Diabetes and Cancer

Epidemiological Evidence and Molecular Links

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Frontiers in Diabetes

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Contents

Preface

This book was made possible by the contributions of leading experimental scientists and clinicians from newly upcoming and interdisciplinary fields of research concerning the common molecular and clinical features of chronic diseases. Chronic disease represents the main cause of mortality in developed countries. The increase in its prevalence is associated with changes in lifestyle habits and related risk factors such as tobacco use, physical inactivity, overweight and obesity, and poor nutrition. Collectively, cardiovascular diseases, cancer and diabetes/metabolic syndrome – ranking first among the ten leading causes of death – are responsible for more than 25 million deaths in the Western world each year. Much of this disease burden could be prevented, however, by controlling the modifiable risk factors.

The present trend of progressively lengthening lifespan in all social groups of Western societies reflects the changing pattern of mortality from formerly untreatable infectious diseases to chronic (degenerative) diseases. Predictions for the continuing lengthening of the lifespan of the class of 2005 and succeeding classes may be jeopardized by the alarming increase in obesity, for example, which worsens the incidence of cardiovascular disorders, diabetes and cancer.

The recent discoveries of epidemiological and molecular links between the diabetes/metabolic syndrome and cancer originated from interdisciplinary-oriented researchers revealing roles in biological processes that are likewise varied. The diabetes/metabolic syndrome is like the wolf in sheep's clothing – by the time it has been diagnosed, most subjects might already have an established chronic disease, like cardiovascular disease and cancer. The most recent findings suggest a connection between inflammation and chronic disease, such as insulin resistance associated with diabetes and cancer, which had not or only inadequately been appreciated previously.

The following distinguished authors guarantee that this book is at the forefront of experimental and clinical research in diabetes and cancer, and offers the reader novel insights into the interdisciplinary approaches of tomorrow: F. Thévenod (Witten, Germany) introduces the state of the art of the pathophysiology of type 2 diabetes. M. Gotthard (Nijmegen, The Netherlands) addresses new issues of in vivo imaging of the β-cell and insulinoma. B. Gallwitz (Tübingen, Germany) reviews the most advanced therapy strategies embarking on incretins and DPP4 inhibitors. K. Masur (Witten, Germany) bridges on a molecular level diabetes and cancer with specific reference to glucose and glucose-regulating hormones. R. Gatenby (Moffitt Cancer Center, Tampa, Fla., USA) clearly demonstrates that the 'Warburg effect' has to be reconsidered to understand the energetic metabolism of tumor cells. A. Schürmann (Potsdam-Rehbrücke, Germany) describes the glucose transporter systems and shows their abnormalities and significance in type 2 diabetes and cancer. K.S. Zänker (Witten, Germany) summarizes the epidemiology and molecular epidemiology of type 2 diabetes and cancer. I. Wolf (Tel-Hashomer, Israel) points at the increased risk of breast cancer in relationship to type 2 diabetes. J. LaValle (Pittsburgh, Pa., USA) describes the metabolic spiral, which leads to chronic disease. Finally, the editors of this book (K.M., F.T., K.S.Z) advocate the efforts of Beaglehole et al. [Lancet 2007;370:2152–2157] who have established the Chronic Disease Action Group to encourage, support, and monitor action on the implementation of an evidence-based effort to promote global, regional, and national action to prevent and control chronic disease.

This book should encourage scientists and physicians – working separately on various aspects of the illnesses with the highest predicted mortality in the 21st century – to come together and combine their therapies and strategies. Since the health problems mentioned may be merged with the overall topic 'metabolic syndrome', the common goal should be early detection at the first signs indicating the onset of a metabolic misbalance in order to prevent the consecutive cascades which lead to metabolic syndrome, resulting in the so-called diseases of modern civilization – cancer, diabetes and hypertension.

This volume of *Frontiers in Diabetes, 'Diabetes and Cancer – Epidemiological Evidence and Molecular Links'*, demonstrates why that it is necessary to reflect on the different aspects of an illness and that it is worthwhile checking for metabolic derangements in order to find an early therapy combining approaches devised by specialists working in different fields.

The Editors of this book are grateful to Karger Publishers, Switzerland, and to F.M. Matschinsky (Philadelphia, Pa., USA) and M. Porta (Turin, Italy), the Editors-in-Chief of the long-standing and well-recognized series of *Frontiers in Diabetes*, for publishing this volume.

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Pathophysiology of Diabetes Mellitus Type 2: Roles of Obesity, Insulin Resistance and β-Cell Dysfunction

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Abstract

The past two decades have seen an explosive increase in the number of people diagnosed with diabetes mellitus worldwide, particularly type 2 diabetes (T2D), which is found associated with modern lifestyle, abundant nutrient supply, reduced physical activity, and obesity. Actually, between 60 and 90% of cases of T2D now appear to be related to obesity. Numerous studies have shown that insulin resistance precedes the development of hyperglycemia in subjects that eventually develop T2D. However, it is increasingly being realized that T2D only develops in insulin-resistant subjects with the onset of β-cell dysfunction. It is therefore important to characterize the mechanisms of insulin resistance and subsequent pancreatic β-cell failure associated with obesity in order to better understand the pathophysiology of T2D and develop approaches to prevent T2D.

Introduction

Diabetes mellitus (DM) encompasses a range of diseases that are characterized by elevation of the blood glucose level and lead to a reduced quality of life and life expectancy, with a greater risk of heart disease, stroke, peripheral neuropathy, renal disease, blindness and amputation. Depending on the etiology, DM can be divided into two principal forms, type 1 (T1D) and type 2 diabetes (T2D). T1D occurs in childhood and is due primarily to autoimmune-mediated destruction of pancreatic β-cell islets, resulting in absolute insulin deficiency. People with T1D must take exogenous insulin for survival to prevent the development of ketoacidosis. The frequency of T1D is low relative to T2D, which accounts for over 90% of cases globally. T2D is more prevalent in adulthood, though it is becoming more common in children

and adolescents. T2D is characterized by insulin resistance and/or abnormal insulin secretion. Individuals with T2D are not dependent on exogenous insulin, but may require it for control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents.

DM has long been considered a disease of minor significance to world health, but is now developing into one of the main public health challenges for the 21st century. The past two decades have seen an explosive increase in the number of people diagnosed with DM worldwide. This DM epidemic relates particularly to T2D, which is taking place both in developed and developing countries. The global figure of people with DM is set to rise from the current estimate of 150 to 220 million in 2010, and 300 million in 2025 [1]. The direct healthcare costs of the disease are also considerable, and have been estimated at around 5% of the total annual expenditure on health in Western societies.

About 80% of T2D patients are overweight. In fact, obesity is a primary risk factor for 'metabolic' diseases, which include coronary heart disease, hypertension, but also T2D. Knowledge of adipocyte physiology is therefore crucial for a better understanding of the pathophysiological basis of obesity and T2D.

Physiology of Adipose Tissues

Adipose tissues are located throughout the body. Some of these depots are structural, providing mechanical support but contributing little to energy homeostasis. Other adipocytes exist in the skin as subcutaneous fat. Finally, several distinct depots are found within the body cavity, surrounding the heart and other organs, associated with the intestinal mesentery, and in the retroperitoneum. This visceral fat drains directly into the portal circulation and has been linked to morbidities, such as cardiovascular disease and T2D. Adipose tissues modulate energy balance by regulating both food intake and energy expenditure. They also have a considerable effect on glucose balance, which is mediated by endocrine (mainly through the synthesis and release of peptide hormones, the so-called 'adipokines') and non-endocrine mechanisms.

Among the endocrine factors, adipocyte-derived proteins with antidiabetic action include leptin, adiponectin, omentin and visfatin. For instance, in addition to its well-characterized role in energy balance, leptin reverses hyperglycemia by improving insulin sensitivity in muscles and the liver. According to the current view that intracellular lipids may contribute to insulin resistance, this occurs most likely by reducing intracellular lipid levels through a combination of direct activation of AMP-activated protein kinase (AMPK) and indirect actions mediated through central neural pathways [2]. Other factors tend to raise blood glucose, including resistin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and retinol-binding protein 4 (RBP4). TNF-α is produced in macrophages and reduces insulin action [3]. IL-6 is produced by

adipocytes, and has insulin-resistance-promoting effects as well [4]. Such 'adipocytokines' can induce insulin resistance through several mechanisms, including c-Jun N-terminal kinase 1 (JNK1)-mediated serine phosphorylation of insulin receptor substrate-1 (IRS-1) (see below), IκB kinase-β (IKK-β)-mediated nuclear factor-κB (NF-κB) activation, induction of suppressor of cytokine signaling 3 (SOCS3) and production of ROS [for review, see 5]. RBP4, a secreted member of the lipocalin superfamily, is regulated by the changes in adipocyte glucose transporter 4 (GLUT4) levels. Studies have shown that overexpression of RBP4 impairs hepatic and muscle insulin action, and *Rbp4*−/− mice show enhanced insulin sensitivity [6]. Furthermore, high serum RBP4 levels are associated with insulin resistance in obese humans and patients with T2D [7]. The exact mechanisms how RBP4 impairs insulin action are, however, not clear.

Adipocytes also release non-esterified fatty acids (NEFAs) into the circulation, which may therefore be viewed as an adipocyte-derived secreted non-endocrine product. They are primarily released during fasting, i.e. when glucose is limiting, as a nutrient source for most organs. Circulating NEFAs reduce adipocyte and muscle glucose uptake, and also promote hepatic glucose output, consistent with insulin resistance. The net effect of these actions is to promote lipid burning as a fuel source in most tissues, while sparing carbohydrate for neurons and red blood cells, which depend on glucose as an energy source. Several mechanisms have been proposed to account for the effects of NEFAs on muscle, liver and adipose tissue, including protein kinase C (PKC) activation, oxidative stress, ceramide formation, and activation of Toll-like receptor 4 [for review, see 5, 8]. Because lipolysis in adipocytes is repressed by insulin, insulin resistance from any cause can lead to NEFA elevation, which, in turn, induces additional insulin resistance as part of a vicious cycle. β-Cells are also affected by NEFAs, depending in part on the duration of exposure; acutely, NEFAs induce insulin secretion (as after a meal), whereas chronic exposure to NEFAs causes a decrease in insulin secretion [9] (see below), which may involve lipotoxicity-induced apoptosis of islet cells [10] and/or induction of uncoupling protein-2 (UCP-2), which decreases mitochondrial membrane potential, ATP synthesis and insulin secretion [10, 11]. The ability to store large amounts of esterified lipid in a manner that is not toxic to the cell or the organism as a whole may therefore be one of the most critical physiological functions of adipocytes.

The Insulin Receptor: Transduction through Tyrosine Kinase

An understanding of insulin resistance requires knowledge of the mechanisms of insulin action in target tissues, such as liver, muscle and adipose tissue. The net responses to this hormone include short-term metabolic effects, such as a rapid increase in the uptake of glucose, and longer-term effects on cellular differentiation and growth [12]. The α-subunits of the insulin receptor are located extracellularly

and are the insulin-binding sites. Ligand binding promotes autophosphorylation of multiple tyrosine residues located in the cytoplasmic portions of β-subunits. This autophosphorylation facilitates binding of cytosolic substrate proteins, such as IRS-1. When phosphorylated, this substrate acts as a docking protein for proteins mediating insulin action. Although the insulin receptor becomes autophosphorylated on tyrosines and phosphorylates tyrosines of IRS-1, other mediators are phosphorylated predominantly on serine and threonine residues. An insulin second messenger, possibly a glycoinositol derivative that stimulates phosphoprotein phosphatases, may be released at the cell membrane to account for the short-term metabolic effects of insulin. The activated β-subunit also catalyzes the phosphorylation of other members of the IRS family, such as Shc and Cbl. Upon tyrosine phosphorylation, these proteins interact with other signaling molecules (such as p85, and Grb2-Sos and SHP-2) through their SH2 (Src-homolog-2) domains, which bind to a distinct sequence of amino acids surrounding a phosphotyrosine residue. Several diverse pathways are activated, and those include activation of phosphatidylinositol 3′-OH kinase (PI_3K), the small GTP-binding protein Ras, the mitogen-activated protein (MAP) kinase cascade, and the small GTP-binding protein TC10. Formation of the IRS-1/p85 complex activates PI3 kinase (class 1A), which transmits the major metabolic actions of insulin via downstream effectors such as phosphoinositide-dependent kinase 1 (PDK1), Akt and mTOR. The IRS-l/Grb2-Sos complex and SHP-2 transmit mitogenic signals through the activation of Ras to activate MAP kinase. Once activated via an exchange of GTP for GDP, TC10 promotes translocation of GLUT4 vesicles to the plasma membrane of muscle and fat cells, perhaps by stabilizing cortical actin filaments. These pathways coordinate the regulation of vesicle trafficking (incorporation of GLUT4 into the plasma membrane), protein synthesis, enzyme activation and inactivation, and gene expression [for further details, see 12, 13]. The net result of these diverse pathways is regulation of glucose, lipid, and protein metabolism as well as cell growth and differentiation.

Pathophysiology of Adipose Tissues: Obesity and Insulin Resistance

Lipid storage in adipose tissue represents excess energy consumption relative to energy expenditure, which in its pathological form has been coined 'obesity'. In recent years, overnutrition has reached epidemic proportions in developed as well as developing countries. This reflects recent lifestyle changes, however there is also a strong genetic component as well. While the biochemical mechanism(s) for this genetic predisposition are still under investigation, the genes that control appetite and regulate energy homeostasis are now better known. For example, adipocytes produce leptin (see above) that suppresses appetite and was initially considered a promising target for drug therapy. However, most overweight individuals overproduce leptin, and no more than 2–4% of the overweight population has defects in the leptin appetite

suppression pathway [14]. In contrast, genetic predisposition to obesity and/or T2D when excess calories are consumed is common in the population: for instance, polymorphisms in the peroxisome proliferator-activated receptor-γ_2 (PPAR-γ_2) gene may have a broad impact on the risk of obesity and insulin resistance. A minority of people is heterozygous for the Pro12Ala variant of PPAR-γ and is less likely to become overweight and less likely to develop DM when overweight than the majority of Pro homozygotes in the population [15].

One striking clinical feature of overweight individuals is a marked elevation of serum NEFAs, cholesterol, and triacylglycerols irrespective of the dietary intake of fat. Obesity is obviously associated with an increased number and/or size of adipose tissue cells. These cells overproduce hormones, such as leptin, and cytokines, such as TNF-α, some of which appear to cause cellular resistance to insulin [16]. At the same time, the lipid-laden adipocytes decrease synthesis of hormones, such as adiponectin, which appear to enhance insulin responsiveness. The insulin resistance in adipose tissue results in increased activity of the hormone-sensitive lipase, which is probably sufficient to explain the increase in circulating NEFAs [17]. The high circulating levels of NEFAs may also contribute to insulin resistance in the muscle and liver (see below). Initially, the pancreas maintains glycemic control by overproducing insulin. Thus, many obese individuals with apparently normal blood glucose control have a syndrome characterized by insulin resistance of the peripheral tissue and high concentrations of insulin in the circulation. This hyperinsulinemia appears to stimulate the sympathetic nervous system, leading to sodium and water retention and vasoconstriction, which increase blood pressure [18]. The excess NEFAs are carried to the liver and converted to triacylglycerol and cholesterol. Excess triacylglycerol and cholesterol are released as very-low-density lipoprotein particles, leading to higher circulating levels of both triacylglycerol and cholesterol. Eventually, the capacity of the pancreas to overproduce insulin declines which leads to higher fasting blood sugar levels and decreased glucose tolerance (see below).

Inflammation: A Process Associated with Obesity-Induced Insulin Resistance

Adipose tissue modulates metabolism by releasing NEFAs and glycerol, hormones – including leptin and adiponectin – and proinflammatory cytokines [19]. There is now clear evidence that obesity associated with or without T2D is an inflammatory state, consistent with the production of TNF-α and other cytokines by adipose tissue. Chronic inflammation of white adipose tissue characterized by macrophage infiltration is thought to contribute to insulin resistance associated with obesity, and in obesity, the production of many of these adipokines is increased. RBP4 induces insulin resistance through reduced phosphatidylinositol-3-OH kinase (PI_3K) signaling in muscle and enhanced expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase in the liver through a retinol-dependent mechanism. By contrast,

adiponectin acts as an insulin sensitizer, stimulating fatty acid oxidation in an AMPK and peroxisome proliferator-activated receptor-α (PPAR-α)-dependent manner [for review, see 5].

In obese animals and humans, bone-marrow-derived macrophages are recruited to the fat pad under the influence of proteins secreted by adipocytes, including macrophage chemoattractant protein-1 (MCP-1) [19]. In addition to adipocyte-derived factors, increased release of TNF-α, IL-6, MCP-1 and additional products of macrophages that populate adipose tissue might also have a role in the development of insulin resistance [20]. TNF-α and IL-6 act through classical receptor-mediated processes to stimulate both the c-Jun aminoterminal kinase (JNK) and the IκB kinase-β (IKK-β)/nuclear factor-κB (NF-κB) pathways, resulting in upregulation of potential mediators of inflammation that can lead to insulin resistance. The adipokine MCP-1 and its receptor CCR2 may play a role in the recruitment of macrophages to white adipose tissue and in the initiation of an inflammatory response in mice. Thiazolidinediones, which are PPAR-γ agonists used clinically as insulin sensitizers, reduce MCP-1 levels and macrophage infiltration into adipose tissue [21]. Increased secretion of MCP-1 from adipocytes may thus trigger such macrophage recruitment, and the infiltrated cells may in turn secrete a variety of chemokines and other cytokines that further promote a local inflammatory response and affect gene expression in adipocytes, resulting in systemic insulin resistance.

NEFAs: A Critical Factor in the Development of Insulin Resistance

The amount of adipokines secreted from adipocytes is correlated with adipocyte size, i.e. with the amount of triglyceride stored in the cells. Such a relation implies that adipokines directly mediate insulin resistance associated with obesity. Given that the release of NEFAs also is correlated with adipocyte size and that an increase in the NEFA concentration in plasma is a common feature of insulin resistance, increased NEFA levels are also associated with the insulin resistance observed in obesity and T2D [22]. The passage of NEFAs across the plasma membrane and into the cell, where they are thought to exert their effects, is mediated in a specific manner by fatty acid transport protein 1 (FATP1), a transmembrane protein that enhances the cellular uptake of NEFAs. Interestingly, FATP1-deficient mice are protected from diet-induced obesity and insulin resistance [23]. The cytosol of cells also contains fatty acid-binding proteins (FABPs), which are thought to facilitate the utilization of lipids in metabolic pathways. Mice that lack both of two related adipocyte FABPs, aP2 and mal1, are also protected from diet-induced obesity and insulin resistance [24].

In fact, it appears that the release of NEFAs may be the single most critical factor in modulating insulin sensitivity. Insulin resistance develops within hours of an acute increase in plasma NEFA levels in humans [22]. Conversely, insulin-mediated glucose uptake and glucose tolerance improve with an acute decrease in NEFA levels

after treatment with the antilipolytic agent acipimox [25]. Increased intracellular NEFAs may result in competition with glucose for substrate oxidation leading to the serial inhibition of pyruvate dehydrogenase, phosphofructokinase and hexokinase II activity [26]. It has also been proposed that increased NEFA delivery or decreased intracellular metabolism of fatty acids results in an increase in the intracellular content of fatty acid metabolites such as diacylglycerol (DAG), fatty acyl-coenzyme A (fatty acyl-CoA), and ceramides, which, in turn, activate a serine/threonine kinase cascade leading to serine/threonine phosphorylation of IRS-1 and IRS-2, and a reduced ability of these molecules to activate PI_3K [27]. Subsequently, events downstream of insulin-receptor signaling are diminished.

These observations thus suggest that the transport of NEFAs into cells and their intracellular availability are important determinants of diet-induced obesity and insulin resistance. NEFAs also bind to and activate members of the G-protein-coupled class of receptors in the plasma membrane. Among these receptors, GPR40 is preferentially expressed in pancreatic β-cells and mediates the stimulatory effect of NEFAs on insulin secretion [28], and mice that lack GPR40 have a reduced susceptibility to the hyperinsulinemia, hepatic steatosis, increased hepatic glucose output, hyperglycemia, and glucose intolerance induced by obesity [29].

This latter finding provides support for a more 'β-centric' perspective of obesity-induced insulin resistance, as opposed to the 'adipo-centric' perspective, with hyperinsulinemia per se possibly contributing to hepatic steatosis, hepatic insulin resistance, and hyperglycemia associated with diet-induced obesity. It remains to be determined what kinds of signals regulate the secretion of adipokines or NEFA release from adipocytes. Oxidative stress and endoplasmic reticulum-associated stress may be candidates for such a signal.

Relationship between Insulin Sensitivity and Insulin Release

Fluctuations in insulin sensitivity occur during the normal life cycle, with insulin resistance being observed during puberty, pregnancy, and with ageing. On the other hand, increased physical activity and increased carbohydrate intake are associated with enhanced insulin sensitivity. Hence β-cells are markedly adaptable in their ability to regulate insulin release in a very precise manner. Obviously, the β-cell is fundamental to ensuring that in healthy subjects, plasma glucose levels remain within a narrow physiological range [for review, see 30].

In healthy individuals, there is a feedback loop between the insulin-sensitive tissues and the β-cells, with β-cells increasing insulin supply in response to demand by the liver, muscles and adipose tissue. The relationship between insulin sensitivity and insulin levels is reciprocal and hyperbolic [31]. In response to changes in insulin sensitivity, insulin release increases or decreases reciprocally to maintain normal glucose tolerance. Insulin sensitivity is almost always decreased in obesity and insulin-resistant

individuals, whether lean or obese, have greater insulin responses and lower hepatic insulin clearance than insulin-sensitive individuals. In contrast, individuals with high risk of developing T2D display inadequate insulin release for any level of insulin sensitivity at any stage of the disease and even when they have normal glucose tolerance, suggesting that β-cell function has already being decreased before the development of hyperglycemia [30]. Hence, failure of this feedback loop seems to contribute to the development of DM.

Another important implication of this feedback loop is that assessment of β-cell function requires knowledge of both insulin sensitivity and the insulin response, in other words the interpretation of the β-cell's secretory response to a given stimulus must take into account the prevailing degree of insulin sensitivity. This ability of the β-cell to adapt to changes in insulin sensitivity seems to result from (1) the functional responsiveness of the cell and (2) β-cell mass. In response to the insulin resistance observed in obesity, puberty and pregnancy, human β-cells can increase insulin release to levels 4- to 5-fold higher than in insulin-sensitive individuals, whereas β-cell volume is only enhanced by about 50%. In individuals with normal β-cells, glucose tolerance is preserved during these periods of insulin resistance as the decrease in insulin sensitivity is matched by a compensatory increase in insulin release. In contrast, in groups of people with T2D and those at increased risk of developing T2D, the decline in insulin sensitivity is not matched by a reciprocal increase in the insulin response. Instead, the insulin response also declines, which is compatible with the idea of β-cell dysfunction [30].

Adaptation of β-Cell Function to Insulin Resistance: Increased Insulin Release

Under physiological conditions, glucose-stimulated insulin secretion requires the metabolism of glucose and thereby the generation of ATP. The resulting increase in the ATP/ADP ratio triggers the closure of the ATP-sensitive potassium (K_{ATP}) channel, depolarization of the cell membrane and influx of calcium through voltage-dependent calcium channels, resulting in insulin granule exocytosis [32]. The β-cell's adaptive response to changes in insulin sensitivity is probably mediated by increased cellular glucose metabolism, NEFA signaling and sensitivity to incretins. Data from animal studies suggest that the increase in β-cell glucose metabolism involves an increase in the activity of glucokinase, the rate-limiting enzyme responsible for glucose phosphorylation after its entry into the cell [33]. Glucose utilization rises as both oxidation and flux of glucose are increased, the latter through pyruvate carboxylase and the replenishment of tricarboxylic acid cycle intermediates in the mitochondria. Increased citrate levels generated by glucose metabolism may lead to generation of malonyl-CoA and increased long-chain acyl-CoA and diacylglycerol levels through inhibition of carnitine palmitoyl transferase 1 [34]. This leads to PKC activation and stimulation of insulin release. In humans, however, the role of increased glucose

levels for the adaptive increase in insulin release in response to decreased insulin sensitivity is still debated [30].

NEFAs are important for normal β-cell function and may mediate increased β-cell output in response to decreased insulin sensitivity. NEFAs potentiate insulin release in response to glucose and non-glucose secretagogues by binding to the G-protein-coupled receptor GPR40 on the cell membrane, resulting in the activation of phospholipase C signaling and a subsequent increase in intracellular calcium and secretory granule exocytosis [28]. Additionally, fatty acyl-CoA may also be generated, which increases insulin release both by directly stimulating secretory granule exocytosis and by PKC activation [30]. A third possible mechanism is increased sensitivity to incretin hormones, such as glucagon-like peptide-1 (GLP-1), that are produced in the intestinal mucosa and are responsible for the enhancement of the insulin response observed after oral – compared with intravenous – glucose administration [35]. The β-cell might become more responsive to the effects of GLP-1 to modulate insulin secretion by G-protein-coupled receptor activation involving stimulation of protein kinase A (PKA) and the guanine nucleotide exchange factor EPAC2. The extensive innervation of the islet by both parasympathetic and sympathetic neurons, and the intimate involvement of the central nervous system (CNS) in the regulation of metabolism suggest that the CNS may also have an important role in the functional adaptation to changes in insulin sensitivity [for review, see 30].

Adaptation of β-Cell Mass to Insulin Resistance: Mechanisms of Growth and Proliferation

Although changes in β-cell function are observed under conditions of increased secretory demand, the volume of β-cells also increases. In rodents fed a high-fat diet for 12 months to induce obesity and insulin resistance, islet size increases as a result of an increase in the number of β-cells rather than a change in β-cell size, and new islets do not form [36]. NEFAs rather than glucose may mediate this increase in β-cell mass [for review, see 30, 37]. In contrast, human studies suggest that β-cell volume is increased by about 50% in healthy obese individuals, which, however seems to be more dependent on hypertrophy of existing cells than proliferation [38, 39]. Interestingly, in the long-term increased dietary fat feeding study in rats, β-cell mass increased but glucose-induced insulin release did not, which indicates a dissociation between β-cell mass and the secretory function [36]. Increased signaling by insulin and/or insulin-like growth factor 1 (IGF-1) could also underlie the modulation of islet mass. Activation of the insulin/IGF-1 receptor leads to phosphorylation of IRS-2 and downstream signaling through pathways including PI_3K/protein kinase-B (PKB/Akt) and Ras, leading to activation of the mitogen-activated protein (MAP) kinases ERK-1 and ERK-2 [40]. IRS-2 appears to play a key role in the cellular processes associated with increased β-cell proliferation, neogenesis and survival.

Finally, the incretin GLP-1 is an insulin secretagogue but is also a β-cell mitogen, capable of increasing β-cell proliferation and reducing β-cell apoptosis in animal models through several pathways, including transactivation of the epidermal growth factor receptor and stimulation of the IRS-2 pathway [35]. Whether GLP-1 has similar effects in humans is not known. Finally, neural signaling could also contribute to increased β-cell mass.

Failed Adaptation to Insulin Resistance and β-Cell Failure: The 'Natural History' of T2D?

Normal pancreatic β-cell responds to a chronic fuel excess and obesity-associated insulin resistance with compensatory insulin hypersecretion in order to maintain normoglycemia. This adaptive response to insulin resistance involves changes in both function and mass, and is so efficient that normal glucose tolerance is maintained. Longitudinal studies of subjects that develop T2D show a rise in insulin levels in the normoglycemic and prediabetes phases that keep glycemia near normal despite the insulin resistance (β-cell compensation), followed by a decline when fasting glycemia surpasses the upper limit of normal of 5.5 mM (β-cell failure). T2D may develop when pancreatic β-cells fail to secrete sufficient amounts of insulin to meet the metabolic demand. Both insulin secretion and insulin action are impaired in T2D [reviewed in 41]. Their relative importance has been hotly debated, but it is now recognized that β-cell dysfunction is crucial for the development of the disease [30, 37]. For example, abnormalities in insulin secretion precede the onset of T2D and may be present even when subjects show normal glucose tolerance [42, 43]. By the time of diagnosis, insulin secretion is significantly reduced and it continues to diminish inevitably throughout the course of the disease [43]. T2D can also occur in the absence of insulin resistance [44] and, conversely, some severe forms of insulin resistance (such as those caused by mutations in the insulin receptor) may not be accompanied by diabetes [45]. Thus, it now appears that insulin resistance only leads to diabetes if combined with a genetically determined tendency to β-cell dysfunction [30, 44]. In these individuals, however, insulin resistance plays an important role in the development of diabetes by placing an increased demand upon the β-cell that it is unable to match.

