Metabolic Syndrome and Robustness Tradeoffs

Hiroaki Kitano,[1,2,3] Kanae Oda,[4] Tomomi Kimura,[2,4] Yukiko Matsuoka,[5] Marie Csete,[6] John Doyle,[7] and Masaaki Muramatsu[4]

The metabolic syndrome is a highly complex breakdown of normal physiology characterized by obesity, insulin resistance, hyperlipidemia, and hypertension. Type 2 diabetes is a major manifestation of this syndrome, although increased risk for cardiovascular disease (CVD) often precedes the onset of frank clinical diabetes. Prevention and cure for this disease constellation is of major importance to world health. Because the metabolic syndrome affects multiple interacting organ systems (i.e., it is a systemic disease), a systems-level analysis of disease evolution is essential for both complete elucidation of its pathophysiology and improved approaches to therapy. The goal of this review is to provide a perspective on systems-level approaches to metabolic syndrome, with particular emphasis on type 2 diabetes. We consider that metabolic syndromes take over inherent dynamics of our body that ensure robustness against unstable food supply and pathogenic infections, and lead to chronic inflammation that ultimately results in CVD. This exemplifies how trade-offs between robustness against common perturbations (unstable food and infections) and fragility against unusual perturbations (high–energy content foods and low–energy utilization lifestyle) is exploited to form chronic diseases. Possible therapeutic approaches that target fragility of emergent robustness of the disease state have been discussed. A detailed molecular interaction map for adipocyte, hepatocyte, skeletal muscle cell, and pancreatic β-cell cross-talk in the metabolic syndrome can be viewed at http://www.systems-biology.org/001/003.html. *Diabetes* 53 (Suppl. 3):S6–S15, 2004

ROBUSTNESS AS A FUNDAMENTAL ORGANIZATION PRINCIPLE

From a systems perspective, all living organisms share a notable feature—a high level of robustness against external and internal perturbations. Robustness is one of the fundamental organizational principles of biological systems and the robust design of biological systems mediates short- and long-term survival, reproduction, and evolution. In a systems biology construct, diseases are viewed as breakdowns of robustness in biological systems, and disease is perpetuated if damage to mechanisms that maintain robustness cannot be repaired. Because robustness mechanisms are at the core of normal function, sustained disease processes that further impact interconnected homeostatic mechanisms that maintain normal function lead to end-organ failure. Similarly, some disease processes can be viewed as taking advantage of evolved robustness strategies to maintain a disease state or further its progression. It is critical to understand that robustness is a foundation of system function in both biology and complex engineered systems. The concept of robustness can be defined as the property of active maintenance of specific function despite external and internal perturbations. The automatic flight control system (or autopilot) in modern aviation is a typical example of robust systemic function. The automatic flight control system maintains a flight path against fluctuations in a wide variety of atmospheric and vehicle conditions, using extensive and carefully designed feedback controls. In modern aircraft, automatic flight control systems are purposefully separated on multiple (often three) computers. The computer systems perform identical functions interpreting data from sensors but implement their responses to the data independently, ensuring redundancy of the control system. Failure of one computer can be detected and compensated for by the remaining functional computers. Heterogeneity of hardware and software implementation is further used to avoid common mode failures in which all three computers fail due to the same cause, such as a software bug.

In general, robustness of the system is manifested as adaptation to the environment and as stability against external and internal disturbances. These properties are enabled by feedback control, redundancy, modularity, and structural stability (1,2). The design features of engineered control systems have parallels in biological systems and are informative for robust properties observed ubiquitously in biological processes across species (3–12). Biological parallels to these engineering examples include massive amounts of feedback control that maintain core body temperature despite changing weather and energy expenditure. As in control systems design, many gene products are functionally redundant. Cellular sensing and repair of misfolded proteins are also highly regulated using control systems and conserved across biological systems. While robustness is generally regarded as a means to protect and maintain normal, complex functions, designs that mediate robustness also have drawbacks.

First, systems that evolved to be robust against various

From the [1]Sony Computer Science Laboratories, Inc., Tokyo, Japan; [2]Systems Biology Institute, Tokyo, Japan; [3]Keio University, Tokyo, Japan; [4]Medicala Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; [5]ERATO-SORST Kitano Symbiotic Systems Project, Japan Science and Technology Agency, Tokyo, Japan; [6]Anesthesiology Department, Emory University, Atlanta, Georgia; and [7]Control and Dynamical Systems, California Institute of Technology, Pasadena, California.

Address correspondence and reprint requests to Hiroaki Kitano, Sony Computer Science Laboratories, Inc. 3-14-13, Higashi-Gotanda, Shinagawa, Tokyo 141-0022 Japan. E-mail: kitano@csl.sony.co.jp.

Received for publication 23 February 2004 and accepted in revised form 12 April 2004.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

CVD, cardiovascular disease; FFA, free fatty acid; IL, interleukin; PPAR, peroxisome proliferator–activated receptor; TNF, tumor necrosis factor; TZD, thiazolidinedione.

but common perturbations exhibit extreme fragility against unusual, unanticipated perturbations (13,14). Aircraft with modern avionics are highly robust against atmospheric perturbations, but are extremely fragile to highly unusual perturbations such as complete power failure or software bugs. Thus, the computers and automatic controllers make aircraft safer but the designs that mediate the robustness and safety introduce new potential flaws that were nonexitent in old-fashioned airplanes. Enhanced robustness of modern aircraft is accomplished with acknowledged tradeoffs, rare fragilities to critical system failures. The universality of robustness in biology suggests that robustness itself is most likely a conserved quantity (15).

A second implication of robustness design is that mechanisms that provide robustness and protect normal functions in a dynamic environment may also be used to maintain abnormal states. When fundamental robustness mechanisms are coopted by the disease process, the disease will be difficult to cure. Cancer, for example, can be viewed as confiscation of cell cycle regulation, which empowers cancerous cells with a highly robust regulatory system with high level of redundancy, and feedback loops for survival and proliferation. The necessary design of cell cycle regulation provides robustness in many forms, including the ability to regenerate after trauma, but these same mechanisms for creating robustness can be hijacked to promote cancer (16,17). In this context, many diseases can be viewed as exposed fragilities in normal biologic design, with disease progression mediated by acquisition of established and emergent robustness mechanisms.

In this article, we argue that the metabolic syndrome (18–21) can be understood in the context of robust system design: a result of physiological and cellular responses to the complex, built-in feedback regulation governing robust maintenance of glucose homeostasis and buffering. These homeostatic mechanisms are first disrupted in the early stages of disease, then used in interlocked positive feedback loops that maintain and aggravate the syndrome, finally leading toward cascading failure of various critical end-organs.

