Perspectives

Odyssey between Scylla and Charybdis through storms of carbohydrate metabolism and diabetes: a career retrospective

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Vranic M. Odyssey between Scylla and Charybdis through storms of carbohydrate metabolism and diabetes: a career retrospective. Am J Physiol Endocrinol Metab 299: E849–E867, 2010. First published September 7, 2010; doi:10.1152/ajpendo.00344.2010.—This research perspective allows me to summarize some of my work completed over 50 years, and it is organized in seven sections. 1) The treatment of diabetes concentrates on the liver and/or the periphery. We quantified hormonal and metabolic interactions involved in physiology and the pathogenesis of diabetes by developing tracer methods to separate the effects of diabetes on both. We collaborated in the first tracer clinical studies on insulin resistance, hypertriglycerideremia, and the Cori cycle. 2) Diabetes reflects insulin deficiency and glucagon abundance. Extrapancreatic glucagon changed the prevailing dogma and permitted precise exploration of the roles of insulin and glucagon in physiology and diabetes. 3) We established the critical role of glucagon-insulin interaction and the control of glucose metabolism during moderate exercise and of catecholamines during strenuous exercise. Deficiencies of the release and effects of these hormones were quantified in diabetes. We also revealed how acute and chronic hyperglycemia affects the expression of GLUT2 gene and protein in diabetes. 4) We outlined molecular and physiological mechanisms whereby exercise training and repetitive neurogenic stress can prevent diabetes in ZDF rats. 5) We and others established that the indirect effect of insulin plays an important role in the regulation of glucose production in dogs. We confirmed this effect in humans and demonstrated that in type 2 diabetes it is mainly the indirect effect. 6) We indicated that the muscle and the liver protected against glucose changes. 7) We described molecular mechanisms responsible for increased HPA axis in diabetes and for the diminished responses of HPA axis, catecholamines, and glucagon to hypoglycemia. We proposed a new approach to decrease the threat of hypoglycemia.

exercise; stress; diabetes pathogenesis; hypoglycemia

SCYLLA AND CHARYBDIS are two sea monsters in Greek mythology described by Homer. They were located close enough to each other that they posed an inescapable threat to passing sailors, including Odysseus. Avoiding Charybdis meant passing too close to Scylla and vice versa. Substitute Scylla for granting agencies and Charybdis for high-impact journals. There is a feedback relationship between problems with granting agencies and the acceptance of papers in important journals. The storms (originality) require the strength of Odysseus to sail safely toward acceptance of a new and original hypothesis.

I am very indebted to Amira Klip (the first non-U.S. editor of the American Journal of Physiology - Endocrinology and Metabolism), who invited me to write a review related to my research endeavors over the past half-century. Since this is a personal history rather than a general review, I have focused primarily on my laboratory work as well as highlighting the innovative and original research that my students and fellows subsequently performed after leaving my lab. It is also exciting to follow my collaborators’ work. A great privilege resulting from this collaborative work reflects lasting close personal friendships with so many who fought the fierce battles with me. The greatest satisfaction that I have is to follow the impact of the research of my previous students and collaborators. I have worked in a number of related research projects and I have collaborated widely, which has resulted in this rather extended text.

I began my research career in Toronto in the mid-1960s by developing tracer methods, which led to studies of hormonal interactions in physiology and diabetes, with a major focus on glucagon-insulin interactions in animal and human subjects and the control of glucoregulation in non-steady state. We also demonstrated that in dogs glucagon is secreted not only from the pancreas but also from the stomach. This finding made it possible to better understand the role of glucagon in physiology and diabetes. Work with hormonal interactions allowed us to concentrate on the pathogenesis of diabetes, exercise, stress, and hypoglycemia, which are fields that we reviewed and researched extensively (158, 192, 206). Some biographical notes pertaining to my life and research have been published (18).

I dedicate this autobiography to my father, Professor Vladimir Vranic, who taught mathematics at the University of Zagreb, Croatia, and to my mother Ana. My father inspired me with his fighting spirit. Before World War II, he taught mathematics, but due to racial discrimination, he could not hold an academic appointment. During World War II, we escaped from Croatia and then spent time in Italian concentration camps, later reaching Taranto, Italy, which was occupied by allied forces. Eventually, my father joined the partisans in Yugoslavia. After the war, he was professor and Dean at the Faculty of Economics and Engineering, respectively, at the University of Zagreb. He managed to bring the first computer to the University despite the fact that his mathematician colleagues believed that computers represented a prostitution of pure mathematics. I have tried to apply the same tenacity to my own research endeavors. In addition, I owe my life to my mother, who saved my family by organizing our escape from fascist Croatia in 1941. Finally, all of my work would not have been possible without my wonderful wife, Linda, whose love and advice have sustained me. I also appreciate the support of my three daughters and of our extended family.

I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference.
[from Robert Frost’s “The Road Not Taken” (66a)]

The way that I interpret this marvelous poem is that a scientist is always facing choices (“Two roads diverged in a wood”), focusing on specific areas of research, selecting students, fellows, and collaborators. The “less traveled” offers the opportunity of originality, which in my opinion is the key goal in all aspects of arts and science.

**Quest to Establish an Accurate and Physiologically Relevant Method to Measure Glucose Turnover out of Steady State**

**Methodology.** The treatment of diabetes concentrates on the liver and/or the periphery. We quantified factors involved in the pathogenesis of diabetes by developing more precise methods to separate the effects of diabetes on both. We collaborated in the first clinical studies on insulin resistance and hypertriglyceridemia and the Cori cycle using non-steady-state tracer methods. These methods are now widely used in animal and clinical research.

The maintenance of homeostasis is a hallmark of life. One mechanism for maintaining homeostasis is reflected by continuous changes of hormones and metabolites in response to the alteration of the internal and the external environment. In the case of glucose, an assessment of homeostasis is not possible by measuring plasma glucose alone, because there are numerous metabolic perturbations where glucose production and its utilization change to the same extent without any change in net plasma glucose concentration. In the steady state, glucose turnover is assessed by a simple equation: relating infusion of radioactive glucose tracer to the specific activity achieved. The main assumption is that an infusion or injection of tracer distributes uniformly through a number of compartments of the body. Prior to the studies by the team of Steele, de Bodo, and Altszuler, it was not possible to assess glucose fluxes outside of steady-state conditions. Yet, this was essential to understand the dynamics of control mechanisms (105). The breakthrough in the assessment of non-steady-state glucose metabolism was Steele’s invention of a new tracer method involving primed-tracer infusion of $^{13}$C at constant rate (4, 7, 170, 171). The assumption was that two compartments could approximately represent the entire body’s glucose pool. This was a very daring idea, because in reality, glucose distributes between plasma and a number of different interstitial compartments. However, because they did not validate this method in vivo, other researchers would not use the tracer method, either in animal or in clinical research. Wrenshall and Hetenyi’s laboratory (209) used successive tracer injections. However, this method was not sufficiently accurate to monitor more rapid changes in glucose turnover. There was animated discussion between the two groups, resulting in some barriers to publication. The first group published mainly in the *American Journal of Physiology* and the second in *Diabetes*. The Toronto group eventually adopted Steele, de Bodo, and Altszuler’s approach. Validation became possible when Katz (99) and others used diverse glucose tracers ($^{2}$H, $^{3}$H, and $^{6}$H) to assess, in addition to glucose turnover, recycling and hepatic futile cycles (related to glucose entering and leaving the liver). Cowan and Hetenyi (37) used an integral method to measure average glucose production and suggested some modification of the original Steele’s equation. John Cowan (at the time an M.Sc. student of Gerry Wrenshall) has since had an illustrious academic career, which has culminated in his appointment as the principal of the Royal Military College in Kingston, Ontario. Geza Hetenyi, who escaped from Hungary in 1976, became Chairman of Physiology, and Vice Dean at the University of Ottawa. He was truly a Renaissance man, and he helped to initiate me into the tracer domain. Finally, with two mathematically trained physiologists, Jerry Radziuk (now Professor of Medicine and Physiology at Ottawa’s Medical School) and Kenny Norris (professor in our department), we (148) compared known infusion rates of $^{13}$C glucose to the tracer infusion method using a variety of tracers. We also validated the method by using insulin (a compound that is not metabolized) with Radziuk, Norris and David Lai (a summer student, and nova professor in the Departments of Medicine and Biochemistry and Molecular Biology, Director of the Julia McFadzean Diabetes Research Centre, and Chair of the Diabetes and Endocrine Group, University of Calgary (136a). We concluded that Cowan and Hetenyi’s corrections applied not only to integrated glucose turnover rates but were also valid on a minute-to-minute basis. This final validation represented a watershed in measurements of non-steady-state glucose turnover, and a large number of laboratories, using human and animal research, began to use this tracer method to assess the physiology of glucoregulation and the consequences of insulin lack and its resistance in diabetes. However, this method was not accurate for glucose clamp studies (as indicated below).

