas a hostile one favors maintenance and survival (11,12). The final scope is to maximize reproduction. Switching toward maintenance and survival programs, as occurs in response to nutrient scarcity, psychosocial stress, and toxins, can threaten health status. As an example of this, low birth weight, as a proxy of in utero nutrient scarcity, not only predicts cardiovascular disease, but also obesity and type 2 diabetes (13). Furthermore, this condition may also reduce cortisol and hypothalamic–pituitary–adrenal axis response to stress in adulthood (14,15). Psychosocial stress itself may profoundly increase cardiovascular risk. In a population-based study with a follow-up of 27 years, exposure to stress was associated with a hazard ratio for any cardiovascular disease of 1.64, with stress-related disorders being more strongly associated with early-onset cardiovascular disease (16). Interestingly, in mouse models, chronic variable stress accelerates atherosclerosis by inducing inflammatory leucocytosis (17).

Stressful conditions are common among people with diabetes, and a strong association between glucose control and stress has been reported (18).

Hyperglycemia, and, consequently, diabetes, can be considered a toxic determinant able to interfere with the basic programs of cell evolution. The consequence of chronic exposure to hyperglycemia is that people affected by diabetes have shorter life expectancy as compared with those without (19,20). Moreover, fertility, as a proxy of reproduction, is reduced in subjects with diabetes. As it has been shown by the Type 1 Diabetes Genetics Consortium (T1DGC), subjects with type 1 diabetes have fewer children than their unaffected siblings (21).

In summary, the diabetic condition is paradigmatic of the response to a hostile metabolic environment with the activation of a maintenance program. The latter can be directed toward the defense program or the dormancy program, the former being characterized by energy consumption and anabolic metabolism, the latter by deferral in nonessential functions, energy conservation, and reliance on catabolic metabolism (22).

THE DEFENSE PROGRAM

The defense program is characterized by activation of the immune response and anabolic metabolism. This program mainly activates intracellular pathways, which mediate cell proliferation of proto-oncogenes and transcription factors such as phosphoinositide 3-kinases (PI3K), mammalian target of rapamycin (mTOR), c-Myc, and mitogen-activated protein kinase (MAPK). The consequence of this activation is (stem) cell proliferation and activation of the immune system.

The clinical implication of switching toward a defense program is threefold. First, an increased level of white blood cells, an independent predictor of incident type 2 diabetes (23,24), as well as acute coronary syndrome, is frequently observed in individuals with obesity (25). Second, in obese subjects, especially those with visceral or ectopic adiposity, there is a condition of subclinical inflammation and immune activation, which is not just the biologic feature of an expanded adipose tissue, but an important process leading to the atherosclerotic lesion (26). In the atherosclerotic plaque, the concerted action of M1-polarized monocytes, T lymphocytes, B lymphocytes, mast cells, and dendritic cells supports inflammation, which is implicated in the pathogenesis of acute coronary syndromes. In the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS), the use of canakinumab, a monoclonal antibody against interleukin-1β, decreased the rate of recurrent cardiovascular events by 15% in patients with previous myocardial infarction and elevated levels of C-reactive protein (27). Similarly, reduction of circulating levels of C-reactive protein with high-intensity statin decreased the incidence of major cardiovascular events by 44% (28). Third, activation of the immune system after an acute myocardial infarction triggers an increased inflammatory response, which further enhances the risk of a recurrent event, an effect mediated by stem cell activation and propagation of inflammatory leukocytes to the atherosclerotic plaques (29).

Activation of the immune system is an exquisite anabolic process. For example, in activated T cells, the PI3K–protein kinase B (Akt) axis promotes plasma membrane translocation of glucose transporter 1 (GLUT1) (30) and increases the activity of the glycolytic enzyme hexokinase so that glucose can fuel several intracellular metabolic pathways (31,32).

In summary, in the context of the defense program, glucose utilization in leukocytes is tightly linked to the systemic proinflammatory response. In addition, increased glycolysis in M1-polarized macrophages within the atherosclerotic plaque allows amplification of the inflammatory response (33).

THE DORMANCY PROGRAM

The dormancy program is an energy-preserving state with high resistance to environmental stressors. It has strong analogy with the situation of animal hibernation where fuel is stored, metabolic rate is suppressed, and insulin secretion is reduced (34). In humans, a switch toward the dormancy program occurs in the myocardium in response to chronic ischemia, where a persistently impaired function of viable cardiac muscle cells is observed in response to chronic starvation due to reduction of coronary blood flow (35). At variance with the defense mechanism, catabolism and stem cell quiescence prevail and the immune system is suppressed. Several pathways mediate this condition, the principal one being the activation of fatty acid oxidation with increased production of ketone bodies, increase of circulating levels of fibroblast growth factor (FGF) 21, and activation of Forkhead box protein (FOXO) and 5′ AMP-activated protein kinase (AMPK) (11).