THE ENERGY SUPPLY SYSTEM AND ITS ROBUSTNESS

The "system" involved in metabolic syndrome is an energy supply system, used by all organs for powering physiologic function. Because brain stores glycogen sufficient only to survive for a few minutes without blood flow, a reliable supply of glucose is vital for sustaining life. Although the relationship between the immune system and glucose is not completely understood, malnutrition is a major risk factor for immune-deficiency in developing countries. Studies relating high level of glucose uptake and stimulated proliferation in macrophages (22,23) indicate potential important target pathways relating glucose homeostasis to immune function. Multiple regulatory feedback loops are involved in control of food intake and satiety, glucose homeostasis, and energy storage to essentially ensure stable glucose supply for widely varying energy demands. Figure 1 illustrates the overall regulatory relationships of these components. This system can be viewed as an integrated system with several interrelated control subsystems, as outlined below.

Energy intake control. Negative feedback loops regulate

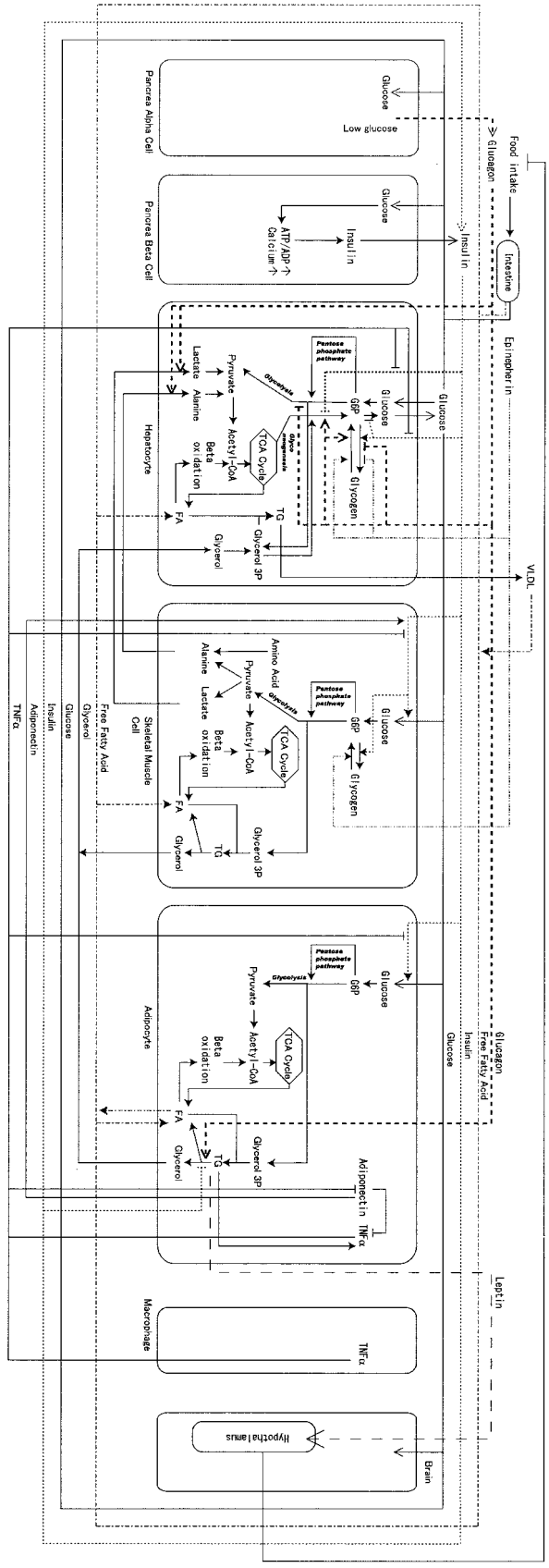

FIG. 1. Schematic illustration of energy control system regulation relevant to diabetes and the metabolic syndrome.

appetite with numerous hormonal messengers. Leptin secreted by adipocytes and ghrelin by the stomach are important peripheral mediators of appetite; they are controlled at the level of the hypothalamus (24–26), which integrates a variety of signals, along with the limbic system and cortex, and then actuates a response to control regulation of stress hormones versus anabolic hormones. This is an obviously simplified description of energy intake control but nonetheless reveals that appetite is a subsystem, complex in itself, controlled by intracellular, intercellular, and inter-organ feedback regulation as well as transcriptional and posttranslational stability strategies.

Energy storage control. Multiple feedback regulations control energy storage. Insulin-dependent glucose uptake in hepatocytes, skeletal muscle cells, and adipocytes forms a negative feedback regulation to control plasma glucose level by promoting energy storage. Insulin is secreted from pancreatic β-cells in response to plasma glucose elevation (27), mediating this regulatory mechanism. The insulin signaling pathway incorporates dynamics of adaptation through a negative feedback loop via SOCS3-mediated downregulation of insulin receptor substrate (IRS)-1 (28–30), but whether such regulation is physiologically relevant has not been confirmed. In the long term, this negative feedback is further modulated by free fatty acids (FFAs) and tumor necrosis factor (TNF)-α, leading to insulin resistance.

Energy production control. At low plasma glucose levels, pancreatic α-cells secrete glucagon that stimulates hepatic glucose production, forming a negative feedback regulation. There is a cascading recycling process, too. Adipocyte lipolysis releases fatty acid (FA) and glycerol into the blood, which are taken up by skeletal muscle cells for FA-based β-oxidation. After metabolism switches to anaerobic glycolysis, muscle secretes lactate and alanine, which are taken up by hepatocytes and eventually converted into glucose via gluconeogenesis.

Emergency energy supply control. As a contingency in emergencies, epinephrine is secreted to promote glycogenolysis in both hepatocytes and skeletal muscle cells to increase plasma glucose and glycolysis-based ATP production, respectively. These back-up reactions, a possible feed-forward regulation, ensure energy supplies in case of extreme physiologic stress.

Long-term control of glucose uptake. TNF-α, adiponectin, and insulin form major known regulatory feedback loops that define the long-term glucose uptake control. The role of TNF-α in metabolic control has been of major interest (31–33). When the balance of energy uptake and consumption shift toward accumulation of triglycerides in adipocytes, adipocytes grow in size. Increased adipocyte volume is associated with enhanced adipose tissue secretion of TNF-α, which in turn inhibits insulin signaling in adipocytes, skeletal muscle, and hepatocytes (34–37). This cascade of events results in insulin resistance. TNF-α inhibits both insulin-suppression of hepatic glucose production and insulin-mediated peripheral glucose utilization. Adiponectin (also known as Acrp30) (38) inhibits TNF-α and promotes insulin-simulated glucose uptake in skeletal muscle cells. TNF-α and adiponectin are mutually inhibitory (39).

Thus, multiple feedback control loops enable stable supply of glucose and maintain glucose homeostasis. The system is robust to changes in food availability and intake. Essentially, complex regulation insulates plasma glucose from intermittency of food intake by controlling storage and production of glucose. Nonetheless, this elaborate control system is overwhelmed by the combination of high-energy foods with low-energy utilization lifestyle.