The knowledge of quantifying insulin secretion and resistance was greatly advanced by the glucose clamp technique (42). It was groundbreaking because it enabled researchers to differentiate between insulin secretion and sensitivity with a single glucose injection. In order to be able to conduct large clinical studies, Richard Bergman et al. (17) developed an ingenious method using “minimal-model calculation” that differentiates between insulin secretion and sensitivity with a single glucose injection. This was a landmark discovery for large epidemiological studies. Bergman is presently the Chair of Molecular Physiology and Biophysics at the University of Southern California.

The next step was the combination of glucose clamps with tracer methods. This made it possible to differentiate the effects of insulin on the liver and other tissues in the body. Unexpectedly, negative glucose production rates were frequently observed during glucose clamps. This was a severe blow to the method, because glucose production can only be positive, and therefore, wrong conclusions were reported. The problem was that during glucose infusions glucose production was a very small component of the total glucose entry into the body, and the accuracy of the tracer methods depended on the accuracy of specific activity. With another bioengineer, Diane Finegood (a postdoctoral fellow), and with Bergman, we resolved this problem (61), using the hot GINF (glucose infusion). (Finegood was the Director of the Institute of Nutrition, Metabolism and Diabetes at the Canadian Institute of Health Research.) At this protocol, and in addition to a constant infusion of tracer, tracer was also added to exogenous glucose for clamping. The novelty consisted of maintaining near constant the glucose specific activity during the nonsteady state and thereby avoiding mistakes due to rapid changes of specific activity (60).
For a number of years, the Federation of American Societies of Experimental Biology (FASEB) held a symposium every year to consider advances in tracer methodology. I became the chairman and organizer of the Tracer Methodology Study Group in 1972. This was an exciting task, since I was asked for the first time, to edit and publish the *Proceedings from the Tracer Symposia in Federation Proceedings*.

**Glucoregulation controlled by hormonal interaction.** The application of non-steady-state glucose turnover enabled us to study the selective effects of metabolic hormones [glucagon, insulin, catecholamines, IGF-I, glucocorticoids (GR)] and their interactions. This is a cornerstone for understanding the physiology of metabolic hormone actions as related to pathogenesis of diabetes, stress, exercise, and hypoglycemia. For the first time, we characterized insulin resistance in lean type 2 diabetics (197). With George Steiner (former head of Toronto Endocrinology Division), we conducted the first clinical studies using the validated tracer infusion method. This collaboration expanded my interest in clinical research and stimulated me to strengthen my ties between physiology and medicine. As chair of our department, I had the opportunity to develop an even closer relationship between our department and several clinical departments. We demonstrated that insulin resistance is present not only in obesity (194) but also in lean hypertriglyceridemics (172). With a double-tracer technique, we also quantified the role of the Cori cycle (recycling of glucose from the liver to the periphery and back) during metabolic adaptations (173). An important mechanism for reducing gluconeogenesis is to recycle glucose through the Cori cycle, thus minimizing the need for carbon from amino acids to produce glucose. During fasting, glucose production decreases, and we showed for the first time that the absolute rate of glucose recycling is unchanged. But since glucose production decreases, the proportion of glucose production that originated by recycling, increases. Radziuk (149) devised a sophisticated computer program for a double-tracer technique to understand the mechanism whereby the drug acarbose (α-glucoside hydrolase inhibitor) delays intestinal absorption of glucose in humans. This drug is proved to be useful for treatment. We also used a double-tracer method with post-doc Murenide Matsuhisa (now a professor at Tokushima University, Japan), to quantify liver and peripheral effects of ploglitazone. This was the first demonstration that this drug not only decreases glucose production, but also increases glucose uptake, both in the periphery and in the liver. This drug is now widely used for treatment (127a).

My mentor, Aron Rappaport (a surgeon who came from Romania), changed our concepts about the structure of liver acini, which was important for our understanding of hepatic physiology and pathophysiology. He also had a special interest in the physiology of the hepatic artery. Working with Wrenshall (my other mentor) and Cowan, we were amazed that ligation of the hepatic artery decreased hepatic glucose production and prevented ketosis and therefore greatly prolonged the life of totally pancreatectized dogs (153). This was an early indication about the importance of the liver in inducing a diabetic coma.

Without the glucose clamp approach, one cannot precisely predict the changes in specific activity. By trial and error, we adapted the methods to minimize the changes in specific activity. We demonstrated that accurate data can be obtained if the change in specific activity is under 25%. This new method was developed with Errol Marliss (head of the Department of Nutrition at McGill University, Montreal). This was essential during strenuous exercise, when rapid increments of glucose turnover occur within minutes (165).

Advances in tracer methodology opened the door for precise investigation of hormonal interactions. With Alan Cherrington (then a graduate student, later Chair of Physiology at Vanderbilt University, and President of the American Diabetes Association), by applying an insulin clamp, we outlined quantitative interactions between glucagon and insulin. Measuring glucose turnover, it was possible, for the first time, to investigate effective rates of glucagon secretion that were previously thought to be much higher (34). This interaction between glucagon and insulin is very precise so that glucose turnover can increase but without changing glucose concentration. In diabetic patients maintained with subnormal insulin infusion, the liver’s hepatic glucose production is so sensitive to glucagon that with a minimal infusion of glucagon glucose production increases markedly, although an increment of plasma glucagon concentration could not be detected (119). Cherrington increased the precision of the clamps by developing a bihormonal clamp. The clamp was used to establish the catabolic effect of glucagon during acute insulin deficiency (35), the role of portal vein glucose delivery on glucose uptake and glycogen storage by the liver (2), and molecular characterization of insulin-mediated suppression of hepatic glucose production in vivo (150).

Then, with Friedrich Kemmer, a post-doc (presently Professor of Medicine in Potsdam, Germany), and colleagues Otto and Anna Sirek, we solved an old problem: Why does hypophysectomy decrease hyperglycemia in diabetes? We demonstrated that this is due to a decrease of glucose production, which is partly due to normalization of glucagon secretion (101). The Sireks, who had emigrated from Slovakia, introduced me to Prof. C. H. Best, and I owe it to them that I was invited to Toronto as a post-doc, and the rest is history. With postdoctoral fellows Kamal El-Tayeb from Sudan, Patricia Brubaker (presently holding a prestigious research chair in our Department), and Lavinia Lickley (eventually the first female full professor of surgery in Canada), we demonstrated that β-endorphins (which have opiate-like effects during stress) are also important regulators of glucose metabolism (57, 58). We established that excessive amounts of IGF-I have similar effects to insulin on glucose production and utilization, but only if glucose concentration is clamped in diabetic dogs (70, 72). This indicates that the effect of IGF-I is modulated by plasma glucose concentration, as is the case with many other hormones. Rappaport developed an ingenious method of grafting the uncinate process of the pancreas wrapped into a plastic bag under the skin; the rest of the pancreas was removed. By clamping the vessel that supplied the uncinate process, we could induce instantaneous diabetes or normalization of glucose by removing the clamp (152, 210). Supplying different glucose infusions when the graft was removed allowed us, for the first time, to measure insulin secretion rates in dogs (189). We were excited to realize that the same rate was subsequently reported for humans.

In summary, as the field of insulin action moves toward understanding the complex insulin signaling cascades that are activated in time and space within insulin-sensitive tissues and the pliable nature of islet tissue renewal, the tracer method and
glucose clamps continue to be mainstays in establishing whole body glucose homeostasis (105).

Resolving the Controversy Regarding the Existence of Extrapancreatic Glucagon

Diabetes reflects insulin deficiency and glucagon abundance. The question we had to address was why depancreatized dogs that do not have pancreatic insulin or glucagon are severely diabetic?

We demonstrated that glucagon in dogs can be secreted in equal amounts from the pancreas and from the gastric mucosa. Therefore, depancreatized dogs are severely diabetic. This work confirmed Unger's hypothesis that insulin lack and glucagon abundance are important in the pathogenesis of diabetes. Depancreatized humans are diabetic despite only a small residual amount of glucagon, but this is a very mild form of diabetes that does not lead to ketosis (169).