A key element in the dormancy program is the role of the transcription factor and tumor-suppressor gene p53,

which not only promotes cell cycle arrest, senescence, or apoptosis, but also plays a significant role in the development of metabolic diseases. For instance, in diet-induced insulin resistance, p53 represses the transcription of GLUT1 and GLUT4, downregulates glycolysis, and promotes gluconeogenesis (36). The activity of p53 is strictly related to autophagy, an evolutionarily conserved lysosomal degradation process, linked to the dormancy program. While autophagy may promote cancer by repressing p53, p53, in turn, activates autophagy-related genes, thus leading to neoplastic cell apoptosis (37).

Although there is still no evidence that SGLT2i can promote the dormancy program, we wish to note that achievement of such effect with antidiabetic therapy is feasible. Peroxisome proliferator–activated receptor (PPAR)-γ agonists used in the treatment of type 2 diabetes recapitulate some features of the dormancy program. Glitazones can stimulate lipid synthesis, counteract inflammation, favor adipose tissue browning, and improve insulin sensitivity. Moreover, p53 is a cofactor of the PPAR-γ coactivator-1 alpha (PGC-1α) in regulating cell cycle arrest, while PPAR-α itself can induce autophagy and apoptosis, thus protecting against immune activation in atherosclerosis (38). PPAR-α has been shown to be an important transcriptional activator of FGF21 expression, a potent regulator with physiological effects on glucose and lipid metabolism that also exerts important antiatherosclerotic and anti-inflammatory effects (39). Circulating levels of FGF21 are elevated in patients with obesity and type 2 diabetes, and FGF21 mRNA expression is twofold higher in the visceral fat of obese individuals as compared with healthy subjects (40). Nonetheless, FGF21 can exert beneficial effects, including weight loss and improved insulin sensitivity, in subjects with obesity or type 2 diabetes (41). Though Eriksson et al. (42) found that dapagliflozin reduced the levels of FGF21 in patients with type 2 diabetes and nonalcoholic fatty liver disease, other studies clearly show that SGLT2i increase both hepatic and plasma FGF21 (43,44). However, it should be emphasized that these last two reports were obtained in mice; therefore, their results should be interpreted with caution, in the light of species-related differences.

Interestingly, the response of FGF21 to SGLT2i appears significantly related to BMI (45).

These findings as well as those from the study by Eriksson et al. (42) performed in humans indicate a decrease rather than an increase in FGF21 levels. Furthermore, the rise in body weight over time was accompanied by an FGF21 elevation, implying that an increase in FGF21 levels may not be beneficial in general.

As mentioned, all responses activated by switching cell programming toward a dormancy program are typically occurring in hibernating animals. Under this condition, ketone bodies become key metabolic substrates favoring glucose sparing. Therefore, in the context of fuel selection, one of the most relevant adaptive mechanism in the dormancy program is the rise of 3-hydroxybutyrate (46).

SGLT2i AND THE DORMANCY PROGRAM

If we consider the metabolic changes that follow treatment with SGLT2i, these are much reminiscent of the metabolic picture characteristic of the dormancy program. Therefore, we hypothesize that the beneficial cardioprotective effects of SGLT2i may be related to their ability to switch cell life programs from defense, as triggered by the metabolically toxic environment (i.e., diabetic hyperglycemia), to dormancy, thus lending additional benefit.

SGLT2i, AMPK, and mTOR

Type 2 diabetes and obesity are conditions characterized by chronic exposure to high-energy substrates and high plasma insulin conditions, disrupting the physiologic diurnal cycling between catabolic and anabolic phases. A corollary condition of this loss of energy fluctuations is the development of metabolic inflexibility (47,48). As a consequence, amino acid concentrations are consistently augmented, resulting in stimulation of mTOR, a central regulator of cell proliferation and of the defense program through regulation of GATOR1 and 2 complexes (49). mTOR also plays a crucial role in innate as well as adaptive immune responses (50). Moreover, mTOR signaling can be activated by a variety of immunological stimuli, including signaling through the Toll-like receptor family of receptors (51). The role of mTOR is also central in macrophage polarization to the proinflammatory M1 state, thus potentiating inflammation (52). As mentioned, the dormancy program foresees activation of several pathways including AMPK (11). Osataphan et al. (44) showed that the SGLT2i canagliflozin increased AMPK activity in obese mice independently of insulin or glucagon sensitivity or signaling. This SGLT2i-mediated activation of AMPK has been confirmed at the cellular level in mesangial (53) and myocardial cells (54). At variance with AMPK, activation of mTOR is essential in the defense mechanism and in mediating the immune response (55). In the work of Osataphan et al., canagliflozin significantly repressed mTOR. In another study, empagliflozin was shown to increase autophagic activities in renal tubular cells under high glucose environment with concomitant mTOR inhibition (56). No data are so far available on the effect of SGLT2i on FOXO.