The number of β-cells is clearly reduced by about 50% in T2D [38, 39], but this degree of β-cell loss cannot fully account for the change in secretory function of existing intact β-cells. The β-cell is unable to release insulin rapidly in response to intravenous glucose, despite the fact that the β-cells in individuals with T2D clearly contain insulin and delivery of non-glucose secretagogues can acutely increase insulin release but does not result in equivalent responses to those seen with similar stimulation in healthy subjects [46–48]. The β-cell failure and T2D that follow β-cell compensation could result from both inadequate expansion of β-cell mass and failure of the existing β-cell mass to respond to glucose, e.g. due to a defect in insulin and

IGF-1 signaling in pancreatic β-cells or impaired incretin signaling in the β-cell. However, experimental evidence for these processes is currently lacking.

The extremely elevated blood glucose levels frequently observed in diabetes may contribute to further disease progression through glucotoxic effects on the β-cell and harmful effects on insulin sensitivity, both of which can be ameliorated by therapeutically lowering the glucose level [49]. By contrast, raising the blood glucose level for 20 h in healthy subjects has exactly the opposite effect: it improves insulin sensitivity and enhances β-cell function [50]. This also suggests that a pre-existing, and perhaps genetically determined, risk is crucial for β-cell dysfunction to occur. It is this pre-existing abnormality that results, with time, in a progressive impairment in insulin release and, ultimately, an increase in glucose levels, the latter of which further aggravates the situation and thereby contributes to β-cell failure.

Groups at increased risk of subsequently developing diabetes exhibit β-cell dysfunction well before they would be considered to have reduced glucose tolerance, in keeping with the idea of a pre-existing risk. Examples include women with a history of gestational diabetes or polycystic ovarian syndrome, older subjects, who frequently develop hyperglycemia as they continue to age, and individuals with impaired glucose tolerance [30]. First-degree relatives of individuals with T2D, who are genetically at increased risk, also have impaired β-cell function, even though they may still have normal glucose tolerance [42]. This has particularly been well studied in the Pima Indians, in whom the prevalence of diabetes is higher than almost any other group in the world, and also been confirmed for non-Hispanic whites, African-Americans and Hispanics participating in the Insulin Resistance Atherosclerosis Study (IRAS) [reviewed in 30].

Pathogenesis of T2D: Genetic Factors

Many genes interact with the environment to produce obesity and/or T2D. In the case of obesity, the most frequent mutation is that in the melanocortin-4 receptor, which accounts for up to 4% of cases of severe obesity. Other rare causes include mutations in leptin and the leptin receptor, prohormone convertase 1 (PC1) and pro-opiomelanocortin (POMC). The gene variant most commonly associated with insulin sensitivity is the P12A polymorphism in *PPARγ*, which is associated with an increased risk of developing T2D [15, 51]. A number of genes associated with β-cell dysfunction have been identified, including two non-coding single-nucleotide polymorphisms in transcription factor 7-like 2 (*TCF7L2*) and mutations in the mitochondrial genome that are also associated with neurosensory hearing loss [51]. Work is ongoing on many candidate genes, including calpain 10, adiponectin, PPAR-γ coactivator 1 (*PGC1*) and the glucose transporter *GLUT2* [51].

Polymorphisms in genes encoding β-cell ion channels are also causative candidates for a reduction in β-cell function that may be associated with T2D. One that has

received much recent attention is a polymorphism (E23K) in the Kir6.2 subunit of the K_{ATP} channel (encoded by *KCNJ11*), with a prevalence of 34% and between 11 and 15% of the population risk of T2D in Caucasians [51]. When heterologously expressed in mammalian cells, the E23K polymorphism leads to a 2-fold reduction in the ATP sensitivity of the K_{ATP} channel [52]. It is therefore expected to reduce β-cell electrical activity and insulin secretion. The functional effects of the E23K polymorphism in man are, however, controversial [53]. Polymorphisms in ABCC8/SUR1 have also been linked to T2D [54].

Because metabolism regulates β-cell function, polymorphisms in metabolic genes may also influence the ability of glucose to stimulate electrical activity and insulin secretion. Support for this idea comes from a number of rare (1–5%) monogenic forms of diabetes referred to as maturity-onset diabetes of the young [6] because they present early in life [51]. The first of these to be identified (MODY2) results from inactivating mutations in glucokinase, the high Km enzyme that phosphorylates glucose in β-cells and is rate-limiting for glucose metabolism. All MODY2 patients are heterozygotes: permanent neonatal diabetes results from homozygous mutations [55]. Other forms of MODY are due to mutations in genes (e.g. HNF1α, HNF4α, IpF1) encoding a transcriptional network that regulates the expression of several genes critical for glucose sensing [51]. Mutations in mitochondrial DNA (most frequently A3243G in the leucine tRNA gene) cause maternally inherited diabetes, probably by impairing β-cell metabolism and so reducing insulin secretion [56]. These account for a further 1–2% of diabetic cases. There is also an overlap between MODY and multifactorial T2D [51].

Mitochondrial metabolism generates substantially more ATP than glycolysis and the production of mitochondrial ATP is critical for both glucose-dependent insulin secretion and K_{ATP} channel closure. It is therefore pertinent that polymorphisms in genes that regulate mitochondrial ATP production are associated with T2D. UCP2 is an uncoupling protein that resides in the inner mitochondrial membrane and uncouples electron transport from ATP synthesis [11]. This suggests that the level of UCP2 expression may influence insulin secretion in man. Consistent with this idea, a common polymorphism in the UCP2 promoter (−866G/A) causes a 2-fold increase in the risk of T2D in obese white Europeans [57]. The frequency of the 866A variant in the European population is 37% [58], suggesting it may make a significant contribution to T2D. The β-cell transcription factor PAX6 preferentially binds to, and transactivates, the −866A/A variant in insulin-secreting (INS-1) cells and is expected to increase UCP2 mRNA expression in islet β-cells, thereby reducing ATP levels, electrical activity and insulin release. Recent studies further indicate that islets from −866A/A homozygous had lower insulin secretion in response to glucose stimulation as compared with −866G/G and −866G/A carriers [59]. A common variant in mitochondrial DNA itself (16189) causes a T to C transition in a region of mtDNA that lies close to control sequences governing replication and transcription. It is associated with increased fasting plasma insulin in several populations, maternal restraint of fetal growth and thinness at birth and is also with T2D [60].

Pathogenesis of T2D: Interactions between Genes and the Environment

Environmental factors are largely responsible for the modern-day outbreak of obesity and T2D. Increased caloric availability and fat consumption in the setting of decreased physical activity lead to overnutrition, increased nutrient storage, obesity and insulin resistance. This has important consequences if β-cell function is already inherently abnormal owing to genetic susceptibility: obesity promotes insulin resistance, which can lead to insulin insufficiency if the secretory capacity of the β-cell is already lower than normal. Another proposed environmental mechanism is thought to occur in utero and/or during the early postnatal period when poor nutrition alters metabolism, resulting in a tissue adaptation that favors the storage of nutrients ('thrifty phenotype hypothesis' of T2D) [61]. The end result of these environmental changes is a deleterious interaction with genes that predispose to the development of obesity and T2D.

Any explanation of T2D must also account for the fact that disease develops with age and that it is enhanced by obesity. There is evidence that the interaction of such lifestyle factors with genetic ones may also occur at the level of β-cell function. This may reflect the decline in mitochondrial function with age that is believed to result from accumulating mutations in mitochondrial DNA [56, 62]. Obese individuals and T2D patients have higher circulating levels of NEFAs [22, 63], which are taken up and metabolized by β-cells. Chronic exposure to NEFAs leads to the accumulation of long-chain acyl CoAs (LC-CoAs) within the β-cell. LC-CoAs both enhance K_{ATP} channel activity and reduce its ATP sensitivity [64], thereby reducing glucose-dependent closure of K_{ATP} channels, electrical activity and insulin secretion. Moreover, changes in β-cell metabolism as a consequence of age and/or obesity will translate into reduced β-cell electrical activity and insulin secretion, not only to glucose but also to incretins and sulfonylureas.

It is well established that T2D is a polygenic disease [51]. However, how these polymorphisms relate to the disease phenotype, has not been established. A current model for T2D suggests that the effect of several gene variants and lifestyle factors combine to produce a small decrease in β-cell function and thus a reduction in insulin secretion. Though the functional consequences of each individual gene variant will be small, so that a single polymorphism, by itself, is unlikely to result in diabetes, the cumulative effect of several such polymorphisms will increase disease risk, and in combination with age and/or obesity lead to overt disease. β-Cell function could serve as a bottleneck at which the effects of many different genes and lifestyle factors converge. Individuals at risk of T2D may carry one or more polymorphisms in ion channel genes, or in genes regulating their activity, membrane targeting or transcription. Because the functional effect of these gene variants is small this does not cause diabetes in early life, because glucose homeostasis is tightly controlled and the β-cell secretory output is adjusted. However, with age, β-cell metabolic function declines, leading to a reduction in glucose sensitivity, electrical activity and insulin secretion. In non-diabetics this does not pose a problem, as the β-cell adjusts its secretory output. However, in individuals who already have genetically determined

reduced β-cell function, the further reduction in electrical activity means that β-cell is no longer able to compensate, so that insulin secretion declines and glucose intolerance, and subsequently overt diabetes, develop. Obesity exacerbates the situation both by causing insulin resistance (increasing the demand on the β-cell) and by further decreasing insulin secretion from the β-cell. As pancreatic β-cells are exposed during β-cell compensation to metabolic changes associated with obesity, so factors commonly associated with obesity – such as insulin resistance (including that in β-cells), adipokines, NEFAs, reactive oxygen species, and endoplasmic reticulum-associated stress – should also be examined as candidates for inducers of β-cell failure.

Current Concepts and Future Directions

Having a single mechanism to explain the link between obesity, insulin resistance and T2D would be ideal. NEFAs induce insulin resistance and impair β-cell function, making them a likely culprit [8, 22, 30, 37]. Although NEFAs are critical for normal insulin release, chronic exposure to NEFAs in vitro and in vivo is associated with marked impairments in glucose-stimulated insulin secretion and decreased insulin biosynthesis [8, 22, 65]. Elevated NEFA levels produced by a lipid infusion in vivo contribute to the development of insulin resistance and also prevent the expected compensatory β-cell response in humans [66]. This dual effect makes them a good candidate to link obesity, insulin resistance and β-cell dysfunction in individuals with T2D and those at risk of the disorder. This lipotoxic effect can also act synergistically with glucose to produce even greater deleterious effects, commonly referred to as 'glucolipotoxicity'.

A genetically determined defect in insulin release by the β-cell could also be crucial [30, 46–48]. Impaired insulin release could result in decreased insulin levels and decreased signaling in the hypothalamus, leading to increased food intake and weight gain, in disordered regulation of glucose levels by decreasing suppression of hepatic glucose production and reducing the efficiency of glucose uptake in insulin-sensitive tissues. Decreased insulin output could also impair adipocyte metabolism, resulting in increased lipolysis in the adipocyte and elevated plasma NEFA levels. Thus the process may slowly feed forward, in keeping with observations that the onset of T2D is usually a slow process that takes many years. Even mild impairments of insulin release may have central effects on metabolic homeostasis. Insulin acts in the hypothalamus to regulate body weight, and impaired insulin signaling is associated with changes in food intake and body weight. Thus, β-cell dysfunction resulting in a relative reduction in insulin release would be expected to result in decreased insulin action in this crucial brain region and be associated with weight gain and an aggravation of insulin resistance [8].

Both of the prominent features of T2D – insulin resistance in peripheral tissues and β-cell failure – may result from a defect in insulin signaling. Mice lacking insulin

receptors or a dominant negative mutant of PI3K activity in the liver exhibit insulin resistance, glucose intolerance, and a failure of insulin to suppress hepatic glucose production and to regulate hepatic gene expression [67, 68]. These observations suggest that insulin resistance in the liver contributes to the pathogenesis of T2D, which is not the case for muscle or adipocytes. Insulin resistance at the level of the β-cell may also have a role in the pathogenesis of defective insulin release. IRS-2-deficient mice exhibit impaired pancreatic β-cell function [69]. Moreover, mice that lack insulin receptors in β-cells also have a defect in glucose sensing and a reduced β-cell mass [70]. Although there is currently no evidence that insulin receptor mutations are commonly associated with T2D, a reduction in insulin signaling in the β-cell remains an interesting possibility in further integrating defects in insulin action into the pathogenesis of obesity and T2D.

Another such unifying hypothesis may be that genetically determined mitochondrial dysfunction may cause both insulin resistance in peripheral tissues and impairment of glucose-induced insulin secretion in β-cells [10]. However, it remains to be proven whether altered mitochondrial oxidative phosphorylation is an underlying genetic element of insulin resistance. In a recent study, muscle- and liver-specific AIF ablation in mice initiated a pattern of oxidative phosphorylation deficiency closely mimicking that of human insulin resistance, but contrary to current expectations, resulted in increased glucose tolerance, reduced fat mass, and increased insulin sensitivity [71]. This finding suggests that the moderate deficiency in oxidative phosphorylation that is observed in peripheral tissues of insulin-resistant humans is not a causative factor in T2D but may instead be a compensatory response.

In summary, current concepts to explain the pathogenesis of T2D propose that genes responsible for obesity and insulin resistance interact with environmental factors (increased fat/caloric intake and decreased physical activity), resulting in the development of obesity and insulin resistance. These increase secretory demand on β-cells. If the β-cells are normal, their function and mass increase in response to this increased secretory demand, leading to compensatory hyperinsulinemia and the maintenance of normal glucose tolerance. By contrast, susceptible β-cells have a genetically determined risk, and the combination of increased secretory demand and detrimental environment result in β-cell dysfunction and decreased β-cell mass, resulting in progression to impaired glucose tolerance, followed, ultimately, by the development of T2D. New insights of the genetic bases of these processes, of the cellular events that underlie them and of their relationship to environmental factors should enhance our ability to devise breakthrough therapies for T2D.

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In vivo β-Cell Imaging in Diabetes, β-Cell Hyperplasia, and Insulinoma

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Abstract

A reliable method for (repeated) non-invasive quantification of β-cell mass in vivo in humans will enhance our understanding of the pathophysiology of both type 1 and type 2 diabetes. Individual patients with type 2 diabetes show large differences regarding the relative contribution of insulin resistance or insulin deficiency to the diabetic state. Also, the deterioration of β-cell function varies. When β-cell mass could be measured in vivo in humans, the effects of different diabetes treatments on β-cell mass could be studied and result in a more individually-tailored therapy, based on the principle underlying defect. Furthermore, quantification of β-cell mass could be used for monitoring in patients with diabetes type 1 undergoing islet transplantation. Recently, new strategies for imaging of β-cell in vivo have been developed. For imaging of transplanted β-cell in vivo, the β-cells can be preloaded with superparamagnetic iron-oxide particles. By this approach, magnetic resonance imaging of β-cell mass was possible in mice. For imaging of pancreatic β-cells, radiolabeled tracers are preferable due to their high sensitivity. A tracer targeting the β-cell-specific transport molecule VMAT-2 has been used in mice for positron emission tomography. Another promising approach is targeting of the glucagon-like peptide-1 receptor with a radiopeptide.

Clinical and Scientific Significance of Non-Invasive Determination of the β-Cell Mass in Diabetes

A reliable method for (repeated) non-invasive quantification of β-cell mass in vivo in humans will enhance our understanding of the pathophysiology of both type 1 and type 2 diabetes (T1D, T2D). Progressive β-cell loss is characteristic for T1D, but the natural history of β-cell loss remains to be determined. β-Cell dysfunction is a hallmark of T2D, but it is not known at which stage of the disease this occurs. Individual patients show large differences regarding the relative contribution of insulin resistance

or insulin deficiency to the diabetic state. Also the deterioration of β-cell function varies. Development of diabetes is thought to occur in steps [1]. At early stages, β-cell mass may even be increased [2]. Development of β-cell mass and β-cell function in the course of disease do not necessarily show a direct correlation, i.e. in particular stages of the disease, the β-cell function may be impaired while the β-cell mass is not significantly reduced or vice versa [1]. If a technique were available for non-invasive quantification of β-cell mass, it would be possible to follow the natural history of the decline of functional and afunctional β-cell mass in both T1D and T2D. A method to non-invasively measure β-cell mass in vivo in humans would also enable us to study the effects of different diabetes treatments on β-cell mass which may result in a more individually-tailored therapy, based on the principle underlying defect. Such a technique would for example enable us to monitor β-cell mass in vivo in patients receiving antidiabetic medication thought to increase β-cell mass in T2D (such as Exenatide or inhibitors of dipeptidyl peptidase IV [3]).

Quantification of β-cell mass could also be used for monitoring in patients with T1D undergoing islet transplantation which is a promising method for restoration of glucose homeostasis. To obtain a sufficient number of functioning islets, islets are typically isolated from two cadaveric donor pancreata and transplanted. As yet, it is unknown how many of these islets will contribute to glucose homeostasis directly after transplantation, i.e. how many islets survive during the first weeks after transplantation. Using a number of approximately 800,000–900,000 islets per patient, the rate of insulin-independent patients is approximately 80% after 1 year, which drops to about 65% after 2 years [4]. Monitoring of the β-cell mass after transplantation may help to optimize immunosuppressive therapy regimens and thus help to increase the rate of insulin-independent β-cell recipients.

In diabetes research, non-invasive methods for quantification of β-cell mass (including β-cell loss and β-cell neogenesis) would help to perform longitudinal studies in animal models addressing the questions related to β-cell mass mentioned above. Such methods would allow researchers to image β-cell mass in vivo by small animal imaging techniques but also to follow individual islets or subgroups of islets in vivo. This would greatly improve monitoring of new therapies in animal models, speed up their translation into clinical trials, and would help to reduce the number of animals required because longitudinal studies would no longer require immunohistochemical determination of β-cell mass in pancreatic specimen from killed animals. Furthermore, the effects of new drugs on individual islets (with respect to blood flow, islets biodistribution of β-cell markers, etc.) could be monitored.

Imaging Methods for Non-Invasive Imaging of β-Cell Mass

The optimal solution for non-invasive imaging of β-cells would be a method with high depth penetration and without radiation exposure, offering high sensitivity and

specificity combined with a resolution allowing to image single β-cells. Current imaging techniques offer all these characteristics, but unfortunately no single technique offers all of them at the same time. Therefore, all imaging methods potentially useful for in vivo β-cell imaging are a compromise. For a given task, such as imaging of pancreatic β-cells versus imaging of transplanted β-cells, an individual approach needs to be chosen dependent on the specific technical characteristics of the imaging modality used as well as characteristics of specific tracers etc. In the following, the most important current imaging techniques with potential for in vivo β-cell imaging will be described together with their specific advantages and disadvantages.

Positron Emission Tomography

Positrons are emitted from tracers labeled with positron-emitting radionuclides. These positrons hit electrons in their close vicinity, which results in annihilation and simultaneous emission of a pair of high-energetic photons at an angle of 180° ('coincidence'). These photons cause light flashes in a crystal ring, which are detected by adjacent photomultipliers. The source of the photons is located on a straight line between the positions in which the signals have been detected in the crystal ring. This information is used to create 3-dimensional (3-D) images of the tracer distribution. The (physical) spatial resolution of modern clinical positron emission tomography (PET) scanners currently reaches about 2 mm.

Single Photon Emission Computed Tomography

For single photon emission computed tomography (SPECT) imaging, low-energy photons (ca. 140–200 keV) emitted by γ-emitting radionuclides are detected by gamma cameras. In comparison to PET, this technique uses thinner crystals for detection of photons. Because the photons detected are not coincident (and therefore the information about the 3-D location of the source is missing), so-called collimators are used to create an image. Collimators are lead plates with bores that let only photons pass which hit the crystal orthogonally and eliminate all others. In comparison to PET, this results in lower detection sensitivity as a part of the emitted radiation is absorbed in the collimators. Since the 3-D images have to be reconstructed from 2-D projection images and the collimators limit the resolution by the size of the bores, SPECT has a lower spatial resolution and quantification is more difficult. The advantages of SPECT are the relative low costs and the wider availability. SPECT radionuclides often have longer half-lives which facilitates labeling and use of tracers.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) relies on the detection of spin relaxation of excited hydrogen nuclei in a magnetic field in water and lipids. In an intensive, uniform magnetic field, the spins of atomic nuclei with a resulting non-zero spin have to arrange in a particular manner with the applied magnetic field according to quantum mechanics. Nuclei of hydrogen atoms align either parallel or antiparallel to the magnetic

field. The magnetic dipole moment of the nuclei processes around the axial field. If exposed to short electromagnetic impulses, some of the magnetically aligned hydrogen nuclei assume a temporary non-aligned high-energy state. When returning to their prior state, a change in a weak magnetic field is induced which can be detected by coils within the magnetic field of the scanner. These signals are then converted into 3-D images. Besides gaining anatomical information, it is also possible for example to measure blood flow. The spatial resolution of clinical MRI scanners reaches ca. 1 mm. The sensitivity of this technique, however, is 3–6 orders of magnitude lower than that of PET.

Optical Imaging

Optical imaging techniques suitable for in vivo β-cell imaging are highly sensitive and will most probably either rely on the detection of a fluorescent tracer or on the reflection of light. For the first technique, a fluorescent probe attached to a suitable tracer molecule is detected by a camera system equipped with a charged-coupled device. An excitation light of a specific wavelength (in the visible light range of 395–600 nm) illuminates the subject which emits light of a shifted wavelength which can be detected. Another option is extended field Fourier domain optical coherence microscopy (xf-FDOCM), a technique with a very high sensitivity and an intrinsic 3-D imaging modality. The underlying contrast mechanism is due to small changes in the refractive index of the investigated tissue layers and translates into a label-free tissue imaging. In addition, due to the intrinsic optical amplification of the low coherence interferometry, tissue structures down to a depth of ca. 2 mm can be visualized. The depth profile of the sample is encoded in the detected interferogram and extracted via a fast Fourier transform. Therefore, FDOCM allows 3-D imaging but needs only 2-D sampling in the x-y direction with an extremely high imaging speed. xf-FDOCM combines advanced illumination concepts based on Bessel beams, which results in a uniform depth-independent lateral resolution close to single cell resolution. FDOCT (Fourier domain optical coherence tomography) in general allows extracting the phase signal, which can be used for fast blood flow measurements. Molecular imaging is an additional option to merge with FDOCM based on molecular tracers with a specifically enhanced absorption. Overall, xf-FDOCM provides a fast 3-D imaging concept with high contrast and high resolution suitable for in vivo imaging of biological tissues and small organ features.

Tracer Development

Development of radiotracers relies on several factors, including size of the tracer molecule, specificity and affinity of binding to a given target, metabolic behavior, metabolic stability, stability of the label, etc. A highly diffusible small tracer molecule rapidly binding to a target with high specificity and affinity is optimal. At the same time, the tracer should be cleared from the background (blood, non-target tissues) as quickly as possible, preferably via the kidneys. Clearance via the liver would lead to

high uptake into the gastrointestinal tract resulting in background activity blurring the target on the images obtained [5]. If the tracer is taken up into the cell, metabolic trapping is one method to obtain high target-to-background ratios. Metabolic trapping means that a tracer is taken up into the cell and stays there if no metabolic pathway for tracer degradation or externalization exists. If metabolic trapping is achieved via the labeling method, the label is called a 'residualizing label'. At the same time, unbound tracer is excreted via the kidney so that the target-to-background ratio increases over time. If a tracer is not internalized, a high affinity is required to obtain a good target-to-background ratio. An example for such a tracer would be a radiolabeled antibody binding with high affinity to an antigen on the surface of the target cells. If a potential tracer molecule would lose its specificity for or binding affinity to a target due to necessary modifications for a residualizing label, it can be labeled with F-18 or C-11, two positron emitters. Labeling with these radionuclides does not (or to a lesser extent) cause major changes to the tracer molecule, in the case of C-11 the radionuclide may even be integrated into the tracer molecule without changing its chemical properties at all. The short half-life of the radionuclides, however, requires efficient and quick labeling procedures. For specific targeting with radiotracers, the choice of the best approach (residualizing label, high-affinity binding, C-11 label to preserve chemical properties) is dependent on the target, the potential tracer molecules and the possible/available synthesis/labeling techniques.

Production of optical tracers can be done based on radiotracers, because the labeling with radiometals can often be replaced by optical dyes. In the case of MRI tracers, paramagnetic particles (such as iron oxide) have to be packed in nanoparticles coated with specific ligands. These ligands may need modification for coating of the nanoparticles. Furthermore, the nanoparticles have to be carefully chosen and optimized concerning their size as this determines diffusibility, residence time in the circulation, and the pathways of elimination.

For clinical β-cell imaging, MRI, PET, and SPECT are currently the most promising imaging techniques. However, none of them is able to resolve single islets or β-cells. Therefore, these techniques have to rely on the quantification of the uptake of tracers into the β-cells. These tracers would have to be specific for β-cells and target the β-cells with a high affinity. Furthermore, quick targeting would allow scanning within a short period of time after tracer injection which would make the technique more convenient for clinical use.

For research purposes, basically the same is true as said above for MRI, PET, and SPECT. However, other imaging techniques with a limited depth of penetration would also be suitable for in vivo β-cell imaging. Optical imaging offers unique possibilities for imaging in animals. Because the penetration depth is less important in animals than in humans, it is possible to use optical imaging techniques in animal research. Fluorescent tracers would allow to image pancreatic β-cells in vivo in animals. Basically, the same tracers as used for PET, SPECT, or MRI imaging could also be labeled with fluorescent dyes. For quantification, 3-D scanning would be preferable

which is currently in a developmental state. This technique would also rely on the specific accumulation of a tracer in β-cells and would not be able to resolve single β-cells or islets. In contrast, due to the high spatial resolution and the short scanning times, xf-FDOCM would allow to image and follow single islets over time. The limitation is the lower penetration depth, which is why in vivo imaging would require laparotomy. Therefore, this technique would for example be suitable for anatomical imaging of islet response to certain stimuli. Furthermore, β-cell tracers changing the refractory index of the tissue in which they have accumulated might also be detectable with this technique. Whether the sensitivity of xf-FDOCM for this application is sufficient will largely depend on the amount of tracer accumulation in the β-cells as well as the improvement of existing techniques.

Different Approaches to in vivo β-Cell Imaging

A large variety of potential β-cell imaging agents has been tested as tracers, including mannoheptulose, glibenclamide, tolbutamide, serotonin, L-DOPA, dopamine, nicotinamide, fluorodeoxyglucose, fluorodithizone, glyburide analogs, and antibodies [6–9]. For most of the agents developed for β-cell imaging it has not been demonstrated that they are able to precisely determine the β-cell mass in vivo [6, 10]. For most agents, uptake into the pancreas was relatively low or uptake into the β-cells was not sufficient in relation to uptake in the exocrine pancreas to allow sensitive and specific imaging of the β-cells [6, 8, 11].

In order to develop a method for more specific β-cell imaging, the use of radiolabeled antibodies against pancreatic β-cells has been proposed [9]. It was shown that these antibodies specifically bind to β-cells in vivo. So far, (small animal) in vivo imaging has not been demonstrated using this antibody preparation. A major obstacle for the use of antibodies for imaging is their long circulatory half-life. Furthermore, the large size of antibodies also hinders diffusion into the target tissues. Therefore, their use for nuclear medicine imaging is limited as the high blood activity decreases the target-to-background ratios [5]. This is especially true if the target is small, consists of solid tissue, and is surrounded by other well-perfused organs and large blood vessels, as is the case in pancreatic β-cell imaging.