Roger Unger (185) postulated that diabetes is caused by insulin deficiency (amount or effect) and glucagon presence or excess. Unger is considered to be the father of glucagon physiology and biochemistry, and Lelio Orci the father of the morphology of the islet cell (186). The main problem with Unger's hypothesis was that diabetes can be induced in animals either by selective chemical destruction of the insulin-producing \( \beta \)-cells or by total pancreatectomy, which removes the \( \beta \)-cells and the glucagon-producing \( \alpha \)-cells. If glucagon were essential for development of diabetes, one would have expected that total pancreatectomy would not induce diabetes. But that was not the case. Unger (Dallas, Texas) developed an immunoassay of glucagon (185). At that time, the laboratories that were best known for measuring glucagon plasma (in addition to that of Unger), were those of Pierre Lefebvre in Liege and Roger Assan in Paris. All three laboratories reported that, after pancreatectomy in dogs, plasma glucagon could not be detected. In order to clarify the issue of extrapancreatic glucagon, I arranged to visit Unger. As if in a Western movie, we sat across a table, but instead of guns, we had selections of our papers. I believe that I convinced Roger of our views regarding extrapancreatic glucagon. A few years earlier, two Nobel Prize winners, DeDuve and Sutherland, had shown that extracts from the mucosa of the dog's stomach have a hyperglycemic effect (176). This could have been glucagon, but a quantitative glucagon assay did not yet exist. My first post-doc, R. Kawamori, came from Japan (later he was Chairman of the Department of Medicine at the Tokyo Juntendo Medical School and President of the Japanese Diabetes Society). This was followed by a number of post-docs from Japanese universities. At the time, we did not yet have a glucagon assay and collaborated with Sumer Pek (190) from the University of Michigan. At that time, our laboratory was interested in glucagon physiology and in isolating the effects of insulin from those of glucagon. Therefore, we depancreatized dogs, believing that they would not only deprived of insulin but also glucagon. To our surprise and in contrast to other laboratories, we could still detect plasma glucagon in insulin-infused depancreatized dogs. Depancreatized dogs can live without insulin for about one week when insulin treatment is discontinued. We noticed, to our great surprise, that plasma glucagon skyrocketed within one week after pancreatectomy (190). These results created a great deal of excitement, because it was contrary to the dogma that depancreatized dogs did not have any glucagon in the plasma. I was extremely active presenting these findings, so that Altszuler heard that I was speaking in two conference rooms at the same time. This led to the stunning discovery that a pancreatic hormone can be produced in large amounts outside its original endocrine gland. Over the next 10 years, using tracer methods to measure glucose fluxes, biochemical, histological, immunological methods, electron microscopy, and purifying gastric glucagon to homogeneity, we determined beyond all doubt that the parietal mucosa of the dog stomach can synthesize and secrete true glucagon (133, 190).

Albert Renold, who was the director of the “Mecca” of diabetes research, and President of the International Diabetes Federation, invited me to spend a sabbatical year in Geneva. Renold stressed the importance of supervising students and fellows and collaborating intensely. I was very honored when I got the Renold Award from the American Diabetes Association for training diabetes scientists. With Cecil Yip (Chair of Banting and Best, Department of Medical Research and Vice Dean in Toronto), and Kunihiro Doi (a postdoctoral fellow and presently the President of the Japanese Diabetes Clinical Society), we purified stomach glucagon. We quantified its effect on isolated liver cells in vitro, together with Mark Prentki (now the head of Montreal University Diabetes Research Centre), Jeanrenaud, and Muller. The effects of the extracts were identical to those of pancreatic glucagon. Measurements of glyco(genolysis, glucoseogenesis, production of lactate and pyruvate, and concentration of cAMP explained why, following pancreatectomy in dogs, diabetes is as severe as with the selective destruction of the \( \beta \)-cells (48). With Orci in Geneva, we showed that, in the gastric mucosa of a depancreatized dog that was maintained on insulin for five years, there was a large hyperplasia of the \( \alpha \)-cells and a large amount of glucagon in the dog’s stomach. By electron microscopy of the parietal mucosa of the stomach it looked like a glucagon-producing endocrine gland (156). We also demonstrated, with labeled tryptophan, leucine, and \( \alpha \)-methylone, the specific biosynthesis of glucagon in mucosa pieces of the stomach (81). These findings challenged classical views of endocrinology and provided further proof that one hormone is not necessarily produced in only one endocrine gland. With Girardier, Miller, Seidoux, Berger, and Renold, we quantified the amount of glucagon-like peptides that are secreted exclusively from the gastrointestinal tract (135). High glucagon plasma levels in depancreatized dogs were also confirmed by others (128). The regulation of extrapancreatic glucagon release was different from that from the pancreas (121). True glucagon was localized exclusively in the stomach because pancreatectomy plus gastrectomy virtually removed glucagon from plasma (134). In contrast to dogs, in totally depancreatized humans, there is only a negligible amount of plasma glucagon (9, 20, 87, 135, 179). We and others who detected glucagon-like peptides in the brain (80, 177, 181) stimulated interest in this field. It was very exciting to present these data at my Banting and Best Award and plenary lecture at the International Diabetes Federation meeting in 1985. Other laboratories discovered that glucagon-like peptide-1 (GLP-1) coencoded in the glucagon gene as a potent stimulator of insulin release (132). Its antidiabetogenic effect in human subjects was first demonstrated by Efendic et al. (75). This peptide has not only pancreatic but also some peripheral effects (160). It was then shown that GLP-2 has an
important effect on the gastrointestinal tract (54, 95). The biological action and therapeutic potential of the proglucagon-derived peptides was extensively reviewed by Drucker (53). In order to study the regulation of the release of the GLP hormones from the gut, Brubaker developed fetal rat intestinal cells in monolayer culture (24). She then characterized the stimulatory effects of M1 agonist, of leptin, of the oleic acid derivative (OEA), and of insulin on GLP-1 release (5, 6, 110, 118).

The most exciting and memorable symposium that I ever attended was the 1974 Conference on Glucagon held at a Santa Ynez ranch in California. The ranch belonged to Mr. Kroc, founder and owner of McDonald’s. At that time, there were a number of major breakthroughs in the field of glucagon research. I spoke about the discovery of extrapancreatic glucagon. Guilleman had discovered somatostatin and some other hypothalamic-releasing hormones, for which he had received the Nobel Prize. Somatostatin suppresses secretion of glucagon and insulin, and therefore it became possible, with the replacement of one or the other pancreatic hormone, to precisely determine the distinct functions of insulin and glucagon. Rodbell suggested that G proteins act as transducers in cell signaling by glucagon and other hormones and also received the Nobel Prize. The highest awards given in North America for diabetes research are the Outstanding Scientific Achievement Award, and the Banting Medal and Lectureship for Life Achievement [including my own (195)]. The participants were also future recipients of seven Banting Medals, five Outstanding Scientific Achievement Awards, and three Claude Bernard Medals.

In summary, the discovery of extrapancreatic glucagon led to a much better understanding of the role of glucagon in physiology and diabetes.

**Combat to Unravel the Roles Between Hormones and Metabolites to Precisely Regulate Metabolic Fluxes Needed to Minimize Changes in Homeostasis and Provide Adequate Fuel: Physiology and Diabetes**

We established the critical role of glucagon-insulin interaction in the control of glucose metabolism during moderate exercise and the role of catecholamines during strenuous exercise. For the first time, deficiencies of the release and defects of the effect of these hormones were quantified in diabetes. We also revealed for the first time how acute and chronic hyperglycemia transiently affects the expression of GLUT2 gene and protein in diabetes.

**Role of insulin and glucagon in the control of glucose metabolism during moderate exercise.** Wrenshall was a type 1 diabetic. He walked after each meal to improve his glucose regulation. We began exercise experiments in depancreatized dogs that were either infused with or totally deprived of insulin. The surprise was that, during light exercise in uncontrolled diabetic dogs, blood sugar did not decrease but instead sharply increased due to an excessive increase in hepatic glucose production (188). The main beneficial effect of exercise is an increase of glucose metabolic clearance. Glucose clearance reflects the efficiency of tissues to extract glucose independently of glucose concentration. We were the first to suggest the importance of assessing glucose clearance in diabetes (188). In diabetic dogs, glucose clearance did not increase during exercise. It was even more disturbing that an intraportal insulin infusion still resulted in an increase of plasma glucose without the increase of glucose clearance during exercise. In later experiments, we increased the work load. Consequently, the response of glucose clearance to exercise was improved even without acute insulin infusion. This is clinically important because it indicates the importance of more intense exercise in insulin treated patients (for review see Ref. 158).