Globally, these studies showed that the SGLT2i can cause modifications in different pathways involved in the switching of cellular reprogramming from defense to dormancy.

SGLT2i and Inflammation

Data obtained in rodent models (43,57–59) and in humans (60,61) consistently indicate that SGLT2i blunt inflammation with a specific protective effect exerted in the kidney and the liver (62). This effect has been shown also in humans, where canagliflozin was able to reverse molecular processes related to inflammation by decreasing interleukin-1, interleukin-6, and tumor necrosis factor 1 receptor (63). In line with these effects, there is mounting evidence showing that SGLT2i can improve vascular stiffness (64,65) and endothelial function (66) and reduce microvascular injury (67), possibly as a result of their anti-inflammatory properties (68). SGLT2i have the ability to modify macrophage polarization from proinflammatory M1 to anti-inflammatory M2 and to stimulate whole-body energy expenditure via activation of AMPK and increased expression of the uncoupling protein 1 in brown and white adipose tissue (43,69). This is reminiscent of the decrease of the number of leukocytes occurring in European ground squirrels during hibernation (70). In conclusion,

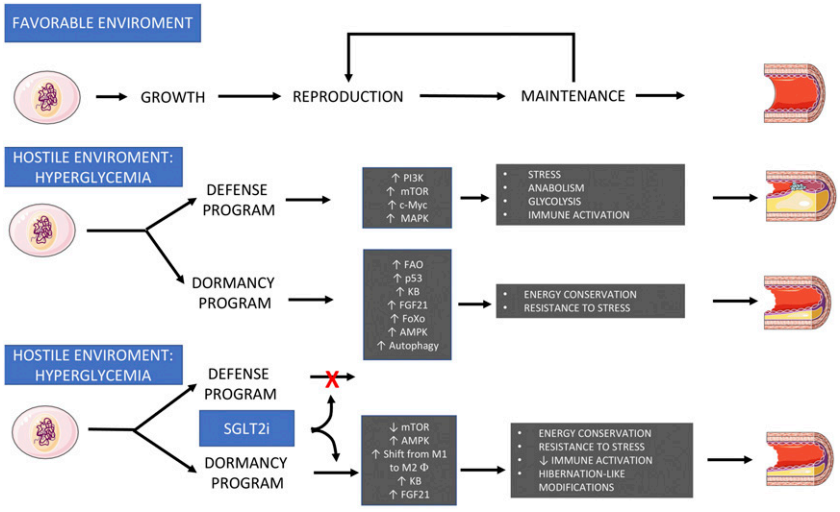

Figure 1—The mechanisms underlying the potential effects of SGLT2i in determining their protection via cellular life history reprogramming. KB, ketone bodies; M1Φ, classically activated macrophages; M2Φ, alternatively activated macrophages.

there is ample evidence that SGLT2i have the ability to decrease the cellular inflammatory responses; however, we acknowledge that the robust data on the potential anti-inflammatory actions of these agents in the human are yet to be obtained.

SGLT2i, Lipid Oxidation, and Ketones

A feature of the dormancy program is the activation of a catabolic state. SGLT2i therapy is associated with an increase in ketone body concentrations (71,72) that occurs, at least according to some reports, mainly under conditions of impaired glucose regulation, i.e., a toxic environment. Stimulation of fatty acid oxidation is due to a switch in energy metabolism from carbohydrate to lipid sources (73). The glucose-lowering effect of SGLT2i results in a reduction of the circulating plasma insulin concentration paralleled by an increase of glucagon secretion. This metabolic adaptation becomes more apparent during interprandial and nocturnal fasting, resulting in the improvement, if not restoration, of the circadian alternation of anabolic (postprandial) and catabolic condition, recovery of metabolic flexibility, and liver–brain–adipose neurocircuitry (74).