Historically the glucose homeostatic mechanisms have been successful (robust) because they mediated stability in the face of food shortage to ensure organism survival. With variable food supply, logical controls theoretically include *1*) maximizing glucose intake from the environment, *2*) maximizing storage of glucose in a stable form, *3*) increasing glucose supply in response to low plasma glucose levels, *4*) recycling resources to maximize glucose production, *5*) tight regulation of glucose supply to avoid wasteful oversupply, and *6*) a built-in mechanism for emergency glucose supply. Not surprisingly, these mechanisms are actually part of the system regulation, each with well-known control loops (19). Thus, the energy control system uses feedback to remain remarkably robust in the face of an unstable food supply. The control system is overwhelmed, however, by the combination of high-energy foods with low-energy utilization lifestyle. In the context of robust energy supply, an interesting hypothesis can be postulated that insulin resistance may actively contribute to glucose homeostasis during malnutrition by preventing glucose uptake in case of infection and other acute needs, so that the immune and cognitive systems can function properly (40). TNF-α is secreted by macrophages and stimulates NK cells to secrete TNF-γ for antipathogenic effects. TNF-α is, thus, a reasonable indicator of mobilization of the innate immune system. As macrophage activation is associated with high glucose uptake (22,41,42), it is a reasonable system design to have TNF-α mediating negative feedback for maintaining plasma glucose by inhibiting insulin-dependent glucose uptake. In this regard, an acute and temporal insulin resistance may be a part of normal defense mechanisms to secure energy supply for an innate immune system. This idea could be generalized to adipose-dependent TNF-α secretion. With obesity, hypertrophied adipocytes attract macrophages, which produce TNF-α (43–45). Adipocytes also secrete TNF-α in response to CD36-mediated uptake of advanced glycation end products (AGEs) (46) and other pro-inflammation factors, which may correlate with a systemic need to mobilize immune responses. In essence, obesity becomes an inflammatory state. Adipose-dependent insulin resistance is a built-in mechanism of robust control of plasma glucose level to ensure energy supply for systemic immunological reactions.

The second priority of the energy system, because it is not an acute survival issue, is to cope with excessive food intake. Our interest in the context of the systems model is on chronic nutritional over-intake beyond equilibrium mechanisms that balance energy intake and consumption. In these historically extreme circumstances of overabundance of food, adipocytes, skeletal muscle cells, and hepatocytes increase triglyceride accumulation. Feedback loops that reduce plasma glucose by insulin-stimulated glucose uptake have a finite capacity, and when exceeded, triglycerides accumulate, generally leading to increased

leptin secretion, the mediator of a negative feedback loop controlling food intake. Appetite suppression is therefore a back-up mechanism for overabundance of glucose and accumulation of triglycerides but is impotent in the face of extreme glucose excess. Ongoing TNF-α secretion inhibits insulin signaling in hepatocytes, skeletal muscle, and adipocytes (47,48). TNF-α receptor engagement activates various signal transduction pathways, ultimately leading to serine phosphorylation of IRS-1 so that insulin signaling is attenuated. This constitutes another mechanism of insulin resistance. This feedback regulation is essential to prevent accumulation of triglycerides sufficient to cause cellular dysfunction. Induction of insulin resistance represents a mechanism to counteract the expansion of body fat and therefore limits obesity (33). Considered as a regulatory strategy, insulin resistance is a mechanism that establishes robustness of a subsystem against glucose overdose. Without this mechanism, cells continue to accumulate triglycerides, which damages adipocytes, skeletal muscle cells, and hepatocytes, the core components of energy storage and production. Insulin resistance then provides a window of opportunity during which reduction of cellular triglyceride content can start a recovery process in the cell and systemically.

ASYMMETRIC RISK PROFILE IN GLUCOSE HOMEOSTASIS REGULATION

The two regulatory feedback systems for glucose homeostasis are not directionally symmetric. Feedback provides tight control for glucose shortfall but loose control for overdosage. Multiple nested feedback loops are conserved across species to prevent low plasma glucose levels, but the feedback loop involving leptin and ghrelin does not counterbalance oversupply of glucose. The obvious question is why such asymmetry in glucose regulation has evolved. One likely possibility is that mammalian evolution has taken place mostly during near-starvation conditions, so that we are adapted to take in as much food as possible in that environment. This view is intuitively appealing and consistent with the idea of highly optimized tolerance (HOT) of complex systems, which postulates that systems robust against frequent perturbations can be extremely fragile to unusual perturbations (13,14). In this context, unstable food supply is a perturbation for which human systems are adapted and robust, but overnutrition is a historically unusual perturbation and exposes the fragility of the system design. Another explanation, not necessarily exclusive of the first, is that the metabolic syndrome is caused by asymmetric profiles of the risk of glucose shortage versus overdosage. Figure 2 illustrates this asymmetry.

Normally, blood glucose is maintained within a narrow range. Hypoglycemia induces neuronal damage within minutes, and irreversible, fatal damage occurs in minutes instead of hours. Thus, for the organism as a system, the effect of glucose deprivation is acute and catastrophic. (In this article, we refer to the glucose threshold at which acute damage occurs as the "glucose floor.") However, the true impact of chronic glucose overdosage emerges only after years of continuous exposure. The risk of damage increases gradually (years) as the glucose overdose persists. (The range of glucose levels that mediate this more chronic risk of damage is referred to as the "glucose ceiling" in this article.) During the course of evolution, mutations to break or change the glucose floor likely faced serious selection pressures because of the very nature of the acute threshold events. In contrast, mutations of glucose ceiling regulation must have received much less pressure due to the chronic and accumulative nature of adverse effects. Naturally, polymorphisms distributed toward the glucose ceiling instead of the glucose floor.

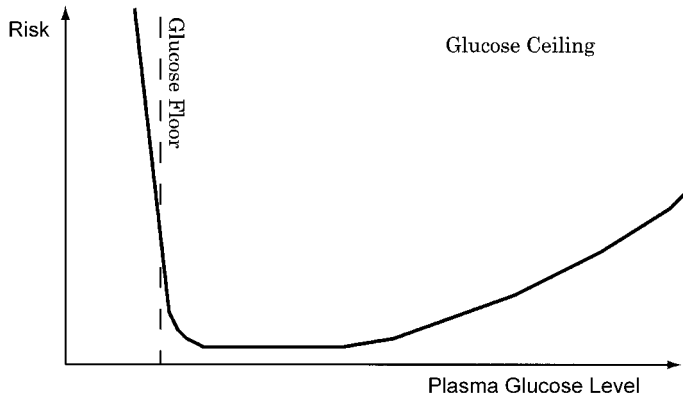

FIG. 2. Glucose floor and glucose ceiling.

METABOLIC SYNDROME AS HIJACKED ROBUSTNESS FOLLOWED BY CASCADING FAILURES

The metabolic syndrome exhibits its own robustness, preserving persistent hyperglycemia and hyperinsulinemia. However, preservation of the syndrome is ultimately confounded by devastating cardiovascular diseases (CVDs) (49). Disease is driven by complex, distinctively system-level dynamics that promote its progression. The implication of looking at disease progression in this light is that possible therapies may be modeled and identified by understanding system dynamics.

Onset: drifted equilibrium. The early changes in the metabolic syndrome are a flux balance drift defined by a discrepancy between energy intake and consumption, marked by gradual accumulation of intracellular triglycerides. Assuming that energy consumption is constant, balance is restored by decreasing food intake, controlled mainly by leptin- and ghrelin-mediated feedback loops. When the feedback gain is insufficient, the system is biased toward excessive energy intake. Genetic polymorphisms that affect the gain of this feedback loop tend to be biased for flexibility in the glucose ceiling instead of the glucose floor.