For imaging of transplanted β-cells, optical imaging methods have been successful in animal models. As stated above, optical imaging may be of very limited use in a clinical situation. For research in animal models, however, it may prove very useful. Park et al. [12] have successfully generated transgenic mice expressing firefly luciferase under control of the mouse insulin I promoter. In this animal model, the pancreatic β-cells can be visualized by whole-body bioluminescence imaging. Whether β-cell imaging in this mouse model may be helpful beyond measurement of blood glucose levels in the evaluation of diabetes remains to be shown. Another example of successful in vivo β-cell imaging is the work of Fowler et al. [13] who were able to follow transplanted islets

by bioluminescence imaging for more than 8 weeks. Prior to transplantation, the islets had been transfected with the luciferase gene by adenovirus-mediated gene transfer.

Recent Advances in in vivo Imaging of Pancreatic β-Cells

Recently, a compound targeting vesicular monoamine transporter-2 (VMAT-2) specifically expressed on β-cells has been labeled for PET imaging of β-cells (dihydrotetrabenazine (DTBZ) labeled with ^{11}C or ^{18}F). In a rat model of spontaneously developing diabetes (BB-DB rat), a significant decline in pancreatic uptake of ^{11}C-DTBZ anticipating the loss of glycemic control, could be found in longitudinal PET studies [14]. Based on comparison of standardized uptake values of ^{11}C-DTBZ and blood glucose concentrations, loss of more than 65% of the original standardized uptake value correlated significantly with the development of persistent hyperglycemia. A high pancreatic uptake was also observed in rodents and non-human primates [15]. However, after a major chemical eradication of β-cells, the pancreatic uptake of DTBZ was reduced by only 30–40%, indicating that the uptake of the compound might lack specificity for eventual clinical use [16]. In clinical studies with optimized dynamic imaging protocols and analyzing several parameters (uptake, pancreatic mass, etc.), the maximal decrease of tracer uptake in patients with longstanding T1D with complete loss of β-cell mass never exceeded 45% in comparison to healthy volunteers [17, 18]. Therefore, at this timepoint it is questionable whether small differences in β-cell mass in T2D or in the early phases of T1D can be detected with this tracer, let alone the small increase of β-cell mass which is anticipated under due conditions promoting neogenesis or after pharmacological treatment.

Currently, a tracer based on the glucagon-like peptide-1 (GLP-1) analog Exendin targeting the GLP-1 receptor is under development in our laboratory. With funding from the National Institutes of Health, it is optimized for imaging in humans. In comparison to DTBZ, this compound also has a high in vivo uptake in the pancreas seems to be more specific, resulting in reduction of pancreatic uptake by $>$90% after chemical eradication of β-cells as opposed to 30–40% for DTBZ [19, 20]. Therefore, this tracer may be more suitable for clinical imaging of pancreatic β-cells than DTBZ.

In comparison to conventional optical imaging strategies for 3-D imaging, xf-FDOCM offers the unique possibility of in vivo dynamic imaging. This technology is currently optimized for the determination of islet size, β-cell mass, and islet blood flow (by Doppler technique). This technique has successfully been used for in vivo imaging of human retina including blood flow [21] on a microscopic level. First results of imaging in pancreatic islets have been very promising. Single islets could be measured in size and the distribution of the islets could be determined in mouse pancreata [M. Villiger and T. Lasser, Lausanne, Switzerland, pers. commun.]. In the future, this technique may allow to follow islet size, islet distribution, and islet blood flow in vivo in animal models of diabetes over time.

Recent Advances in in vivo Imaging of Transplanted β-Cells

In vivo imaging of transplanted islets prelabeled with superparamagnetic iron-oxide nanoparticles using MRI has successfully been achieved in animal models by two groups [22, 23]. The islets are incubated with superparamagnetic iron-oxide nanoparticles before transplantation. Not incorporated nanoparticles are removed by washing before transplantation. After transplantation into the liver, the loaded islets are visible as voids on the T_2-weighted image. The signal seems to be dependent on the number of living islets because in rejection models of islet transplantation the signal decreases with the decreasing number of islets [24, 25]. It has been shown that the labeled islets preserve their function [23]. Another major concern may be that not only living islets are imaged but also deposits of superparamagnetic iron-oxide nanoparticles remaining after the islets themselves have died. This theoretical problem has not been reported to have hampered the experiments so far and localization of signal and islets as determined immunohistochemically has been reported to correlate well [23, 25]. With this technique, living islets have been followed in vivo up to 6 months after transplantation. Therefore, this technique may be suitable for clinical imaging of transplanted islets. Currently, the National Institutes of Health (USA) are supporting research aiming to make the technique of prelabeling of human islets available for clinical imaging (for further information about the funded projects, please see 'CRISP' (Computer Retrieval of Information on Scientific Projects), a searchable database of all biomedical research projects federally funded by the USA under 'http://crisp.cit.nih.gov/').

PET imaging has also successfully been used to image transplanted β-cells. Prior to transplantation, islets were transfected with HSV1 (herpes simplex type 1) thymidine kinase (HSV1-TK) phosphorylating derivatives of thymidine and acylguanosine. These are retained within the cells and further metabolized by cellular kinases to di- and triphosphates. After systemic administration of the HSV1-TK ligand 9-(4-[^{18}F]-fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG) to mice after islet transplantation, islet uptake of the tracer could be quantified by PET imaging and correlated with the number of transplanted β-cells [26, 27].

Recent Advances in Imaging of Insulinoma and β-Cell Hyperplasia

Current imaging techniques for the detection of insulinomas in patients with hyperinsulinemic hypoglycemia include endosonography, which is considered the most sensitive imaging technique, followed by MRI or CT. Somatostatin receptor scintigraphy (SRS) is a valuable imaging technique in most neuroendocrine tumors of the gastrointestinal tract. In the case of benign insulinomas, however, its value is limited due to the fact that many insulinomas do not express the somatostatin receptor subtypes binding octreotide resulting in a sensitivity of 10–50%. Therefore, SRS plays a more important role in the staging and follow-up of malignant insulinomas which more commonly

express the respective somatostatin receptor subtypes [28]. Despite technical improvements in sonography, CT, and MRI, the detection of insulinomas in the pancreas still remains a challenge. Kauhanen et al. [29] reported a series of 10 consecutive patients with clinically suspected insulinoma that have been imaged by PET using ^{18}F-DOPA (dihydroxyphenylalanine) as tracer molecule. Because neuroendocrine tumors belong to the group of 'APUDomas' (APUD – amine precursor uptake and decarboxylation), insulinomas are able to take up and decarboxylate L-DOPA, converting it to dopamine [30]. In all of the patients that had been included into the study, the cause of hyperinsulinemic hypoglycemia could be detected. In 8 cases, this was an insulinoma which could be visualized by ^{18}F-DOPA while CT, MRI and sonography were negative. In 2 patients, nesidioblastosis (β-cell hyperplasia) was diagnosed. In all patients, histological confirmation of the findings was available. This study, despite the low number of patients caused by the rarity of the disease, impressively shows the potential power of ^{18}F-DOPA as tracer for the detection of neuroendocrine tumors and especially insulinomas. This study also shows that a considerable part of the patients diagnosed with hyperinsulinemic hypoglycemia may suffer from β-cell hyperplasia instead of insulinoma. These results support the finding that about 5% of adult patients with hyperinsulinemic hypoglycemia suffer from β-cell hyperplasia and not from insulinoma [31]. In two studies in children suffering from congenital hyperinsulinism, PET with ^{18}F-DOPA was able to differentiate those patients with focal β-cell hyperplasia from those with diffuse β-cell hyperplasia. As a consequence, the children with focal β-cell hyperplasia underwent resection of the focus and were cured. In diffuse β-cell hyperplasia, medical treatment is performed and in case of non-response, subtotal pancreatectomy is the remaining therapeutic option [32, 33]. From 49 children included in one study, 15 were recognized as suffering from focal β-cell hyperplasia while from the remaining 34 patients, 24 underwent partial pancreatectomy due to insufficient response to medical treatment. From these, only 3 had focal β-cell hyperplasia false negative in ^{18}F-DOPA PET [32]. These results demonstrate that ^{18}F-DOPA PET provides additional value in the diagnosis of insulinoma as well as β-cell hyperplasia. In β-cell hyperplasia, it is the only imaging technique available to differentiate between focal and diffuse disease with high sensitivity and specificity.

In preclinical studies, a new radiopharmaceutical targeting the GLP-1 receptor has been tested for imaging of pancreatic insulinomas in a transgenic mouse model [34]. This tracer showed an unusually high specific uptake [35] and in a clinical situation may offer advantages over ^{18}F-DOPA because it has no uptake into the exocrine pancreas. Therefore, it may even become possible to image diffuse β-cell hyperplasia with this tracer. This tracer molecule has also been tested for peptide receptor radiotherapy in the same transgenic mouse model and showed very promising results [36]. Therefore, it may also offer a therapeutic option in (malignant) insulinoma and in diffuse β-cell hyperplasia. Whether uptake into the exocrine pancreas is present in humans (which may disturb quantification of uptake and detection of diffuse β-cell hyperplasia) has to be evaluated in the future [37].

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Incretin-Based Therapies for the Treatment of Type 2 Diabetes – DPP-4 Inhibitors and Incretin Mimetics

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Abstract

The current treatment options for type 2 diabetes do not achieve the glycemic goals. Improving islet function by incretin hormone action is a novel and attractive therapeutic approach. There are two different approaches to utilize incretin action in the treatment of type 2 diabetes: dipeptidyl-peptidase IV (DPP-4) inhibitors inhibit the degradation of the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide. The DPP-4 inhibitors sitagliptin and vildagliptin are orally active and have been shown to be efficacious and safe. They reduce hemoglobin A1c (HbA1c), fasting and postprandial glucose by glucose-dependent stimulation of insulin secretion and inhibition of glucagon secretion. They are weight neutral. Indirect measures show an improvement of β-cell function. DPP-4 inhibitors do not cause a higher rate of hypoglycemia in comparison to metformin or placebo. The second option is using GLP-1 receptor agonists, called incretin mimetics. Exenatide is available for subcutaneous injectable therapy, liraglutide is in phase III clinical trials. Both compounds are peptides. They reduce HbA1c sustainedly, lead to weight loss and also show an improvement in β-cell function in man and an increase in β-cell mass in animal models.

Introduction

Current Therapies in Type 2 Diabetes

The prevalence of type 2 diabetes is rising dramatically consecutively leading to an increase of complications of the disease. It is predicted that the total number of people with diabetes may be 370 million worldwide by the year 2030, along with a substantial rise in prediabetic conditions [1]. Since type 2 diabetes is increasing and most patients do not reach their therapeutic goals, novel treatment options are needed.

While insulin resistance is constant in the course of type 2 diabetes, islet function continuously declines over time. Disease progression of type 2 diabetes is characterized by the loss of islet function. Hyperglycemia, free fatty acids, cytokines, adipokines and toxic metabolic products may lead to a loss of β-cell function and β-cell mass in the islets. The α cells in the islet additionally develop a disturbance of glucagon secretion. In healthy subjects, glucagon secretion is suppressed under hyperglycemic conditions, whereas in type 2 diabetes glucagon secretion is elevated, leading to excessive glucose production by the liver [2].

The therapeutic options currently available do not address the problem of islet-cell dysfunction. Sulfonylureas and glinides both exclusively stimulate insulin secretion from the β cells; metformin and glitazones act on insulin resistance, and α-glucosidase delays the breakdown of complex carbohydrates. Exogenous insulin replaces the endogenous secretory insulin deficit, although it potentially causes weight gain and hypoglycemia. The progressive loss of islet function observed in type 2 diabetes is not ameliorated by any of the current therapeutic options [3].

Glucagon-Like Peptide-1

The gastrointestinal hormones 'glucose-dependent insulinotropic peptide' (GIP) and 'glucagon-like peptide-1 (GLP-1)' stimulate insulin secretion after a meal. They are responsible that orally administered glucose evokes a greater insulin response than an intravenously administered glucose infusion calculated to lead to identical serum glucose excursions. The difference in the insulin response was called the 'incretin effect' [4]. GIP and GLP-1 are important 'incretins'. The incretin effect is reduced or even absent in patients with type 2 diabetes [5].

The promising therapeutic potential of GLP-1 as a pharmacological tool for treating type 2 diabetes was discovered in the 1990s. In contrast to other insulinotropic agents, e.g. the sulfonylureas, the insulinotropic effect of GLP-1 depends even more closely on the actual glucose concentration providing the possibility of glucose normalization without the risk of hypoglycemias. In patients with type 2 diabetes, exogenous GLP-1 increases insulin secretion and normalizes both fasting and postprandial blood glucose. It further has the ability to restore the blunted first phase of insulin secretion in type 2 diabetes [6].

Besides the glucose-lowering effects, GLP-1 has a variety of additional 'non-insulinotropic' physiological actions that may be advantageous in type 2 diabetes therapy: it suppresses glucagon secretion from the α cells and slows gastric emptying. It therefore contributes to satiety and to a slower passage and resorption of carbohydrates. Additionally, GLP-1 acts as a mediator of satiety in the hypothalamus, where it is also found as neurotransmitter [3]. Patients with type 2 diabetes having received GLP-1 as a continuous infusion have lost body weight [7]. Furthermore, GLP-1 stimulates β-cell formation from precursor cells and also inhibits their apoptosis leading to an increase in β-cell mass and to an improvement in β-cell function [8]. Table 1 summarizes the biological effects of GLP-1 that are favorable in view of the pathophysiolgical findings in type 2 diabetes.

Table 1. GLP-1 actions in type 2 diabetes

Parameter	Type 2 diabetes	GLP-1 action
Insulin secretion	disturbed	increased
First phase	diminished	restored
Incretin effect	diminished	restored
Glucagon	hypersecretion	inhibition of secretion
β-Cell mass	diminished	increased (animal data, in vitro)
Appetite/body weight	increased	decreased
Gastric emptying	normal/(accelerated?)	slowed

Due to dipeptidyl-peptidase IV (DPP-4) action, the biological half-life of exogenous GLP-1 is only 1–2 min, therefore treatment with native GLP-1 is not feasible. In order to utilize GLP-1 effects, long-acting GLP-1 analogues or 'incretin mimetics' have been developed as an injectable therapy. The other alternative to utilize GLP-1 action is the inhibition of the degrading enzyme DPP-4 by orally active DPP-4 inhibitors [6].

DPP-4 and DPP-4 Inhibitors

DPP-4 is a ubiquitous enzyme that can be detected in the endothelium of different organs and that is measurable as circulating enzymatic activity in plasma. Besides GLP-1 and GIP, additional peptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP) and gastrin-releasing peptide, are substrates of DPP-4 (table 2). However, the affinity of DPP-4 is higher toward GLP-1 than towards other peptides including GIP. DPP-4 cleaves and inactivates GLP-1 within a few minutes [9]. For this reason, a therapy with native GLP-1 given parenterally is not feasible for the continuous treatment of type 2 diabetes. DPP-4 preferentially cleaves peptides with the amino acid alanine or proline in position 2 of the N-terminus of the peptide chain. The degradation products of GLP-1 are a dipeptide (His-Ala) and GLP-1(9–36)amide, which has GLP-1-antagonistic properties under various conditions [9]. DPP-4 is also expressed on the cell membrane of activated T lymphocytes as CD26. Here, the enzymatic properties of the DPP-4/CD26 molecule do not seem to be important. The influence of DPP-4 inhibitors on immunological CD26-mediated functions is therefore not expected. In clinical studies with DPP-4 inhibitors, no serious side effects or adverse events on immunological regulatory mechanisms were observed [6].

The increase of uncleaved, biologically active GLP-1 by DPP-4 inhibitors offers an alternative therapeutic option. DPP-4 inhibitors are orally active in contrast to incretin

Table 2. Important substrates of DPP-4 [data modified from 9]

With influence on activity and elimination	Without influence on activity or elimination
GLP-1	GRH
GIP	Interleukin-1β
GLP-2	Interleukin-2
PACAP	IGF-1
Neuropeptide Y	Prolactin
Peptide YY	HCG
Substance P	Bradykinin
RANTES	
GRH	

GLP = Glucagon-like peptide; GRH = growth hormone-releasing hormone; GIP = glucose-dependent insulinotropic peptide; PACAP = pituitary adenylate cyclase-activating polypeptide; IGF = insulin-like growth factor; hCG = human chorionic gonadotropin; RANTES = regulated on activation normal T cell expressed and secreted.

mimetics [6]. DPP-4 inhibitors also inhibit the degradation of GIP, PACAP and other peptides involved in regulating glucose homeostasis. They could therefore also have additional effects that are favorable in diabetes treatment. DPP-4 belongs to a whole enzyme family of endopeptidases, therefore DPP-4 inhibitors need to have a high selectivity to inhibit exclusively DPP-4 and not other DPPs. The DPP-4 inhibitors sitagliptin and vildaglitpin are two compounds of the DPP-4 inhibitor class that have been approved in various countries. The structures of both molecules are shown in figure 1. Further DPP-4 inhibitors are in development.

Pharmacology of DPP-4 Inhibitors

DPP-4 inhibitors are orally active. Sitagliptin and vildagliptin both have a very good selectivity over other proline-selective peptidases. In humans, the pharmacokinetic and pharmacodynamic properties and tolerability of multiple oral once- or twice-daily doses have been assessed in extensive studies. After a standard meal, active endogenous GLP-1 concentrations are increased two- to threefold by DPP-4 inhibitors. Across doses and multiple clinical and preclinical studies, no apparent adverse effects, including hypoglycemia, have been found or reported so far, and tolerability and safety data are good. Both sitagliptin and vildagliptin inhibited plasma DPP-4 activity and increased active GLP-1 concentrations without producing hypoglycemia. They also have a low propensity to be involved in drug-drug interactions as either a perpetrator or a substrate for metabolism, especially with other antihyperglycemic oral agents [10, 11].

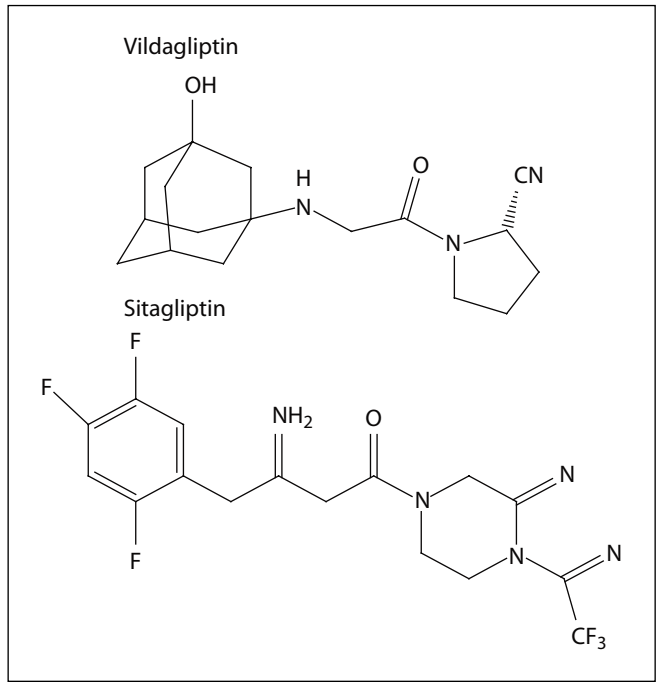

Fig. 1. Structural formulas of sitagliptin and vildagliptin.

β-*Cell Function and* β-*Cell Mass in Animal Studies with DPP-4 Inhibitors*

An important discussion focuses on whether DPP-4 inhibitors are able to influence the disease progression of type 2 diabetes advantageously by slowing or even inhibiting the loss of β-cell mass and function. Therefore the effect of sitagliptin and vildagliptin was investigated extensively in animal models. Diabetic mice treated with DPP-4 inhibitors in long-term studies showed a significant, dose-dependent reduction of glycemic parameters (postprandial and fasting hyperglycemia, hemoglobin A1c (HbA1c)) as well as lipid parameters (plasma triglycerides and free fatty acids). DPP-4 inhibitors increased the number of insulin-positive β cells in islets and the β- to α-cell ratio in different diabetic animals was normalized. Furthermore, islet insulin content was found to be increased and glucose-stimulated insulin secretion in isolated islets was found to be improved in comparison to glipizide-treated mice. According to these experimental results, DPP-4 inhibitors may have the potential to delay or prevent disease progression in type 2 diabetes and to improve β-cell mass and function [10, 11]. In mice deficient in DPP-4 (CD26−/−; DPP-4 knockout mice) concentrations of circulating intact GLP-1 and GIP are elevated and these animals are resistant to streptozotocin-induced β-cell destruction [12]. Detailed studies on the mechanisms of β- and α-cell mass regulation and the regulation of insulin production and insulin secretion in this regard will be necessary to clarify the role of DPP-4 inhibitors in influencing the course of type 2 diabetes.

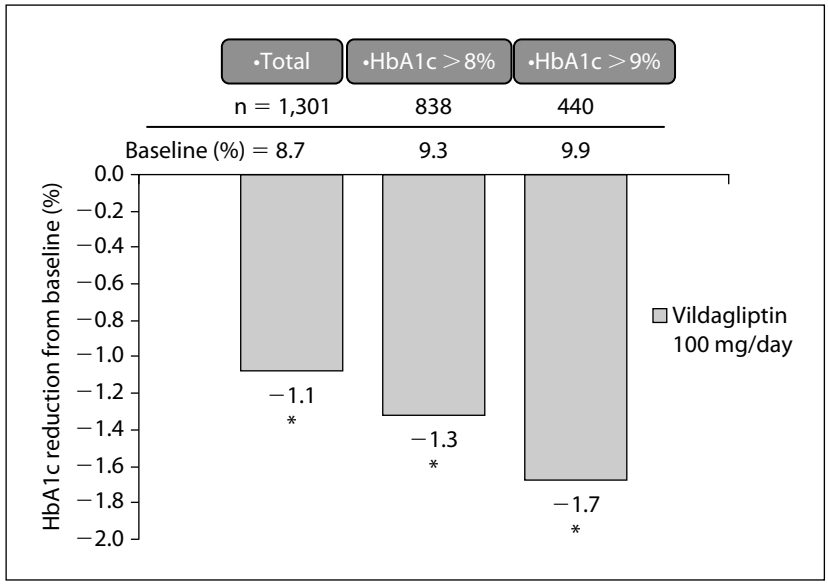

Fig. 2. Effect of a monotherapy with vildagliptin in drug-naive patients with type 2 diabetes not well controlled with diet and exercise. Pooled data of various studies with vildagliptin in monotherapy are shown [reproduced from 11, with permission]. *denotes a significant change vs. baseline ($p < 0.05$ vs. baseline).

Clinical Efficacy and Safety of DPP-4 Inhibitors

In monotherapy, sitagliptin and vildagliptin improved glycemic control both in the fasting and postprandial states as well as β-cell function in patients with type 2 diabetes. Both lead to a significant reduction in HbA1c compared to placebo and to fasting plasma glucose reductions in clinical studies up to 52 weeks (fig. 2). In a meal tolerance tests, 2-hour postprandial plasma glucose concentrations were also significantly reduced. Parameters for β-cell function (postprandial insulin and C-peptide responses, HOMA-B, proinsulin/insulin ratio) were improved. Treatment with DPP-4 inhibitors was weight neutral. In head-to-head comparisons of a vildagliptin monotherapy with either metformin or rosiglitazone monotherapy in drug-naive patients, efficacy in improving glycemic parameters was comparable [10, 11].

As an add-on combination to ongoing metformin therapy in patients with type 2 diabetes not reaching therapeutic goals, both DPP-4 inhibitors reduced HbA1c, fasting plasma glucose, and 2-hour postprandial plasma glucose (fig. 3). The reduction in HbA1c after adding sitagliptin was identical to the reduction after adding the sulfonylurea glipizide to an ongoing therapy to metformin [13]. The above-mentioned β-cell function parameters were improved. The additional DPP-4 inhibitor therapy was generally well tolerated and no increased incidence of hypoglycemia or adverse events was observed.

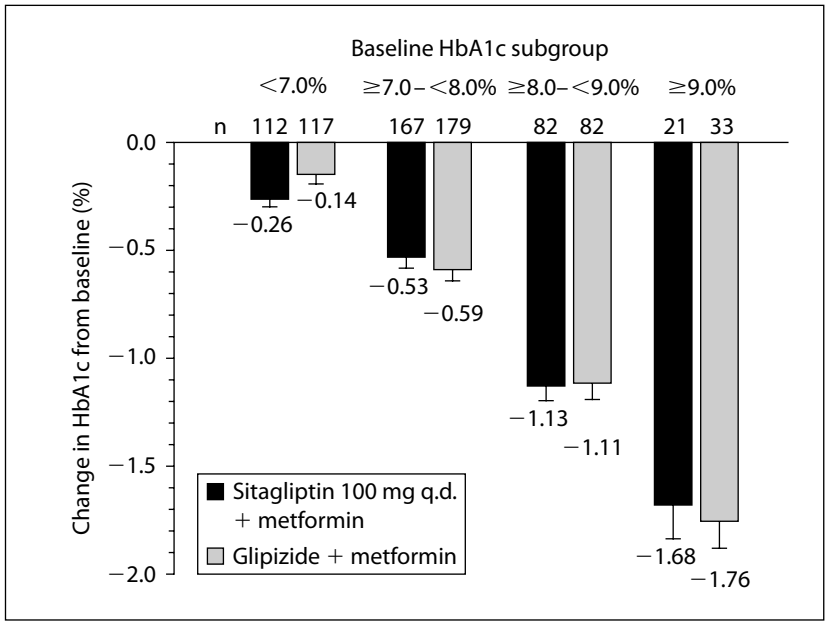

Fig. 3. Mean HbA1c changes (±SEM) from baseline at week 52 in patients on ongoing metformin therapy treated with sitagliptin 100 mg o.d. or glipizide by baseline HbA1c subgroups [reproduced from 13, with permission].

In further studies, DPP-4 inhibitors as add-on therapy to pioglitazone monotherapy was investigated in patients. Similarly, the mean HbA1c was reduced and a significantly higher percentage of patients reached a target HbA1c <7% in the groups receiving the combination therapy with a DPP-4 inhibitor. β-Cell function parameters also improved with the add-on therapy. Sitagliptin and vildagliptin were well tolerated in terms of the number of adverse events and the incidence of hypoglycemia. The incidence of total adverse events as well as hypoglycemic episodes were similar in the treatment and in the placebo groups. The additional DPP-4 inhibitor therapy was weight neutral [10, 11].

A direct comparison of sitagliptin added to an ongoing treatment with metformin showed a similar efficacy to the addition of glipizide to metformin. Sitagliptin was non-inferior in this 52-week study compared to glipizide. HbA1c and fasting glucose decreased equally in both groups. Expectedly, the occurrence of hypoglycemic episodes was much larger in the glipizide group than in the sitagliptin group. The development of body weight showed an increase of 1.1 kg in the glipizide-treated patients, whereas the patients on sitagliptin experienced a weight loss of 1.5 kg [13].