The increased availability of ketone bodies has been suggested as a main mechanism of cellular protection, due to a highly favorable ratio between oxygen consumption and energy production (75). As mentioned previously, ketone bodies are key in the metabolic reprogramming during hibernation and play a fundamental role in the organ-specific switches to tissue-protective, dormant metabolic states (46). During hibernation, metabolic fluxes limit carbohydrate oxidation and increase free fatty acid availability and oxidation. The latter is more efficient as, on a per-gram basis, it provides more than twice as much energy as carbohydrate oxidation (76). These metabolic differences are similar to the ones promoted by SGLT2i. Ketone bodies may also link SGLT2i to their potential anti-inflammatory action. High ketone body levels, as occurs for diabetic ketoacidosis, can trigger a proinflammatory condition, whereas at levels commonly reached during SGLT2i treatment, they can repress inflammation (77,78).

Obata et al. (79) have shown that the SGLT2i tofogliflozin increased the phosphorylation of hormone-sensitive lipase and adipose triglyceride lipase protein levels in white adipose tissue, with concomitant increase of the expressions of genes related to β-oxidation, such as CPT1α, in the liver. It is not readily intuitive to reconcile the increased lipid oxidation triggered by SGLT2i and their positive cardiovascular action, as lipid oxidation has been regarded as a main source of oxidative stress paralleled by increased atherogenic oxidized lipoproteins (80,81). However, the increase in lipid oxidation accompanying SGLT2i therapy is similar to that observed with low-intensity endurance exercise or daily physical exercise, which facilitate weight loss, increase lean body mass, and ameliorate all traits of the metabolic syndrome (82,83).

On a more chronic basis, the increased fat oxidation associated with SGLT2i therapy may prevent the impaired oxidation of these substrates occurring with aging (84).

Finally, increased lipid oxidation is the main source of the energy necessary for M2 macrophage polarization that confers anti-inflammatory properties. On the other hand, inhibition of fatty acid oxidation impairs polarization to M2 and, consequently, reduces their anti-inflammatory response (85).

In summary, SGLT2i can offset the toxic insult of hyperglycemia in a direct manner by increasing elimination of glucose excess through the urine and in an indirect manner by modifying the metabolic milieu of the cell, switching from a negative, defense program to a positive, dormancy one. Then, this set of actions can play a positive effect in the biology of the cellular phenotype of the arterial wall, thus preventing atherosclerotic process (86).

SGLT2i, Hormones, and Cellular Programs

The role of hormones is of paramount importance for the response to a toxic environment and hence for the maintenance of cellular homeostasis. Hormones play a fundamental role in growth and reproduction, with the GH–IGF axis and sex hormones promoting anabolism and maintenance and glucocorticoids being the main activators of catabolism (11).

Therapy with SGLT2i is associated with increased plasma glucagon concentrations, which, at the organismal level, promote maintenance and dormancy. Of interest, the dormancy program activated at the time of hibernation of the golden-mantled ground squirrel is characterized by a reduction in the circulating levels of insulin, pancreatic polypeptide, and somatostatin but not of glucagon, suggesting an important role for this hormone in the dormancy/hibernation programs (87). Similar findings have been observed in the seasonal adaptation of the little brown bat (88). Unfortunately, no data are available on direct effects of SGLT2i on GH–IGF axis, sex hormones, and glucocorticoids. However, it was shown that canagliflozin

can attenuate proinflammatory gene expression in the hypothalamus, an integration sensor of environmental quality and glucose metabolism (89,90).

CONCLUSIONS

We have screened the available literature to identify arguments supporting the hypothesis that, in the presence of pathologic elevation of plasma glucose levels, glycosuria develops as the clinical expression of glucose concentrations above the renal threshold, and, teleologically, as a way to remove cellular (glucose) toxins. SGLT2i increases glucosuria by lowering the renal glucose threshold: this effect positively modifies both hemodynamic and metabolic milieu, through which cardiovascular protection is conferred.

Based on experimental data, largely obtained in animal models, we now offer the hypothesis that these effects are, at least in part, the result of cellular life history programming induced by SGLT2i, i.e., deviation of cellular life trajectory in response to the toxic environment, to a dormancy rather than a defense program (Fig. 1). Although we acknowledge that most of the data presented herein are largely derived from animal studies, these modifications remain highly reminiscent of those occurring in hibernating animals as well as those in the hibernation-like state observed in chronically ischemic myocardium and in the brain after stroke (91), all paradigmatic conditions of a dormancy program.

A consistently emerging effect of SGLT2i is represented by kidney protection, which is believed to be accounted for largely by restoration of the tubular-glomerular feedback and constriction of the afferent arteriole, with reduction of intraglomerular pressure. Of interest, in hibernating animals, even in the presence of perfectly preserved glomerular structure, a significant reduction in renal blood flow and glomerular filtration rate develops (92). Finally, in keeping with our hypothesis also is the potential oxygen sparing effect (93).