During exercise in nondiabetic subjects, insulin in plasma decreases up to 50%. We hypothesized that, during exercise, since blood flow in the muscle can increase 20-fold or more, the perfusion of muscle with insulin is still maintained. Other laboratories indicated that muscle contraction increases glucose uptake independently of insulin (136, 143, 200). However, we suggested that during exercise in vivo insulin is necessary to counterbalance factors that would decrease glucose uptake (206), such as catecholamines and increased fatty acids (151). We therefore examined the mechanism of exercise-induced hypoglycemia in depancreatized dogs that were maintained on long-acting insulin. We were very surprised to find that, during exercise in insulin treated, depancreatized dogs, rats, and humans, plasma insulin increased dramatically from its subcutaneous depot (15, 100, 216). (A friend remarked that what we observed was highly unlikely, since it would surely have been noticed before). Now for the first time we could explain, in part, a mechanism of the development of hypoglycemia during exercise in insulin-treated dogs or patients (100, 216). With increased insulin levels during exercise, glucose production did not increase to match the increased glucose uptake by muscle. During my sabbatical leave in Geneva, working with Michael Berger (previous vice president of the International Diabetes Federation and president of the European Association for the Study of Diabetes), Philippe Halban, (president of the European Association for the Study of Diabetes) and Renold, we injected tritiated insulin (which Halban synthesized for the first time) (14, 76–78), which gave us the unique opportunity to study insulin kinetics during exercise (15). Previous studies with iodinated insulin were misleading because of the heavy label of iodine and/or its deiodination. Clinically, the observation that exercises can increase the release of subcutaneously injected insulin has an important impact on insulin treatment in relation to exercise (217). It stimulated exciting discussions, because the effect of the enhanced release of insulin depends on the site of injection and on time relationships. Finally, it was very exciting to be able to reveal the mechanisms whereby exercise in diabetics could result in unchanged glucose levels and in hyperglycemia or hypoglycemia, and this hypothesis has been accepted. With insulin deficiency or resistance, there is overproduction and underutilization of glucose and hyperglycemia (191) [Fig. 1 (206)] and ketosis develops (13). Clinical investigations on humans were performed with Bernie Zinman (previous head of Endocrinology and of the Banting and Best Diabetes Centre, University of Toronto) and by Marliss. Under those conditions, exercise is not recommended. On the other hand, when there is excessive insulin due to enhanced mobilization from its subcutaneous depot, glucose production is inhibited, its utilization is enhanced, and hypoglycemia ensues. I received a phone call from a trainer who was involved in exercise training of diabetic patients. He indicated that after reading our papers he now realized when exercise is beneficial and under which condi-
Today, diabetic athletes with proper guidance are fully competitive in their achievements with nondiabetic athletes.

During a sabbatical in Oxford, UK, experiments using phosphorous nuclear magnetic resonance spectroscopy offered a unique opportunity to study intracellular pH and phosphocreatine continuously and noninvasively in rat muscle. This was done in collaboration with Challis and Rada, and it was the first NMR spectroscopy of muscle in diabetes (26). During muscular contractions in diabetic rats that were not insulin treated, the bioenergetics were deficient; insulin-treated rats were normal. The big surprise was that if insulin treatment was discontinued for as long as three days most of the parameters studied remained normal. This supported the view that the continued presence of normal insulin concentration is not needed for glucose metabolism during contractions in isolated muscle. The deficiency of diabetic rats was therefore a consequence of more chronic effects of the diabetic state. We postulated that in vivo insulin is needed during exercise to counteract the effects of increased catecholamines and FFA.

The discovery of somatostatin made it possible, in collaboration with Bela Issekutz, to study the role of glucagon in the regulation of glucose production (93). Issekutz, from the University of Dalhousie, left his native Hungary during the Russian aggression and was a pioneer in studying the effects of exercise on glucose and FFA turnover. A suppression of glucagon greatly decreased the increment of glucose production, resulting in hypoglycemia during exercise. It is now widely accepted that the main regulator of glucose production during moderate exercise is the ratio of glucagon to insulin. With a glucose clamp, we determined with Wasserman, and Lickley, that glucagon controls most of the glucose production increment (203, 204). However, in normal humans, glucagon does not always increase during exercise, but the ratio of glucagon to insulin increases. The effect of glucagon during exercise is one of the most important physiological metabolic roles of this hormone. With Ole Bjorkman (a post-doc and now managing director of Upsalla Science Park in Sweden), we studied the effect of exercise in depancreatized dogs (19). Depancreatized dogs have the same amount of glucagon in the stomach as normal dogs have in the pancreas; however, exercise does not stimulate glucagon release from the stomach. Therefore, it was possible to study the regulation of glucose production, when glucagon has not been released. Indeed, the excessive release of catecholamines can compensate indirectly for the glucagon lack. These dogs are insulin deprived, and therefore, it was possible to find out whether insulin was needed to increase glucose uptake in vivo. This was a controversial issue because there was considerable evidence that the translocation of glucose transporters in striated muscle during exercise is regulated by a different signaling cascade than that of insulin (for review see Ref. 158). This was also our conclusion when we studied the contractions of a diabetic muscle in vivo (26). Most importantly, we could demonstrate that glucose utilization during exercise can increase independently of insulin, but this increase was very small, since insulin was necessary to achieve a full increase of glucose uptake by decreasing the flux of fatty acids and the effect of catecholamines on the exercising muscle (26). This topic created heated controversy, because, in isolated muscle, contractions...
can increase glucose uptake without insulin. That paper provided a definitive answer for the first time. David Wasserman (a professor at Vanderbilt University and a world leader in the field of metabolic and molecular effects of exercise) extended this work by quantifying the role of exercise-induced increase in glucagon on specific pathways in the liver. As he indicated, exercise is a powerful tool that permits us to study phenotypes and reveals functional limitations in diseases. He showed that the rise in glucagon is necessary for exercise-induced stimulation of glycogenolysis and gluconeogenesis (204, 205), control of pathways for nitrogen disposal (107), and fatty acid oxidation. During exercise, glucagon is sixfold more effective at stimulating glucose production than under sedentary conditions (202). He also demonstrated that prior exercise significantly accelerates the repletion of liver glycogen by increasing the rate of glucose absorption from the gut (79, 141) and the extraction of glucose by the liver (68) and by directing more of the glucose to liver glycogen (140). In his role as director of the Vanderbilt-NIH Mouse Metabolic Phenotyping Center (MMPC), he adapted procedures developed for the study of large animals to the mouse. Particularly important is the development of glucose clamp techniques in mice (8, 16).

Role of catecholamines during strenuous exercise. In studies of moderate and strenuous exercise, we collaborated with Marliss. He discovered the first animal model of spontaneous diabetes (BB rat). This model reflects most of our present knowledge about the immunological mechanisms inducing diabetes. Following our collaborative work on moderate exercise in human subjects, we examined the effect of strenuous exercise in non-diabetics. Since glucose production increases rapidly and maximally, we used a modification of the tracer methods (as indicated in Methodology) (124). We postulated that, in contrast to moderate exercise, the primary direct or indirect regulators of glucose production are catecholamines and not glucagon. The problem is that in human subjects it would be too dangerous to examine this hypothesis by the total blockade of α-receptors and β-receptors. Our evidence about the importance of catecholamines is based on the following observations. 1) During strenuous exercise, catecholamines increase sixfold, but only threefold in moderate exercise. 2) When we clamped glucagon, insulin, and growth hormone by an analog of somatostatin, the increase of glucose production was not affected, and, importantly, it was proportional to the concentration of the catecholamines. 3) When we infused catecholamines during moderate exercise, it converted the metabolic response of moderate exercise into that of intense exercise (124, 147). Since there has been controversy about this statement, it is up to the reader to decide whether our evidence is conclusive, as we believe it is. In nondiabetic subjects, during strenuous exercise, glucose concentration increases because glucose production increases much more than glucose utilization. This also increases insulin release, which is important for regenerating muscle glycogen as rapidly as possible. The absence of this response in insulin-treated type 1 diabetes leads to sustained hyperglycemia. Clinically, under those conditions some extra insulin may be needed (147).