In a study of patients with type 2 diabetes with impaired renal function including end-stage renal disease, dose-adjusted sitagliptin (25 mg/day for patients with

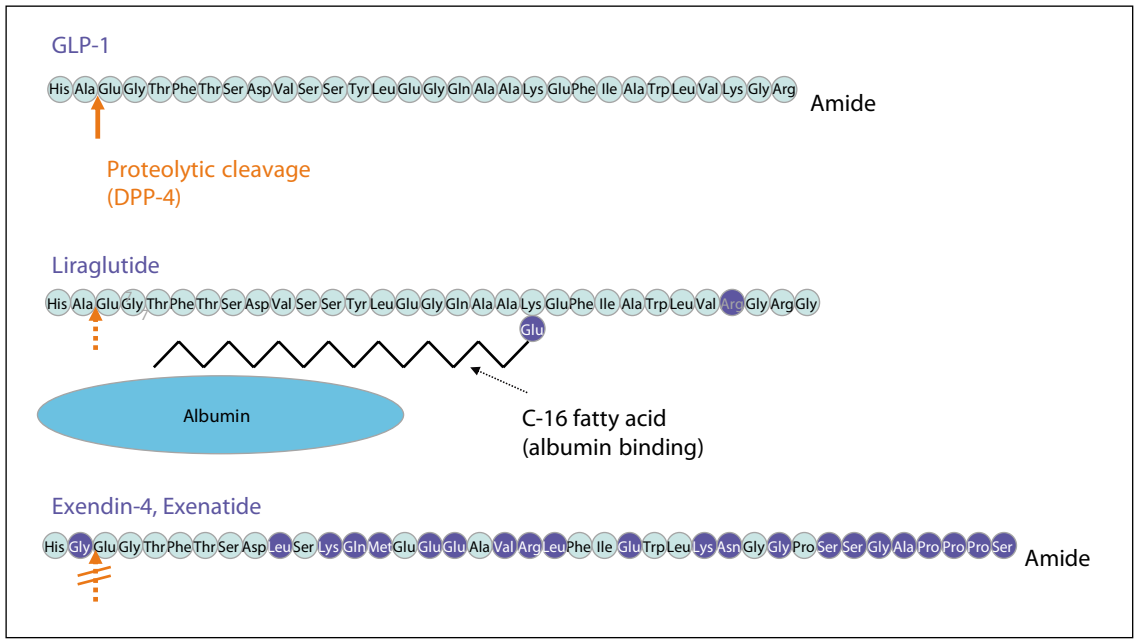

Fig. 4. Amino acid sequences of native GLP-1 (top), liraglutide (middle) and exenatide (bottom). The sequence homologies are highlighted in green. The cleavage site by DPP-4 is indicated by a red arrow.

severely impaired renal function [creatinine clearance <30 ml/min or end-stage renal disease or 50 mg/day for moderately impaired patients]) was generally well tolerated and appeared to be effective [10].

Incretin Mimetics

Exenatide and Its Pharmacological Profile

In 1992, exendin-4, a peptide with a 52% amino acid sequence similarity to GLP-1, was isolated from the salivary gland of the lizard *Heloderma suspectum* (Gila monster). Exendin-4 acts as a high potency agonist at the GLP-1 receptor on β cells and the fragment exendin 9-39 is a high potency antagonist [14]. Synthetic recombinant exendin-4 was named exenatide. Exenatide shares all effects of native GLP-1. Figure 4 shows the amino acid sequences of GLP-1, exenatide and liraglutide in comparison.

A single subcutaneous injection of 10 μg exendin-4 has biological effects for approximately 5–7 h in humans. Exenatide is not enzymatically degraded by DPP-4. Based on this prolonged in vivo half-life compared to GLP-1, with the twice

daily subcutaneous administration sufficient plasma concentrations can be reached to obtain the desired GLP-1 like therapeutic effects in type 2 diabetic patients [15].

Exenatide application in humans leads to antibody formation against this lizard peptide in more than 30% of the cases after prolonged administration. However, this has not yet been associated with any reduction in efficacy, nor do those antibodies appear to cross-react with native human GLP-1. Adverse reactions after subcutaneous administration include nausea and vomiting, and sometimes headache, in a dose-dependent manner. Nausea is transient and most pronounced at the beginning of exenatide treatment. For this reason, a dose titration from 5 μg twice daily to 10 μg twice daily after 4 weeks is recommended when starting therapy. The recommended dose for long-term treatment is 10 μg twice daily before breakfast and before dinner. Hypoglycemia has not been reported during monotherapy, but can occur when exenatide is administered in combination with sulfonylureas [15].

Exenatide was the first incretin mimetic and the first drug using the principle of GLP-1 action to be approved for the treatment of type 2 diabetes in patients not sufficiently controlled with an oral therapy of sulfonylureas, metformin or a combination of both.

Exenatide in Clinical Studies

Exenatide has been tested in several clinical trials. In three placebo-controlled studies, exenatide was given as an add-on therapy to type 2 diabetic patients inadequately treated with sulfonylureas, metformin or a combination of metformin and sulfonylureas. In these studies, fasting blood glucose concentrations decreased, HbA1c levels were reduced by approximately 1.0% overall (fig. 5). Adverse effects were mild, mostly at the beginning of the study and generally gastrointestinal (nausea and fullness). Mild hypoglycemia was noted only in patients receiving sulfonylureas in combination. A significant, dose-dependent and progressive weight loss from baseline was observed amounting to approximately 5.0 kg in 2 years (fig. 5). In addition, exenatide treatment produced clinically significant improvements in cardiovascular risk factors in long-term treatment. Exenatide thus represents an efficacious supplement to failing conventional oral antihyperglycemic agents, and the sustained effect observed in the extension studies and its continued weight-lowering effects must be considered very promising [15, 16].

In comparative studies, exenatide therapy as add on to metformin or a sulfonylurea or a combination of these oral antidiabetic drugs was compared to the add on of a long-acting insulin. Both exenatide and insulin reduced HbA1c levels by approximately 1.0%. Exenatide reduced postprandial glucose excursions more than insulin, while insulin reduced fasting glucose concentrations more than exenatide. Body weight decreased 2.3 kg with exenatide and increased 1.8 kg with insulin glargine in one study. Rates of symptomatic hypoglycemia were similar, but nocturnal hypoglycemia occurred less frequently with exenatide [15, 16].

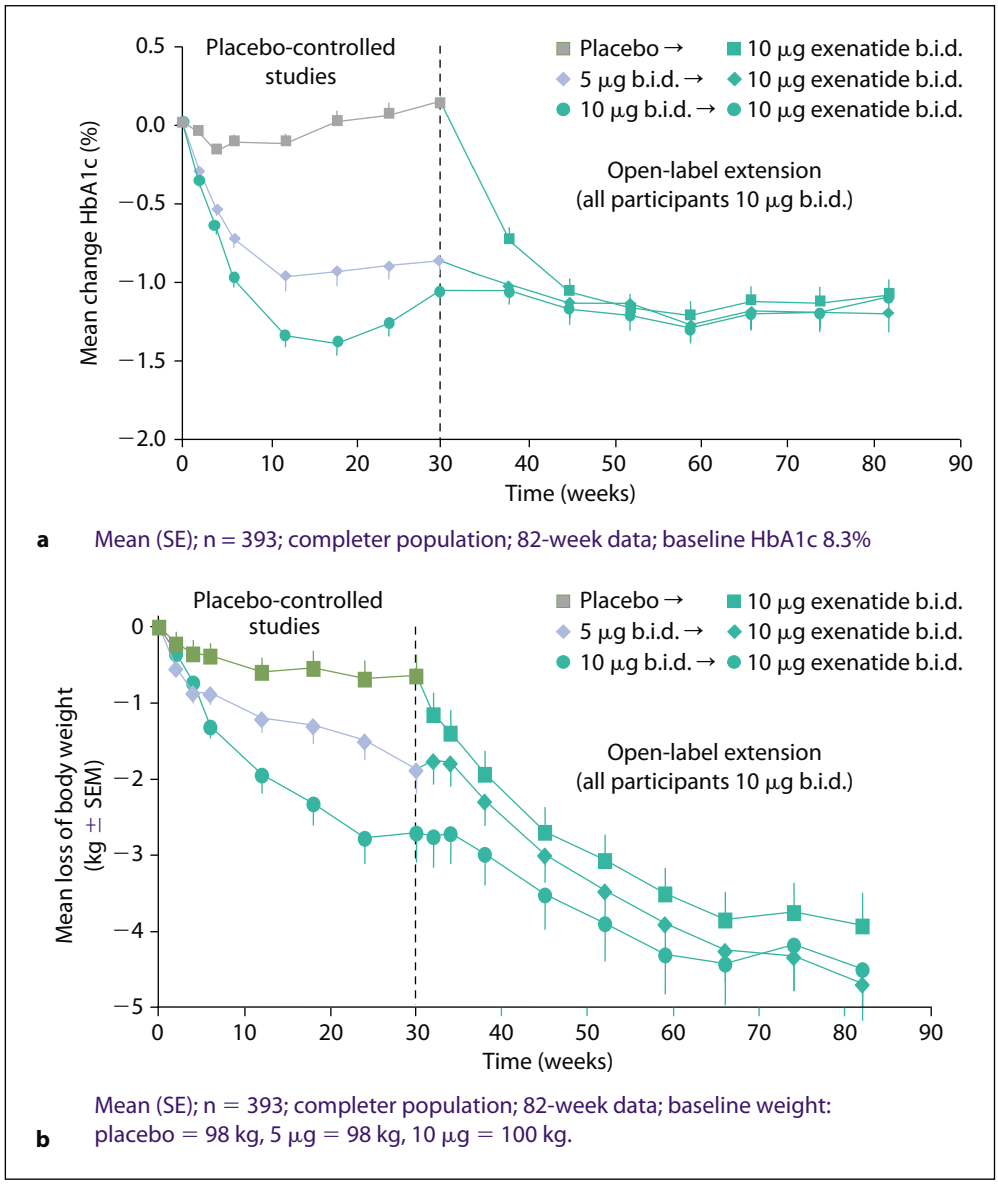

Fig. 5. Clinical efficacy of an add-on therapy with exenatide in type 2 diabetic patients not sufficiently controlled with a therapy with metformin and/or sulfonylureas. Effect of exenatide on HbA1c over 82 weeks (**a**) and on body weight (**b**) [adapted with permission from 16].

Animal and in vitro Studies with Exenatide

In animal studies with rodents as well as in vitro studies, GLP-1 and exenatide cause an increase of β-cell mass due to a stimulation of islet cell neogenesis from precursor cells on the one hand and due to an inhibition of apoptosis of β cells on

the other [17]. The improvement of some functional parameters of β cells can be detected indirectly from the increased insulin secretory capacity in humans receiving GLP-1, however an increase of β-cell mass cannot be directly quantified in man so far [18]. In isolated human islets, glucose-dependent insulin secretion and islet cell morphology is significantly improved, when the islets are incubated with GLP-1 [19]. The reason for the expansion of β-cell mass by GLP-1 is due to an inhibition of apoptotic signaling pathways and a stimulation of signaling pathways leading to a proliferation of β cells [17]. The mRNA levels for Bcl-2 and caspase 3 as markers for apoptotic activity are downregulated in human islets incubated with GLP-1 [19].

Pharmacology and Clinical Profile of Liraglutide

Liraglutide is a long-acting GLP-1 analogue with two modifications in the amino acid sequence of the native GLP-1 involving an amino acid substitution (replacement of the naturally occurring lysine with an arginine in position 34) and an attachment of a C16 acyl chain via a glutamoyl spacer to the lysine residue in position 26 of the peptide chain. Liraglutide is injected subcutaneously once daily. It has a half-life of 11–13 h. Liraglutide is stable towards enzymatic degradation by DPP-4 due to formation of micelles and as a consequence of binding to albumin [20]. It has the above-described effects of GLP-1 in vivo.

In animal studies involving diabetic rodent models, liraglutide has been shown to increase β-cell mass. Liraglutide lowers blood glucose, body weight and food intake in a broad selection of animal models [21].

Clinical Efficacy of Liraglutide

Liraglutide lowers HbA1c, fasting and postprandial plasma glucose dose-dependently. In monotherapy, a decrease in HbA1c of up to 1.7 percentage points was observed with the liraglutide doses of 1.25 and 1.9 mg/day (fig. 6). Liraglutide (1.9 mg/day) provoked a weight loss from baseline by approximately 3.0 kg [22].

As a parameter for β-cell function, liraglutide significantly increased the maximal β-cell secretory capacity compared to placebo, by 114 and 97% for the 1.25- and 1.90-mg doses, respectively. The same doses significantly increased the first phase insulin secretion by 124% (1.25 mg) and 107% (1.90 mg).

Clinical Efficacy of Liraglutide in Combination Therapies

In combination therapy, the clinical efficacy and safety of liraglutide in combination with metformin was assessed and compared to a combination of metformin and glimepiride [23]. The primary endpoints were changes in HbA1c and fasting plasma glucose. Liraglutide added to metformin monotherapy was associated with a significant reduction in fasting plasma glucose by 70 mg/dl and HbA1c levels by 0.8%. The combination of metformin and liraglutide was significantly more effective than the traditional combination of metformin + glimepiride. In addition, the body weight reduction

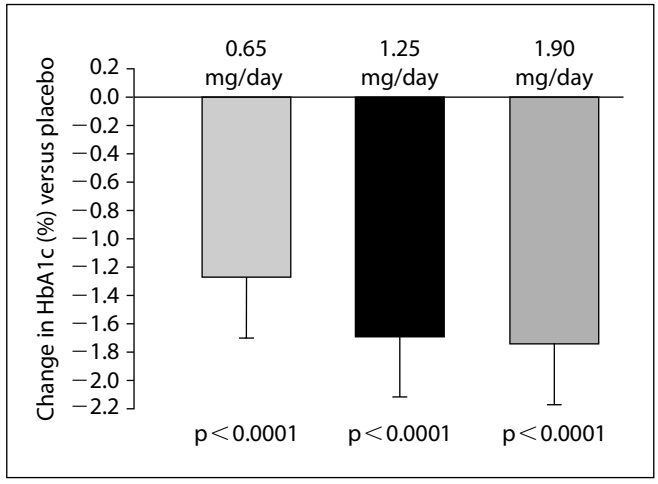

Fig. 6. Dose-dependent effect of liraglutide in lowering HbA1c in monotherapy [reproduced from 24, with permission].

(2.9 kg) was significantly better in the metformin + liraglutide group compared to the metformin + glimepiride group.

Adverse Events

So far, in all clinical trials with liraglutide hypoglycemic events were rare and the incidence of hypoglycemic episodes was similar to that found in patients on metformin treatment or placebo. No major hypoglycemic episodes have been reported by any subject treated with liraglutide [24]. The most frequently reported side effects during liraglutide treatment were gastrointestinal in nature corresponding to GLP-1 receptor activation. Gastrointestinal complaints often occurred within the first week of treatment and with diarrhea and nausea being the most frequent. The majority of subjects reported adverse events of mild to moderate severity [24]. So far, no liraglutide antibodies have been found in clinical studies.

Conclusions

The therapeutic principle of GLP-1 with multiple modes of action, in addition to its glucose-normalizing effect, adds a completely novel and attractive perspective to diabetes therapy. The inhibition of glucagon secretion and the improvement of β-cell function address unmet and important needs in type 2 diabetes therapy. DPP-4 inhibitors are oral agents as opposed to injectable incretin mimetics. In contrast to incretin mimetics, DPP-4 inhibitors do not exclusively act via pharmacological concentrations of GLP-1-like activity, but raise endogenous levels of GIP and other peptide hormones possibly involved in metabolic control within the physiological range [25]. The DPP-4 inhibitors sitagliptin and vildagliptin have been shown to be effective,

well tolerated and safe. Long-term safety based on CD26 effects remains unknown, but to date no significant alterations of immune function have been observed. Both DPP-4 inhibitors have been effective in monotherapy and combination therapies with metformin or glitazones. They did not show an increased incidence of hypoglycemic events in mono- or combination therapies, and the incidence of adverse events was comparable to the incidence observed in the control groups.

The incretin mimetics exenatide and liraglutide act primarily by activation of the GLP-1 receptor. They also offer a wide range of beneficial glucoregulatory effects, including enhancement of glucose-dependent insulin secretion, restoration of first-phase insulin response, suppression of inappropriately elevated glucagon secretion, slowing of gastric emptying, and reduction of food intake. Hypoglycemias only occur in the combination with other insulin secretagogues, since incretin mimetics stimulate insulin secretion only under hyperglycemic conditions. These effects make them attractive for the treatment of type 2 diabetes in patients not sufficiently controlled any more by oral antidiabetic agents. Clinically, besides the improvement in glycemic parameters, a reduction in body weight is observed as well as an improvement in the insulin secretion capacity.

Whether a therapy with DPP-4 inhibitors or incretin mimetics is capable of influencing the natural progressive course of type 2 diabetes with β-cell failure is not known yet. If the effects of DPP-4 inhibitors observed on β-cell mass and function in preclinical studies also apply to human studies, DPP-4 inhibitors could eventually be used in prediabetic stages and the very early stages of diabetes to slow or prevent the progression of type 2 diabetes. Incretin mimetics as injectable therapies are also effective in later stages of type 2 diabetes, where they may also halt disease progression. Further studies are needed to characterize the potential of incretin based therapies to influence the natural course of type 2 diabetes in man.

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Janus Face of Glucose and Glucose-Regulating Hormones

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Abstract

Recently we have discovered that the incretin hormone glucagon-like peptide-1 (GLP-2) is an important mediator of cell migration and that GLP-2 is also able to increase proliferation of colon cancer cells. We found that dipeptidyl peptidase IV inhibitors – thought to be a treatment of type 2 diabetes – enhance these GLP-2-mediated effects, and therefore may elevate the risk for diabetic colon cancer patients. Following this intriguing line of evidence, we discovered that most cancer cell lines display enhanced features of tumor progression when cultured under diabetic conditions. Not only the established stimulus for cell proliferation – insulin – increased the activity of tumor cells, but also hyperglycemic glucose concentrations per se enhanced proliferation and migratory activity of tumor cells. Many tumors display a switch towards aerobic glycolysis – known as the Warburg effect – which is accompanied by a high rate of lactate secretion, leading to an acidification of the surrounding tissues. As a result of an imbalance in the multifunctional interrelationship of different metabolic and signaling pathways, tumor cells profit from diabetic conditions such as elevated levels of blood glucose-regulating hormones (insulin, incretins, etc.) as well as raised glucose concentrations (as a source of energy and for metabolic by-products for synthesis processes), and therefore display increased proliferation and migration rates when compared to non-diabetic conditions. All effects acted in an additional manner, so that cancer cells growing under hyperglycemic conditions with elevated levels of insulin and other glucose-regulating hormones do have an advantage compared to healthy cells and even over the same cancer cells in a non-diabetic setting.

Background

An average person on a normal western diet consumes >0.5 kg of sugar-related carbohydrates each week, consisting of highly refined sugars in the forms of sucrose (table sugar), dextrose (corn sugar), and high-fructose corn syrup, which comes from many processed foods such as bread, breakfast cereals, pastries, candies, ketchup, and a plethora of soft drinks.

Since the 1850s, sugar consumption in Germany has risen 10-fold to 34 kg per person per year. Even more dramatic findings can be made in the USA, with an explosion in the average consumption of pure sugar from 5 lb per person per year (1887–1890) to 135 lb of sugar per person per year in the late 1990s! This goes hand in hand with the consequence that cardiovascular diseases and cancer was virtually unknown in the early 1900s, but today are leading causes of death and the main reason for an increasing mortality in the industrialized world. Therefore, it is necessary to provide a deeper insight into the role of glucose and possible changes in the glucose metabolism in oncogenesis, especially with respect to proliferation, cell signaling and cell survival.

Metabolic Syndrome

The latest statistical studies have revealed that patients with type 2 diabetes bear a higher risk for various kinds of cancer (e.g. breast, colon, kidney, liver, and pancreas) [1–3]. Type 2 diabetes can be seen as an extreme state of glucose intolerance, and is associated with elevated plasma levels of glucose as well as insulin, but also other glucose-regulating hormones are influenced by this disease. More important, this misbalanced glucose metabolism appears both a long time before and after its diagnosis, and is associated with multiple risk factors, such as increased triglyceride levels and reduced HDL cholesterol. Since most of the patients are obese, the complications mentioned are not solely specific for type 2 diabetes, but also indicators for hypertension and other cardiac diseases. All these metabolic disorders can be summarized and are best described as *metabolic syndrome* [4, 5].

The causes of the metabolic syndrome are still not completely understood, and up to now there is no conclusive definition (no ICD-10 code). The actual definition for Germany is adapted from the International Diabetes Foundation IDF [http://www.ipm-praevention.de/docs/Metabolisches_Syndrom_2005.pdf]. A common premise is adiposity or generally a visceral obesity. Therefore, the diagnosis of the metabolic syndrome is existent when a visceral obesity is associated with at least two additional risk factors, such as increased triglyceride levels, diabetes, and reduced HDL cholesterol (or increased LDL and cholesterol).

Excess body weight is the sixth most important risk factor contributing to the overall burden of disease worldwide. In the UK, 12 million adults and 10% of children are now classified as overweight or obese. Average life expectancy is already diminishing; the main adverse consequences are cardiovascular disease, type 2 diabetes and several cancers. Obesity with its array of comorbidities requires careful clinical assessment to identify underlying factors and to allow coherent management. The epidemic reflects progressive secular and age-related decreases in physical activity, along with substantial dietary changes combined with passive overconsumption of energy, despite the neurobiological processes controlling food intake. Effective long-term

weight loss depends on permanent changes in dietary quality, energy intake, and activity. Neither the medical management nor the societal preventive challenges are currently being met [6].

It is still not clear whether obesity or insulin resistance is the reason for the metabolic syndrome or if they are the outcome of a more complex metabolic misbalance. A number of markers of systemic inflammation, including C-reactive protein, are often increased, as are fibrinogen, interleukin-6 (IL-6), tumor -α (TNF-α) and others. Some have pointed to oxidative stress due to a variety of causes including increased uric acid levels caused by dietary fructose. However, a surplus of glucose or nutrition in general not only leads to an increased body weight due to an elevated proliferation of adipose tissue, but also to a permanent elevated serum glucose level which directly influences tumor progression since glucose is the main source of energy. Therefore, also the source of nutrition generating this glucose is of importance in the meaning of bioavailability – influencing digestion regulated by different hormones.

Carbohydrate Degradation and Glycemic Index

Once food has entered the body, the gustatory sense and stomach will signal the arrival of nutrition via the nervous system. Since most people in the western hemisphere do have a regulated daily routine, they are somewhat conditioned with regard to meal times. Even before the first morsel, hormones are secreted ready to digest the next meal. Depending on the source of carbohydrates the circulating glucose will be released at a different velocity. Therefore, blood glucose will also rise in dependency to the origin of carbohydrates in connection to all carbohydrate-degrading enzymes as well as hormones regulating the intestinal uptake of glucose. Because of this, the glycemic index plays an important role in respect to the peak of blood glucose levels. The glycemic index of a meal is defined by the area under the curve of a 2-hour blood glucose response following the uptake of a predetermined portion of carbohydrate. Food rich in easily degradable carbohydrates release glucose much faster into the bloodstream than sources with a high content of fibers. However, meals with a lower glycemic index will increase blood glucose levels slower (but longer), so that the liver as well as muscles and fatty tissue will be able to extract more glucose from the blood. On the other hand, a high glycemic index means that blood glucose levels are increased quickly, which stimulates the pancreas to secrete insulin in order to decrease the level of blood glucose. These expeditious alterations of blood glucose levels are not healthy because the body is forced to react promptly. Since pancreatic β cells secrete insulin in response to an increase in blood glucose, nutrition with a low glycemic index implies less stress to β cells. Therefore, a lower glycemic response is often thought to equate to a lower insulin demand, better long-term blood glucose control and a reduction in blood lipids.

Not only the kind of carbohydrates contained in a meal but also the nutrients themselves will have an influence on further digestion. Directly after the food has reached the stomach, enzymes will start to degrade carbohydrates, and after having left the stomach, intestinal hormones are secreted in response to the nutrients. A functional connection between the small intestine and endocrine pancreas was proven in the 1960s, after it had become possible to determine the exact amount of insulin in plasma. In addition to the classical incretin hormones gastric inhibitory polypeptide-1 (GIP) and glucagon-like peptide-1(GLP-1) are very interesting for investigators today [7]. The peptide hormones GLP-1 and GLP-2 are processed by the prohormone convertase 1/3, and cleaved out from a shared proglucagon precursor and will be released after processing conjointly from intestinal L cells [8]. While GLP-2 promotes intestinal proliferation and hexose uptake, GLP-1 increases insulin production as well as secretion from pancreatic β cells. Furthermore, GLP-1 decreases plasma glucagon levels and delays gastric emptying [9]. Already at this stage, nutrition has an influence on hormone secretion and its own degradation, since the time of gastric degradation is prolonged by the incretin GLP-1. However, GLP-1 stimulates glucose-induced insulin secretion by binding to a specific G-protein-coupled receptor (GLP-1R) linked to activation of the adenylyl cyclase pathway [10]. Moreover, the mechanism of insulin secretion by β cells is very complex and involves different aspects such as glucose sensing and uptake via GLUT-2, cAMP production, but also an ATP-dependent membrane depolarization.

Since GLP-1-induced insulin secretion depends on increased blood glucose levels, recent drug developments have focused on GLP-1-like agonists, because GLP-based medications would prevent hypoglycemic shock situations [for details, see B. Gallwitz, this volume, pp. 30–43]. However, GLP-1 displays a short half-life of about 5 min, caused by degradation by dipeptidyl peptidase IV (DPPIV). Therefore, another aspect of diabetes research was the development of either DPPIV-resistant GLP-1 analogous or DPPIV inhibitors [11]. Finally, both approaches have led to new drugs currently released on the market. However, especially DPPIV inhibitors seem to have a potential of side effects based on their fairly unspecific cleaving actions. Sedo et al. [12] report that DPPIV activity and/or structure homologs may be contributing factors in the pathogenesis of rheumatoid arthritis, since many substrates of DPPIV are proinflammatory peptides such as RANTES, SDF-1α and substance P. By inhibiting DPPIV activity, these peptides display different or prolonged actions than usually found, and therefore may increase the risk of influencing rheumatoid arthritis. Additionally, we found that inhibition of DPPIV activity causes a prolonged action of GLP-2 on colon cancer cells. Typically, GLP-2 promotes cell proliferation of the intestine. We were able to should that this is also the case for tumor cells expressing the GLP-2R. Furthermore, GLP-2 was able to increase migratory activity of colon cancer cells, which was even further elevated by the addition of DPPIV inhibitors [13]. Our

results also support the findings of Kajiyama et al. [14], who described that overexpression of DPPIV/ CD26 induced upregulation of E-cadherin, resulting in a decreased invasive potential in ovarian carcinoma cells. In return this would mean that inhibiting DPPIV activity leads to an increased invasiveness by downregulation of E-cadherin. In earlier studies we have shown a direct correlation between a high expression of PCK-α and low E-cadherin levels and an increase in tumor cell migration [15]. Furthermore, Mizokami et al. [16] report on a SDF-1α-induced tumor cell proliferation and its possible regulation by DPPIV. Since SDF-1α is expressed in various solid tumors and is involved in tumor progression and the development of metastasis, inhibition of DPPIV could increase the risk of promoting such tumors. In addition, Boonacker and Noorden [17] describe in their review that decreased levels of serum DPPIV activity can be found in cancer patients.

Summarizing, both incretin hormones GLP-1 and GLP-2 are secreted in a nutrient-dependent manner and directly influence blood glucose levels, by either increasing the amount of glucose available to the body (GLP-2), or stimulating insulin secretion (GLP-1). Moreover, GLP-2 promotes intestinal tumor proliferation as well as cancer cell migration. All these effects displayed elevated actions when inhibitors of DPPIV were given additionally.

Glucose Transporters

While GLP-2 increases the uptake of hexoses in the small and large intestine, the general glucose uptake is mediated by a certain group of transporter molecules – the glucose transporter families SGLT and GLUT [18]. The organ with the highest consumption of glucose is the brain. Although it only represents maximally 2% of overall body weight, it utilizes up to 25% of total body glucose. Because the brain needs constant energy, the uptake is secured even at low blood glucose levels by GLUT-1 glucose transporters with a high affinity for glucose even at low blood glucose concentrations. However, the major part of blood glucose during the peaks after a mealtime is taken up from peripheral tissues (mainly muscle or fat) via the insulin-dependent GLUT-4 glucose transporters [or details, see A. Schürmann, this volume, pp. 71–83]. Consequently, the peripheral tissues extract glucose from the bloodstream when glucose as well as insulin concentrations are elevated. In healthy subjects it causes blood glucose to drop to normal levels (between 90 and 120 mg/dl – or around 5.5 mM) within 2 h. A good portion of this glucose will be stored as glycogen in the liver and muscle, and will be released again when blood glucose drops below 5.5 mM. Since liver and skeletal muscles can only store limited amounts of glycogen, the remaining blood glucose will be accumulated in the adipose tissue as triglycerides. Since there is a well-organized balance between adipocytes and the bloodstream, a permanent uptake and release of triglycerides occurs depending on the nutritional stage. Therefore, a prolonged period of increased blood glucose will also

lead to permanent elevated levels of triglycerides. Not only adipocytes, but also other tissues have the ability to store triglycerides.