An important point is whether this purported effect of SGLT2i on cellular reprogramming may occur with other non–insulin dependent reductions in plasma glucose concentration such as exercise or very-low calorie diet. Low-intensity endurance exercise or daily physical exercise may induce cellular reprogramming by increasing lipid oxidation (82). Very-low calorie diet reduces the proinflammatory state, i.e., defense program, in right ventricular function in healthy men (94) and overall in obese subjects (95). Recently, Jordan et al. (96) have shown that caloric restriction not only activates AMPK and PPAR-α but also reduces monocyte polarization, a paramount condition of switching from a defense to a dormancy program. However, the effects on cellular reprogramming are probably not only related to the scarcity of caloric intake, per se, but also to the nutritional composition of the diet (97).

In conclusion, we notice a remarkable resemblance of the metabolic changes occurring in subjects with diabetes in response to treatment with SGLT2i and conditions dictated by activation of the cell dormancy program and speculate it is through such a switch that SGLT2i may provide cardiorenal protection. Dedicated studies will be needed to test this hypothesis experimentally and gather further support from human studies.

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References

1. Ferrannini E. Sodium-glucose co-transporters and their inhibition: clinical physiology. Cell Metab 2017;26:27–38
2. DeFronzo RA, Norton L, Abdul-Ghani M. Renal, metabolic and cardiovascular considerations of SGLT2 inhibition. Nat Rev Nephrol 2017;13:11–26
3. Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2016;374:1094
4. Neal B, Perkovic V, Mahaffey KW, et al.; CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 2017;377:644–657
5. Wiviott SD, Raz I, Bonaca MP, et al.; DECLARE–TIMI 58 Investigators. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2019;380:347–357
6. Perkovic V, Jardine MJ, Neal B, et al.; CREDENCE Trial Investigators. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med 2019;380:2295–2306
7. Vettor R, Inzucchi SE, Fioretto P. The cardiovascular benefits of empagliflozin: SGLT2-dependent and -independent effects. Diabetologia 2017;60:395–398
8. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care 2016;39:1115–1122
9. Inzucchi SE, Zinman B, Fitchett D, et al. How does empagliflozin reduce cardiovascular mortality? Insights from a mediation analysis of the EMPA-REG OUTCOME trial. Diabetes Care 2018;41:356–363
10. Baartscheer A, Schumacher CA, Wüst RC, et al. Empagliflozin decreases myocardial cytoplasmic Na^+ through inhibition of the cardiac Na^+/H^+ exchanger in rats and rabbits. Diabetologia 2017;60:568–573
11. Wang A, Luan HH, Medzhitov R. An evolutionary perspective on immunometabolism. Science 2019;363:363
12. Lee YS, Wollam J, Olefsky JM. An integrated view of immunometabolism. Cell 2018;172:22–40
13. Kajantie E, Osmond C, Barker DJ, Forsén T, Phillips DI, Eriksson JG. Size at birth as a predictor of mortality in adulthood: a follow-up of 350 000 person-years. Int J Epidemiol 2005;34:655–663
14. Kajantie E, Feldt K, Räikkönen K, et al. Body size at birth predicts hypothalamic-pituitary-adrenal axis response to psychosocial stress at age 60 to 70 years. J Clin Endocrinol Metab 2007;92:4094–4100
15. Phillips DI, Barker DJ, Fall CH, et al. Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? J Clin Endocrinol Metab 1998;83:757–760
16. Song H, Fang F, Arnberg FK, et al. Stress related disorders and risk of cardiovascular disease: population based, sibling controlled cohort study. BMJ 2019;365:l1255
17. Heidt T, Sager HB, Courties G, et al. Chronic variable stress activates hematopoietic stem cells. Nat Med 2014;20:754–758
18. Hilliard ME, Yi-Frazier JP, Hessler D, Butler AM, Anderson BJ, Jaser S. Stress and A1c among people with diabetes across the lifespan. Curr Diab Rep 2016;16:67
19. Livingstone SJ, Levin D, Looker HC, et al.; Scottish Diabetes Research Network epidemiology group; Scottish Renal Registry. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. JAMA 2015;313:37–44
20. Grover SA, Kaouache M, Rempel P, et al. Years of life lost and healthy life-years lost from diabetes and cardiovascular disease in overweight and obese people: a modelling study. Lancet Diabetes Endocrinol 2015;3:114–122
21. Wiebe JC, Santana A, Medina-Rodríguez N, et al.; T1DGC. Fertility is reduced in women and in men with type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium (T1DGC). Diabetologia 2014;57:2501–2504
22. Kültz D. Molecular and evolutionary basis of the cellular stress response. Annu Rev Physiol 2005;67:225–257
23. Lorenzo C, Hanley AJ, Haffner SM. Differential white cell count and incident type 2 diabetes: the Insulin Resistance Atherosclerosis Study. Diabetologia 2014;57:83–92
24. Fadini GP, Marcuzzo G, Marescotti MC, de Kreutzenberg SV, Avogaro A. Elevated white blood cell count is associated with prevalence and development of the metabolic syndrome and

