To provide an understanding of both the preclinical and clinical aspects of closed-loop artificial pancreas systems, we provide a discussion of this topic as part of this two-part Bench to Clinic narrative. Here, the Bench narrative provides an in-depth understanding of insulin-glucose-glucagon physiology in conditions that mimic the free-living situation to the extent possible in type 1 diabetes that will help refine and improve future closed-loop system algorithms. In the Clinic narrative, Doyle and colleagues compare and evaluate technology used in current closed-loop studies to gain further momentum toward outpatient trials and eventual approval for widespread use.

The concept of a system that responds automatically to changing blood glucose concentrations by modulating insulin delivery in patients with type 1 diabetes (T1D) was born decades ago as the pioneering but cumbersome Biostator (1) and involved intravenous routes for both glucose sensing and insulin delivery. Ideally, such a system performs without human interventions operating as a “closed” process; however, attempts at “closing the loop” with Biostator were suboptimal in normalizing postprandial glucose excursions despite modulation of prandial rates of insulin delivery. For the system to be truly closed, technologic, algorithmic, and physiologic limitations must be overcome. While the Biostator had the ideal intravascular route for closing the loop, the lack of portability of the entire system precluded widespread use in patients with T1D in the free-living situation.

Over the past 15 years, the concept of closed-loop control (CLC) has been re-kindled, thanks to significant advances in technological and algorithmic capabilities, renewed interest by funding agencies (both governmental and nongovernmental), and regulatory bodies, including the U.S. Food and Drug Administration, replacing lengthy preclinical animal studies with in silico (computer-based) simulations and “simulators” (2,3) to expedite the approval processes with integrated efforts of all parties involved. Consequently, several investigative teams worldwide have successfully conducted and published outpatient and inpatient clinical trials (4–6) that have demonstrated safety and efficacy in relatively short-term CLC using the subcutaneous route for both glucose sensing and insulin delivery in adults and children with T1D. While the subcutaneous compartment for glucose sensing and insulin delivery is nonphysiological and suboptimal involving definitive time delays at both ends, the practicality, usability, and simplicity of this route makes current efforts and realization of CLC promising to a potentially large segment of the T1D patient population.

Refinements in algorithms (proportional integral derivative, model predictive control, fuzzy logic), platforms (artificial pancreas system, Diabetes Assistant), continuous glucose monitors (CGMs), and insulin pumps, along with continuing
miniatuization and portability of these devices, have enhanced the practicality and usability of contemporary CLC systems by adults and adolescents with T1D (7). Furthermore, recent studies (8) incorporating both insulin and glucagon infusions have extended the entire concept from an artificial β-cell closer to an artificial endocrine pancreas system.

Formidable challenges remain before such a system can be safely used in free-living situations in T1D. These include adaptation (with possible individualization) of algorithms based on changes in physiological parameters (e.g., insulin sensitivity [SI], glucagon sensitivity) related to multiple natural perturbations induced by, among others, physical activity (varying types and intensities), meals (varying sizes and composition), intercurrent illness, dawn phenomenon, circadian variability, and biological factors (puberty, menstrual cycles, pregnancy, menopause) (Table 1). The logical way forward therefore would be to systematically determine the effect sizes of these relevant scenarios to perturbations on postprandial SI has been challenging primarily due to methodological limitations. A better assessment of the effect size of these perturbations on SI could then be incorporated into existing control algorithms and tested for safety and efficacy in next-generation artificial pancreas systems. A challenge with integrating SI into control algorithms is variability in SI during the day, as well as within and between individuals. Using a triple tracer technique (14), the existence of a diurnal pattern of SI in T1D that differs from healthy non-diabetic subjects (15) has recently been reported (16). Although these studies underscored a threefold greater intra-individual variability of SI in T1D compared with anthropometrically matched control subjects despite identical experimental conditions, careful analyses of SI diurnal patterns suggest that approximately half of T1D subjects demonstrated rising SI as the day progressed. Unchanging SI or falling SI comprised the remaining half in roughly equal proportions. Such variability of diurnal SI is currently being tested in the University of Virginia-Padova simulator (2), which has been accepted by the U.S. Food and Drug Administration, before incorporation into algorithms for clinical testing. Investigations exploring the effects of varying meal composition on SI are needed to adequately inform control algorithms. While there has been an attempt to measure postprandial glucose turnover after complex carbohydrate ingestion (11) in T1D, the interpretation was confounded by limitations in the applied methods where the biochemical backbones of the meal tracer and tracee were dissimilar. However, such efforts will be further enhanced if prandial insulin dosing is determined not only by the meal carbohydrate content, as is the current practice, but also guided by the proportions of other noncarbohydrate nutrients, including fat and proteins.