However, cancer cells are not only able to take up high amounts of glucose under hyperglycemic conditions. There are medical findings that even at fasting glucose concentrations tumor cells demonstrated an increased glucose uptake. One of the mechanisms proposed for the elevated glucose consumption in malignant cells is the overexpression of glucose transporters. There are various reports that overexpression of GLUT-1 transporters have been detected in different tumor cell lines [19, 20]. Already in the early 1990s, Brown et al. [21] described in an immunohistochemical study high expression levels of GLUT-1 transporters in breast cancer cells and their descended lymph node metastasis. One common feature in all these studies is that the healthy surrounding tissue did not show any or only very little expression of GLUT-1. This is of great importance, because GLUT-1 transporters have the highest affinity for glucose of all GLUTs, and therefore raise the ability of these cancer cells to have a high proliferation rate, even when blood glucose is at a physiological normoglycemic level. This gives the tumor the advantage to grow permanently and more aggressively and sooner or later to oust the healthy surrounding tissue. Therefore, tumors with high GLUT-1 expression levels do have an additional advantage because they can proliferate in times of normoglycemic conditions and (even at times of starvation) as well as when an excess of glucose is found under diabetic conditions. Especially when cancer patients are obese they may develop a type 2 diabetes eventually. In this case the elevated blood glucose concentration (eventually in combination with increased insulin levels) would be an advantage for the tumor they are bearing.

We could show that both SW480 colon carcinoma cells as well as MDA MB468 breast cancer cells displayed an increased amount of stored triglycerides when cultured under hyperglycemic conditions (see also book cover illustration). The amount of lipid drops accumulated in fat vacuoles was even more elevated when insulin was given in addition to hyperglycemic glucose concentrations. We found for MDA MB468 breast cancer cells an increase in Nile red-positive fat-filled vacuoles of more than 30% for cells cultured in 11 mM glucose plus 100 ng/ml insulin, compared to cells grown under normoglycemic conditions (5.5 mM glucose). These results show that not only typical tissues such as adipose tissue, liver and muscles stored energy-rich compounds for times of undernourishment, but also tumor cells may exhibit a comparable mechanism. This may be of great importance during the treatment of cancer patients, when tumors should be starved out by glucose restriction or during the treatment with antiangiogenic drugs.

Since adipose tissue plays an important role in glucose homeostasis under normoglycemic conditions, it becomes more important under diabetic circumstances. It is known that adipocytes also affect insulin sensitivity in other tissues. Carvalho et al. [22] showed that in obesity and type 2 diabetes – GLUT-4 is down regulated in fatty tissue, and glucose transporters of skeletal muscles are also expressed less, when compared to non-diabetic conditions.

Adipose tissue is also a major source of inflammatory cytokines. The group of You et al. [23] investigated the relationship of abdominal subcutaneous adipose tissue cytokine gene expression to body composition, fat distribution, and metabolic risk during obesity. They determined body composition, abdominal fat distribution, plasma lipids, and abdominal subcutaneous fat gene expression of leptin, TNF-α, IL-6 and adiponectin in 20 obese, middle-aged women and concluded that abdominal subcutaneous adipose tissue expression of inflammatory cytokines is a potential mechanism linking obesity with its metabolic comorbidities.

Those inflammatory cytokines secreted from adipose tissue may play an important role in the progression of cancer. While most of the cytokines originate from immune cells, or tumor cells themselves, cytokines secreted from adipose tissue could play a more central role in the treatment of obese patients. The increased release of cytokines from adipose tissue may play a role in the progression of cancer during the inflammatory state that is associated with obesity as well. Furthermore, leptin is also one of the many products produced by fat cells and has given rise to the ideas that the fat cell is an endocrine cell and that adipose tissue is an endocrine organ [24]. The inflammatory interactions between adipose tissue and cancer cells further promote the progression of cancer since it is a known fact that cytokines promote cancer [25] and that tumor cells themselves may be able to produce inflammatory cytokines [26–28]. However, the interplay between tumors and immune cells is not completely understood, but there is increasing evidence that cytokines play a central role in tumor progression. Azenshtein et al. [29] demonstrated that the expression of RANTES in breast tumor cells is elevated significantly and in a synergistic manner by IFN-γ and TNF-α. Balkwill and Mantovani [25] summarized the role of cytokines tellingly: If genetic damage is the 'match that lights the fire' of cancer, some types of inflammation may provide the 'fuel that feeds the flames'.

Besides that, especially TNF-α seems to have an additional and direct negative influence on insulin-induced glucose uptake. Miura et al. [30] could show that preincubation with TNF-α reduced insulin-stimulated activation of tyrosine kinase, IRS-1 and phosphatidylinositol 3-kinase (PI_3K), and similarly glucose uptake, without affecting basal glucose uptake. This mechanism would further affect glucose uptake and may be one factor of insulin resistance.

Furthermore, numerous studies revealed a direct link between hyperglycemia, high insulin levels and high concentrations of insulin-like growth factor (IGF) and an increased risk of various types of cancer. In 2001, Giovannucci [31] reviewed the evidence which associates high levels of insulin and IGF-1 with increased risk of colon cancer in humans. Clinical conditions associated with high levels of insulin (type 2 diabetes mellitus and hypertriglyceridemia) and IGF-1 (acromegaly) are related to an increased risk of colon cancer, and increased circulating concentrations of insulin and IGF-1 are related to a higher risk of colonic neoplasia. Determinants and markers

of hyperinsulinemia (physical inactivity, high body mass index, central adiposity) and high IGF-1 levels (tall stature) are also related to a higher risk.

Since then, many studies revealed that dietary patterns that accelerate insulin resistance or secretion, including high consumption of sucrose, various sources of starch, a high glycemic index and high saturated fatty acid intake, are associated with a higher risk of colon cancer [32, 33]. Bray [24] also found that the predominant cancers associated with obesity have a hormonal base and include breast, prostate, endometrium, colon and gallbladder cancers.

Glucose Metabolism: Oxidative Phosphorylation versus Aerobe Glycolysis

However, even once glucose has entered the cell, its fate is not the same when normal tissue is compared to malignant cells. Glucose in healthy cells will be metabolized via the glycolytic pathway until pyruvate (see fig. 1, highlighted in light blue), which will be converted into acetyl-CoA, replenishing the concomitant tricarboxylic acid cycle (TCA) (see fig. 1, highlighted in green). The resulting NADH+H+ (and $FADH_2$) will further be used in the mitochondrial respiratory chain to recycle ATP from ADP. Since most cells do not proliferate, they will mainly use glucose for energy (ATP) production and less for synthesis of macromolecules. Optimally, using oxidative glycolysis, 1 mol of glucose will generate 38 mol of ATP.

However, the metabolism of high proliferating malignant cells differs from normal cells by a high rate of glycolysis, accompanied by a massive lactate production. The main difference is manifested in the fate of glucose. While normal cells almost completely convert glucose into ATP (plus CO_2 and H_2O), tumor cells use most of their glucose for biosynthesis of lipids, RNA, DNA and other macromolecules necessary for the production of daughter cells [34]. Since not all of the produced pyruvate will enter the TCA, an accumulation of pyruvate occurs which, after LDH-driven conversion into lactate, will be secreted from the malignant cells into the surrounding extracellular matrix. This permanent lactate secretion induces an acidification of the tissue surrounding the growing tumor, which gives the tumor a further advantage. Low pH will cause the death of normal cells of the surrounding tissue by acidosis and thereby making room for those growing tumors with an acquired ability to increase H+/Na+ exchanger. Additionally, the acidification will increase the activity of matrix metalloproteinases and therefore promote tumor cell migration/metastasis.

Nevertheless, because of the increased glucose flux in malignant cells, ATP production by aerobe glycolysis can exceed the amount of ATP produced by their ancestral cells. The reason for this is that glycolysis is much faster than oxidation of pyruvate via TCA and the respiratory chain [34]. ATP is the standard energy currency of the cell and the AMP/ATP ratio signals the actual energy demand of a cell. Usually, ATP concentrations in cells are around 100 times higher than AMP levels, but the concentration of ATP does not change more than about 10% under physiological

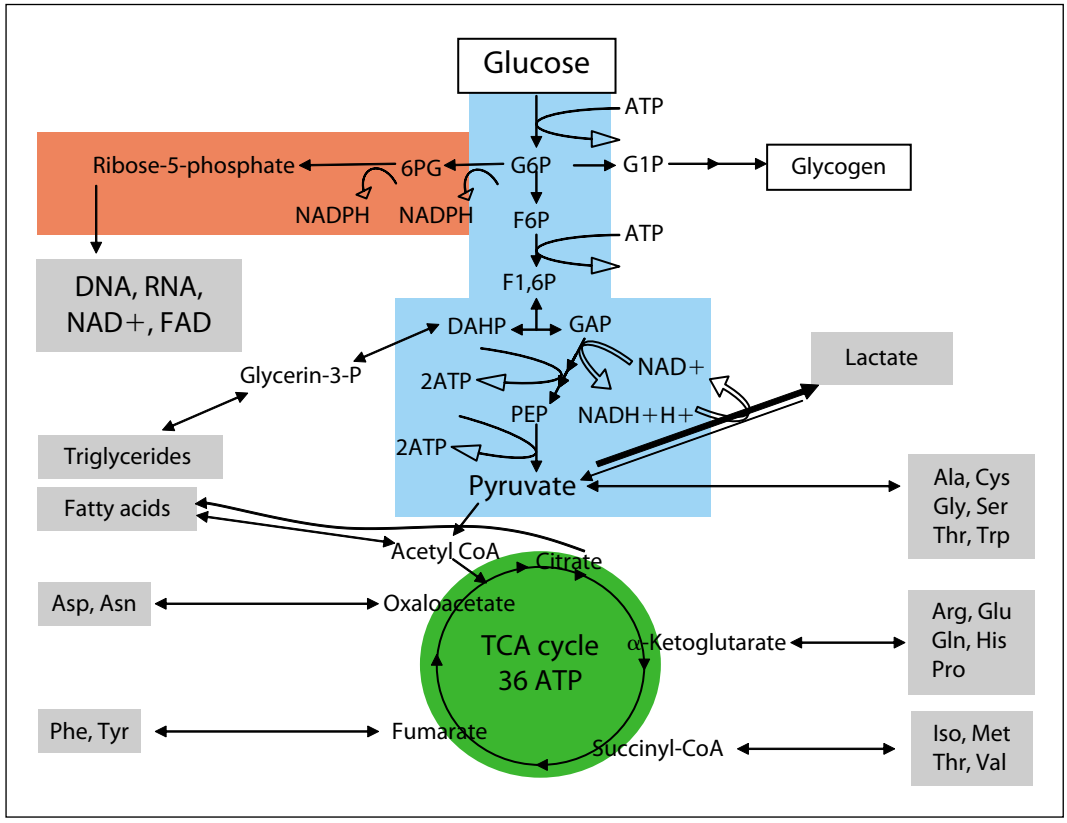

Fig. 1. Scheme displaying the various aspects of glucose metabolism – supporting catabolic as well as anabolic pathways. Non-proliferating (healthy) cells consume glucose mainly for ATP production, via glycolysis (light blue) followed by TCA cycle (green) – accompanied by respiratory chain – resulting in 38 mol ATP per mol glucose. However, cancer cells are highly active in proliferation and therefore exhibit increased glucose consumption. But contrary to non-proliferating cells, cancer cells use major parts of glucose for anabolic processes. Especially the TCA intermediates will be used for the synthesis of non-essential amino acids as well as fatty acids, instead of being the main source for energy. Also the glycolysis intermediate GAP will be used in large parts for the synthesis of triglycerides. A further difference of tumor glucose consumption is the enormous increase of PPP activity (red). The PPP is the source of supply for RNA and DNA production, and secondly supplies cancer cells with NADPH – the main source to regenerate the glutathione complex, giving those cells the ability to fight high concentrations of ROS, caused by the high rate of glucose consumption. Despite this elevated activity of PPP, there is a tailback within the glycolysis at the level of pyruvate. The high throughput of glucose via glycolysis and PPP – in combination with a relatively slow TCA cycle – results in the accumulation of large amounts of pyruvate which will be converted into lactate. The secreted lactate lowers the pH of the tissue surrounding the tumor, leading to acidosis and therefore giving place for further tumor proliferation. G1P = Glucose-1-phosphate; G6P = glucose-6-phosphate; 6PG = 6-phosphogluconolactone; F6P = fructose-6-phosphate; F1,6P = fructose-1,6-bisphosphate; DHAP = dihydroxyacetone phosphate; GAP = glyceraldehyde-3-phosphate; PEP = phosphoenolpyruvate.

conditions, whereas a 10% drop in ATP results in a 6-fold increase in AMP [35]. Thus, the relevance of ATP as a signaling molecule is questionable. However, an increase in the AMP concentration is a result of a decrease in the energy content of the cell. In the case of an AMP increase, AMPK will be activated – inducing an elevated glucose uptake [36]. Thus, glucose consumption of high proliferating cancer cells can be up to 100-fold higher compared to cells of their normal tissues of origin.

The Warburg Effect

Over eight decades ago, Otto Warburg [37] discovered that many tumors exhibit a high rate of glycolysis under aerobic conditions – a phenomenon known as the Warburg effect. He identified a particular metabolic pathway in carcinomas characterized by the anaerobic degradation of glucose even in the presence of oxygen (aerobic glycolysis) that leads to the production of large amounts of lactate. Warburg [37] originally hypothesized that the cause of cancer is primarily a defect in energy metabolism. This hypothesis was based on the observation, repeated and verified many times ever since, proving that cancer cells show clear differences in energy metabolism when compared to normal cells. Although Warburg's hypothesis has been declared for obsolete by the concept of carcinogenesis (including the discovery of oncogenes and tumor suppressor genes), his original interpretations still have their significance, and recent discoveries concerning the participation of mitochondria in the phenomenon of apoptosis again raise the question of the role energy metabolism plays in the process of carcinogenesis.

A better understanding of cancer at the molecular level will provide insights into the causes of altered metabolism of cancer cells. So far, different metabolic alterations have been detected in tumors. Mainly there are oncogenic changes that affect glucose metabolism and cellular responses to hypoxia, as well as physiological responses by tumor cells in order to adapt to hypoxia [38]. In general, hypoxia leads to the induction of the hypoxia-inducible transcription factor, HIF-1, in tumors as well as in normal tissues to the activation of genes that encode glycolytic enzymes (e.g. aldolase A, enolase 1, lactate dehydrogenase A, phosphofructokinase L, phosphoglycerate kinase 1 and pyruvate kinase M) and vascular endothelial growth factor.

Pentose Phosphate Pathway

Especially in tumor cells a second pathway – the pentose phosphate pathway (PPP) (see fig. 1, highlighted in red) – becomes more important when focused on the synthesis of primary by-products. The PPP is logically divided into two components: an oxidative component in which two moles of NADPH are produced for each mole of glucose-6-phosphate that enters the pathway; the second portion of the PPP is the

non-oxidative phase in which the product of the oxidative phase is reorganized into glucose-6-phosphate.

Starting from glucose-6-phosphate, the PPP has two important products that are of great importance in specific tissues. First the pentose pathway yields ribose-5-phosphate which is used in the synthesis of RNA, DNA, and ATP as well as the coenzymes such as NAD+, FAD, and coenzyme A. Particularly, fast proliferating cancer cells need large amounts of ribose-5-phosphate to satisfy their demand on RNA and DNA. The other major product is the reducing equivalents of NADPH which are required for reductive biosynthetic reactions and for protection of tissues from damage due to reactive oxygen species (ROS). The NADPH/NADP ratio plays a central role in the glutathione redox system of all cells and is necessary to survive under aerobe conditions. Especially in tumor cells with a high turnover of substrates, where the formation of ROS is dramatically increased, the demand on NADPH is on a much higher level.

Secondly, NADPH as well as the PPP intermediate product GAP is also required for the synthesis of fatty acids in liver and adipose tissue. Consequently, since NADPH is also the key for the synthesis of fatty acids, it becomes of more concern to cancer cells grown under the above-mentioned hyperglycemic conditions (see book cover). As already described, we have seen a dramatic increase in vacuoles filled with lipid drops (positive staining with Nile red), when tumor cell lines were cultured under permanent high glucose concentrations (11 mM). When insulin was given additionally, the amount of triglycerides stored within those vacuoles was further increased. Since these cells were grown in standard culture medium, most of these fatty acids had to be synthesized by the tumor cells themselves. This indicates that those cells will have a shift towards the PPP followed by a noticeably increase in NADPH turnover. Indeed, first results showed a distinctive increase in glucose-6-phosphate dehydrogenase enzyme activity, indicating a shift towards the PPP. Kuhajda [39] described that aggressive cancer cells display increased lipogenic enzymes, including acetyl-CoA carboxylase β and fatty acid synthase. Also the Warburg effect, which is characterized by a high rate of glycolysis, a lowered pyruvate oxidation rate combined with an increased production of lactate, causes a diminished fatty acid oxidation rate. This restricted fatty acid oxidation is caused by a PI_3K-dependent suppressed expression of carnitine palmitoyl transferase-1 enzyme and aided by the fact that the cancer is successfully limiting the availability of L-carnitine [40]. L-Carnitine is known for its essential role in fatty acid transport and metabolism in mitochondria, but it is also well known for its antiapoptotic activity [41]. It was found that palmitoyl-L-carnitine administration was able to prevail over the control of PI_3K and redirect the metabolism to include a functioning citrate cycle and thus again allowing the oxidation of fatty acid [42]; a reduction in triglyceride synthesis was also observed [43].

Type 2 diabetes is characterized by abnormal metabolism of glucose and fat, in part due to resistance to the actions of insulin in peripheral tissues. The benefit of exercise in diabetic patients is well known and recent research indicates that

AMP-activated protein kinase (AMPK) plays a major role in this exercise-related effect. AMPK is considered as a master switch in the regulation of glucose and lipid metabolism. AMPK is described as a cellular energy sensor because its activity is increased when AMP levels raise. In its activated form it affects increased catabolism and ATP generation. To accomplish this, AMPK exerts an acute regulatory role on numerous metabolic processes including fatty acid oxidation. The AMPK is an enzyme that works as a fuel gauge, being activated in conditions of high-energy phosphate depletion. AMPK is also activated robustly by skeletal muscle contraction and myocardial ischemia, and is involved in the stimulation of glucose transport and fatty acid oxidation produced by these stimuli. In liver, activation of AMPK results in enhanced fatty acid oxidation and decreased production of glucose, cholesterol, and triglycerides. The two leading diabetic drugs, namely metformin and rosiglitazone, show their metabolic effects partially through AMPK. Misra and Chakrabarti [44] showed data that chemical activation of AMPK in vivo with 5-aminoimidazole-4-carboxamide ribonucleoside improves blood glucose concentrations and lipid profiles, which make this enzyme an attractive pharmacological target for the treatment of type 2 diabetes and other metabolic disorders.

Summarizing, the activation of PI_3K plays a central role in the connection of cancer progression and hyperglycemia as well as type 2 diabetes mellitus. Both increased glucose concentrations as well as elevated insulin levels lead to an activation of PI_3K, which in turn is essential for initiating different signaling pathways. For example, the serine/threonine protein kinase Akt/PKB is activated by various growth and survival factors such as insulin. Therefore, the activation of the PI_3K/Akt pathway directly increases the glucose uptake as well as its metabolism, and thereby supports various biosynthetic processes. Wiemann et al. [45] described the connection between cytokines produced by adipose tissue from obese subjects in combination with the increased levels of glucose and insulin and an elevated glucose uptake via PI_3K-dependent upregulation of GLUT-1. Furthermore, growth factors activating the PI_3K/Akt pathway are also able to induce genes necessary for lipogenesis [46].

Conclusion

Glucose is much more than just a sweet nutrition serving as the main source of energy when metabolized via glycolysis, TCA and the respiratory chain. Permanent elevated glucose levels induce an orchestra of metabolic alterations, finally leading to obesity – eventually followed by insulin resistance, the metabolic syndrome and type 2 diabetes mellitus. Obesity goes hand in hand with an accumulation of visceral fat – a major source of various stimuli of tumor progression, such as cytokines and hormones. Furthermore, nutrition itself induces the secretion of different gut hormones promoting degradation and uptake of glucose, finally leading to increased serum levels of glucose and insulin. The fatal combination of an almost infinite glucose source

and growth signal such as insulin, incretins and cytokines promotes tumor progression. However, the same ingredients are important factors in the development of diabetes mellitus. Additionally, recent advances in diabetic research have led to a new group of medications based on the inhibition of DPPIV. These drugs for diabetic patients may in turn support the progression of cancer, since many DPPIV substrates are known mediators of tumor cell proliferation.

Epidemiological data have revealed that over the past decades a dramatic increase could be noticed in both cases of type 2 diabetes and various kinds of cancer. These studies revealed a direct connection between nourishment, obesity and alarming incidents in both cancer and diabetes with a wide range of intersection. There is growing evidence that elevated blood glucose and aerobic glycolysis contribute to cancer cell growth and tumor progression, an observation first described by Otto Warburg in the early 20th century. Aerobic glycolysis may provide cancer cells a growth advantage in the tumor microenvironment. Although ATP production through the glycolytic pathway is much less efficient stoichiometrically in comparison to mitochondrial oxidative phosphorylation, activation of AKT by insulin and increased glucose levels could provide cells with sufficiently high glycolytic fluxes to maintain a higher than adequate level of ATP [34]. Furthermore, malignant cells with a high rate of glucose consumption will accumulate high amounts of pyruvate, which can be used to fuel the TCA for biosynthetic processes and even earlier by-products of glycolysis will enter the PPP for the maintenance of DNA, RNA and fatty acids. Taken together, the switch to glycolytic metabolism may contribute to tumor development through enhanced glycolytic flux accompanied by elevated levels of intermediates of glycolysis as well as TCA for the biosynthesis of macromolecules necessary for the dramatic increase in proliferation seen in the majority of malignant cells.

We hypothesize that this Janus-faced relation between the altered glucose consumption accompanied with various biosynthetic processes contributes to tumorigenesis – and therefore would have significant implications for public health. We conclude that prevention of obesity by well-balanced nutrition as well as physical activity could reduce the predisposition to both cancer and type 2 diabetes mellitus.

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Role of Glucose Metabolism in Carcinogenesis and Cancer Progression

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Abstract

Epithelial malignancies develop through a series of steps often described as somatic evolution. Conceptual models of carcinogenesis typically focus on heritable changes in genes controlling proliferation, apoptosis, and senescence. However, recent work has demonstrated the critical role of cellular metabolism and substrate limitation in the later stages of carcinogenesis. This is based on recognition that somatic evolution of epithelial cancers occurs entirely within a space contained by a basement membrane. This anatomic constraint results in separation of the evolving tumor cells from the underlying stroma including blood vessels so that carcinogenesis occurs in an avascular environment. This enforces diffusion-reaction kinetics that limits substrate delivery to and metabolite removal from tumor cells as proliferation carries them progressively into the lumen. As a result, tumor cell proliferation even following multiple oncogene and tumor suppressor gene mutations will be limited by regional variations in oxygen, glucose, and H+. In the proposed carcinogenesis model, cellular adaptations to these environmental selection forces drive additional somatic evolution that is necessary for formation of an invasive cancer. Specifically, regional hypoxia promotes upregulation of glycolysis to maintain ATP production despite hypoxia, and acidosis promotes upregulation of Na+/H+ exchangers (NHE) or mutations in apoptotic pathways to reduce acid-mediated toxicity. This population is shown to possess a strong proliferative advantage because it produces a toxic, acidic environment through upregulated glycolysis that is lethal to other competing populations but not itself. Retention of the glycolytic phenotype in primary and metastatic human cancers has been convincingly demonstrated by FdG-PET imaging which has shown significantly increased glucose uptake in the vast majority of clinical malignancies but not in benign tumors. This indicates a continuing proliferative advantage and has led to the acid-mediated tumor invasion model. The key role of glucose metabolism and acid concentrations during carcinogenesis and cancer progression provides a mechanism by which systemic metabolic disorders such as diabetes can alter the lifetime risk of carcinogenesis and cancer progression.

Invasive cancer develops over a prolonged period through intermediate premalignant lesions that result from the accumulation of multiple heritable genetic changes [1–4]. This process is often characterized as 'somatic evolution' because it appears formally analogous to darwinian processes wherein phenotypic properties arise through

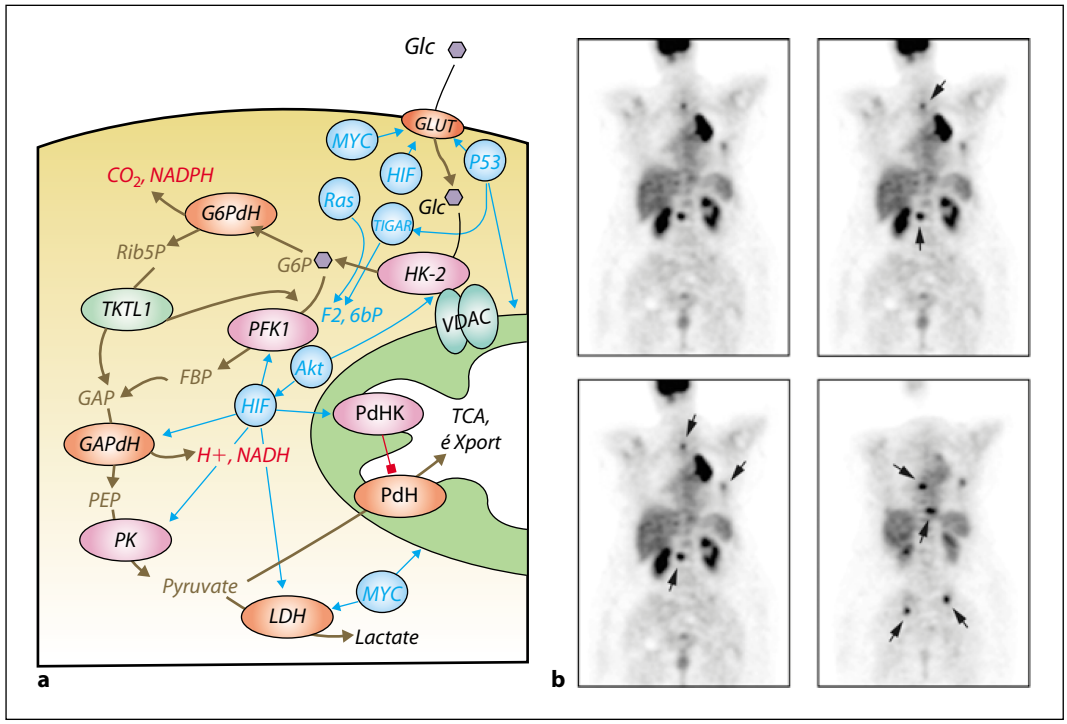

Fig. 1. **a** The major pathways for metabolism of glucose along with key enzymes and control mechanisms. Normal mammalian cells under aerobic conditions will shunt pyruvate into the mitochondria where it is oxidized to H_2O and CO_2 producing about 36 mol of ATP/mol glucose. Under anaerobic conditions, glucose is metabolized to lactic acid producing only 2 mol of ATP/mol glucose and increasing acid excretion into the microenvironment. Almost a century ago, Warburg first noted that cancer cells use the anaerobic pathways and excrete lactic acid even in the presence of oxygen. **b** Clinical imaging with FdG-PET has demonstrated that the vast majority of human primary and metastatic cancers take up far more glucose than normal tissue reflecting the need to increase flux to compensate for diminished energy yield. The arrows point to abnormal areas of increased glucose uptake in primary and metastatic tumors.

mutations and epigenetic events and are then retained or lost depending on their contribution to individual fitness. According to this conceptual model, traits found in invasive cancers must arise as adaptive mechanisms to environmental proliferative constraints within the evolutionary dynamics of carcinogenesis. Conversely, the common appearance of a phenotypic property in most cancers is presumptive evidence that it *must* confer a selective growth advantage [5].