its components in the general population. Acta Diabetol 2012;49:445–451
25. Sabatine MS, Morrow DA, Cannon CP, et al. Relationship between baseline white blood cell count and degree of coronary artery disease and mortality in patients with acute coronary syndromes: a TACTICS-TIMI 18 (Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis in Myocardial Infarction 18 trial) substudy. J Am Coll Cardiol 2002;40:1761–1768
26. Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol 2012;32:2045–2051
27. Ridker PM, Everett BM, Thuren T, et al.; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377:1119–1131
28. Ridker PM, Danielson E, Fonseca FA, et al.; JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195–2207
29. Dutta P, Courties G, Wei Y, et al. Myocardial infarction accelerates atherosclerosis. Nature 2012;487:325–329
30. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol Biol Cell 2007;18:1437–1446
31. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005;5:844–852
32. Tawakol A, Singh P, Mojena M, et al. HIF-1α and PFKFB3 mediate a tight relationship between proinflammatory activation and anerobic metabolism in atherosclerotic macrophages. Arterioscler Thromb Vasc Biol 2015;35:1463–1471
33. Bories GFP, Leitinger N. Macrophage metabolism in atherosclerosis. FEBS Lett 2017;591:3042–3060
34. Campbell MA. Dormancy and the cell cycle. Genet Eng (N Y) 2006;27:21–33
35. Wijns W, Vatner SF, Camici PG. Hibernating myocardium. N Engl J Med 1998;339:173–181
36. Kung CP, Murphy ME. The role of the p53 tumor suppressor in metabolism and diabetes. J Endocrinol 2016;231:R61–R75
37. White E. Autophagy and p53. Cold Spring Harb Perspect Med 2016;6:a026120
38. Chinetti G, Griglio S, Antonucci M, et al. Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998;273:25573–25580
39. Kokkinos J, Tang S, Rye KA, Ong KL. The role of fibroblast growth factor 21 in atherosclerosis. Atherosclerosis 2017;257:259–265
40. Xie T, Leung PS. Fibroblast growth factor 21: a regulator of metabolic disease and health span. Am J Physiol Endocrinol Metab 2017;313:E292–E302
41. Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring HU. Fibroblast growth factor 21-metabolic role in mice and men. Endocr Rev 2017;38:468–488
42. Eriksson JW, Lundkvist P, Jansson PA, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. Diabetologia 2018;61:1923–1934
43. Xu L, Nagata N, Nagashimada M, et al. SGLT2 inhibition by empagliflozin promotes fat utilization and browning and attenuates inflammation and insulin resistance by polarizing M2 macrophages in diet-induced obese mice. EBioMedicine 2017;20:137–149
44. Osataphan S, Macchi C, Singhal G, et al. SGLT2 inhibition reprograms systemic metabolism via FGF21-dependent and -independent mechanisms. JCI Insight 2019;4:4
45. Kosugi R, Nakatani E, Okamoto K, Aoshima S, Arai H, Inoue T. Effects of sodium-glucose cotransporter 2 inhibitor (dapagliflozin) on food intake and plasma fibroblast growth factor 21 levels in type 2 diabetes patients. Endocr J 2019;66:677–682
46. Krilowicz BL. Ketone body metabolism in a ground squirrel during hibernation and fasting. Am J Physiol 1985;249:R462–R470
47. Apostolopoulou M, Strassburger K, Herder C, et al.; GDS group. Metabolic flexibility and oxidative capacity independently associate with insulin sensitivity in individuals with newly diagnosed type 2 diabetes. Diabetologia 2016;59:2203–2207
48. Esterline RL, Vaag A, Oscarsson J, Vora J. MECHANISMS IN ENDOCRINOLOGY: SGLT2 inhibitors: clinical benefits by restoration of normal diurnal metabolism? Eur J Endocrinol 2018;178:R113–R125
49. Zhu J, Thompson CB. Metabolic regulation of cell growth and proliferation. Nat Rev Mol Cell Biol 2019;20:436–450
50. Jones RG, Pearce EJ. MenTORing immunity: mTOR signaling in the development and function of tissue-resident immune cells. Immunity 2017;46:730–742
51. Snyder JP, Amiel E. Regulation of dendritic cell immune function and metabolism by cellular nutrient sensor mammalian target of rapamycin (mTOR). Front Immunol 2019;9:3145