Phase I: triglyceride accumulation. As triglycerides continue to accumulate, TNF-α and leptin secretion also increases. Leptin does downregulate appetite via hypothalamic receptors but is impotent in the face of positive regulators of the metabolic syndrome. Elevated TNF-α reduces the efficacy of insulin signals, so that plasma glucose and insulin levels are both elevated. However, at early stages of disease, the dynamics of the system is kept relatively linear, so that reducing energy intake may result in reversing adipocyte hypertrophy, TNF-α secretion is reduced, and efficient insulin signaling is ultimately restored. Even with reversal of the insulin-resistant state, however, the natural history of the disease has already

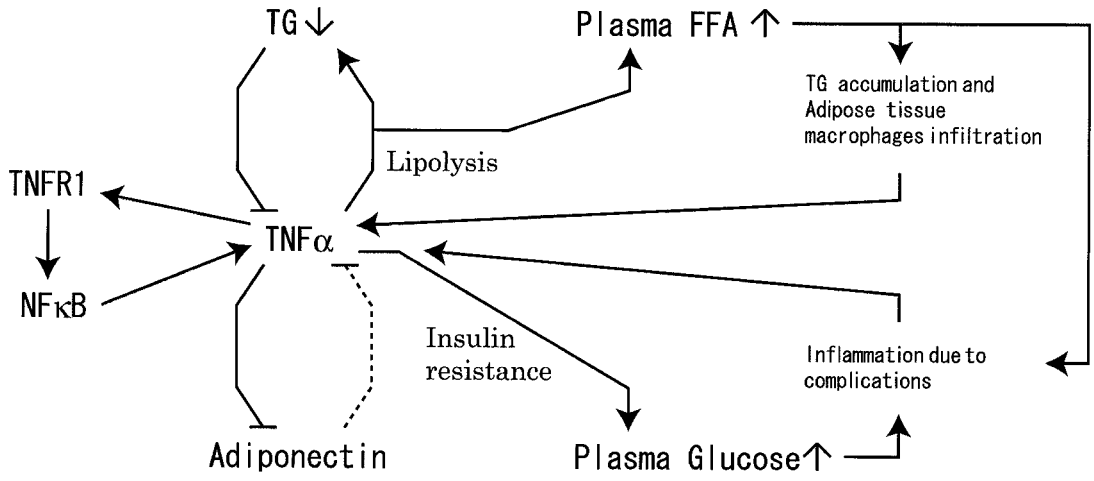

FIG. 3. Multiple feedback regulations involving TNF-α. TG, triglyceride.

been impacted by sustained elevated plasma glucose and insulin levels, increasing risk for cardiovascular disease.

Phase II: TNF-α switch activation. There are three regulatory feedback loops involving TNF-α that define the dynamics of this subsystem. First, there is mutually inhibitory regulation between TNF-α and adiponectin. As TNF-α secretion increases, it suppresses adiponectin secretion (50). Adiponectin may, directly or indirectly, inhibit TNF-α expression and/or secretion (51); however, the more potent TNF-α eventually inhibits adiponectin secretion (39). Second, there are multiple paracrine/autocrine positive feedback loops to upregulate TNF-α transcription via TNF-R1 cascades that eventually activate nuclear factor (NF)-κB, which in turn increases TNF-α transcription. This is a positive feedback loop to upregulate TNF-α. Third, TNF-α secretion enhances lipolysis in adipocytes, so that triglyceride accumulation is limited and may define the upper limit of TNF-α secretion from adipose tissue. Lipolysis, however, increases plasma FFAs, which increases triglyceride accumulation in skeletal muscle and hepatocytes and increases TNF-α secretion from these tissues (50). Feedback loops with this specific arrangement may, depending on specific rate constants, result in a characteristic behavior that is A nonlinear bistable switch with wide swings in effector levels instead of tightly controlled levels. This switch-like behavior at adipocytes, however, could be masked due to enhanced TNF-α at skeletal muscle and hepatocytes caused by enhanced released of FFAs at high TNF-α secretion.

The timing of the switch to high TNF-α expression and positive feedback of deleterious compensatory responses is complex. Identification of genetic polymorphisms in the regulatory circuit, however, provides some insight into the regulation of metabolic changes that mark the threshold of irreversible onset of diabetes. A study of 224 type 2 diabetic subjects found frequent polymorphisms in the adiponectin gene associated with reduced adiponectin levels. This group of patients also demonstrated higher insulin resistance independent of BMI (52). In the systems view outlined here, polymorphisms associated with reduced adiponectin levels reduce the threshold for the switch to high TNF-α functional effect. It should be noted, however, that other conflicting reports indicate that TNF-α polymorphism and obesity, insulin resistance, and other characteristics of the metabolic syndrome are unrelated (53).

Phase III: emergent TNF-α positive feedback. Once insulin resistance is established with TNF-α switched on, hyperglycemia and hyperinsulinemia also persist. This triggers cascading complications of endothelial dysfunction (49,52). Another deleterious positive feedback loop involving TNF-α likely emerges during disease progression. Robust immune mechanisms recognize oxidative LDL and advanced glycation end products, resulting in macrophage infiltration of affected microvascular systems. These macrophages secrete more TNF-α and interleukin (IL)-6 in the inflammatory response (54). Insulin resistance is robustly maintained now by both adipose-secreted and inflammation-based TNF-α. By this stage, hyperglycemia and hyperinsulinemia are robustly locked in by these positive feedback loops (Fig. 3). At the same time, glucose toxicity starts to impact vital organs and pancreatic β-cells and eventually causes irreversible damage and apoptosis. These late events in the disease process make usual therapeutic interventions futile. Because the plasma TNF-α level is locked by positive feedback loops, reduction of adipocyte mass would no longer efficiently reduce insulin resistance, as inflammation-based TNF-α continues to maintain insulin resistance. In fact, Rhesus monkeys with dramatic reduction in BMI during the advanced stage of disease do not demonstrate increased plasma adiponectin (55).

The emergent positive feedback that gave rise to robustness of insulin resistance at this stage is caused by inflammation, a defense mechanism to robustly protect the body from infection and injury. A hypothesis connecting these observations proposes that insulin resistance caused by TNF-α and IL-6 secretion due to inflammation was conserved to ensure effective defense against infection in times of food shortage (40). This evolutionary adaptation to enhance robustness against malnutrition and associated infectious risk is a fragility now exposed in conditions of food oversupply and low general infection risk.