A curious but extremely common property of invasive cancers is 'aerobic glycolysis'. Figure 1 demonstrates that in mammalian cells, glucose active transported into the cell by membrane transporters (GLUT) and is initially metabolized to pyruvate. In the presence of oxygen, pyruvate is shunted into the mitochondria where it is oxidized to H_2O and CO_2. In the absence of oxygen, pyruvate is reduced to lactic acid which is exported from the cell. The inhibition of glycolysis by oxygen is known as

the 'Pasteur effect' and presumably reflects the increased efficiency of aerobic glycolysis which converts 1 mol of glucose into approximately 36 mol of ATP while anaerobic metabolism yields only 2 mol of ATP/mol glucose.

Normal mammalian cells maintain aerobic metabolism except under conditions of ischemia and hypoxia. However, tumor cells typically metabolize a much higher percentage of their ingested glucose to lactic acid even in the presence of oxygen – a phenomenon known as *aerobic glycolysis*, or the 'Warburg effect'. Increased aerobic glycolysis in tumors was first observed by Warburg [6] in the 1920s, who noted a 'remarkable ability of tumors to ferment glucose, even in the presence of oxygen'. This led to the 'Warburg hypothesis', which postulated that cancer is caused by impaired mitochondrial metabolism [7]. While the Warburg hypothesis has been proven incorrect, the experimental observations of increased glycolysis in tumors even in the presence of oxygen have been repeatedly verified [8]. Subsequent interest in the metabolic property of cancers has been periodic. Intense investigation in the 1960s was followed by a steep decline concomitant with the widespread application of new molecular techniques. This was summarized by Sidney Weinhouse' [8]: *'Since our perspectives have broadened over the years, the burning issues of glycolysis and respiration in cancer now flicker only dimly.'*

Recently, however, interest in tumor metabolism has been rekindled due in large part to the widespread clinical application of the imaging technique, positron emission tomography (PET) using the glucose analog tracer, fluoro-18-deoxyglucose (FdG) [9–12]. FdG-PET imaging of thousands of oncology patients has extended the glycolytic phenotype from a laboratory oddity to the clinical mainstream, demonstrating the vast majority (perhaps all) invasive human cancers exhibit significantly increased glucose uptake (fig. 1). Furthermore, glucose metabolism is related to prognosis as tumors with higher rates of uptake are generally more aggressive than those with lower values.

At first glance, these results might seem at odds with an evolutionary model of carcinogenesis, since cells using aerobic glycolysis will be energy-inefficient. Furthermore, the metabolic products of glycolysis, such as lactic acid and hydrogen ions, cause a spatially heterogeneous but consistent acidification of the extracellular space which will result in cellular toxicity. Intuitively, it would seem the Darwinian forces prevailing during the somatic evolution of invasive cancers would select *against* a metabolic phenotype that is more than an order of magnitude less efficient than its competitors and environmentally poisonous. In other words, the accepted tenet of 'survival of the fittest' would appear to generally favor populations with ever more efficient and sophisticated substrate metabolism. So, why do many tumors have upregulated glycolysis?

Recently it has been proposed [13, 14] that, in fact, the remarkable prevalence of upregulated glycolysis in clinical cancers is neither random nor accidental. Rather, it represents an evolved solution to common environmental growth constraints during carcinogenesis. Its near-universal persistence in clinical metastases indicates the phenotype contributes significantly to subsequent evolution of invasive populations

that breach their confining basement membranes and intravasate into metastatic routes.

Carcinogenesis Reconsidered

In healthy tissue, constraints to tumorigenesis exist in the form of hard-wired barriers that prevent disruption of tissue architecture and function by inappropriate cellular proliferation. These controls include highly-regulated activation by specific pro-growth signals and growth-inhibitory barriers through anoikis (see below) and interactions with other cells and the extracellualr matrix [15–18]. Classical theoretical models focus on these normal tissue controls and propose that carcinogenesis is the result of a sequence of heritable events that upregulate oncogenes and downregulate tumor suppressor genes. However, these changes cannot directly account for the consistently altered metabolism observed in the vast majority of human cancers. This has led to the hypothesis of a previously unrecognized era in carcinogenesis dominated by substrate limitation. It is proposed that cellular adaptation during this period of limited resource availability results in adoption of aerobic glycolysis and is critical for the transition to invasive cancer.

As illustrated in figure 2, cellular hyperproliferation on epithelial surfaces occurs in a spatially limited milieu. That is, normal epithelial cells are bound to the basement membrane which separates them from the underlying stroma including blood vessels. In normal tissue, proliferation is constrained by the interactions of the epithelial cells with each other and the basement membrane. For example, an epithelial cell that loses contact with the basement membrane will typically undergo apoptosis – a phenomenon labeled *anoikis* [19, 20]. Thus, early tumor growth requires mutations in the tumor suppressor genes that control anoikis and other normal inhibitory phenomenon such as contact inhibition. However, as demonstrated in figure 2, these hyperproliferative cells are constrained by the basement membrane to grow outward (or into the lumen). As the tumor grows, cells that are most able to proliferate (i.e. the ones with the greatest available potential space) are also most distant from the basement membrane and, therefore, the underlying stroma. One consequence of this may be a decrease in the availability of growth promoters derived from the blood or produced by mesenchymal cells. This microenvironment will promote cellular phenotypes that are independent of external growth promoters. Adaptive strategies might include autocrine production of growth factors, increased numbers of growth factor receptors or upregulation of signal transduction pathways.

For the purposes here, the most interesting consequence of tumor growth distant to the basement membrane is the potential reduction in available substrate. As illustrated in figure 2, as tumor cell proliferation carries them further from the basement membrane, the distance underlying blood vessels also increases. The diffusion-reaction kinetics of this system will inevitably result in a decline in substrate concentration with distance

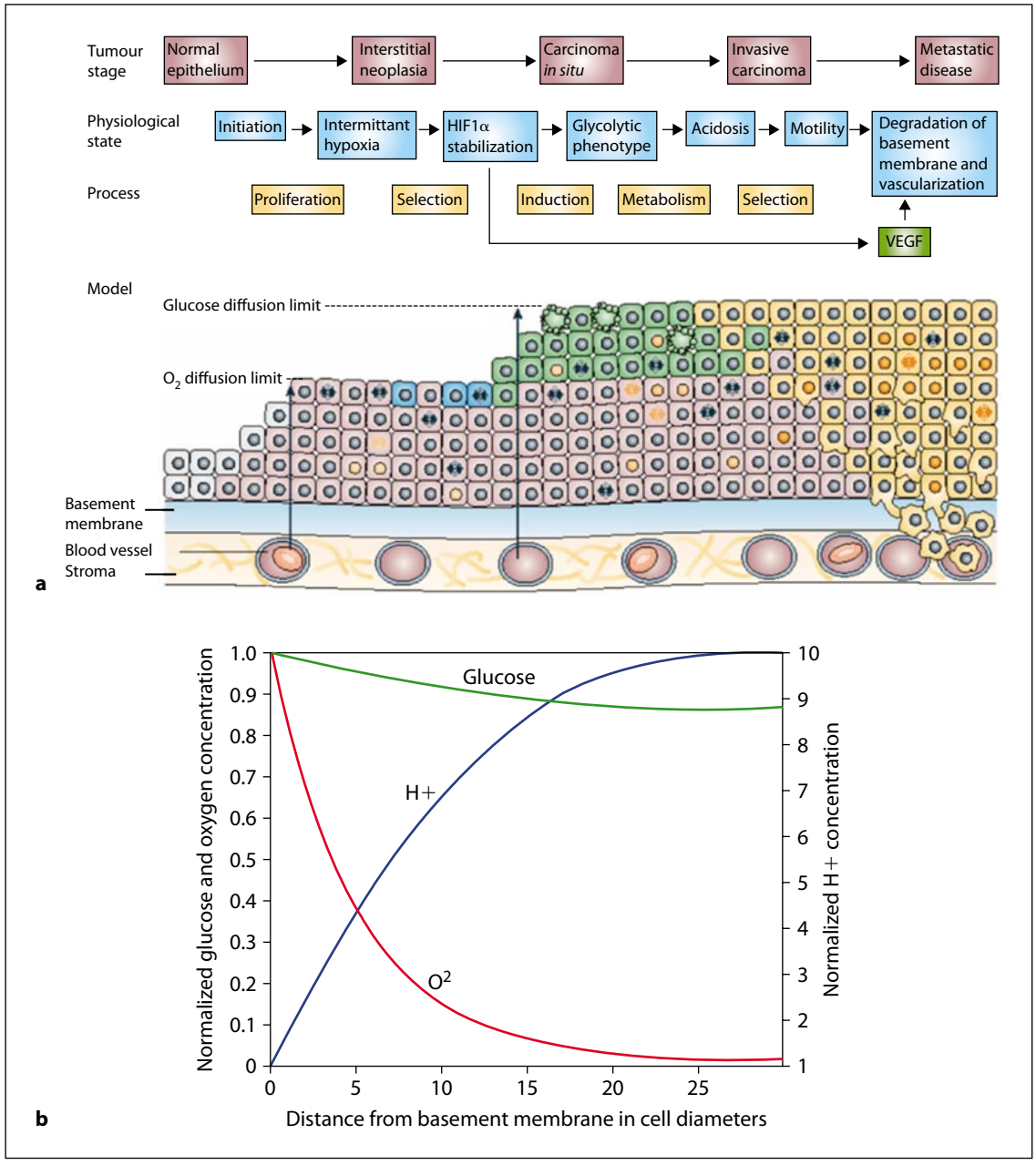

Fig. 2. A proposed model to explain in vivo evolution of aerobic glycolysis during carcinogenesis [from 13, 23]. The key point of this model (**a**) is that in in situ tumors, the proliferating cells are separated from their blood supply by the intact basement membrane. As a result, the proliferating cells are carried further and further into the lumen increasing the distance required for diffusion of substrate. Reaction-diffusion mathematical models demonstrate that significant hypoxia (**b**) will occur within about 5 cell diameters while glucose concentrations remain only mildly decreased. The subsequent increase in anaerobic metabolism of glucose results in a corresponding rise in acid concentrations. This supports the hypothesis that there is a previously unknown era in the later stages of carcinogenesis dominated by cellular adaptations to hypoxia and acidosis.

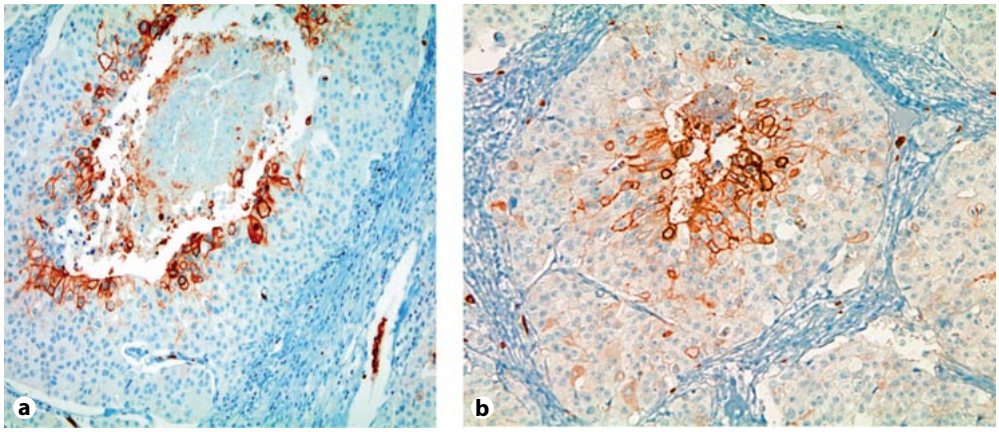

Fig. 3. **a**, **b** Immunohistochemistry stains for glucose transporter 1 (GLUT-1) from clinical specimens with ductal carcinoma in situ (DCIS). Both show upregulation of GLUT-1 in the regions of the tumor farthest from the basement membrane indicating regional hypoxia as predicted by the theoretical model and mathematical simulations in figure 2.

from the blood vessel. This observation led to the hypothesis that regions of premalignant tumors would experience substantial reduction in substrate concentrations [21].

The specific effects on the microenvironment were first examined using diffusion reaction mathematical models. Using typical values and the Krogh equation for oxygen diffusion [22], it can be readily calculated that significant hypoxia will occur in regions >50–100 μm from the basement membrane. However, as demonstrated in figure 2, these models also show that glucose concentrations will remain fairly high in these hypoxic regions. Thus, the microenvironmental selection forces will favor adoption of the glycolytic phenotype. These predictions are consistent with experimental results in tumor spheroids as well as clinical observations in breast ductal carcinoma in situ (DCIS) (fig. 3) in which GLUT-1 and carbonic anhydrase IX are upregulated in the central regions of DCIS [23, 24].

Evolutionary Consequences of Hypoxia and Acidosis

Recognition of the reliable development of hypoxia in premalignant lesions has allowed the Warburg effect to be derived from modeling of cancer populations in terms of Darwinian selection [21, 25]. It is proposed that aerobic glycolysis arises through two evolutionary steps and represents a critical development in the transition from in situ to invasive cancer.

The first evolutionary step is adoption of upregulated glycolysis as a result of fixed or cyclical hypoxia. This transition is observed in many organisms, suggesting that hypoxia-sensitive transitions between glycolysis and aerobic metabolism represent a

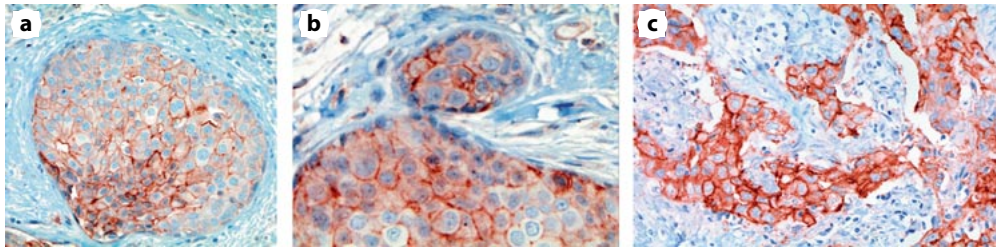

Fig. 4. a–c Immunohistochemical stains [23] for Na+/H+ exchanger-1 (NHE-1) in human DCIS showing upregulation and therefore resistance to an acidic environment in a region invasive breast cancer (**c**). These observations support the proposal that creation of an acidic microenvironment is critical for transition from in situ to invasive cancer.

fundamental metabolic signaling pathway common to air-breathing vertebrates and is subverted during the progression of carcinomas. In normal tissues, cellular response to hypoxia is largely mediated by stabilization of intranuclear HIF-1α [26, 27]. Not surprisingly, increased expression of HIF-1α is very frequently observed in malignant cells [28–34] that also exhibit constitutively upregulated glycolysis.

As noted above, a constitutive increase in glycolysis also results in acute and chronic acidification of the local environment. This produces an additional environmental selection pressure because an acidic microenvironment is ordinarily toxic to mammalian cells typically resulting in apotosis due to increased expression of caspase [35, 36]. Thus, the acidosis produced by the glycolytic flux, in turn, produces a selective pressure benefiting cells with resistance to acidosis, such as cells with increased H+ transporter activity (e.g. N+/H+ exchanger) [23] (fig. 4).

The phenotype that emerges following this evolutionary process is, thus, both glycolytically upregulated and resistant to extracellular acidosis. This cell population possesses a powerful selective advantage not only because they are more resistant to the hypoxia of the tumor microenvironment, but also because they actually produce toxic (acidic) conditions for surrounding cells that have not developed an acid-resistant phenotype. This advantage permits this transformed population to invade first into the normoxic regions of the in situ tumor and then through the basement membrane as the tumor transitions from in situ to microinvasive cancer (figs. 4, 5).

Finally, the intermediates of the glycolytic pathway are involved in the pentose phosphate pathway, which plays an essential role in recycling NADPH, a reducing agent critical in anabolic processes such as nucleic acid synthesis. NADPH is also involved in counteracting reactive oxygen species, ROS, which are produced during reperfusion or increased iron metabolism [37]. Therefore, the transition to a glycolytic metabolism strategy may also be favored by the selective advantage conferred to cells by increased NADPH recycling, thus allowing for enhanced anabolism and/or resistance to ischemic-reperfusion induction of ROS.

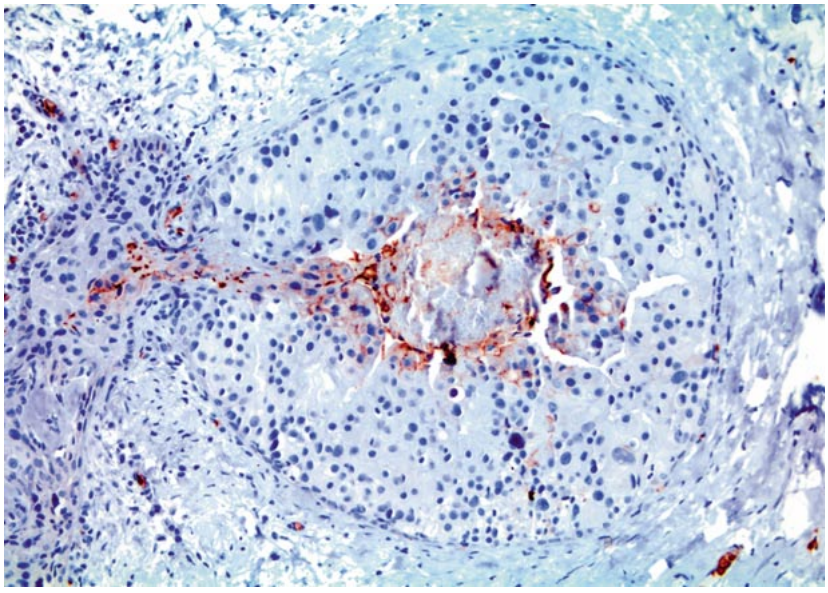

Fig. 5. Immunohistochemical stain for GLUT-1 from a clinical specimen [23]. Note that there is increased expression of GLUT-1 in the central regions of the DCIS as predicted by the model. In addition the population with increased GLUT-1 also invades into the adjacent normoxic region of the DCIS and then through the basement membrane into a focus of microinvasion.

Acid-Mediated Invasion

The persistence of upregulated glycolysis in clinically evident primary and metastatic tumors indicates that this phenotype continues to confer a proliferative advantage beyond the transition from in situ to invasive cancer. This may be explained by the acid-mediated tumor invasion hypothesis [38–39] (fig. 6). It has been demonstrated both mathematically and empirically that intratumoral acidosis that results from upregulated glycolysis results in diffusion of $H+$ ions along concentration gradients into peritumoral normal tissue [39]. Normal cells, which lack a mechanism to adapt to extracellular acidosis (such as a p53 mutation), are unable to survive under such conditions while the tumor populations continue to proliferate. In addition, acidosis itself can be mutagenic and clastogenic, possibly through inhibition of DNA repair [40, 41]. It has also been demonstrated that prolonged acidosis can lead to inhibition of gap junction conductance and spontaneous transformation of normal diploid fibroblasts [42]. The resulting phenotypic diversity enhances the evolutionary potential of the tumor population accelerating malignant progression and adaptation to therapeutic strategies. Finally, under some conditions, low pH stimulates in vitro invasion and in vivo metastasis [43, 44]. The mechanisms of

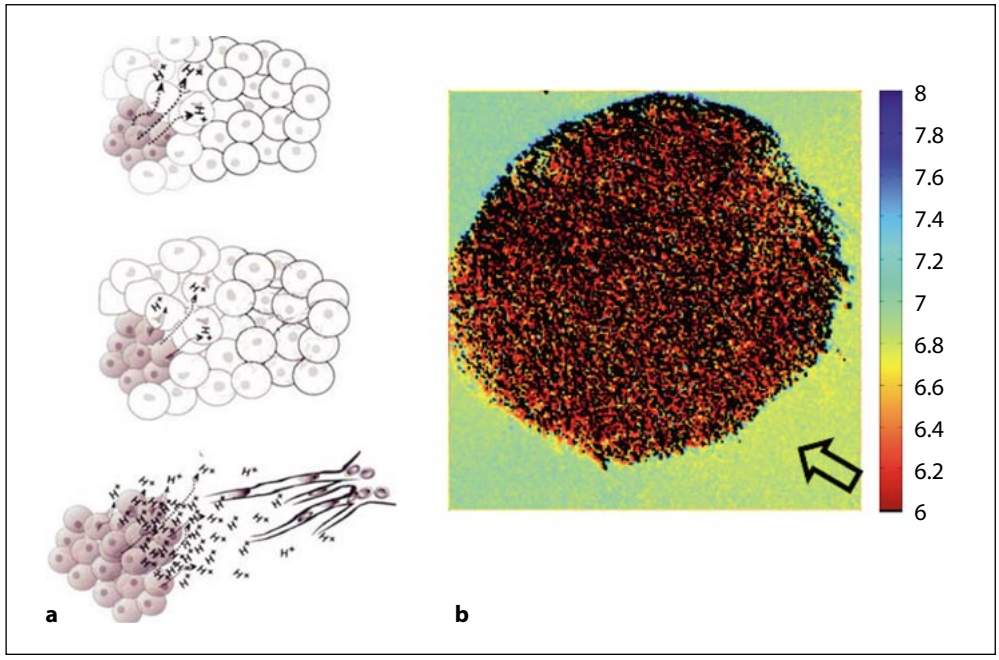

Fig. 6. The acid-mediated invasion model [38, 39] proposes that the acidic tumor microenvironment causes H+ ions to diffuse along concentration gradients into peritumoral normal tissues. As discussed in the text, this results (**a**) in normal cell death through acid-mediated apoptosis, extracellular matrix degradation due to acid-induced release of proteases, and increased angiogenesis through acid-mediated release of VEGF. **b** The pHe distribution within an in vivo tumor and the peritumoral normal tissues using fluorescent ratio imaging. Using the color code it is apparent that the tumor pHe is generally $<$6.5 while the normal tissue immediately adjacent to the tumor (arrow) has a higher but still quit acidic pHe in the range of 6.8–7.0. More distant normal tissue had a pHe of 7.3–7.4.

such induction are not known but probably involve the metalloproteinases and/or cathepsins which promote the degradation of the extracellular matrix and basement membranes [45].

Metastasis

Thus far, we have focused on the role of upregulated glycolysis and resistance to extracellular acidosis in adaptation to conditions in early premalignant lesions and in evolution of invasive primary cancers. However, we note that this phenotype is also likely to be critical in development of metastases. Migratory cells invading the stromal tissue and distant sites probably also experience periodic hypoxic-ischemic episodes favoring cells that are glycolytic and resistant to acid-induced apoptosis.

Consistent with this, intratumor lactate levels predict the probability of metastases in cervical cancer and a correlation between GLUT-1 expression levels and metalloproteinase expression has been reported [46, 47]. Furthermore, circulating tumor cells, when they arrive in new environments, are subject to a new and potentially harsh adaptive environment and selection pressures. For example, in a typical lung colonization assay, as many as 10^5 cells are injected into tail veins, while fewer than 100 generally survive to form colonies. Cells pretreated with hypoxia (which stabilizes HIF-1α) for 24 h are four times more likely to survive than their normoxic counterparts [48]. This suggests the glycolytic phenotype may also contribute significantly to the efficiency of metastasis, perhaps due to their ability invade into the tissue at the site of metastasis and to survive in harsh conditions caused by vascular plugging that result following impaction of tumor emboli in terminal arterioles.

Conclusion

Abnormally increased glucose uptake is demonstrated by FdG-PET in the vast majority of human primary and metastatic cancers. This is due to increased anaerobic metabolism which requires increased glucose flux to offset the decreased efficiency in ATP production. Since Warburg's pioneering research nearly one century ago, it has been clear that tumor cells selectively use the anaerobic glycolytic pathways even in the presence of oxygen – a phenomenon described as aerobic glycolysis or the Warburg effect. This observation initially seems at odds with the more modern concept of carcinogenesis as somatic evolution. That is, there is no clear evolutionary advantage and, in fact there seems to be a substantial disadvantage, to aerobic glycolysis which is both energetically inefficient and produces an acidic and potentially toxic microenvironment.

Recent analyses of the evolutionary dynamics of carcinogenesis using mathematical models, in vitro experiments, and clinical observations have specifically addressed this paradox. This work proposes a previously unknown era in carcinogenesis in which environmental selection forces are largely generated by hypoxia and acidosis. Cellular adaptations in the premalignant lesions favors phenotypes with constitutively upregulated glycolysis and resistance to acid-induced toxicity. This population is shown to possess a powerful adaptive advantage because it can create an acidic environment (through aerobic glycolysis) that is toxic to its competitors but not itself.

The acid-mediated tumor invasion model proposes that the glycolytic phenotype is retained in invasive primary and metastatic cancer because the intratumoral acid diffuses along concentration gradients into adjacent normal tissue causing normal cell death and degradation of the extracellular matrix. The acid-resistant tumor cells are then readily able to migrate into and proliferate within this damaged tissue.

The key role of glucose metabolism and substrate concentrations during carcinogenesis provides a mechanism by which systemic metabolic disorders such as diabetes can alter the lifetime risk of cancer development. However, many questions

remain and much work will be necessary to definitively understand and quantify the roles of diabetes and obesity in carcinogenesis and cancer progression.

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Glucose Transporters: Their Abnormalities and Significance in Type 2 Diabetes and Cancer

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Abstract

Glucose, the major substrate for energy production in mammalian cells, is transported into the cell via facilitative glucose transporters (GLUT). The GLUT family consists of 14 members, which differ in their tissue distribution and substrate specificity. Expression of several GLUTs is controlled by hormones and environmental factors and differential expression is involved in various disease states such as diabetes and cancer. Insulin-stimulated glucose uptake in skeletal muscle and adipose tissue is critical for reducing post-prandial blood glucose concentrations and the dysregulation of this process is one hallmark of insulin resistance and type 2 diabetes. Type 2 diabetes is also associated with β-cell failure that is characterized by the inability to secrete sufficient insulin in response to glucose. Impairment in glucose-sensing contributes to β-cell dysfunction. GLUT2, and the glucose phosphorylating enzyme glucokinase, are key elements for glucose-sensing of the pancreatic β-cell, the initial event in the pathway for glucose-stimulated insulin secretion. In the insulin-resistant state, e.g. induced by a high-fat diet, expression of both GLUT2 and glucokinase is reduced thereby impairing glucose-stimulated insulin secretion. The majority of cancers and isolated cancer cell lines overexpress the GLUT family members which are present in the respective tissue of origin under non-cancerous conditions. Moreover, due to the requirement of energy to feed uncontrolled proliferation, cancer cells often express GLUT proteins which would not be present in these tissues under normal conditions. This overexpression is predominantly associated with the likelihood of metastasis and hence poor patient prognosis.

The existence of an organism depends on the continuous provision of energy for metabolic processes. Glucose is the main source of energy in eukaryotic organisms. It is obtained directly from the diet, principally following the hydrolysis of ingested disaccharides and polysaccharides, and by synthesis from other substrates in organs such as the liver. Glucose derived from the diet is transferred from the lumen of the small intestine, and both dietary glucose and glucose synthesized within the body have to be transported from the circulation into target cells. These processes involve the transfer of glucose across plasma membranes, which occur via integral transport

proteins. These transporters comprise two structurally and functionally distinct groups, whose members have been identified over the past two decades, namely (i) the sodium-dependent glucose cotransporters (SGLT, members of a larger family of sodium-dependent transporters, gene name *SLC5A*) [1] and (ii) the facilitative sugar transporters (GLUT family, gene name *SLC2A*) [2]. Elevated glucose levels are rapidly returned to normal (5–6 mM) even after huge caloric ingestions, and they are maintained at only slightly lower levels during long-term starvation. Such control prevents severe dysfunctions such as loss of consciousness due to hypoglycemia and toxicity to peripheral tissues in response to the chronic hyperglycemia of diabetes.