Regulation of glucose transporters. The factors that control glucose transport in muscle have been a subject of intense research in humans and in animals. Recently, Holloszy splendidly reviewed his work and the work of others as a part of an autobiographical paper (86). It is well known that glucose is transported into various tissues by specialized glucose transporters. A stunning discovery by Cushman (39, 201) and Kono (106) was that the adipose tissue cell has by far the largest number of transporters in the cytoplasm, but insulin translocates the transporters to the plasma membrane where they exert their function. It was very exciting to collaborate with Amira Klip, who is a world leader in studies of the control of glucose transporters in the muscle. She was the first to show the translocation of glucose transporters in rat hindquarter muscles (104), induced by both insulin and exercise (52). The first confirmation using rat diaphragms was by Wardzala, Jeanrenaud, and Salans (201). More recently she explored the role of actin filaments and their remodeling (94, 184), the differential contributions of GLUT4 and GLUT1 (159), and the intracellular segregation of phosphatidylinositol 3,4,5-triphosphate (139). She explored how insulin regulates the membrane arrival, fusion, and unmasking of GLUT4 (92). We demonstrated, together with a graduate student, Dimitrios Dimitrakoudis, that in mildly diabetic rats glucose transporter number is decreased both in the plasma membrane and inside the cell and that this is not due to insulin deficiency but to hyperglycemia. When we treated diabetic rats with phlorizin for several days, glucose concentration normalized because of excessive glycosuria, and the number of glucose transporters and their genetic expression became near normal (47). Glucose regulates the number of glucose transporters not only chronically but also acutely (123). The mechanism of this critical effect of glucose is still not resolved.

With Andre Marette (then a post-doc and now Professor of Physiology at Laval University, Quebec, and an FRQ National Scientist), we showed for the first time that most of the glucose transporters are translocated not to the plasma membrane but to the transverse tubules of the skeletal muscle (122). This is important since transverse tubules represent a much larger surface area than the plasma membrane. After leaving our laboratory, Marette developed a novel procedure to precisely measure GLUT4 in the plasma membrane and T tubules in diabetes (49). Insulin receptors are internalized in GLUT4 vesicles, which permit their sustained activation (51). Interestingly, the AMPK activator AICAR increases muscle glucose uptake by two mechanisms: 1) inducing selective recruitment of GLUT4 to the plasma membrane and 2) activating p38 MAPKα and β, which may be involved in the activation of GLUT4 (112). Peripheral insulin resistance in diabetic rats is caused by the selective impairment of GLUT4 translocation to skeletal muscle T tubules (50). His pioneering paper demonstrated that cytokines modulate glucose transport in skeletal muscle by inducing the expression of inducible nitric oxide synthase (12).

With a PhD student, Theos Tsakiridis, we demonstrated that the actin network is not only an important part of the cytoskeleton but is also part of the insulin signaling cascade that controls stimulation of GLUT4 translocation during muscle contraction, but not during hypoxia, which mimics the effect of exercise. This was also one of the early indications that exercise and insulin recruit glucose transporters with different mechanisms (182, 183).

In summary, revealing the deficiencies in diabetes during moderate and strenuous exercise was important and has resulted in intensive work in this area, so that diabetic patients can now compete even in Olympic events. Insulin resistance
seen both during rest and exercise in diabetes is partly due to the effect of hyperglycemia on muscle glucose transporters. It is an important challenge to reveal the mechanisms of this intriguing interaction.

**Battle to Establish Beneficial Effects of Exercise and Some Stress in Diabetes**

We outlined molecular and physiological mechanisms whereby exercise training and surprisingly, repetitive neurogenic stress can prevent diabetes in ZDF rats.

**Role of exercise training.** Our work regarding metabolic regulations in physiology and diabetes during exercise has attracted wide attention. It was very exciting to be invited to organize the first symposium on “Exercise and Diabetes,” sponsored by the Kroc Foundation in 1978, which was held in the same place as the previously described Glucagon Symposium (193). It is generally agreed upon that this conference greatly stimulated further research and symposia in the field of diabetes and exercise. Most importantly, epidemiologists concluded that exercise can not only improve but prevent type 2 diabetes (83, 137, 178) with proper insulin treatment (as reviewed in Ref. 158). However, the mechanism of such prevention is not fully understood.

With Michael Riddell (a post-doc and now an associate professor at York University, Toronto) and Michael Kiraly, we explored the mechanisms of preventing hyperglycemia in an animal model of obese type 2 diabetes (ZDF rat) (102). These animals have a defective leptin receptor and therefore eat excessively. Concurrently with the development of obesity, they also develop type 2 diabetes. Finegood (62, 63) and others (36, 142, 180) have studied in detail the dynamics of β-cell mass in ZDF rats. Indeed, in diabetic subjects it was also shown that exercise enhances the insulin response to hyperglycemia or arginine infusion in type 2 diabetics (43). It is amazing that, with only one hour of exercise per day five times a week, the rats, although obese, did not develop diabetes. This is similar to clinical recommendations that prevention of type 2 diabetes can result from moderate, one-hour exercise five times per week. The puzzling question concerns how such short periods of exercise can sustain their beneficial effect? It is remarkable that this effect is not a result of decreased body weight, although some of the fat is replaced by muscle mass.

This provided an opportunity to study the function of the β-cells, which was forming a new topic. I owe it to my student Kiraly, who helped us enter this new field with excitement and difficulty. The improvement of blood sugar occurred in both the fasted and fed states and during an intravenous glucose tolerance test. With exercise, there was a considerable increase of insulin release, β-cell mass expansion, proliferation, and neogenesis, and preservation of islet structure (102). Thus, exercise promoted both β-cell mass and its function. In contrast, sham exercise (rats confined to containers with shallow water) also promoted increased β-cell mass but not its function. This indicates that measurement of β-cell mass by itself cannot always provide information about its function. The other criteria indicating improved function of the β-cells are preservation of GLUT2 (glucose sensing) and β-cell Akt/PKB expression. Diabetes-induced oxidative stress leads to protein misfolding and to degradation by the ubiquitin-proteasome system in many tissues. With Kiraly, Kaniuk, Volchuck, and Brummel, we demonstrated for the first time that these toxic aggregates are removed by autophagy. We hypothesized that autophagy acts as a defense to cellular damage incurred during diabetes (97). Oxidative stress was due to high glucose levels; therefore, it was not surprising that exercise in diabetic rats prevented misfolding and ubiquitination of proteins in the β-cells. It is well known that exercise improves uptake of glucose in the muscle (increased insulin sensitivity) (144). Swimming as a modality of exercise is accompanied by considerable stress. We then investigated the effect of volitional exercise that does not have that component of neurological stress. As long as the rats exercised voluntarily (~3 wk), diabetes was prevented, in part by decreasing hypothalamic-pituitary-adrenal (HPA) hyperactivity (protein expression of hippocampal GR) and preventing decreased feedback mechanism. It is interesting that in this way the hippocampus plays a major protective role. In addition, there is lower adrenal sensitivity to ACTH (adrenal ACTH receptor MC2R, and stereogenic acute regulatory protein) (25). This resulted in the maintenance of plasma corticosterone concentration that was markedly elevated in ZDF controls.

Low-grade infection in obesity (23, 44, 67, 85, 211) is an important factor that induces insulin resistance. During volitional exercise, in association with improvement in glycemia, we also observed decreased circulating markers of inflammation and oxidative stress [circulating IL-6, haptoglobin, MDA levels, and hepatic protein oxidation, phosphorylated JNK (pJNK)]. Ser^Thr-phosphorylated IRS-1 and PEPCK were also reduced in the liver of exercised rats (103). During each bout of exercise, plasma corticosterone increased, but, due to adaptation, basal cortisol is decreased. We hypothesized that the beneficial effects of exercise are due, in part, to the fact that the magnitude of corticosterone decreased to exercise with repetitive stimuli, leading to a decrease of basal corticosterone. Thus, adaptive mechanisms that normalize plasma corticosterone, increased muscle utilization of glucose and decreased inflammation, all contribute to the preservation of β-cell function and the prevention of diabetes (Fig. 2) (25, 97 and 103).

**Effect of a single bout of stress on glucoregulation.**

We established that one bout of intense stress (infusion of carbachol into the third ventricle of the brain) in dogs can increase glucose utilization. This is lacking in diabetes, leading to excessive hyperglycemia. However, in contrast to intense continuous stress, adaptation to mild, repetitive, neurogenic stress can even prevent or delay the onset of diabetes in animal models of obesity and diabetes.