Analysis of system dynamics allows construction of a simple model of TNF-α–adiponectin regulation, which shows that steady states of this system depend on TNF-α regulation at several levels. The simple model incorpo-

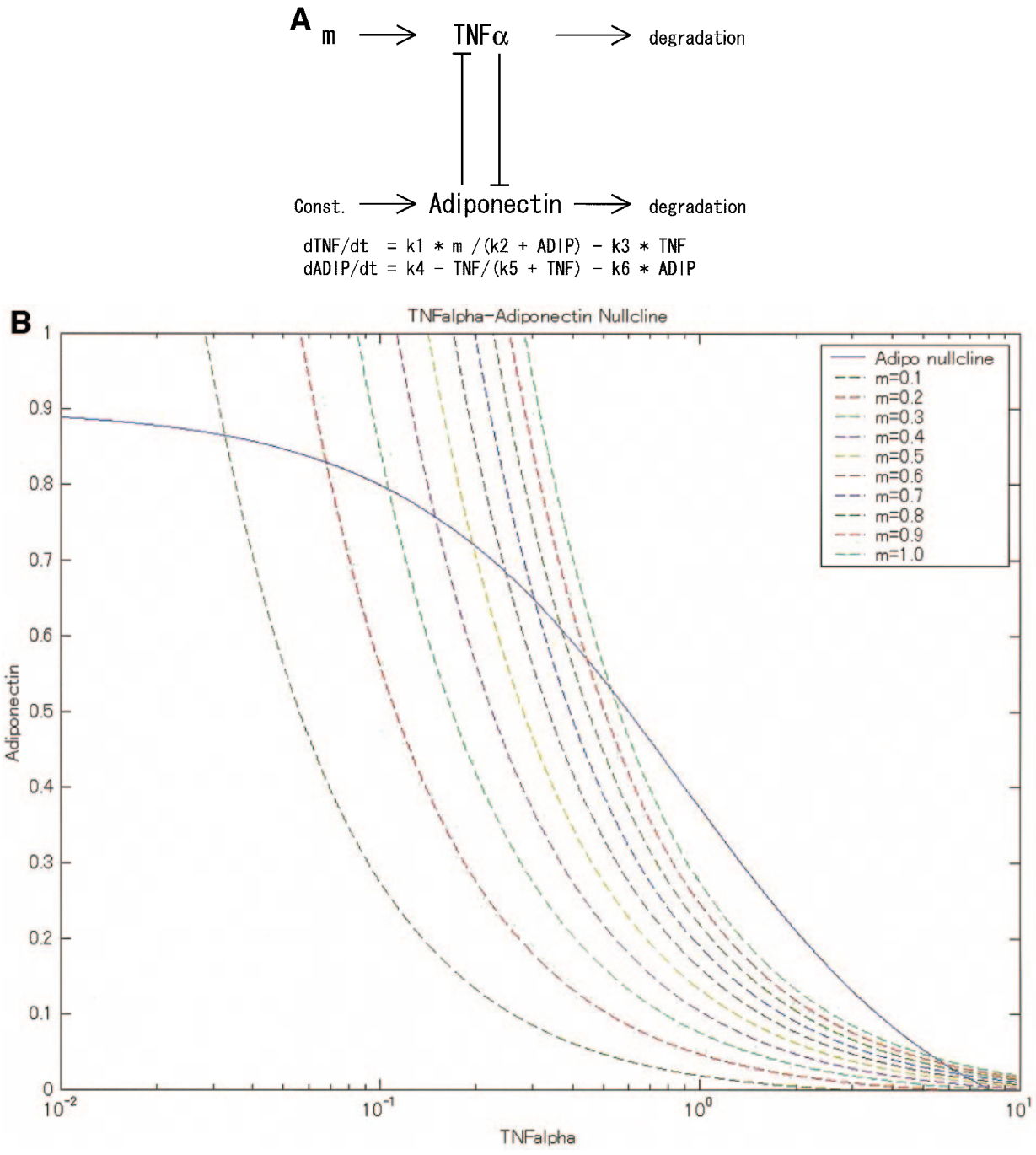

FIG. 4. Nullcline analysis of a simple model of TNF-α–adiponectin regulatory system. The graphs are generated from a simple model consisting from two differential equations, representing mutual inhibition of TNF-α and adiponectin, and the rate of TNF-α is dependent on hypothetical BMI (indicated as "m"). Nullcline curvatures indicate steady state derived from the equations. This is a highly abstract and simplified model, and more accurate models should have different formulations, but it helps us capture overall behaviors of the system. In particular, how TNF-α is regulated is largely unknown and certain to be revised; thus, the equation only represents "m" and adiponectin involved antagonistically. The reader should be aware that this model is created for the sake of explanation on how a mathematical approach can provide insights into complex biological processes and therefore should not consider it a faithful modeling. Detailed models will be created and verified for further investigations.

rates TNF-α secretion correlates with triglyceride accumulation, mutual inhibition of TNF-α and adiponectin, and varying stability of TNF-α, reflecting a possible positive feedback (Fig. 4*A*). Figure 4*B* indicates changes of TNF-α and adiponectin levels at different total adipocyte mass ("m" in arbitrary units) when TNF-α turnover is high. The intersection of two lines ("Adipo nullcline" and "m = x.x") is the steady-state point of this system. In this case, increase of TNF-α secretion and reduction of adiponectin secretion correlate with increased total adipocyte mass in a switch-like manner. Figure 4*C* shows how steady-states change as the rate of TNF-α reduction is reduced in the model. Such changes may take place if there is a mechanism for stabilizing secreted TNF-α or via positive feedback to sustain plasma TNF-α levels. In this case, the system's state may transit from one with low TNF-α and