The Family of Sodium-Dependent Glucose Cotransporters

SGLT are located on small-intestine and kidney brush-border membranes. SGLT1, SGLT2, and SGLT3 are structurally different sodium-glucose cotransporters with 59–75% identity, and exhibit no homology with GLUT proteins [3]. They catalyze glucose transport into the cell against a concentration gradient. This transport process is a cotransport of one glucose and of one (for SGLT2) or two (for SGLT1 and SGLT3) $Na+$ ions in the same direction. The energetically favored movement of a $Na+$ ion through the plasma membrane into the cell, driven both by its concentration gradient and by the membrane potential, is coupled to the movement of the glucose molecule. SGLT proteins exhibit binding sites for glucose and $Na+$ on their exofacial surface. The simultaneous binding of $Na+$ and glucose to these sites induces a conformational change, generating a transmembrane pore that allows both $Na+$ and glucose to pass into the cytosol. After this passage, the proteins revert to their original conformation. In the steady state, $Na+$ ions transported from the intestinal lumen into the cells are pumped by a $Na+/K+$-ATPase across the basolateral membrane. Glucose concentrated inside the cell by the symport moves outward through the basolateral membrane via GLUT proteins. Unlike SGLT1 and SGLT2, SGLT3 does not transport glucose and rather acts as a glucosensor. In the presence of glucose, SGLT3 depolarizes the membrane potential because of an uncoupled inward $Na+$ current at pH 7.5 mediated by a transporter and/or channel mechanism. This glucose-activated inward $Na+$ current is not accompanied by glucose transport [4]. Additional members (SGLT4–6) have been assigned but await complete functional and structural characterization. Amino acid comparisons of human SGLTs range between 57 and 71% sequence identity; they exhibit no homology with the facilitated glucose transporters [3].

The Family of Glucose Transport Facilitators

Glucose transport facilitators (GLUT proteins) are uniporters which catalyze the diffusion of glucose into (or out of) cells along the concentration gradient [2, 5]. Within most

cells, glucose is rapidly phosphorylated and metabolized. Thus, under normal conditions the influx of glucose into cells does not alter its concentration gradient. In liver, kidney, and intestinal mucosa, GLUT proteins catalyze the efflux of glucose from cells, when the intracellular glucose concentration exceeds the serum glucose concentration.

The family of GLUT proteins comprises 14 structurally related members, GLUT1–12, HMIT, GLUT14 (29–65% identity). Among these, there are glucose (GLUT1–3, 4, 8, 10, 14), fructose (GLUT5, 7, 11), polyol (GLUT12), and myoinositol (HMIT) transporters [2, 5]. At present, the function of the other family members is incompletely characterized. The presumed secondary structure of all GLUT proteins is similar, with 12 membrane-spanning helices, intracellular N- and C-termini and a large cytoplasmic loop. GLUT proteins carry charged residues at the intracellular surface of the proteins which are believed to provide the proper orientation and anchoring of the helices in the membrane, and to participate in the conformational changes during the transport process. Several sequence motifs, the sugar transporter signatures, are conserved in all family members, and are essential for the function of the proteins [2].

According to a comparison of the sequences, the GLUT family can be divided into three subclasses [2, 5]. Class I comprises the thoroughly characterized members GLUT1–4 and GLUT14 that are distinguished mainly by their tissue distribution (GLUT1, erythrocytes, brain microvessels; GLUT2, liver, pancreatic islet (β-cells); GLUT3, neuronal cells; GLUT4, muscle, adipose tissue; GLUT14, testis), their affinity to glucose, and their hormonal regulation. Class II comprises the fructose-specific transporter GLUT5 (testis, intestine, muscle) and three related proteins, GLUT7 (intestine, testis, and prostate), GLUT9 (pancreas, kidney, liver), and GLUT11 (heart, muscle, pancreas, placenta, kidney). For GLUT7, GLUT9, and GLUT11, fructose-inhibitable glucose transport activity has been demonstrated after expression of their mRNA in *Xenopus* oocytes. Class III comprises 5 isotypes: GLUT6 (brain, spleen, leukocytes), GLUT8 (testis, brain, adipocytes), GLUT10 (pancreas, liver), GLUT12 (heart, prostate), and HMIT, a myoinositol transporter (brain). Glucose transport activity has been shown for GLUT6, GLUT8, GLUT10, and GLUT12.

Glucose Homeostasis and Hormonal Regulation of Glucose Transport

Blood glucose concentrations need to be maintained within narrow limits and are therefore kept at a steady level of about 80–110 mg/dl. The liver plays a central role in maintaining glucose homeostasis by balancing the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis. Several substrate cycles in the major metabolic pathways of the liver play key roles in the regulation of glucose production. For instance, glucose-6-phosphatase (Glc-6-Pase) is a substrate cycle enzyme that catalyzes the terminal step in both the gluconeogenic and glycogenolytic pathways, and is opposed by the glycolytic enzyme glucokinase. Glc-6-Pase activity is known to be inhibited by various metabolites of carbohydrate, lipid, and protein catabolism

as well as products of PI_3 kinase. In addition, Glc-6-Pase expression is altered due to various nutritional and hormonal states. Glc-6-Pase mRNA was low in the fed and refed states, where insulin levels were elevated while in diabetic rats exhibited higher Glc-6-Pase expression than control animals [6].

The final step in the transport of glucose out of the liver and into the bloodstream was assumed to be mediated by the facilitator GLUT2, which has low affinity and high capacity for glucose, fructose and galactose [6]. Mice lacking GLUT2 were hyperglycemic, relatively hypoinsulinemic, had elevated plasma glucagon levels, severe glycosuria and died around weaning [7]. Interestingly, freshly isolated hepatocytes from 15-day-old knockout mice exhibited normal glucose production [8], indicating that an alternative pathway for hepatic glucose output exists.

Beside the brain, muscle and adipose tissue preferentially utilize glucose. Here, glucose uptake is regulated by insulin and the principle glucose transporter protein that mediates this uptake is GLUT4, a major mediator of glucose removal from the circulation and a key regulator of whole-body glucose homeostasis. In basal cells, most transporters are sequestered in intracellular compartments. In the presence of insulin, the translocation of GLUT4-containing vesicles to the plasma membrane is markedly accelerated, resulting in an increase in glucose transport uptake. Several intensive studies reveal that multiple steps in the GLUT4 trafficking pathway are regulated by insulin [9]. Newly synthesized and glycosylated GLUT4 enters a continuously recycling pathways that concentrates GLUT4 in intracellular compartments. In the case of adipocytes, a highly insulin-responsive, specialized GLUT4 sequestration compartment is described in which GLUT4 accumulates after internalization from the plasma membrane into the endosomal recycling compartment. In heart and skeletal muscle, GLUT4 traffics through uniquely structured membranes [10, 11].

Binding of insulin to its receptor induces the activation of the insulin receptor tyrosine kinase leading to tyrosine phosphorylation of insulin receptor substrate proteins and their recruitment of PI_3 kinase, which catalyzes conversion of phosphatidylinositol $(4,5)P_2$ to phosphatidylinositol $(3,4,5)P_3$ (PIP_3). PIP_3 triggers the activation of the protein kinase Akt (Akt2 rather than Akt1 or Akt3) through the actions of PDK1 and mTOR. For translocation of GLUT4-containing vesicles to the cell surface the phosphorylation of AS160 is believed to play a major role. AS160 (TBC1D4) is a GTPase-activating protein (GAP) that catalyzes the inactivation of Rab2a, 8A, 10 and 14 in vitro [12]. Rab proteins are critical organizers of intracellular membrane trafficking [13]. Inhibition of AS160 expression by RNA interference was shown to increase basal GLUT4 levels at the plasma membrane of adipocytes [14].

In muscle, a second stimulus exercise stimulates GLUT4 recruitment to the cell surface via elevation of AMP/ATP ratios and intracellular Ca^{2+} concentration. These second messengers activate downstream kinases (AMPK, Akt, aPKCλ/ς, CaMKII, cPKC) that phosphorylate putative effectors to induce GLUT4 trafficking. One such substrate is the GAP AS160 which was shown to be phosphorylated after contraction mediated through the AMPK pathway [15].

In addition, a variety of other cellular stress signals enhance glucose uptake in skeletal muscle. Hypoxia and inhibitors of cellular metabolism signal at least partially through AMPK by decreasing the cellular energy supply and increasing AMP/ATP ratios. Hyperosmolarity also promotes GLUT4 translocation via the AMPK pathway or by activating Gab-1-dependent signaling pathway in adipocytes [9].

Glucose-Stimulated Insulin Secretion

Glucose uptake into the pancreatic β-cell is the initial step in glucose-stimulated insulin secretion (GSIS). This glucose transport is mediated by GLUT2, a low-affinity (K_M = 17 mmol/l) glucose transporter [16]. Glucose is then phosphorylated by the high-K_M glucokinase, which is rate-limiting for glucose metabolism and of insulin release and is therefore viewed as a glucose sensor. This sensor couples changes in physiological glucose concentration in the pancreatic β-cells and in the liver to the intermediary metabolism, i.e. glycolysis, the citrate cycle and respiratory-chain phosphorylation and increases the ATP levels in the β-cells. Changes in the ATP/ADP ratio within the β-cells inhibits ATP-sensitive K+ channels (subunits Kir6.2 and SUR1), resulting in activation of voltage-gated Ca^{2+} channels which triggers the release of insulin granules. Exocytosis of insulin-containing secretory vesicles in pancreatic β-cells is crucial to maintenance of plasma glucose levels [17].

Decreased expression and cell surface localization of GLUT2 occurs simultaneously with the loss of GSIS in numerous animal models of type 2 diabetes [18]. The significant role of GLUT2 for the regulation of GSIS was confirmed by the *Slc2a2* null mice which lack a first phase of insulin secretion. However, a second phase of secretion was still present and dependent on glucose metabolism [7]. As mentioned above, *Slc2a2*−/− mice are diabetic and die around weaning.

Diseases Caused by Mutations of Glucose Transporters

Some diseases are described that are associated with loss-of-function mutations of glucose transporters. In addition, genome-wide scans identified SNPs in glucose transporter genes associated with a disease or altered serum parameter.

SGLT1 Deficiency

SGLT1 deficiency results in the development of glucose-galactose malabsorption (GMM) through the intestinal brush border, an autosomal-recessive disease characterized by neonatal onset of severe watery and acidic diarrhea, which is fatal within a few weeks unless nutrients containing glucose and galactose, including polycose and products derived from corn syrup, are removed from the diet [19, 20]. The mutations responsible for GMM include missense, nonsense, frame shift, splice site, and promoter

mutations of the *SLC5A1* gene [19, 21]. Most mutations result in either truncated SGLT1 protein or in mistargeting of the transporter in the cell [20].

SGLT2 Deficiency

Congenital defects in SGLT2 which is located in the apical membrane of the S1 segment in proximal renal tubule cells lead to a primary renal glucosuria [22]. Patients with this disease have normal blood glucose levels, normal oral glucose tolerance, and persistent glucosuria. In the most severe cases, patients may excrete a major portion of the filtered glucose [23].

Glucose Transporter Type 1 Deficiency Syndrome

The GLUT1-deficiency syndrome defines a group of disorders resulting from impaired glucose transport across blood-tissue barriers. In 1991, de Vivo et al. [24] described 2 children with infantile seizures, developmental delay and acquired microcephaly. Analysis of the cerebrospinal fluid (CSF) showed an unexplained hypoglycorrhachia (low glucose concentration in CSF) in the presence of normoglycemia (CSF/blood glucose ratio <0.4), while lactate concentrations were low to normal in the CSF, suggesting intact intracellular pathways for glucose utilization. Based on these findings, a defect in GLUT1-mediated glucose transport across the blood-brain barrier was assumed. Since 1991, more than 70 patients and numerous heterozygous mutations resulting in GLUT1 haploinsufficiency have been identified [25]. Clinical features are variable and include seizures, delayed development, acquired microcephaly, hypotonia, and motor disorders including elements of ataxia, dystonia and spasticity [25]. The disease is treated effectively with a ketogenic diet, as ketone bodies easily penetrate the blood-brain barrier and serve as an alternative fuel for the brain [26].

Fanconi-Bickel Syndrome

The Fanconi-Bickel syndrome (FBS) is based on congenital defects within the *SLC2A2* gene and a rare autosomal-recessive inborn error of metabolism, which resembles type I glycogen storage disease [22, 27]. 112 FBS patients and 34 different GLUT2 mutations have been reported in the recent literature. Since GLUT2 is the predominant glucose transporter in hepatocytes, pancreatic β-cells, enterocytes and renal tubular cells, a loss of GLUT2 leads to a typical combination of hepatorenal glycogen accumulation, glucose and galactose intolerance, fasting hypoglycemia, a characteristic tubular nephropathy, and severely stunted growth [27]. While no specific therapy is available for FBS patients, the symptomatic treatment is directed towards a stabilization of glucose homeostasis and compensation for renal losses of various solutes [27].

Association of a Polymorphism in the SLC2A9 *Gene with Serum Uric Acid Levels*

Serum uric acid levels are frequently elevated in conjunction with several disorders, including obesity, hyperlipidemia, atherosclerosis, and hyperinsulinemia. Several

metabolic defects that may increase uric acid levels have been described such as variations in the activity of enzymes in purine metabolism [28] or impaired clearance of uric acid in the kidney due to genetic variants in the urate/anion transporter [29]. Recently genome-wide scans in a genetically isolated Sardinian population identified varians within the GLUT9 encoding *SLC2A9* gene associated with reduced uric acid levels. This finding was replicated in an unrelated cohort from Tuscany [30]. However, from there data it can only be speculated that GLUT9-mediated glucose transport or glucose metabolism affects uric acid synthesis and/or renal reabsorption.

Genetic Variations of GLUT10 and Type 2 Diabetes

The *SLC2A10* gene encoding GLUT10 is mapped on chromosome 20q12-q13.1, a region that has been shown to be linked to type 2 diabetes [31]. Andersen et al. [32] examined the gene in more than 60 type 2 diabetic patients and identified six variants. However, in an association study, which included about 500 type 2 diabetic patients and about 500 glucose-tolerant controls, none of the variants were associated with diabetes. But carriers of one SNP (Ala206Thr) had significantly lower fasting serum insulin levels (18%) and 20% lower insulinogenic index than the controls [32]. Three additional association studies performed in a Finish cohort [33], in a cohort of Caucasian Americans [34] and of the Taiwanese population [35] published the evaluation of *SLC2A10* as putative diabetes gene but did not confirm the association between Ala206Thr and fasting insulin levels or found any other SNP associated with type 2 diabetes.

Genetic Variations of GLUT10 and Arterial Tortuosity Syndrome

Arterial tortuosity syndrome (ATS) is a rare autosomal-recessive connective tissue disease, characterized by widespread arterial involvement with elongation, tortuosity, and aneurysms of the large and middle-sized arteries owing to disruption of elastic fibers in the medial layer of the arterial wall. A candidate locus for ATS was identified in a 4.1-Mb region on chromosome 20q13.1 by homozygosity mapping [36]. Subsequently, the candidate region was narrowed to 1.2 Mb containing seven genes. Loss-of-function mutations in one of these genes, *SLC2A10*, were identified in ATS families [37]. Here, GLUT10 deficiency was shown to be associated with upregulation of the TGF-β pathway in the arterial wall, a finding also observed in Loeys-Dietz syndrome, in which aortic aneurysms associate with arterial tortuosity [37].

Glucose Transporters as Components of the Glucose-Sensing Machinery

It is a long-established phenomenon that feeding is regulated by hypothalamic neurons that can sense blood glucose levels [38]. Whereas all neurons require glucose to fuel their metabolic demands, glucose-sensing neurons also utilize glucose as a signaling molecule to regulate their membrane potential and firing rate triggering counterregulatory impulses, e.g. activation of the sympathic nervous systems [39]. The existence of

specialized glucose-sensing neurons has been known for many years. As brain glucose levels rise, glucose-responsive (GR) neurons increase and glucose-sensitive (GS) neurons decrease their firing rate. Little is known about the mechanism by which GS neurons sense glucose. GR neurons appear to function much like the pancreatic β-cell with glucokinase modulating the K_{ATP} channel, leading to membrane depolarization, calcium influx and increased cell firing. In addition, in neurons and astrocytes the AMP-activated protein kinase (AMPK) acts as an energy sensor [40]. When glucose concentrations decline, AMP levels rise and activate AMPK, which alters neuronal activity. Many glucose-sensing neurons also respond to and integrate signals from other metabolites (e.g. fatty acids) and hormones such as leptin and insulin [39, 41, 42].

The arcuate hypothalamic POMC and NPY/AgRP neurons play significant roles in the regulation of energy and glucose homeostasis. Some POMC neurons utilize AMPK and K_{ATP} channel to increase their activity when glucose concentration rises [41, 42]. NPY/AgRP neurons are altered by rising glucose levels [39, 41] but they do not utilize the K_{ATP} channel for glucose-sensing. In POMC neurons, glucose-sensing was disrupted by mutation of the Kir6.2 subunit of their K_{ATP} channel [42]. This genetic manipulation impaired the whole-body response to a systemic glucose load, demonstrating a role for glucose-sensing by POMC neurons in the overall physiological control of blood glucose. Parton et al. [42] also found that glucose-sensing by POMC neurons became defective in obese mice on a high-fat diet, suggesting that loss of glucose-sensing by neurons has a role in the development of type 2 diabetes. The mechanism for obesity-induced loss of glucose-sensing in POMC neurons involves uncoupling protein 2 (UCP2), a mitochondrial protein that impairs glucose-stimulated ATP production. UCP2 negatively regulates glucose-sensing in POMC neurons and deletion of *Ucp2* was shown to prevent obesity-induced loss of glucose-sensing [42].

Deletion of α_2-subunit of AMPK in both POMC and NPY/AgRP neurons disrupted response to changes in glucose concentration in both neurons. Mice lacking AMPK activity in POMC neurons developed obesity due to reduced energy expenditure and dysregulated food intake but remained sensitive to leptin. In contrast, AgRP neuron-specific *Ampk* α_2 null mutants developed an age-dependent lean phenotype with increased sensitivity to a melanocortin agonist. Electrophysiological studies of both mouse models revealed normal leptin or insulin action. In contrast, responses to alterations in extracellular glucose levels were absent, showing that glucose-sensing signaling mechanisms in these neurons are distinct from those pathways utilized by leptin or insulin [41].

The glucose transporter involved in the neuronal glucose-sensing unit is still not known. Possible candidates are the high-affinity glucose transporter isoforms GLUT3 and GLUT8 because of their distinct expression in neurons of the hypothalamus, but also the low-affinity transporter GLUT2 [43]. Recently, a member of the SGLT family (SGLT3) was predicted to be a glucose sensor rather than a Na+/glucose cotransporter [44]. In cells expressing SGLT3, glucose caused a specific Na+-dependent depolarization of the membrane potential, whereas no sugar transport could be

detected. The authors concluded that SGLT3 might be involved in glucose-sensing in both the central nervous system and the gastrointestinal tract.

Recently, an intestinal glucose-sensing has been elucidated which regulates intestinal glucose absorption [45]. Dietary sugars are transported from the intestinal lumen into absorptive enterocytes by the sodium-dependent glucose transporter isoform 1 (SGLT1). Regulation of this protein is important for the provision of glucose to the body and avoidance of intestinal malabsorption. Expression of SGLT1 is regulated by luminal monosaccharides via a luminal glucose sensor, which consists of the sweet taste receptor T1R3 and the taste G protein gustducin, expressed in enteroendocrine cells. Dietary sugar and artificial sweeteners increased SGLT1 mRNA and protein expression, and glucose absorptive capacity in wild-type mice, but not in knockout mice lacking *Tir3* or *α-gustducin*. Therefore it was suggested that intestine-expressed taste-signaling elements which are involved in regulating SGLT1 expression might provide novel therapeutic targets for modulating the gut's capacity to absorb sugars, with implications for the prevention and/or treatment of malabsorption syndromes and diet-related disorders including diabetes and obesity [45].

Role of Glucose Transporters for the Development of Cancer

Cancer cells require a steady source of metabolic energy in order to continue their uncontrolled growth and proliferation. Accelerated glycolysis is one of the biochemical characteristics of cancer cells. Recent work indicates that glucose transport and metabolism are essential for the post-treatment survival of tumor cells, leading to poor prognosis [46]. Glycolytic breakdown of glucose is preceded by the transport of glucose across the cell membrane, a rate-limiting process mediated by facilitative glucose transporter proteins belonging to the facilitative glucose transporter/solute carrier GLUT/SLC2A family. Tumors frequently show overexpression of GLUTs, especially the hypoxia-responsive GLUT1 and GLUT3 proteins. There are also studies that have reported associations between GLUT expression and proliferative indices, whilst others suggest that GLUT expression may be of prognostic significance [47].

Malignant cells have accelerated metabolism and need more ATP. It was first observed by Warburg [48] that cancer cells have high rates of aerobic glycolysis which can result in an accumulation of lactate in cancer cells. Increased activity of enzymes involved in glycolysis has been observed in malignant cells and tissues [46]. In addition, activities of hexokinase and other glycolytic enzymes were higher in metastasis than in primary breast tumors, indicating that glycolytic activity is associated with tumor progression. In addition to energy generation, higher rates of glucose is needed in cancer cells for increased production of precursor molecules that lead to biosynthesis of nucleotides, phospholipids for new cell membranes, and other components required during cell division [49]. The pentose phosphate pathway for instance uses glucose-6-phosphate to produces ribose-5-phosphate for use in RNA and DNA synthesis.

Hatanaka [50] hypothesized that transport across the plasma membrane is the first rate-limiting step for sugar metabolism in cells and glucose transport, therefore an important regulator of glucose metabolism in transformed cells. Increased glucose transport in malignant cells is associated with increased expression of GLUT proteins. Transformation of rat fibroblasts by *ras* and *scr* oncogenes resulted in elevated glucose transport and increased GLUT1 expression [51] due to *ras*- and *scr*-responsive enhancer elements within the *Slc2a1* (GLUT1 encoding gene) promoter [52]. In carcinoma cell lines (e.g. Caco-2 cells and breast cancer cells) transformation resulted in increased expression of GLUT1 and GLUT3 while other transporters like GLUT2 and GLUT5 were repressed [53]. In addition, Rogers et al. [54] described GLUT12 to be expressed in breast cancer cells (MCF-7 cells) and detected the transporter in an intracellular compartment when cells were grown under serum-free conditions. This finding was confirmed in prostate cancer cells [55] suggesting that GLUT12 could undergo regulated protein trafficking in a similar manner to the translocation of GLUT4. Movement of GLUT12 to the cell surface could then contribute to increased glucose uptake, but the specific trigger for such movement in cancer cells is still unknown. The upregulation of glucose transporters may be a fundamental part of the neoplastic process. It is also discussed that an increased GLUT expression is an indirect effect. Increased glucose consumption could result in decreased intracellular glucose concentration, which itself would result in an enhanced GLUT1 and GLUT3 expression. However, increased GLUT levels at the plasma membrane and enhanced transport activity contribute significantly to tumor growth [46].

Consistent with results obtained in cancer cells, elevated expression of GLUT1 has been described for many cancers, including hepatic, pancreatic, breast, esophageal, brain, renal, lung, cutaneous, colorectal, endometrial, ovarian, and cervical carcinoma. Increased GLUT1 but also GLUT3 expression was shown to correlate with reduced survival rate of the patients [46]. GLUT12, which was first discovered in a human breast cancer line, was also detected in human breast tumors in a preliminary study of 10 patients. While GLUT12 was found in 9 invasive tumors, less or no GLUT12 staining was detected in adjacent normal breast tissue [54]. GLUT12 was also detected in prostate cancer where it was located in the plasma membrane and intracellulary [55].

Use of ^{18}F-Labeled 2-Fluoro-2-Deoxy-D-Glucose as Diagnostic Tool

Molecular imaging of the body involves new techniques to image cellular biochemical processes, which results in studies with high sensitivity, specificity, and signal-to-background ratio. For instance, glucose uptake is visualized in many tumors in vivo by the use of labeled glucose analogs [56]. Injected ^{18}F-labeled 2-fluoro-2-deoxy-D-glucose (FDG) accumulated in tumor cells at much higher levels than in normal surrounding tissue, and thus will render the tumor visible by positron

emission tomography (PET). By now, FDG-PET has become the method of choice for the staging and restaging of many of the most common cancers, including lymphoma, lung cancer, breast cancer, and colorectal cancer. FDG-PET has also become extremely valuable in monitoring the response to therapeutic drugs in many cancers. New PET agents, such as fluorothymidine and acetate, have also shown promise in the evaluation of response to therapy and in the staging of prostate cancer [57].

Regulation of GLUT Expression in Cancer

Some regions in tumors have low oxygenation due to poor perfusion [58] and GLUT1 expression has been shown to be upregulated in hypoxic regions surrounding necrotic foci of breast tumors and colorectal cancer [22]. Hypoxic tumors are significantly more malignant, metastatic, radio- and chemoresistant and have a poor prognosis. Hypoxia, induced in vitro, has been shown to activate uptake of FDG and to increase GLUT1 protein levels [59]. The discovery of the oxygen-sensitive transcription factor hypoxia-inducible factor (HIF-1) has led to a better understanding of the molecular link between hypoxia and deregulated glucose metabolism. Beside the expression of GLUT1, HIF-1 induces a number of genes integral to angiogenesis, e.g. vascular endothelial growth factor, a process intimately involved with metastatic spread. This knowledge may enhance existing chemotherapeutic strategies so that treatment can be more rationally applied and personalized for cancer patients [47].

Hypoxia hormones, particularly estrogen, were described to alter GLUT expression. Treatment of MCF-7 cells with 17β-estradiol stimulates tumor formation and glucose metabolism [60]. In contrast, incubation of cells with tamoxifen, an estrogen receptor antagonist, resulted in lower rates of glucose consumption [61]. Interestingly, estrogen treatment of MCF-7 cells did not alter GLUT12 mRNA but protein level indicating that translation or half-life is increased [46].

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The Epidemiologic Relationship between Diabetes and Cancer

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Abstract

There is a growing amount of epidemiologic literature suggesting an association between history of metabolic syndrome/diabetes and risk of developing a variety of cancers. Data of populations from different hospital- and community-based case-control studies, from cancer registries and a health survey suggest that metabolic syndrome/diabetes mellitus type 2 are associated with an increased risk of cancer or are even independent predictors of mortality from cancer of the colon, pancreas, hepatocellular carcinoma, female breast and endometrium, and in men of liver and bladder. However, the association is still complex because some studies show controversial results of even a lower risk of cancer, e.g. for prostate cancer, in diabetic than in non-diabetic subjects. An association between diabetes, hyperinsulinemia, insulin resistance, insulin-like growth factors, lipotoxicity, obesity, adipokines, Western-style dietary habits and carcinogenesis appears plausible, yet clusters of increased or reduced risk factors need to be confirmed in future studies. Evidence from the intensive care literature indicates that achieving glucose control leads to a better outcome in clinical oncology. If so, continued improvement of cancer outcomes may also depend upon improved diabetes control. As the general population ages, the magnitude of both health problems continues to grow and could overwhelm health systems. It is prime time to break the growing tsunami of both diseases by community-based prevention programs.

The metabolic syndrome (MeS) encompasses a constellation of metabolic disorders that places patients at high risk for the development of cardiovascular diseases and diabetes mellitus type 2, and possibly cancer. The MeS is the concurrence of hypertension, abdominal obesity, impaired fasting blood glucose, dyslipidemia, e.g. low levels of high-density lipoproteins (HDL) cholesterol, and insulin resistance. The diagnostic concept of MeS is still controversially discussed, which is illustrated on the basis of recent primary-care data from Germany and the Centers for Disease Control and Prevention. In Germany, the prevalence ranges from 19 to 31% [1] and in the USA about 22% of the adults have MeS according to the currently existing definitions. The book on MeS as a clinically valuable indicator for estimating the risk of diabetes

mellitus type 2, cardiovascular morbidity and mortality is not yet closed. However, many people afflicted by MeS will develop type 2 diabetes. By 2010 it is suggested that 30 million people in Europe will suffer from diabetes, creating a huge burden on the health service and the economy, not to forget the individuals themselves; to close this circuit of syndromes, many of these people will have features of the MeS.

Although the pathogenesis of MeS is under debate, it is now realized that insulin resistance plays a principle role in initiating and perpetuating the pathological manifestations of the MeS [2]. Studies have shown that MeS and its consequent biochemical derangements in the various phases of diabetes may contribute to carcinogenesis and clinical study protocols are designed to understand in more detail the role of insulin and insulin resistance in cancer-struck subjects [3]. Therefore, basic and clinical science will face a twofold problem in the future, timely summarized by the following questions: (1) how does a clinically manifested diabetes or prediabetes (comorbidity) influence the outcome of a cancer disease (mortality), and (2) how is type 2 diabetes associated with cancer incidence?