The effects of acute and chronic stress are wide-ranging, and it is well known that they can markedly offset metabolic control in diabetes. Together with graduate students Miles and Lekas and post-docs Zhi-Qing Shi (Medical Director at Am phstar Pharmaceuticals in California), and Keiichi Yamatani (Ni gata University, Japan), we induced acute stress in dogs by infusing carbachol into a cannula placed into the third ventricle of the brain (icv) (129, 130). Carbachol injections mimic the muscarinic action of acetylcholine. Such an injection induces a release of all counterregulatory hormones (cortisol, catecholamines, and glucagon). Intracerebroventricular infusion of somatostatin decreased all counterregulatory responses (130), indicating that somatostatin is an important regulator of counterregulation in the brain. In normal dogs, this stress induces a large increase in hepatic glucose production, with only a 5%
change in glucose concentration. Glucose homeostasis is maintained because, paradoxically, stress induces an increase in glucose utilization that is independent of insulin. This was the first demonstration of a putative neuroendocrine pathway that can increase peripheral glucose utilization independently of insulin. In contrast, in diabetic dogs, stress induced a major increase in glucose concentration, because glucose production increased but glucose uptake remained unchanged. Resistance to stress in this model of diabetes reflects a chronic defect that could not be improved by acute hyperinsulinemic euglycemic clamps. However, similarly to exercise, β-blockade partly restored the response of glucose uptake to stress, as shown with Lekas and Rashid (111, 154). This opened the question whether β-blockade may be useful in diabetes during certain stress conditions.

Paradoxically, in contrast to one bout of stress, repeated intermittent stress can also prevent diabetes. Very much to our surprise, we found that adaptations to intermittent, mild, neurogenic stress can prevent the onset of diabetes, similarly to exercise training in the ZDF rats. This was very difficult to explain to clinicians, and the challenge still remains how to translate these findings with respect to clinical practice. It is known that stress can worsen diabetes and can induce HPA axis hyperactivity. However, the differences between chronic and intermittent stress are seldom considered. We have shown (much to our surprise), together with Holly Bates (a PhD student) that intermittent restrained stress (daily 1-h confinement in a plastic case 5 days/wk) delays development of hyperglycemia in ZDF rats while normalizing basal corticosterone. A beneficial effect of the reduction of food intake due to stress was previously reported in Otsuka Long-Evans Tokushima fatty rats (96). It was important to explore whether stress by itself, in addition to the reduction of food intake, further delays the onset of hyperglycemia. We assessed the HPA axis and found that, initially, intermittent restrained stress increased glycemia and plasma corticosterone levels temporarily, but this response adapted over time. Intermittent restraint further delayed hyperglycemia independently of food intake. This was mediated by increased hippocampal mineralocorticoid (MR) mRNA, reduced anterior pituitary proopiomelanocortin (POMC) mRNA levels, and lower adrenal sensitivity to ACTH. This prevented basal and stress-induced hypercorticosteronemia. In contrast, 24-hour catecholamines were unaltered, indicating that corticosterone, but not catecholamines, is responsible for this beneficial effect. Thus, rather than playing a causal role, intermittent stress delayed deterioration in glycemia and ameliorated HPA hyperactivity in the ZDF rats (10, 11). This concept may have implications for many diseases and not only for diabetes. The first researcher that indicated the beneficial effects of some stresses (ustress) was Hans Selye from McGill University (161).

Intermittent stress also had a beneficial effect on pancreatic morphogenesis. Initially, as expected, in controls the pancreata of the obese rats had islet hyperplasia and neogenesis, but eventually the β-cell mass declined. Interestingly, both stress and food restriction partially preserved β-cell mass via islet hyperplasia and hyperplasia. With stress, improved glucoregulation was due to the preservation of β-cell mass, maintenance of insulin response to glucose, and reduction of α-cell mass. We could demonstrate that part of the β-cell hyperplasia (BrDU + β-cells) originates from the duct cells (11), as we had already observed 50 years before (187). Food restriction is a mild stressor and worsens the diabetic-induced glucocorticoid (GR) excess. In contrast, adaptation to intermittent psychogenic stress prevents the worsening of GR levels. This illustrates that intermittent exposure to stressors and adaptations is important for normal physiological functioning by preparing the body to deal with stress (10) (Fig. 3 and Ref. 10). A number of studies suggest that some stressors are deleterious to glycemic control in diabetics (3, 73, 175); however, positive life stress such as job promotion or a wedding, is associated with improved glycemic control (120, 174).

In summary, we hypothesized that the beneficial effect of exercise in improving β-cell function in diabetes includes the
prevention of HPA hyperactivity, increased glucose utilization, and decreased inflammation in the liver and blood. In contrast to a single bout of stress, paradoxically, repetitive, mild, neurogenic stress can also prevent the onset of hyperglycemia in ZDF rats.

**Struggle to Quantify the Relative Importance of Portal and Peripheral Insulin in Regulating Glucose Production**

Our work and the work of others have conclusively established that the indirect effects of insulin play an important role in the regulation of glucose production in dogs, and we provided the evidence in human subjects. Most importantly, we demonstrated that in type 2 diabetes the indirect effects of insulin play the main role in the control of glucose production.

The isolated liver is relatively insensitive to insulin. Levine and Olefski indicated that the indirect effect of insulin could play an important role in vivo (113, 145). Bergman published a watershed paper in this field, indicating that in normal dogs the peripheral infusion of insulin is more important than the portal infusion of insulin in controlling glucose production (1). At that time, this was considered unorthodox, and he had great difficulty in publishing his paper until he sent it to the American Journal of Physiology (AJP) at the time when I was an associate editor. Finally, his ground-breaking hypothesis was generally accepted, but there are still some open questions about the relative importance of peripheral and portal insulin infusion in regulating glucose production (116). It was important to find out whether this also applied to diabetic and nondiabetic dogs and human subjects. Together with Adria Giacca (from Italy and now a professor in our Department), we demonstrated for the first time that, in diabetic dogs, a peripheral infusion of insulin is more effective in suppressing hepatic glucose production than a portal infusion and that the peripheral effects include the suppression of FFA and glucagon release (71). This was surprising, since the general belief was that increased glucose production in diabetes is partly due to peripheral insulin injections, which deliver relatively less insulin to the liver. We were fortunate that Gary Lewis (presently Director of the Endocrinology Division in our medical school) was interested in investigating this problem with Giacca and me. Previously, he had developed a new methodology to assess the effect of portal insulin infusion, since in humans it is not possible to infuse insulin intraportally (114). Although a number of laboratories investigated this problem in dogs, we were the only ones testing it in normal and diabetic humans. We concluded that in normal humans both peripheral signals and direct hepatic insulin effects are important (115). The main peripheral signal is insulin suppression of glucagon and FFAs in dogs and humans (115). For the first time, we also concluded that in type 2 diabetes the sustained suppression of glucose production is due exclusively to peripheral signals (117). This is of considerable clinical importance. Further studies of the mechanisms of the effect of portal and peripheral infusion of insulin in dogs have been conducted by Bergman (131, 157) and by Cherrington (167, 168). Giacca further demonstrated that FFAs have a direct effect on glucose production via increasing glucose-6-phosphatase activity and an indirect effect via inducing insulin resistance (108). FFA activates PKC ε (109), decreases hepatic insulin clearance (208, 212), and impairs inflammatory pathways, insulin signaling (138), and β-cell function (125). In an elegant study, MD/PhD student Simon Fisher (presently an assistant professor at Washington University, St. Louis) demonstrated that, in liver-specific insulin receptor knockout mice, insulin fails to suppress glucose production, indicating that both the direct and indirect effects of insulin require an intact liver insulin signaling pathway. This opened up a new aspect of the relative roles of direct and indirect insulin effects on the liver (65).

In summary, it is interesting that both peripheral and portal effects of insulin control glucose production and that in diabetes it is mainly the peripheral effects. It is still a challenge to characterize in more detail this defect in type 2 diabetes, which can be of considerable clinical importance.

**Disputes Regarding the Protection Against Excessive Hyperglycemia in Muscle and Liver**

We explored the mechanisms whereby the liver and muscle are protected against hyperglycemia and hypoglycemia. In the muscle this is due to the effect of hyperglycemia on GLUT-2 gene and protein, and in the liver, to increased glucose cycling.