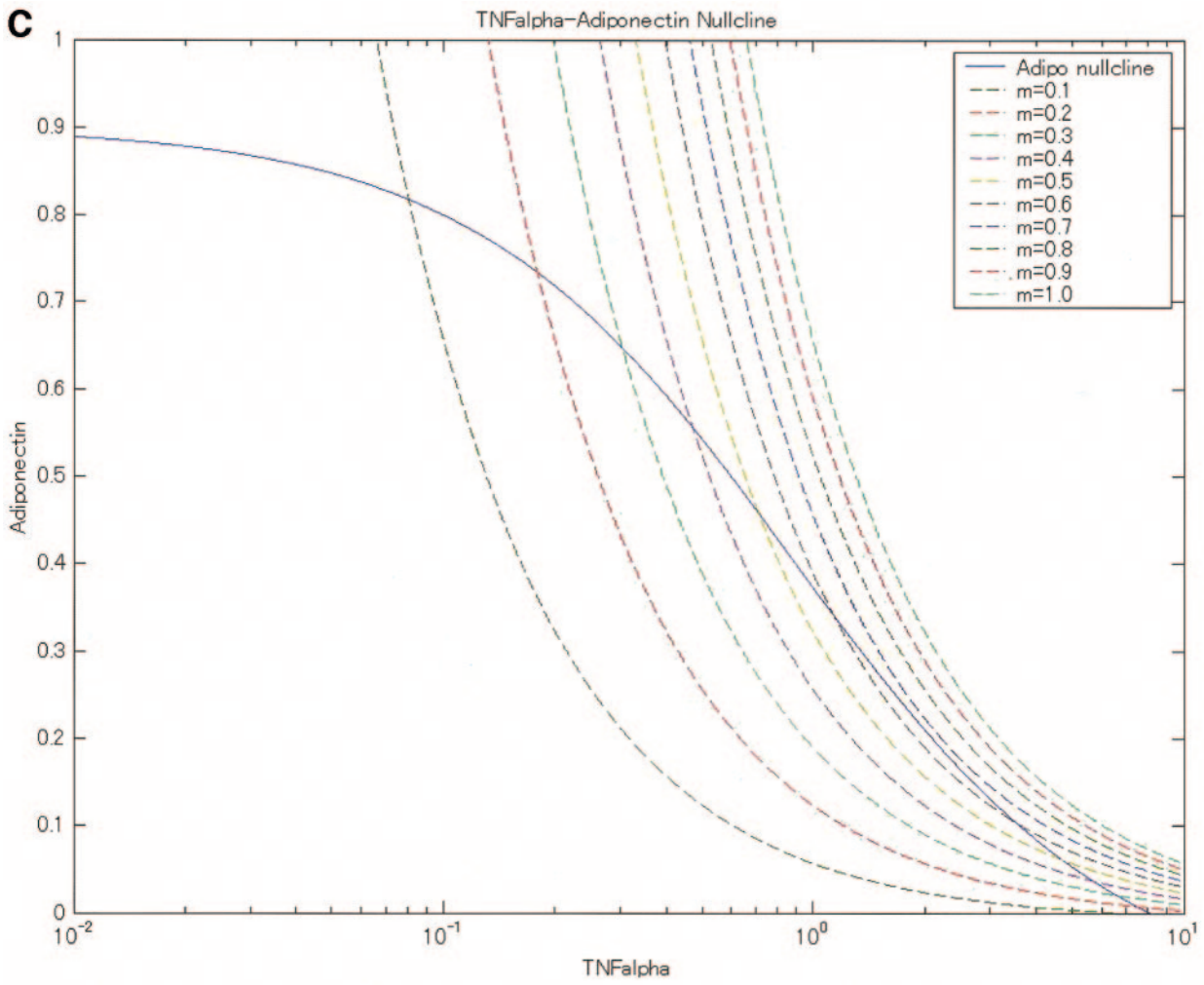

FIG. 4—*Continued.*

high adiponectin to high TNF-α and very low adiponectin, because there are converging steady-state points. The remarkable fact is that for high TNF-α steady state, reduction of "m" increases TNF-α levels, whereas the reverse occurs in low TNF-α steady state. If the model accurately reflects disease progression, reduction of adipocyte mass, most likely correlated with BMI, has adverse effects. The model implies that the goal of therapy should be to forcefully move steady state to the low TNF-α point where reduction of "m" correlates with reduction of TNF-α and increased adiponectin secretion.

In this view, it is interesting to note that TNF-α is also recognized as cachexin, which together with other multiple factors promote cachexia. Thus, the metabolic syndrome may be interpreted as an extreme overdose of TNF-α, reinforced by interlocked feedback, such that anabolic hormones (e.g., insulin) can no longer function.

In summary, from the system dynamics perspective, the metabolic syndrome is triggered by drift of the set-point for TNF-α secretion, followed by stages dominated by linear dynamics, then switch-like activation of TNF-α, and reinforcement of disease state by positive feedback loops. It is now clear that as the metabolic syndrome progresses, it acquires new robustness from an emergent positive feedback loop. In addition, insulin resistance, which is considered harmful today, may have had beneficial functions during malnutrition, preserving plasma glucose levels to support vital functions such as cognition, innate immune defense, and inflammation. This systems view provides us a perspective that the metabolic syndrome is caused by hijacking the robustness of long-standing glucose regulatory systems and immune systems, with emergence of a new robust dynamical state, which contributes to irreversible organ damage. Essential therapy, thus, needs to address the extreme fragility intrinsic to the emergent robustness of metabolic syndromes. The systems framework is one to which details derived from experimental and clinical research can be added to model the global effects of disease interventions.

SYSTEMS APPROACH TO DRUG AND THERAPY DESIGN

Finding appropriate treatments for such complex and robust diseases is a challenging task, and the goal of systems-level approaches is to provide new insight into therapeutic strategies. Identification of therapeutic targets may be possible by looking globally at the dynamics that requires correction, then focusing on its molecular underpinnings. Systems models of sufficient density can be used to not only identify targets of therapy but also predict the number and degree of side effects emerging from interactions within the modeled pathways. Contrary to conven-

tional drug discovery processes, therapeutic interventions may be analyzed initially by using computational models to find a set of perturbations that may correct offset dynamics to normal dynamics, followed by biological experiments to verify predictions. The use of large-scale genome scanning and expression profiling combined with detailed molecular interaction models (see below) can be used to predict and identify possible genes and polymorphisms associated with disease susceptibility. Systems models depict disease as abnormal dynamics of the system, and therefore, therapeutic efforts are targeted at correcting global shifts in dynamics. The models help define the specific targets from high-throughput data in order to identify the appropriate tools that change system dynamics (i.e., disease).

INTERVENTION ON THE EMERGENT ROBUSTNESS OF THE METABOLIC SYNDROME

Complex disease processes, according to systems biology strategies, are optimally treated by identification of disease-related acquired robustness mechanisms. A rational approach is to identify system fragility and target it to restore normal dynamics. In the case of the metabolic syndrome, one of the reasons pathologic hyperglycemia and hyperinsulinemia persist is because of a positive feedback loop involving TNF-α. With disease progression and vascular complications, a secondary positive feedback loop emerges that reinforces the robustness of the metabolic syndrome. The central mediator of this robust property is again TNF-α. This model suggests that the priority of therapeutic strategies is to suppress TNF-α expression and secretion as well as to enhance adiponectin secretion, so that the TNF-α switch can be turned off.

Among currently available therapies, the peroxisome proliferator–activated receptor (PPAR)-γ agonist thiazolidinedione (TZD) (56–58) elevates PPAR-γ expression to supraphysiological levels to inhibit TNF-α and enhance adiponectin secretion, as well as to promote differentiation of mature hypertrophic adipocytes into small adipocytes that secrete less TNF-α and more adiponectin (59). Theoretically these agents should turn off the TNF-α switch, although it is unclear to what extent this affects systemic TNF-α, which is secreted also from normal immunological activities. The drugs could be clinically useful if combined with strict dietary control (60,61), but they induce a range of side effects, including increased appetite and liver failure. Both of these clinical observations and the systems model predict that PPAR-γ activation is unlikely to completely ameliorate the metabolic syndrome because the fundamental problems of biased equilibrium of food intake and energy consumption are unaffected.

Although TNF-α is highlighted in this discussion, the system may utilize several alternative cytokines to achieve redundancy of this feedback regulation. A systems approach suggests that multiple therapies with multiple targets are required to completely "cure" the metabolic syndrome.