Prior reports (13) have examined the effects of delayed gastric emptying on postprandial glucose turnover in healthy and T1D subjects. Research studies are under way to investigate the impact of pramlintide to delay gastric emptying on glucose fluxes and SI. Ongoing studies in T1D subjects will help
Exercise and exercise in generation control algorithms (17). Contrasting effects on the duration and exercise (resistance vs. aerobic) have concentrations (20). Different types of grade exercise mimicking activities of exercise leading to late evening and noc- exercise but also several hours after concentrations in T1D not only during in the management of T1D (24), the delineate the effects of delayed gastric postexercise hypoglycemia. A recent re- cent evidence to recommend exercise of endogenous glucose production in con- exercise. Recently, Fahey et al. (29) demonstrated that a short sprint in- increased plasma glucose levels due to a decline in glucose uptake in both healthy and T1D individuals.

Exercise increases glucose uptake through both insulin-dependent and in- dependent mechanisms and that endogenous glucose production (EGP) must increase to meet the increased metabolic demands of the exercising muscle to prevent hypoglycemia (33–36). These changes in glucose fluxes are facilitated by falling insulin and ris- ing glucagon and catecholamine levels during exercise in healthy individuals (37). In the only human study comparing individuals with and without T1D during moderate and high intensity exercise, Petersen et al. (30), applying magnetic resonance technology, determined that compared with healthy individuals T1D had higher rates of EGP, which was en- tirely due to increased gluconeogenesis. However, as in prior investigations, lack of development of physiological models of insulin action and glucose uptake dur- ing exercise of varying intensities pre- cludes quantification of the effect of exercise on SI, especially in T1D. This repre- sented a significant knowledge gap with potentially large effect size to enh- ance currently available CLC algo- rithms. A recent study in healthy individuals undergoing moderate grade
exercise (50% $V_{O2\max}$) 2 hours after a mixed-meal reported 75% increase in SI (38). This was due to an exercise-induced increase in peripheral SI and was accompanied by a doubling of glucagon concentrations and an eightfold increase in rates of hepatic glucose production (Fig. 3). The increase in SI with exercise has been incorporated into the CLC simulator and experiments run in silico on virtual patients with T1D simulating identical experimental conditions as above. Various bolus and basal insulin adjustments are being simulated with responses analyzed using control-variability grid analyses (39) for refinement of current generation CLC. Sophisticated models are needed to determine the extent of insulin-independent effects of exercise on glucose excursions.

However, there are currently limited data on exercise physiology in T1D and in particular, there are no studies that have measured whole-body SI in T1D during sustained or intermittent exercise before and after a training period. Such studies are therefore sorely needed to better understand exercise effects on carbohydrate physiology in T1D that could help further inform and refine future CLC algorithms.

**Stress/Intercurrent Illness**

Mental stress and intercurrent illnesses affect glucose control through peripheral and hepatic insulin resistance leading to hyperglycemia induced by cytokine and stress hormones (e.g., catecholamine, glucocorticoids). Figure 2 shows anticipated changes in metabolic parameters induced by stress. Systematic investigations are necessary to assess the effect size, reproducibility, and interindividual variabilities on glucose concentrations and the remedial factors (e.g., increasing insulin delivery rates based on changes to SI) to inform and refine next-generation CLC algorithms.

**BIOLOGICAL FACTORS**

**Dawn Phenomenon**

Although perturbations induced by meals and exercise are a major factor for daytime glucose variability, the putative dawn phenomenon could contribute to nocturnal glucose variability in T1D. A better understanding of the frequency (every night), prevalence (every patient with T1D), and causes (cortisol, growth hormone) of this phenomenon (40), and additional investigations of modulators that could determine the effect size and predictability of dawn (sleep pattern, antecedent exercise, bedtime snacks, antecedent hypoglycemia, etc.) would inform next-generation algorithms if effect size is substantial. We are currently conducting studies in T1D subjects that are designed to examine the effect sizes of some of these factors. Subsequently, based on effect sizes and simulation, decisions will be made regarding further human studies before individualizing the control algorithm or proceeding directly to control system use.