52. Duval C, Chinetti G, Trottein F, Fruchart JC, Staels B. The role of PPARs in atherosclerosis. Trends Mol Med 2002;8:422–430
53. Inoue MK, Matsunaga Y, Nakatsu Y, et al. Possible involvement of normalized Pin1 expression level and AMPK activation in the molecular mechanisms underlying renal protective effects of SGLT2 inhibitors in mice. Diabetol Metab Syndr 2019;11:57
54. Sayour AA, Korkmaz-Icöz S, Loganathan S, et al. Acute canagliflozin treatment protects against in vivo myocardial ischemia-reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation. J Transl Med 2019;17:127
55. Weichhart T, Hengstschläger M, Linke M. Regulation of innate immune cell function by mTOR. Nat Rev Immunol 2015;15:599–614
56. Lee YH, Kim SH, Kang JM, et al. Empagliflozin attenuates diabetic tubulopathy by improving mitochondrial fragmentation and autophagy. Am J Physiol Renal Physiol 2019;317:F767–F780
57. Tahara A, Kurosaki E, Yokono M, et al. Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice. Eur J Pharmacol 2013;715:246–255
58. Han JH, Oh TJ, Lee G, et al. The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE $^{-/-}$ mice fed a western diet. Diabetologia 2017;60:364–376
59. Birnbaum Y, Bajaj M, Yang HC, Ye Y. Combined SGLT2 and DPP4 inhibition reduces the activation of the Nlrp3/ASC inflammasome and attenuates the development of diabetic nephropathy in mice with type 2 diabetes. Cardiovasc Drugs Ther 2018;32:135–145
60. Scheen AJ, Esser N, Paquot N. Antidiabetic agents: Potential anti-inflammatory activity beyond glucose control. Diabetes Metab 2015;41:183–194
61. Prattichizzo F, De Nigris V, Micheloni S, La Sala L, Ceriello A. Increases in circulating levels of ketone bodies and cardiovascular protection with SGLT2 inhibitors: Is low-grade inflammation the neglected component? Diabetes Obes Metab 2018;20:2515–2522
62. Wang D, Luo Y, Wang X, et al. The sodium-glucose cotransporter 2 inhibitor dapagliflozin prevents renal and liver disease in Western diet induced obesity mice. Int J Mol Sci 2018;19:137
63. Heerspink HJL, Perco P, Mulder S, et al. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. Diabetologia 2019;62:1154–1166
64. Solini A, Giannini L, Seghieri M, et al. Dapagliflozin acutely improves endothelial dysfunction, reduces aortic stiffness and renal resistive index in type 2 diabetic patients: a pilot study. Cardiovasc Diabetol 2017;16:138
65. Lee DM, Battson ML, Jarrell DK, et al. SGLT2 inhibition via dapagliflozin improves generalized vascular dysfunction and alters the gut microbiota in type 2 diabetic mice. Cardiovasc Diabetol 2018;17:62
66. Shigiyama F, Kumashiro N, Miyagi M, et al. Effectiveness of dapagliflozin on vascular endothelial function and glycemic control in patients with early-stage type 2 diabetes mellitus: DEFENCE study. Cardiovasc Diabetol 2017;16:84
67. Zhou H, Wang S, Zhu P, Hu S, Chen Y, Ren J. Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. Redox Biol 2018;15:335–346
68. Bonnet F, Scheen AJ. Effects of SGLT2 inhibitors on systemic and tissue low-grade inflammation: the potential contribution to diabetes complications and cardiovascular disease. Diabetes Metab 2018;44:457–464
69. Miyachi Y, Tsuchiya K, Shiba K, et al. A reduced M1-like/M2-like ratio of macrophages in healthy adipose tissue expansion during SGLT2 inhibition. Sci Rep 2018;8:16113
70. Bouma HR, Strijkstra AM, Boerema AS, et al. Blood cell dynamics during hibernation in the European Ground Squirrel. Vet Immunol Immunopathol 2010;136:319–323
71. Taylor SI, Blau JE, Rother KI. SGLT2 inhibitors may predispose to ketoacidosis. J Clin Endocrinol Metab 2015;100:2849–2852
72. Kuhadiya ND, Ghanim H, Mehta A, et al. Dapagliflozin as additional treatment to liraglutide and insulin in patients with type 1 diabetes. J Clin Endocrinol Metab 2016;101:3506–3515
73. McPherson PA, McEneny J. The biochemistry of ketogenesis and its role in weight management, neurological disease and oxidative stress. J Physiol Biochem 2012;68:141–151
74. Sawada Y, Izumida Y, Takeuchi Y, et al. Effect of sodium-glucose cotransporter 2 (SGLT2)