SYSTEMS DRUG DISCOVERY

The notion of targeting dynamics instead of specific molecules has interesting implications for drug discovery. In postgenomic drug discovery, a specific molecule may be selected as target, and compounds for activating or inhibiting it may be developed. However, as is often the case, modulating the target molecule incurs unexpected side effects. Because the goal of drug administration is to correct ill-posed dynamics, there may be several different approaches to accomplish this without targeting specific molecules and their substantial side effects. PPAR-γ, for example, may be a viable target for changing TNF-α–related dynamics, but there are notable side effects. An alternative approach suggested by modeling is to use multiple drugs that cause similar desired effects but at doses that do not impact dynamics in other places. TNF-α downregulation, adiponectin upregulation, and temporal inhibition of systemic inflammation may be accomplished by multiple drugs that work on different parts of the pathways. For example, infliximab is an antibody for TNF-α used to treat rheumatoid arthritis (62), and the model above suggests that it may be useful in metabolic syndrome in combination with other anti–TNF-α strategies, although the sole use of TNF-α antibody failed to reverse insulin resistance in humans (63). Theoretically, such combinations of drugs shall have therapeutic effects equivalent to PPAR-γ agonists. Side effects often take place because the targeted protein has pleiotropic roles in organ function. Use of multiple drugs may, ideally, create synthetic effects exclusively on targeted organs.

LARGE-SCALE MAPPING OF HIGH-THROUGHPUT DATA ON A MOLECULAR INTERACTION MAP

The heterogeneous and complex nature of the metabolic syndrome mandates search for individual genetic differences in susceptibility, progression, and likely complications. Although several critical regulatory circuits of disease progression have been identified, recognition of individual differences of transcription-level and metabolic profiles will both enrich the systems models and ultimately allow individualization of therapy. For example, identification of a possible threshold for the onset of TNF-α switch is an important index that impacts the therapeutic strategy for each patient. Polymorphisms in adiponectin (52) point to the importance of understanding individual differences of the TNF-α and disease-switch threshold. As more relevant polymorphisms are identified, the construction of detailed regulatory and biochemical network models is enhanced, and proper mapping of the transcription level of genes and proteomic and metabolome profiles can be used to change disease course.

We have created a molecular interaction map that describes most interactions involved in the metabolic syndrome for adipocyte, hepatocyte, skeletal muscle cell, and pancreatic β-cell. Due to the size and complexity, the map is published online at http://www.systems-biology.org/001/003.html and is continuously updated. This interaction map is created from published literature, and it is available in a standard model representation format known as Systems Biology Mark-up Language (SBML) (64) with diagrams created using CellDesigner (65,66). Models represented in SBML can be handled by software that complies with the standard, as well as an extensible software workbench called Systems Biology Workbench (SBW) (67,68). Numerical simulations using such large

molecular interaction maps are a greater challenge as most kinetic constants, particularly in vivo, are unknown. A computational model that can reproduce some important clinical observations can be built with extensive effort, but quantitative accuracy would not be easily reproducible. Because the computation is more manageable, efforts are under way to construct a theory that can analytically identify a set of parameters that makes the system stable and unstable (69,70), but this effort is currently in its infancy. Nevertheless, such a model, combined with theoretical analysis of robustness and fragility of the glucose regulatory system, is a reasonable goal of systems biology efforts.

FUTURE OF METABOLIC SYNDROME RESEARCH

Analysis of the metabolic syndrome as dysregulation of systemic glucose regulatory mechanisms allows new insight into disease progression. Systems models depict metabolic syndrome progression as a drift in regulatory equilibrium, causing the breakdown of fundamental robustness mechanisms, followed by hijacking of these mechanisms to provide emergent robustness to the disease. With this total breakdown of robustness regulation, disease is end-stage and vital organs fail. A switch-like behavior of TNF-α–adiponectin regulation and positive feedback loops contribute to maintenance and progression of the disease. Development of therapy for such robust disease has to identify and target extreme fragilities that are hidden in the mechanisms that give rise to the acquired robustness. The benefits of such a system-level theory are that it provides us with a global view of complex and often counterintuitive pathologic changes and it focuses research targets and clinical practice. Improvement in systems theories requires incorporation of experimental and clinical data into models and feedback between experimentalists and modelers to verify model predictions. The goals of the models are to create an integrated view of the metabolic syndrome, based on principles of normal regulation, and to point to novel approaches to diabetes therapy.

ACKNOWLEDGMENTS

H.K. is supported by the ERATO-SORST Program (Japan Science and Technology Agency, Ministry of Education, Culture, Sports, Science, and Technology), the Center of Excellence program and the special coordination funds (Ministry of Education, Culture, Sports, Science, and Technology), and the Air Force Office of Scientific Research (AFOSR/AOARD). M.C. is supported by grant no. P01AG20591 from the National Institute of Aging/National Institutes of Health. H.K. and J.D. are supported, in part, by a NEDO Grant (Japanese Ministry of Economy, Trade, and Industry).

REFERENCES

1. Kitano H: Computational systems biology. *Nature* 420:206–210, 2002
2. Kitano H: Systems biology: a brief overview. *Science* 295:1662–1664, 2002
3. Alon U, Surette MG, Barkai N, Leibler S: Robustness in bacterial chemotaxis. *Nature* 397:168–171, 1999
4. Barkai N, Leibler S: Robustness in simple biochemical networks. *Nature* 387:913–917, 1997
5. Eldar A, Dorfman R, Weiss D, Ashe H, Shilo BZ, Barkai N: Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning. *Nature* 419:304–308, 2002
6. Little JW, Shepley DP, Wert DW: Robustness of a gene regulatory circuit. *EMBO J* 18:4299–4307, 1999
7. McAdams HH, Arkin A: It's a noisy business! Genetic regulation at the nanomolar scale. *Trends Genet* 15:65–69, 1999
8. Meir E, von Dassow G, Munro E, Odell GM: Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. *Curr Biol* 12:778–786, 2002
9. Morohashi M, Winn AE, Borisuk MT, Bolouri H, Doyle J, Kitano H: Robustness as a measure of plausibility in models of biochemical networks. *J Theor Biol* 216:19–30, 2002
10. Ueda HR, Hagiwara M, Kitano H: Robust oscillations within the interlocked feedback model of *Drosophila* circadian rhythm. *J Theor Biol* 210: 401–406, 2001
11. von Dassow G, Meir E, Munro EM, Odell GM: The segment polarity network is a robust developmental module. *Nature* 406:188–192, 2000
12. Yi TM, Huang Y, Simon MI, Doyle J: Robust perfect adaptation in bacterial chemotaxis through integral feedback control. *Proc Natl Acad Sci U S A* 97:4649–4653, 2000
13. Carlson JM, Doyle J: Highly optimized tolerance: a mechanism for power laws in designed systems. *Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics* 60:1412–1427, 1999
14. Carlson JM, Doyle J: Complexity and robustness. *Proc Natl Acad Sci U S A* 99 (Suppl. 1):2538–2545, 2002
15. Csete ME, Doyle JC: Reverse engineering of biological complexity. *Science* 295:1664–1669, 2002
16. Kitano H: Cancer robustness: tumour tactics. *Nature* 426:125, 2003
17. Kitano H: Cancer as a robust system: implications for anticancer therapy. *Nat Rev Cancer* 4:227–235, 2004
18. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
19. Porte D, Sherwin R, Baron A: *Diabetes Mellitus*. New York, McGraw-Hill, 2003
20. Zimmet PZ, Alberti KG: The changing face of macrovascular disease in non-insulin-dependent diabetes mellitus: an epidemic in progress. *Lancet* 350 (Suppl. 1):S1–S4, 1997
21. Zimmet P, Alberti KG, Shaw J: Global and societal implications of the diabetes epidemic. *Nature* 414:782–787, 2001
22. Newsholme P, Curi R, Gordon S, Newsholme EA: Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem J* 239:121–125, 1986
23. Liu YJ, Saini A, Cohen DJ, Ooi BS: Modulation of macrophage proliferation by hyperglycemia. *Mol Cell Endocrinol* 114:187–192, 1995
24. Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998
25. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
26. Inui A: Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nat Rev Neurosci* 2:551–560, 2001
27. Saltiel AR, Kahn CR: Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414:799–806, 2001
28. Rui L, Yuan M, Frantz D, Shoelson S, White MF: SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem* 277:42394–42398, 2002
29. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E: SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *J Biol Chem* 276:47944–47949, 2001
30. Emanuelli B, Peraldi P, Filloux C, Sawka-Verhelle D, Hilton D, Van Obberghen E: SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J Biol Chem* 275:15985–15991, 2000
31. Warne JP: Tumour necrosis factor alpha: a key regulator of adipose tissue mass. *J Endocrinol* 177:351–355, 2003
32. Bullo-Bonet M, Garcia-Lorda P, Lopez-Soriano FJ, Argiles JM, Salas-Salvado J: Tumour necrosis factor, a key role in obesity? *FEBS Lett* 451:215–219, 1999
33. Argiles JM, Lopez-Soriano J, Busquets S, Lopez-Soriano FJ: Journey from cachexia to obesity by TNF. *FASEB J* 11:743–751, 1997
34. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
35. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor alpha: a key component of the obesity-diabetes link. *Diabetes* 43:1271–1278, 1994
36. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM: Tumor necrosis