**Sex Steroids**

A large gap currently exists in our understanding of the effects and effect sizes of physiological and pharmacological changes in sex steroids on carbohydrate metabolism in T1D. These include physiological hormonal changes that occur during puberty, menstrual cycle, pregnancy, and menopause, and pharmacological changes due to hormone-replacement therapies. As clinicians, we routinely encounter patients with T1D with changes in insulin requirements that occur often with puberty, menstruation, and menopause, and always occur during pregnancy. It is also widely known that due to fetal and maternal risks, tight maternal glucose control is required from conception to delivery in all pregnant women with T1D. To do so, considerable resources and expertise are used to care for a mother with T1D throughout pregnancy and in the postpartum period. While some studies in healthy women have shown no effects of menstruation on insulin action (41), others (42) have noted alterations in insulin action and secretion and glucose effectiveness. Studies in T1D have been sparse (43), showing subtle decrease in insulin action during the follicular phase. A recent study using CLC and dual isotope technique to measure postprandial glucose turnover (44) demonstrated hepatic and peripheral insulin resistance during late versus early pregnancy in a cohort of T1D. However, systematic examination, using robust state-of-the-art techniques,
to estimate the effect sizes of puberty, menstruation, pregnancy, menopause, and hormone-replacement therapy on parameters of insulin action are needed before individualized next-generation control algorithms in T1D could reliably and safely be used, especially during pregnancy. While there has been emerging interest on use of CGM in pregnancy and labor (45), widespread use of modern diabetes technology has been limited in the pregnant adult.

**HYPOGLYCEMIA**

Hypoglycemia is a major limiting factor for optimal management of T1D with conventional insulin therapies. While short-term CLC clinical trials with single hormone (insulin) and dual hormone (insulin and glucagon) therapies have shown reduction in time spent in hypoglycemia (4–6,8) compared with conventional therapy, we need to better understand the factors that determine hepatic glucagon sensitivity in T1D. These factors include hepatic glycogen content, antecedent hypoglycemia, and prevailing glucose and insulin concentrations, etc. Investigations exploring the effects of such modulators of hepatic glucagon action (by stimulating glycogenolysis) are needed so that algorithms could be adequately informed for not only treatment of (impending) hypoglycemia but also prevention of hypoglycemia in the first place. A recent approach (46) has highlighted the efficacy of discontinuing insulin infusion based on CGM trends to lower hypoglycemia frequency.

**GLUCOSE SENSOR ISSUES**

A necessary prerequisite of an effective CLC algorithm is accurate continuous glucose sensing accomplished by CGMs that measure interstitial fluid (ISF) glucose concentrations through subcutaneously placed glucose-sensing probes. While algorithms, insulin delivery devices, and faster insulin preparations are being constantly refined, such initiatives are limited in the glucose-sensing arena—a critical initial component of any effective artificial pancreas system. In fact, some current control algorithms for artificial pancreas include adjusting for inaccuracy and the putative delay inherent to CGM sensing as an independent module.

While there have been reports (47,48) that have attempted to examine the temporal relationship between changes in plasma glucose concentrations to ISF glucose concentrations in subjects with and without diabetes (suggesting a time delay of 4–50 min, but with significant variability across published reports), detailed analyses of the kinetics of glucose from the intravascular compartment across the vessel wall and into the ISF compartment has not been performed in humans. There is one report (49) that has used fluorescein kinetics between vascular and ISF compartments and found a 2–4 min delay between the two compartments after a bolus dose. Others (50) have used microdialysis to examine the effects of insulin on adipose tissue glucose disposal without examining glucose kinetics. Hence, the relevance of these observations to glucose kinetics across the vascular wall is speculative at best.

An assessment of these physiological variables is crucial before the accuracy of CGM devices could improve and help shorten the delay that is purported to occur across compartmental barriers (48,51). A better understanding of the kinetics of subcutaneous glucose transport (i.e., delay of glucose transport from the vascular system across the capillaries into the ISF) will lead to the development of next-generation CLC algorithms. In a study using glucose isotopes and microdialysis techniques, the estimated mean time lag of appearance of tracer glucose into the ISF after intravenous bolus was between 5.3 and 6.2 min in healthy adults in the overnight fasted state (52). Furthermore, the effects of meals, exercise, insulin administration, hypoglycemia, hyperglycemia, obesity, and other variables on the putative time lag between blood and CGM glucose changes are currently being systematically examined in ongoing studies, and will need to be tested in silico before conducting clinical trials when appropriate to enable development of next-generation algorithms incorporating these changes. Nevertheless, based on analyses of glucose patterns derived from CGM and insulin infusion rates derived from insulin pump, a recent report tested the validity of a novel index of postprandial SI in T1D subjects (53). This index requires further field testing before incorporation into algorithms.

Furthermore, medications like Tylenol are known to interfere with CGM measurements, thereby providing erroneous ISF glucose readings. Ongoing experiments are currently examining the effect size of such interferences in T1D. Further studies are necessary to better understand the cause and nature of this interference, not only with Tylenol but with other pharmaceuticals commonly used in the management of T1D, for next-generation CGM devices that use enzymatic and nonenzymatic approaches for CGM.