**Muscle.** In my Berson lecture to the American Physiological Society (1955), I discussed how the ancient Chinese philosophy of Yin and Yang can be used as a metaphor for the balance between positive and negative feedbacks. In a way, this is the basis of physiology, where regulation of homeostasis is defined by a series of negative and positive feedbacks at the level of the organism, the organ, and the cell. It is well known that in

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**Fig. 3. Mechanisms of the effect of intermittent stress in the prevention of hyperglycemia in ZDF rats. A: reduced food intake alone (pair feeding) ameliorates hyperglycemia, but this is counteracted by the hyperglycemic effects of increased adrenal secretion of nadir corticosterone. B: intermittent stress ameliorates hyperglycemia through reducing food intake but further improves glycemia by preventing the increase in adrenal sensitivity and increasing hippocampal glucocorticoid feedback, thus preventing the increase in nadir corticosterone.**
diabetes glucose tolerance is decreased unless insulin supply mirrors insulin release following food intake. At the time, it was considered that these insulin spikes were essential for increased glucose uptake. For the first time, we showed that glucose concentration in depancreatized insulin-infused dogs during glucose tolerance tests was gradually decreasing, showing that glucose itself, without an insulin spike, can promote glucose uptake (glucose efficiency) (189). Bergman extensively reviewed the importance of glucose efficiency that is equally important in humans as it is in dogs (17).

Our proposal was that, in some organs, decreased glucose efficiency is not a defect but rather a protective mechanism against diabetic complications. Glucose utilization is a result of opposing forces related to the effect of glucose itself and to the interaction between the effects of glucose and insulin. These interactions are dynamic and reflect a continuum of synergistic and contrasting processes. Most of the complications associated with diabetes are due to chronic elevation of plasma glucose. Through mass action, an excessive amount of glucose enters a variety of tissues, resulting either in the glycosylation of many proteins or in the augmentation of otherwise insignificant metabolic pathways.

In diabetes, glucose uptake in the muscle is not necessarily decreased. This hypothesis can be tested by measuring metabolic glucose clearance (MCR), which represents the ratio of glucose utilization to plasma glucose concentration, and it reflects the efficiency of glucose extraction by the tissues. Together with Hetenyi and post-doc Clement Gauthier (now Executive Director of the Coalition for Biomedical and Health Research, Ottawa), we demonstrated that in diabetic dogs the chronic application of phlorizin normalizes glucose concentration and that this leads to the normalization of defective glucose clearance (84). In experiments performed with Fisher, we also demonstrated that during exercise the severe defect of MCR in diabetic dogs is fully restored by an acute normalization of glucose by phlorizin and that this is independent of FFA turnover and insulin levels. As in other experiments, we concluded that in diabetic dogs the impaired metabolic clearance is an adaptive phenomenon, because the correction of hyperglycemia corrects MCR despite partial insulin deficiency and high FFA turnover. This confirmed the concept that constant glucose uptake, despite hyperglycemia in diabetes, protects the muscle from excessive exposure to glucose (64). With Klip, Shi, and graduate student Mathoo, we demonstrated that the same mechanisms also work in the isolated hindquarter of the rat. In the total absence of insulin and by appropriate infusions of glucose, we maintained plasma glucose concentrations normal, hypoglycemic, or hyperglycemic. With hyperglycemia, the number of glucose transporters in the plasma membrane decreased, whereas with hypoglycemia it increased. This was reflected by increased or decreased rates of MCR while glucose uptake remained constant. Thus, during rest and exercise, the muscle is protected against hypoglycemia or hyperglycemia (Fig. 4) (126 and 127). Protection of muscle against hyperglycemia can result in overall increased blood sugar, which could affect other organs to postdiabetic complications.

Liver and glucose cycling. Glucose uptake in the liver is modified by three non-equilibrium reactions: the glucose cycle, the fructose 6-phosphate cycle, and the phosphoenolpyruvate cycle. Together with Suad Efendic (former Chair of the Department of Molecular Medicine at the Karolinska Institute, Stockholm), we investigated the effect of diabetes in the regulation of these cycles. We were particularly interested in the glucose cycle, in which entry of glucose is accelerated by the enzyme glucokinase, which yields glucose 6-phosphate. Some of the glucose 6-phosphate is then cycled back into glucose through the enzyme glucose-6-phosphatase. Therefore, through this mechanism a small amount of glucose taken up by the liver is recycled into the blood stream. We found that the activity of glucose cycling was increased in depancreatized and alloxan-induced diabetic dogs, in lean and obese type 2 diabetic subjects (55, 56), and in acromegaly (98) and hyperthyroidism. It is particularly important that dexamethasone increases glucose cycling but not glucose production (198). In type 2 diabetic subjects, the percent increase in postabsorptive glucose cycling is more marked than the increase in glucose production. Therefore, we suggested that measurement of glucose cycling in addition to glucose production could be a valuable tool to assess the early metabolic derangements of glucose intolerance. This is still a topic to be further investigated. The mechanism of increased glucose cycling
in depancreatized dogs was then investigated (162). With Shi, Giacca, and van der Werve (former Chairman of the Department of Nutrition, University of Montreal), we measured liver enzymes by biopsy and glucose cycling by the double-tracer method during anesthesia. We concluded that increased hepatic glucose cycling from diabetes is mainly due to the increase of substrates for glucokinase and glucose-6-phosphatase originating from hyperglycemia rather than from changes in the total amount of enzymes. This has some similarity to autoregulation in muscle, because, again, glucose concentration by itself can regulate part of its rate of entry into the liver. The most memorable event related, in part, to our collaboration with our colleagues at Karolinska was the honorary doctorate that I received from their prestigious university.

In summary, the fact that the muscle and the liver can be protected against changes in plasma glucose is important, but the mechanisms need to be further explored.

Skirmish Regarding the Mechanisms of Hypoglycemia and Its Prevention

We describe molecular mechanisms responsible for the activation of the HPA axis in diabetes and for diminished responses of HPA axis, catecholamines, and glucagon to hypoglycemia. We propose a new approach to decrease the threat of hypoglycemia.

Deficient counterregulatory responses to hypoglycemia. A major acute complication of diabetes is a defective response of glucagon, catecholamines and GRs to insulin-induced hypoglycemia. This occurs more frequently with antecedent hypoglycemia and hypoglycemia unawareness, and it is in part due to poor counterregulatory responses (21, 38, 40, 41, 45, 59, 69, 74, 207). The threat of hypoglycemia has increased since the treatment for diabetes has aimed for tight blood glucose control to decrease the risk of diabetic complications. In order to avoid hypoglycemia, many diabetic patients reduce their blood glucose control. Thus, hypoglycemia is a limiting factor for proper control of glycaemia. Therefore, it is important to develop a treatment strategy that would decrease the risk of hypoglycemia. The defect of glucagon and epinephrine responses to hypoglycemia in diabetes is puzzling, because both counterregulatory responses are normal or even excessive during some stresses, such as moderate and strenuous exercise, in both dogs and humans (124, 204). With Rastogi and Efendic, we showed that, although in each islet the number of glucagon cells is greatly increased, the total amount of glucagon in the pancreas remains unchanged because of the reduction in the number of islet cells. Clearly, alloxan or streptozotocin not only destroys β-cells but also reduce the total number of islet cells. It is well known that the release of glucagon by the pancreas is inhibited by both insulin and somatostatin. Since most β-cells have been destroyed, somatostatin is the main paracrine inhibitor of the α-cell in diabetes. That is why it was of particular interest that in diabetic dog islets the ratio of somatostatin to glucagon is markedly increased. An acute insulin injection increased this ratio further. This was the first demonstration that part of the defective mechanism in hypoglycemia may reflect alterations of this ratio in diabetes (155). We hypothesize that in diabetes, islet α-cells are overly sensitive to insulin and are exposed to increased somatostatin and therefore do not respond to hypoglycemia. However, α-cells respond to other stimuli in the absence of hyperinsulinemia and despite increased somatostatin. Presently, we are investigating with a graduate student, Jessica Yue, the effect of a specific antagonist (SSTR2) of the somatostatin receptor of the α-cells. We have demonstrated that infusion of this antagonist can fully normalize glucagon responses to insulin-induced hypoglycemia in diabetic rats (214) (Fig. 5 and Ref. 214). We filed a patent (196) for the prevention of hypoglycemia, and we hope that the results obtained in the rats can be applied to human diabetics and thereby diminish or prevent hypoglycemic episodes. This could permit diabetic patients to adhere more strictly to an intensive insulin treatment and lessen the risk of diabetic complications.

Another factor that affects α-cells is chronic hyperglycemia. With Shi (163), we demonstrated that the defective glucagon responses are in part due to chronic hyperglycemia. Normalization of hypoglycemia without, but not with, insulin restored, in part, glucagon’s responsiveness in diabetic rats. This occurs because hyperinsulinemia can offset the beneficial effect of normalization of glucose.