factor alpha inhibits signaling from the insulin receptor. *Proc Natl Acad Sci U S A* 91:4854–4858, 1994
37. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389:610–614, 1997
38. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
39. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50:2094–2099, 2001
40. Fernandez-Real JM, Ricart W: Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. *Diabetologia* 42:1367–1374, 1999
41. Newsholme P, Costa Rosa LF, Newsholme EA, Curi R: The importance of fuel metabolism to macrophage function. *Cell Biochem Funct* 14:1–10, 1996
42. Healy DA, Watson RW, Newsholme P: Glucose, but not glutamine, protects against spontaneous and anti-Fas antibody-induced apoptosis in human neutrophils. *Clin Sci (Lond)* 103:179–189, 2002
43. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
44. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr.: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808, 2003
45. Wellen KE, Hotamisligil GS: Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 112:1785–1788, 2003
46. Kuniyasu A, Ohgami N, Hayashi S, Miyazaki A, Horiuchi S, Nakayama H: CD36-mediated endocytic uptake of advanced glycation end products (AGE) in mouse 3T3–L1 and human subcutaneous adipocytes. *FEBS Lett* 537:85–90, 2003
47. Hotamisligil GS: Molecular mechanisms of insulin resistance and the role of the adipocyte. *Int J Obes Relat Metab Disord* 24 (Suppl. 4):S23–S27, 2000
48. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271:665–668, 1996
49. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
50. Ruan H, Hacohen N, Golub TR, Van Parijs L, Lodish HF: Tumor necrosis factor-alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3–L1 adipocytes: nuclear factor-kappaB activation by TNF-alpha is obligatory. *Diabetes* 51:1319–1336, 2002
51. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
52. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T: Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes* 51:536–540, 2002
53. Lee SC, Pu YB, Thomas GN, Lee ZS, Tomlinson B, Cockram CS, Critchley JA, Chan JC: Tumor necrosis factor alpha gene G-308A polymorphism in the metabolic syndrome. *Metabolism* 49:1021–1024, 2000
54. Libby P: Inflammation in atherosclerosis. *Nature* 420:868–874, 2002
55. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y: Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 50:1126–1133, 2001
56. Moller DE: New drug targets for type 2 diabetes and the metabolic syndrome. *Nature* 414:821–827, 2001
57. Moller DE, Greene DA: Peroxisome proliferator-activated receptor (PPAR) gamma agonists for diabetes. *Adv Protein Chem* 56:181–212, 2001
58. Willson TM, Brown PJ, Sternbach DD, Henke BR: The PPARs: from orphan receptors to drug discovery. *J Med Chem* 43:527–550, 2000
59. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T: The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. *J Biol Chem* 276:41245–41254, 2001
60. Fonseca V: Effect of thiazolidinediones on body weight in patients with diabetes mellitus. *Am J Med* 115 (Suppl. 8A):42S–48S, 2003
61. Abrahamson MJ: Clinical use of thiazolidinediones: recommendations. *Am J Med* 115 (Suppl. 8A):116S–120S, 2003
62. Bondeson J, Maini RN: Tumour necrosis factor as a therapeutic target in rheumatoid arthritis and other chronic inflammatory diseases: the clinical experience with infliximab (REMICADE). *Int J Clin Pract* 55:211–216, 2001
63. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R: Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 45:881–885, 1996
64. Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP, Bornstein BJ, Bray D, Cornish-Bowden A, Cuellar AA, Dronov S, Gilles ED, Ginkel M, Gor V, Goryanin II, Hedley WJ, Hodgman TC, Hofmeyr JH, Hunter PJ, Juty NS, Kasberger JL, Kremling A, Kummer U, Le Novere N, Loew LM, Lucio D, Mendes P, Minch E, Mjolsness ED, Nakayama Y, Nelson MR, Nielsen PF, Sakurada T, Schaff JC, Shapiro BE, Shimizu TS, Spence HD, Stelling J, Takahashi K, Tomita M, Wagner J, Wang J: The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* 19:524–531, 2003
65. Kitano H: A graphical notation for biochemical networks. *Biosilico* 1:169–176, 2003
66. Funahashi A, Kitano H: CellDesigner: a process diagram editor for gene-regulatory and biochemical networks. *Biosilico* 1:159–162, 2003
67. Hucka M, Finney A, Sauro HM, Bolouri H, Doyle J, Kitano H: The ERATO Systems Biology Workbench: enabling interaction and exchange between software tools for computational biology. *Pac Symp Biocomput* 450–461, 2002
68. Sauro HM, Hucka M, Finney A, Wellock C, Bolouri H, Doyle J, Kitano H: Next generation simulation tools: the Systems Biology Workbench and BioSPICE integration. *Omics* 7:355–372, 2003
69. Prajna S, Papachristodoulou A: Analysis of switched and hybrid systems: beyond piecewise quadratic methods. In *Proceedings of American Control Conference*. New York, IEEE, 2003, p. 2779–2784
70. Prajna S, Papachristodoulou A, Parrilo PA: Introducing SOSTOOLS: a general purpose sum of square programming solver. In *Proceedings of IEEE Conference on Decision and Control*. New York, IEEE, 2002, p. 741–746