inhibition on weight loss is partly mediated by liver-brain-adipose neurocircuitry. Biochem Biophys Res Commun 2017;493:40–45
75. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. Prostaglandins Leukot Essent Fatty Acids 2004;70:309–319
76. Williams CT, Barnes BM, Buck CL. Integrating physiology, behavior, and energetics: biologging in a free-living arctic hibernator. Comp Biochem Physiol A Mol Integr Physiol 2016;202:53–62
77. Youm YH, Nguyen KY, Grant RW, et al. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med 2015;21:263–269
78. Gambhir D, Ananth S, Veeranan-Karmegam R, et al. GPR109A as an anti-inflammatory receptor in retinal pigment epithelial cells and its relevance to diabetic retinopathy. Invest Ophthalmol Vis Sci 2012;53:2208–2217
79. Obata A, Kubota N, Kubota T, et al. Tofogliflozin improves insulin resistance in skeletal muscle and accelerates lipolysis in adipose tissue in male mice. Endocrinology 2016;157:1029–1042
80. Cominacini L, Pasini AF, Garbin U, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species. J Biol Chem 2000;275:12633–12638
81. Navab M, Ananthramaiah GM, Reddy ST, et al. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J Lipid Res 2004;45:993–1007
82. Dumortier M, Brandou F, Perez-Martin A, Fedou C, Mercier J, Brun JF. Low intensity endurance exercise targeted for lipid oxidation improves body composition and insulin sensitivity in patients with the metabolic syndrome. Diabetes Metab 2003;29:509–518
83. Trenell MI, Hollingsworth KG, Lim EL, Taylor R. Increased daily walking improves lipid oxidation without changes in mitochondrial function in type 2 diabetes. Diabetes Care 2008;31:1644–1649
84. Toth MJ, Tchernof A. Lipid metabolism in the elderly. Eur J Clin Nutr 2000;54(Suppl. 3):S121–S125
85. Huang SC, Everts B, Ivanova Y, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol 2014;15:846–855
86. Malandrino MI, Fucho R, Weber M, et al. Enhanced fatty acid oxidation in adipocytes and macrophages reduces lipid-induced triglyceride accumulation and inflammation. Am J Physiol Endocrinol Metab 2015;308:E756–E769
87. Bauman WA, Meryn S, Florant GL. Pancreatic hormones in the nonhibernating and hibernating golden mantled ground squirrel. Comp Biochem Physiol A Comp Physiol 1987;86:241–244
88. Bauman WA. Seasonal changes in pancreatic insulin and glucagon in the little brown bat (Myotis lucifugus). Pancreas 1990;5:342–346
89. Naznin F, Sakoda H, Okada T, et al. Canagliflozin, a sodium glucose cotransporter 2 inhibitor, attenuates obesity-induced inflammation in the nodose ganglion, hypothalamus, and skeletal muscle of mice. Eur J Pharmacol 2017;794:37–44
90. Ross R, Wang PY, Chari M, et al. Hypothalamic protein kinase C regulates glucose production. Diabetes 2008;57:2061–2065
91. Forreider B, Pozivilko D, Kawaji Q, Geng X, Ding Y. Hibernation-like neuroprotection in stroke by attenuating brain metabolic dysfunction. Prog Neurobiol 2017;157:174–187
92. Zancanaro C, Malatesta M, Mannello F, Vogel P, Fakan S. The kidney during hibernation and arousal from hibernation. A natural model of organ preservation during cold ischaemia and reperfusion. Nephrol Dial Transplant 1999;14:1982–1990
93. Suh HN, Lee YJ, Kim MO, Ryu JM, Han HJ. Glucosamine-induced Sp1 O-GlcNAcylation ameliorates hypoxia-induced SGLT dysfunction in primary cultured renal proximal tubule cells. J Cell Physiol 2014;229:1557–1568
94. Widya RL, Hammer S, Boon MR, et al. Effects of short-term nutritional interventions on right ventricular function in healthy men. PLoS One 2013;8:e76406
95. Hagen I, Schulte DM, Müller N, et al. Soluble receptor for advanced glycation end products as a potential biomarker to predict weight loss and improvement of insulin sensitivity by a very low calorie diet of obese human subjects. Cytokine 2015;73:265–269
96. Jordan S, Tung N, Casanova-Acebes M, et al. Dietary intake regulates the circulating inflammatory monocyte pool. Cell 2019;178:1102–1114.e17
97. Lyons CL, Roche HM. Nutritional modulation of AMPK-impact upon metabolic-inflammation. Int J Mol Sci 2018;19:3092