Abnormalities of the HPA axis in insulin-treated or uncontrolled diabetic rats. The main feedback mechanism of the HPA axis is the effect of cortisol on the receptors in the hypothalamus and pituitary. An equally important mechanism is the inhibition that the hippocampus exerts on corticosterone-releasing hormone (CRH) release (Fig. 6 and Ref. 29). The collaboration with Steve Matthews (presently Chairmain of our Department) gave me the exciting opportunity to study the expression of the stress gene by using the method of in situ hybridization. Together with graduate students Owen Chan (presently at Yale) and Karen Inouye (presently at Harvard), we investigated by in situ hybridization the gene expression of GR and MR receptors in the hippocampus, CRH and GR in the paraventricular nucleus (PVN), GR and POMC in the pituitary, and the expression of rate-limiting enzymes involved in catecholamine synthesis in the adrenal medulla. Activation of the HPA axis in diabetes (28) is due in part to decreased GR

1. Normal

[Diagram of Normal

2. Diabetes: hypoglycemia

[Diagram of Diabetes: hypoglycemia

3. Diabetes: Hypoglycemia + Somatostatin Antagonist

[Diagram of Diabetes: Hypoglycemia + Somatostatin Antagonist

Fig. 5. In the nondiabetic islet, the α-cell is inhibited by insulin from the β-cell and somatostatin from the δ-cell. In diabetes, during hypoglycemia, there is increased sensitivity to the α-cell by insulin and an increased inhibition by somatostatin; therefore, the response of the α-cell to hypoglycemia is greatly diminished. We hypothesize that somatostatin receptor antagonist can at least partially restore the response of the α-cell to hypoglycemia despite the inhibitory effect of insulin (214).
negative feedback sensitivity. In addition, impaired stress responsiveness of diabetic HPA involves decreased pituitary and adrenal sensitivity (28). Insulin treatment normalizes the concentration of ACTH and corticosterone (28). In contrast, normalization of glucose with phlorizin (inhibition of glucose reabsorption in the kidney) does not normalize the HPA axis. Most importantly, this defect is not due to hyperglycemia per se, but it is due to insulin lack with or without hyperglycemia (30). This could have clinical implications.

Normally, hypoglycemia stimulates the release of glucagon, catecholamines, and corticosterone (counterregulation). It is remarkable that in diabetic rats the increased activation of the HPA axis (gene expression of CRH and POMC) is mainly due to a decrease of MR, thus alleviating the inhibition from the hippocampus on the hypothalamus. In diabetes, responses of all counterregulatory hormones to hypoglycemia are decreased. Interestingly, normalization of glycemia with phlorizin normalized the response of the HPA axis to hypoglycemia. Thus, this defect in diabetes is not due to insulin lack but only to chronic hyperglycemia (30). During hypoglycemia, and in contrast to control animals, there is no increase in CRH or POMC, and MR is not suppressed in diabetic rats.

Antecedent hypoglycemia reduces counterregulation both in normal and diabetic rats. One hypothesis was that this is due to antecedent cortisol bouts during the antecedent episodes of hypoglycemia (41). However, we could not confirm this in normal rats. We hypothesize that the effects of repeated bouts of antecedent hypoglycemia are complex, including excessive insulin concentration, hypoglycemia itself, and increases of catecholamines and cortisol. Therefore, cortisol in hypoglycemia would only be a team player (164). One important defect of antecedent hypoglycemia is a very poor response of epinephrine to hypoglycemic episodes (88, 89). We have shown that the gene expression of tyrosine hydroxylase in the adrenal medulla is decreased in diabetic rats, which could, at least in part, explain the defective epinephrine release. During antecedent hypoglycemia there is additional decrease of phenylethanolamine-\(N\)-methyltransferase (PNMT), which further explains the augmented epinephrine defect, due to antecedent hypoglycemia (89).

Chronic insulin treatment of diabetic rats prevents most counterregulatory defects but not defects in HPA function. We suggested that the protective effect on epinephrine counterregulation is due to restored adrenal catecholamine synthesizing capacity (90). This raises the possibility that targeting adrenal catecholamine’s synthetic capacity may be a means of improving hypoglycemic counterregulation in type 1 diabetes. The absent corticosterone response to a bout of hypoglycemia was associated with an impaired rise in CRH mRNA, which presumably resulted from the inability to decrease the inhibitory input from hippocampal MR. In contrast, hippocampal GR mRNA decreased. This suggests that the central MR receptor system plays a prominent role in modulating the stress response to hypoglycemia (91).

When Chan moved to Yale, he demonstrated that, in addition to the paraventricular nucleus, the ventromedial hypothalamus plays a key role in the control of the release of glucagon. He demonstrated that the inhibitory neurotransmitter \(\gamma\)-aminobutyric acid (GABA), acts within a crucial glucose-sensing region of the brain, the ventromedial hypothalamus (VMH), to suppress glucagon and epinephrine release (31). The release of GABA in the VMH is governed by ATP-sensitive potassium channels in a similar manner to insulin secretion in pancreatic \(\beta\)-cells and is directly correlated to ambient glucose levels (32, 215). During hypoglycemia, the fall in plasma glucose levels decreases VMH GABA release, and this allows for activation of the counterregulatory hormone responses (33). Most importantly, he now has evidence that an increase in GABAergic neurotransmission within the VMH contributes to counterregulatory failure in diabetes (33). Fisher outlined a number of factors important in responses to hypoglycemia, such as insulin signaling (22, 46, 66), and central lipid infusion (82). As
previously mentioned, recurrent hypoglycemia greatly increases the threat of hypoglycemia. On the other hand, Fisher indicated that it also provides some beneficial adaptive responses by preconditioning the brain (146).

Effect of recurrent stress on responses to hypoglycemia.

Together with Yue and Goche Montes, we explored whether recurring restraint stress also increases the threat of hypoglycemia similarly to the effect of episodes of antecedent hypoglycemia. It is of clinical importance to understand the differences between antecedent hypoglycemia and antecedent stress. Indeed, in diabetic rats, these two effects (antecedent hypoglycemia and antecedent stress) were comparable with respect to decreasing responses of the HPA axis to hypoglycemia. The defect correlated with decreased basal gene expression of PVN arginine vasopressin and the anterior pituitary POMC mRNA. However, in contrast to antecedent hypoglycemia, recurrent restraint stress did not impair catecholamines counterregulation (213).

Epilogue

This career retrospective has described how our glucose turnover studies permits us to study the effect of hormonal interactions on glucoregulation, both in animals and in human subjects with or without diabetes. We concentrated on the pathogenesis of diabetes, exercise, stress, and hypoglycemia. There are still many questions that remain unanswered and hypotheses to be tested. From a clinical point of view, it is vital to further explore the mechanisms whereby adaptations to short bouts of exercise and stress have beneficial effects. From a clinical point of view, it would be important to clarify how a daily short bout of exercise can have such long-term beneficial effects. There is some clinical evidence showing a difference between continuous negative effects of stress and positive effects of adaptation to stress; however, these clinical observations are not yet substantiated. Our hypothesis of the beneficial effect of decreased basal cortisol and of adaptation to short spurts of cortisol release still needs to be further explored. The effects of the direct and indirect effects of insulin on glucose production in type 2 diabetes should be further explored. One aspect of insulin resistance could be clarified if we knew more about how glucose by itself affects translocation of glucose transporters in the muscle. Clinically, it is still not ascertained how important increased hepatic glucose cycling is as an early marker of the metabolic syndrome. The prevention of hypoglycemia remains a key goal in insulin-treated diabetics in order to facilitate the intense treatment modalities. The complexities that control the function of the pancreatic α-cell have not yet been fully explored. In addition to the work in our laboratory, I have tried to show how my students and fellows did incisive research after leaving the laboratory. They were able to incorporate molecular methods of signaling and genetics with a large variety of methods including transgenic and knockout models. I consider that the success and impact of my students and fellows reflect the most important achievement of my scientific career. It is also with the greatest pleasure that I follow the work of my previous collaborators. In the years to come, I plan to address some of the unexplored questions by collaborating with my colleagues.

With respect to Frost’s poem, my mother would have preferred if I had taken the more traveled road as a “real” doctor. Nevertheless, her confidence in the road I chose had no limits. My father taught me how to survive the dangers of Scylla and Charybdis.

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