**DIFFERENT REMEDIES FOR HYPER- AND HYPOGLYCEMIA**

As mentioned above and as depicted in Figs. 1 and 2, the physiological consequence of both meals and stress is hyperglycemia. Meal-induced hyperglycemia results predominantly due to the digestive process leading to meal glucose appearance, stimulation of glucose uptake due to insulin secretion (in a nondiabetic person) or infusion (in a T1D subject), and suppression of endogenous glucose production brought about by the normal postprandial suppression of glucagon secretion resulting in net improved SI. In contrast, stress induces hyperglycemia through stimulation of stress hormones and cytokines secretions that increases hepatic glucose production and reduces peripheral glucose uptake leading to worsening SI. Hence, although the net effect of both meals and stress is hyperglycemia, in the former scenario, SI increases; while in the latter, SI decreases. Hence, the controller will need to be adequately informed and trained to distinguish between the different situations by appropriate rapid changes to SI, insulin on board, or correction factors based on multimodality inputs or cues (e.g., meal announcement and/or heart rate or cutaneous sweat sensors).

On the other hand, as shown in Figs. 3 and 4, hypoglycemia can be a consequence of either exercise or alcohol consumption, respectively. The former is due to enhanced exercise-induced insulin-dependent and -independent peripheral glucose uptake leading to increased peripheral SI as simultaneously hepatic glucose production increases due to glucagon counterregulatory effects. Alcohol effect is, however, predominantly due to suppression of hepatic glucose production by alcohol inhibition of gluconeogenesis, thereby enhancing hepatic SI. Hence, although the net effect...
of both exercise and alcohol is hypoglycemia, in the former scenario, peripheral SI increases but hepatic SI decreases, while in the latter, hepatic SI increases with little or no effects on peripheral insulin action. Hence, the controller once again will need to be adequately informed to distinguish between the different scenarios by appropriate changes to SI, insulin on board, or correction factors, with or without glucagon delivery, based on modality inputs or cues (e.g., exercise announcement, accelerometer/heart rate inputs, and alcohol announcement). Control strategies could also exploit the rate of change of glucose that may be unique. For example, the rate of decrease in glucose achieved by moderate or strenuous physical activity is unique and not matched by any other physiological situations.

HORMONE DELIVERY FACTORS

Current limitations in single- and dual-hormone CLC systems include delay in insulin action after subcutaneous administration and the instability of glucagon in solution. Apart from the inherent delay in insulin kinetics due to time lag of insulin absorption from the subcutaneous depot, factors that potentially aggravate the delay in insulin action include local lipohypertrophy, edema, and fibrosis at insulin injection sites. Instability of glucagon in solution relates to fibril formation and consequent loss of biological efficacy. Such drawbacks will hopefully soon be overcome with faster-acting insulin from local heating of insulin or by modification of the controller for adjustments in rates of insulin and/or glucagon delivery. It is important to underscore that the ideal CLC algorithm may ultimately need to account for all or some of these perturbations occurring simultaneously. Each perturbation could be introduced into “simulations,” and ultimately only key ones with large effect sizes would be included in the control system, a working model that engineers do for most or all machine systems.

Development, validation, and incorporation of such physiological models into control algorithms after adequate in silico testing followed by randomized clinical trials will pave the way for fully automated systems to treat patients with T1D as we approach a century after the discovery of insulin!

CONCLUSIONS

There is a critical need for a better and more complete understanding of insulin-glucagon-glucagon physiology in conditions that mimic the free-living situation to the extent possible in T1D that will help refine and improve future CLC algorithms. Further studies directed at understanding the role of the nervous system (brain, autonomic system) on glucose regulation in T1D should also enhance future generation CLC algorithms. Perturbations with large effect sizes, for example on SI, then need to be tested in the simulator to provide the algorithm with stipulations and guidelines that would help inform and modify the controller for adjustments in insulin-delivering systems.

Acknowledgments. The authors are indebted to Mr. Brent McConahey and Dr. Ling Hinshaw of the Endocrine Research Unit, Mayo Clinic, Rochester, MN, for assistance with the figures.

Funding. This work was supported by National Institutes of Health grants DK-R01-085561 to A.B. and Y.C.K., DK-DP3-094331 to A.B., and DK-029953 to R.B.; Helmsley Charitable Trust 2012PG-T1D005 to R.B.; and grant U1L-TR000135 from the National Center for Advancing Translational Sciences, a component of the National Institutes of Health, to the Mayo Clinic. C.C. is partially funded by Italian Ministero dell’Istruzione, dell’Università e della Ricerca (Progetto FIRB 2009).

Duality of Interest. This work is partially supported by a grant from Dexcom, Inc. to R.B. No other potential conflicts of interest relevant to this article were reported.

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