A recently published study on the glucose tolerance status and 20-year cancer incidence within a sample of a Jewish Israeli population (n = 2,780) showed an increased long-term cancer risk for individuals with impaired fasting glucose or diabetes [4]. The Vasterbotten Intervention Project of Northern Sweden showed an association of hyperglycemia with total cancer risk in women and in women and men combined for several cancer sites, independently of obesity [5]. A case-control study of 306 colorectal cancer cases and 595 matched controls nested in the Northern Sweden Health and Disease Cohort supports the view that the presence of obesity, hypertension and hyperglycemia increase the risk of colorectal cancer [6].

A cross-sectional study investigated the question of comorbidity and reduced health-related quality of life (HRQL) in patients that have either diabetes or cancer. The data from the Public Use File of the Canadian Community Health Survey revealed that individuals with diabetes and cancer had a clinically important and significantly lower HRQL than those with either conditions alone [7].

An association between diabetes mellitus type 2 and cancerogenesis appears plausible, considering the complexity of the mode of action of insulin (pro- and pre-insulin), insulin-like growth factor and the appropriate receptors, including the type of oral antidiabetic drugs. It was found that patients with type 2 diabetes exposed to sulfonylureas and exogenous insulin had a significantly increased risk of cancer-related mortality compared with patients exposed to metformin; the cause of this in-/decreased risk-related effect remains speculative [8]. Interestingly, an evaluation of the General Practitioner Research Data in the UK suggests that patients with diabetes have a reduced risk for prostate cancer when using insulin or sulfonylureas [9].

All the epidemiological studies involving diabetes mellitus type 2 and malignancies do have limitations, e.g. differences in treatment modalities, lifestyle and nutrition behavior, race and genetics of participants. A promising way to give some

answers is to focus on specific tumor entities in order to develop tumor-specific and individualized management strategies.

Breast Cancer Risk and Diabetes Mellitus Type 2

Upper body obesity and the related metabolic disorder type 2 diabetes have been identified as risk factors for breast cancer, and associated with late-stage disease and poor prognosis. Components of the MeS, including visceral adiposity, insulin resistance, hyperglycemia and hyperinsulinemia, with or without clinically manifest diabetes mellitus type 2, low serum HDL cholesterol and hypertension have all been related to an increased risk of breast cancer [10]. One of the hallmarks of aggressive cancer cells is a high rate of energy-consuming anabolic processes driving the synthesis of lipids, proteins, and DNA. The breast cancer gene 1 (BRCA-1) stabilizes the inactive (phosphorylated) form of the acetyl coenzyme A carboxylase α, the rate-limiting enzyme catalyzing de novo fatty acid biogenesis. Therefore, one mode of action of BRCA-1 is a tumor suppressor activity which depends on its ability to mimicry a cellular low-energy status, which is also known to block tumor cell anabolism and suppress the malignant phenotype. It is interesting to see that physical activity and lack of obesity in adolescence have been associated with significantly delayed breast cancer onset for Ashkenazi Jewish women carrying BRCA-1 gene mutations [11].

The adipocytes, forming the belly fat, are now in the focus of metabolic research in oncology. Adipocytes produce adipocytokines, which are biologically active polypeptides and act by endocrine, paracrine, and autocrine mechanisms; most have been associated with MeS. Six adipocytokines – vascular endothelial growth factor, hepatocyte growth factor, leptin, tumor necrosis factor-α, heparin-binding epidermal growth factor-like growth factor, and interleukin-6 – promote angiogenesis. Obesity and insulin resistance, again, have been identified as risk factors for breast cancer and are associated with late-stage disease and poor prognosis [12]. However, the picture is not as clear as to be expected because a case-control study in Chile did not show any association between obesity and breast cancer at any age, although the same study revealed that insulin resistance was independently associated with breast cancer in postmenopausal women, but not in premenopausal women [13].

Insulin growth factors (IGFs) are important mediators of growth, development, differentiation and survival of normal and transformed cells. Recent studies confirmed the association between serum levels of IGF-1 and diverse malignant diseases, while some relationships with other pathologies since diabetes mellitus type 2 have been described. Currently, IGFs are considered important targets for the study of new therapeutic drugs and strategies for cancer treatment [14].

A meta-analysis of case-control (n = 5) and cohort studies (n = 15) to assess the evidence regarding the association between diabetes and risk of breast cancer yielded a summary RR of 1.24 for women with (versus without) diabetes. Findings from this

meta-analysis indicate that diabetes is associated with an increased risk of breast cancer [15]. However, it is important to know for diagnostic purposes that, although the breast cancer risk is increased among women with type 2 diabetes, type 2 diabetes does not significantly influence mammographic breast density [16].

The role of diabetes in the etiology of breast cancer in Asian-Americans is of special interest because of their consumption of soy. A population-based case-control study in Los Angeles County that included 1,248 Asian-American women with incident, histologically confirmed breast cancer and 1,148 control women, who were frequency matched to cases on age, Asian ethnicity and neighborhood of residence, showed that the diabetes-breast cancer association was observed only in low/intermediate soy consumers but not among high soy consumers [17].

Another question is becoming increasingly common: How does gestational diabetes relate to future risk of disease? The Jerusalem Perinatal Study, including 37,926 women, suggests that gestational diabetes may be an important early marker of breast cancer risk among postmenopausal women; however, the authors clearly state that these results need to be confirmed in future studies [18]. Another study in Israel revealed after adjustment for body mass index that breast cancer among diabetic patients was more often hormone receptor negative [19]. Population-based health databases from Ontario, Canada, used for retrospective cohort studies showed that diabetes was associated with a close to 40% increase in mortality within the first 5 years following breast cancer, which means that early survival following breast cancer is reduced in women with diabetes [20]. Results from the same databases indicate that women with diabetes were less likely to have a mammogram during a 2-year period than were women without diabetes, despite more healthcare visits. These findings highlight the need for better organization of primary care for patients with chronic diseases, like diabetes and/or cancer [21].

It has been recently shown that activation of the AMP kinase pathway is necessary for metformin to inhibit gluconeogenesis in hepatocytes. This pathway is also involved in metformin-induced growth inhibition of epithelial cells. Breast cancer cells escape metformin-induced growth inhibition by small interfering RNA against AMP kinase. These results provide evidence for a mechanism that may contribute to the antineoplastic effects of metformin suggested by some population studies and stress the potential role for activators of AMP kinases in (breast) cancer prevention and treatment [22, 23].

Risk of Endometrial Cancer and Diabetes Mellitus Type 2

A meta-analysis based on 16 studies (3 cohort and 13 case-cohort studies) showed for 12 studies a statistically significantly increased risk and for 4 studies a non-significant increased risk of endometrial cancer [24]. The association between diabetes and incidence of endometrial cancer and the potential effect of modification by obesity and physical activity was prospectively examined in the Swedish Mammography Cohort

Study. Diabetes was associated with a twofold increased risk, and combination of diabetes with obesity and low physical activity was associated with a further increased risk for endometrial cancer [25]. Therefore, interventions to reduce body weight and increase physical activity may have important implications in terms of endometrial cancer and future management of diabetic subjects.

In 1986, Folsam et al. [26] obtained risk factor information on 41,836 women aged 55–66 years living in Iowa. They followed those initially free of cancer through to 2000 and identified incident endometrial cancers via linkage to a cancer registry. Diabetes was associated with poorer survival after incident endometrial cancer, independent of tumor stage and grade. The findings support the possibility of a diabetes-related condition, such as hyperglycemia or hyperinsulinemia, contributing to poorer endometrial cancer survival. A case-control study, which was nested within three cohorts in New York (USA), Umeå (Sweden) and Milan (Italy), investigated for the first time prospectively the association of prediagnostic blood concentrations of C-peptide, a marker of pancreatic insulin production, IGF-1, (insulin-like growth factor binding protein, IGFBP) IGFBP-1, -2 and -3 with endometrial cancer risk. Chronic hyperinsulinemia, as reflected by increased circulating C-peptide, was associated with increased endometrial cancer risk. Risk was unrelated to levels of IGF-1, IGFBP-2 and IGFBP-3 [27].

A German study (charts abstracted from patients with endometrial cancer from 1985 to 1995) investigating the influence of diabetes mellitus type 2 and nodal distribution in endometrial cancer showed a univariate correlation between lymph node involvement and diabetes [28]. The extension of this study to the year 2003 revealed by multivariate analysis that diabetes mellitus type 2, FIGO stage and depth of myometrial invasion were significantly associated with overall survival [29].

A case-control study performed in Italy and Switzerland found a supramultiplicative effect for obese diabetic women and risk of endometrial cancer [30]. Obesity is a well-known risk factor for the development of endometrial cancer, however weight alone does not account for all cases. Insulin resistance also contributes to an increased risk for endometrial cancer. Adiponectin is a protein secreted by adipose cells and has been shown to be a surrogate marker for insulin resistance, with low levels of adiponectin correlated with hyperinsulinemia and a degree of insulin resistance. Indeed, women with endometrial cancer were more likely to have low adiponectin levels than controls, even after adjusting for body mass index. This suggests that insulin resistance is independently associated with endometrial cancer [31] and insulin resistance/hyperinsulinemia is associated with poorly differentiated endometrial adenocarcinomas and a more aggressive course of the disease [32].

Metabolic Syndrome and Risk of Prostate Cancer

Currently, there is a debate whether MeS predicts the incidence of prostate cancer. The hypothesis was tested using the 27-year follow-up of the prospective cohort of

the Oslo Study in 1972–1973. MeS was found to predict prostate cancer during 27 years of follow-up, indicating an association between insulin resistance and the incidence of prostate cancer [33]. Features of the MeS, specifically abdominal obesity and hypertension, are also associated with prostate cancer in African-American men [34], a population which is more prone to developing MeS symptoms.

A conducted nested case-control study within the Northern Sweden Health and Disease Cohort Study found an increased risk of prostate cancer in men with elevated IGF-1, suggesting that circulating IGF-1 may be specifically involved in the early pathogenesis of prostate cancer [35]. An extension of this study by measuring levels of IGF-1 and IGFBP-3 in prediagnostic blood samples from a total of 281 men who were subsequently diagnosed with prostate cancer supported that IGF-1 is an etiologic factor in prostate cancer. The circulating IGF-1 levels measured at a comparatively young age may be most strongly associated with prostate cancer risk [36].

Despite a growing amount of epidemiologic literature suggesting an association between MeS and prostate cancer risk, there is also growing evidence of an inverse association between history of diabetes – one feature of MeS – and risk of incident prostate cancer. The CaPSURE Study, a community-based prostate cancer registry study, revealed that a history of diabetes was not associated with any diagnostic clinical parameter or with treatment-specific recurrence rates for prostate cancer. Only among men with a low prognostic risk or who were younger at prostate cancer diagnosis, being diabetic (versus not being diabetic), was there a tendency of association in a multivariate analysis with an elevated risk of recurrence after radiation therapy [37]. The NIH-AARP Diet and Health Study disclosed an inverse association between diabetes and prostate cancer which was particularly strong among men in the highest category of routine physical activity at work or at home [38]. This relationship strengthens the importance of high levels of routine physical activity, either for the prevention of prostate cancer and/or cancer in general [39] and MeS. Gonzalez-Perez and Garcia Rodriguez [9] used the General Practitioner Research Database in the UK and found that diabetic patients had a decreased risk of prostate cancer. Interestingly, this association was observed among treated diabetics but not among untreated diabetics. They discussed the possibility that the observed risk could be restricted to users of insulin or sulfonylureas, a very provocative hypothesis considering the mode of action of these therapeutics.

Werny et al. [40] investigated the association between diabetes and prostate-specific antigen levels, controlling for potential confounders, in a nationally representative cross-sectional survey of the US population (National Health and Nutrition Examination Survey, 2001–2002). The reported results are consistent with the hypothesis that long-term diabetes is associated with a lower risk of prostate cancer.

Diabetes seems to be associated with a reduced risk of prostate cancer, but whether the MeS is also associated with prostate cancer is marginally established. Therefore, Tande et al. [41] assessed this association in the Atherosclerosis Risk in Communities (ARIC) Study. When diabetic participants were excluded, the inverse association between MeS and prostate cancer incidence was slightly strengthened.

Diabetes may be a protective factor for prostate cancer since both were found to be negatively associated. Based on the same genetic background, parents of diabetic patients might show similar risks concerning cancers. Meyer et al. [42] investigated family history in as far as genetic factors may play an important role in the negative association between diabetes and prostate cancer. Mothers of diabetic patients showed an increased history of cancers of the liver and biliary tract. Fathers of patients suffering from type 2 diabetes were diagnosed less frequently with prostate cancer compared to fathers of non-diabetic controls. The first genome-wide association scan to search for sequence variants conferring risk of prostate cancer was performed within a population of 1,501 Icelandic men with prostate cancer and 11,290 controls. It was found that two variants on chromosome 17 confer prostate cancer risk; one of the variants is in TCF2 (HNF1β), a gene known to be mutated in individuals with maturity-onset diabetes of the young type 5. However, results from eight case-control groups, including one West African and one Chinese, demonstrate that this wild-type confers protections against type 2 diabetes [43].

Prostate cancer is an example of the complexity of carcinogenesis associated with MeS and/or diabetes. On the one hand, an association between diabetes, IGF-1, hyperinsulinemia and insulin resistance appears plausible, but on the other, these features can be somewhat counterbalanced as well and reduce hereby the risk for the development of one of the leading cancer entities worldwide.

Metabolic Syndrome and Risk of Colorectal Adenoma Development

Two Asian-Pacific Epidemiologic Studies showed an increased risk of colorectal adenoma development associated with MeS: (1) the Self-Defense Forces Health Study, carried out between 1995 and 2002 at two Self Defense Forces hospitals in Japan [44] and (2) one study carried out at the Center for Health Promotion, Samsung Medical Center, Seoul, Korea, between March 2004 and December 2005 [45]. Both studies included subjects who underwent colonoscopy as a screening examination for polyps. Apart from the association of MeS with colorectal adenoma, an increased risk for MeS was more evident for proximal than distal colon, for multiple ($\geq$3), and for advanced adenoma. Abdominal obesity of the individual components of MeS was an important risk factor for colorectal adenoma. Thus, the MeS appears to be a crucial entity with regard to the prevention of colorectal adenoma and consequently colorectal cancer.

Hyperinsulinemia and Insulin Resistance Linked to Colorectal Cancer

Non-insulin-dependent diabetes mellitus (NIDDM) seems to be one risk factor for colorectal cancer. Usually, in the pre-NIDDM state, hyperinsulinemia is seen for several years. Insulin is a growth factor of epithelial and cancer cells of colon and rectum.

At the Department of General Surgical Science, Gumma University, Gumma, Japan [46], patients suffering from colorectal cancer but never diagnosed for diabetes, were tested for glucose tolerance. Serum glucose and insulin levels were found to be higher in cancer patients than in controls. The authors concluded that hyperinsulinemia may be one of the causes of colorectal cancer and should be controlled to prevent recurrence of colorectal cancer even after curative resection. Mechanistically, hyperinsulinemia has been associated with insulin resistance, increased levels of growth factors, including IGF-1, and alterations in NF-κB and peroxisome proliferator-activated receptor signaling, which may promote colon cancer through their effects on colonocyte kinetics. The insulin resistance colon cancer hypothesis, stating that insulin resistance may be associated with the development of colorectal cancer, represents a significant advance in colon cancer, as it emphasized the potential for this cancer to become a modifiable disease [47]. This hypothesis is supported by results from a prospective study including anthropometric and clinical measurements associated with insulin resistance syndrome and colorectal cancer in male smokers [48].

A cohort study on the impact of diabetes within a large randomized adjuvant chemotherapy trial of 3,759 patients with high-risk stage II and stage III colon cancer was carried out in 2003 at the Dana-Farber Cancer Institute, Eastern Cooperative Oncology Group Statistical Center, and Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Mass., USA [49]. Patients with diabetes and high-risk stage II and stage III colon cancer experienced a significantly higher rate of overall mortality and cancer recurrence. Median survival was 6.0 and 11.3 years for diabetics and non-diabetics, respectively.

In various animal models, it could be shown that insulin and insulin-like growth factor axes and insulin resistance are major determinants of proliferation and apoptosis and thus may influence carcinogenesis. Clinical conditions associated with hyperinsulinemia and increased IGF-1 levels are related to an increased risk of colon cancer. Global nutrition studies [39] indicate that dietary patterns that stimulate insulin secretion and resistance, including a high consumption of sucrose, various sources of starch, a high glycemic index and high saturated fatty acid intake, are associated with a higher risk of colon cancer. Efforts to counter these patterns are likely to have the most potential to reduce colon cancer incidence and tumor recurrence.

The Metabolic Syndrome, Diabetes and Steatosis and Incidence of Hepatocellular Carcinoma

The incidence of hepatocellular carcinoma (HCC) is increasing, but the temporal changes of risk factors remain unclear. A significant proportion of HCC develops in cryptogenic cirrhosis, and may present the most worrisome complication of non-alcoholic

steatohepatitis. Non-alcoholic steatohepatitis is tightly linked to insulin resistance and several features of the MeS, e.g. obesity, diabetes and dyslipidemia.

A systemic review and meta-analysis of a total of 26 studies revealed that diabetes is associated with an increased risk for HCC [50]. A population-based case-control study in the USA documented that diabetes increases the risk of HCC two- to three-fold, regardless of the presence of other major HCC risk factors. Findings from this study suggest that diabetes is an independent risk factor for HCC [51]. Databases from the Surveillance & Risk Assessment Division of Health Canada & Statistics Canada were analyzed for trends in both age-adjusted incidence of and mortality due to HCC from 1984 to 2001 [52]. The incidence of HCC in Canada has increased in the past 20 years and is associated with a rise in the incidence of hepatitis C, obesity and diabetes. Similar results are reported by two studies from Taiwan [53] and Japan [54].

It is likely that the association of HCC with obesity and diabetes represents the progression of underlying non-alcoholic fatty liver disease to cirrhosis. The mechanisms most likely involve replicative senescence of steatotic mature hepatocytes and compensatory hyperplasia of progenitor cells as a reaction to chronic injury due to ongoing non-alcoholic steatohepatitis [55] and inflammation [56].

Diabetes and Its Relationship to Pancreatic Carcinoma

The link between pancreatic cancer (PC) and diabetes mellitus is recognized, however controversy still exists because no criteria have been established for the efficient selection of a high-risk group among patients with diabetes mellitus. Regulation of endocrine cell mass is thought to have a central role in the pathogenesis of both diseases. The processes that operate during pancreatic adaptation to a changing hormonal milieu are important in pancreatic carcinogenesis. There is evidence that somatostatin and its receptors are fundamental regulators of endocrine cell mass and are involved in islet tumorigenesis [57].

A hospital-based case-control study revealed that cigarette smoking, family history of PC, heavy alcohol consumption (>60 ml ethanol/day) and diabetes mellitus are significant risk factors for PC. The significant synergy between these risk factors suggests a common pathway for carcinogenesis of the pancreas [58].

Because of the poorly understood temporal association between diabetes mellitus and PC, a research group at the Mayo Clinic College of Medicine, Rochester, Minn., USA [59] compared temporal patterns in diabetes prevalence in PC and controls. Diabetes has a high prevalence in PC and frequently is now onset. Longstanding type 2 diabetes increases the risk of PC by approximately 50%. Furthermore, there seems to be a positive association between obesity and PC [60]. However, as the mechanisms for these associations remain speculative, further studies are deserved. Above all, there is an urgent need for the identification of

specific biomarkers for PC-induced diabetes, which may allow screening for PC in new-onset diabetes.

Diabetes Mellitus and Cancer – A Conclusion

Many studies have suggested that diabetes mellitus type 2 may alter the risk of developing a variety of cancers, and the associations are biologically plausible. One of largest prospective studies worldwide, enrolling 467,922 men and 588,321 women who had no reported history of cancer at the time of enrollment, revealed after 16 years of follow-up that diabetes was significantly associated with fatal colon cancer in men and women, and with PC in men, and significantly associated with liver cancer and bladder cancer. In addition, diabetes was significantly associated with breast cancer in women [61]. These findings strongly suggest that diabetes is an independent predictor of mortality from these cancer entities.

When treating cancer patients who have diabetes, clinicians must consider the cardiac, renal, and neurologic complications commonly associated with diabetes; continued improvement of cancer outcomes may also depend upon improved diabetes control [62].

Diabetes rates continue to skyrocket – nearly 21 million people in the USA are afflicted by diabetes and roughly 250 million worldwide. Health analysts project that by 2025, 50 million Americans and up to 380 million globally will have diabetes. The International Diabetes Federation, which tracks global diabetes, says the disease will cause 3.8 million deaths worldwide in 2007, about equal to HIV/AIDS and malaria combined. In the USA, the Centers for Disease Control and Prevention state that diabetes is the sixth leading cause of death, contributing to nearly 225,000 deaths in 2002, up from 213,064 in 2000 – there is a 'growing tsunami of diabetes'.

Considering the numerous results of epidemiologic and clinical studies involving diabetes mellitus and malignancies, clinicians must also consider the increased risk of new-onset and longstanding diabetics for some tumor entities by regularly screening diabetic patients for early development of tumors.

The association between diabetes and cancer is complex and warrants further and differentiating types of clinical studies – from molecular epidemiology to clinical interventions. The general population ages and the magnitude of both health problems continues to grow. As one consequence, scientists, clinicians and politicians have to develop national policies for early diagnosis and prevention of diabetes mellitus and cancer more effectively, otherwise both diseases and their biologic and sociologic relationships could likely overwhelm health systems.

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Diabetes Mellitus and Breast Cancer

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Abstract

Over the past decades, type 2 diabetes mellitus has become a major health problem and is now affecting more than 7% of the adult population in developed countries. Diabetes mellitus commonly occurs together with breast cancer and two of the major risk factors for type 2 diabetes, older age and obesity, are also associated with breast cancer. At least four mechanisms may associate diabetes mellitus and breast cancer: activation of the insulin pathway, activation of the insulin-like growth factor pathway, altered regulation of endogenous sex hormones and altered regulation of adipocytokines. Comparative cohort studies and case-control studies suggest that type 2 diabetes mellitus is associated with 10–20% excess risk of breast cancer. Gestational diabetes mellitus, but not type 1 diabetes mellitus, might also be associated with excess risk of breast cancer. Diabetes mellitus and its complications can adversely affect screening utilization and cancer therapy, and clinical studies suggest an association between diabetes and adverse breast cancer characteristics and inferior outcome. Interestingly, several antidiabetic therapies, including the biguanides and the peroxisome proliferator-activated receptor γ ligands may also have activity against breast cancer and are being tested in clinical trials.

Breast cancer is the most common malignant neoplasm in women, affecting 1 of every 8 women. The estimated new breast cancer cases and deaths among women in the USA in 2007 are 178,000 and 40,000 respectively [1]. Type 2 diabetes is another major health problem in developed countries, and affects about 7% of adults and about 15% of people older than 60 years [2]. The main risk factors for type 2 diabetes are old age, obesity, and genetic predisposition. Similarly to type 2 diabetes, the incidence of breast cancer rises with age, and the cumulative incidence in Western Europe and the USA is about 2.7% by age 55, about 5.0% by age 65, and about 7.7% by age 75. Breast cancer is associated with multiple risk factors, which are commonly divided into modifiable and non-modifiable. Non-modifiable risk factors include family history of breast cancer, germline mutations in breast cancer susceptibility genes including BRCA1, BRCA2,

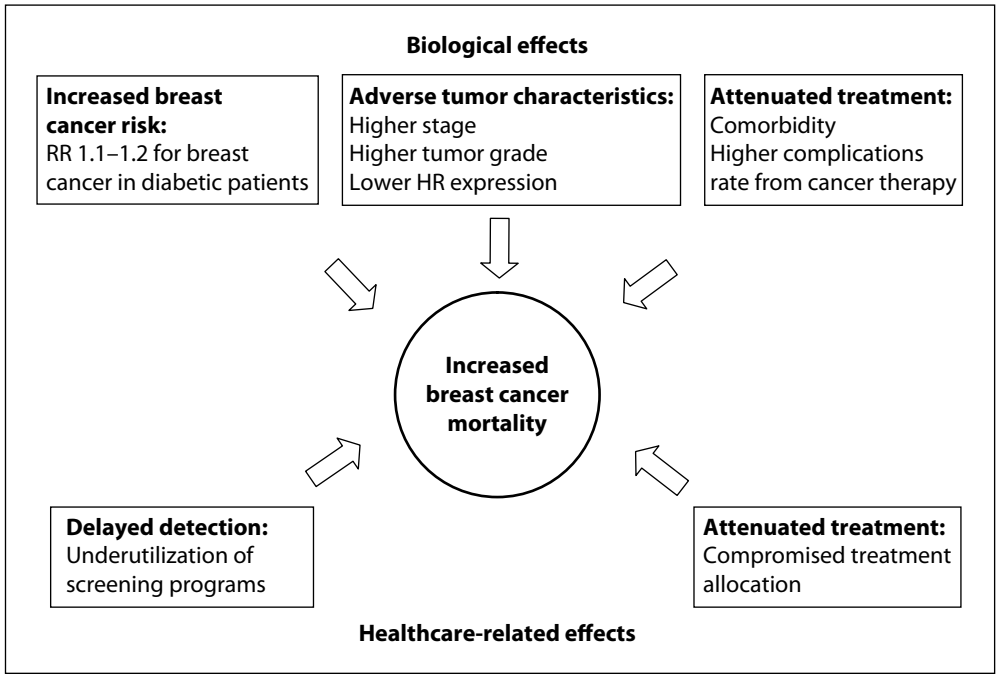

Fig. 1. Schematic representation of biological and health-related factors on breast cancer outcome. RR = Relative risk; HR = hormone receptors.

P53, PTEN, and ATM, hormonal factors such as younger age at menarche and older age menopause, and the presence of benign breast disease [3]. Modifiable risk factors include low parity, use of oral contraceptives and hormone replacement therapy, alcohol consumption, obesity, and lack of physical activity [3].

Breast cancer and diabetes commonly occur together, and up to 16% of older breast cancer patients may suffer from diabetes [4]. An association between diabetes and various types of cancer was first reported more than 100 years ago and diabetes is now recognized as a risk factor for several types of cancer, including endometrial and pancreatic carcinoma [5]. In recent years, a growing number of data, both laboratory and clinical, suggest complex associations between type 2 diabetes mellitus and breast cancer (fig. 1). Diabetes may have direct biologic effects on breast cancer risk, clinical and pathological characteristics, and outcome. Moreover, certain antidiabetic therapies may have direct activity against breast cancer. Diabetes may also affect breast cancer outcome indirectly, and have been shown to influence medical decision-making regarding screening and management of breast cancer.

Obesity, which affects more than 20% of the population in developed countries, is a major risk factor for the development of type 2 diabetes. It is also a well-established risk factor for breast cancer and is associated with increased risk for the development

of postmenopausal breast cancer, but with reduced breast cancer risk among premenopausal women [6]. Obesity is also a poor prognostic factor and is associated with adverse outcomes in both pre- and postmenopausal women with breast cancer. Mechanisms connecting obesity to postmenopausal breast cancer include altered regulation of estrogen and adipocytokines levels, and increased insulin synthesis. Thus, obesity is a major confounding factor in many studies regarding the association between diabetes and breast cancer.

Diabetes Mellitus and Breast Cancer: Possible Associating Mechanisms

Four major mechanisms may contribute to the association between type 2 diabetes mellitus and breast cancer (fig. 2): activation of the insulin pathway, activation of the insulin-like growth factor (IGF)-1 pathway, altered regulation of endogenous sex hormones, altered regulation of adipocytokines.

The Insulin Pathway and Breast Cancer

Insulin is a polypeptide hormone secreted from pancreatic β-cells in response to elevation in glucose levels [7]. The first step in activation of the insulin pathway is binding of insulin to the insulin receptor (IR). The primary targets for insulin are skeletal muscle, adipose tissue and the liver, however many other tissues, including normal breast tissue and breast cancer, express the IR. The IR is a tyrosine kinase receptor, composed of two extracellular α-subunits and two transmembrane β-subunits. Insulin binding leads to autophosphorylation of tyrosine residues in the intracellular subunits and thus activates the tyrosine kinase. Once activated, the IR phosphorylates a number of intracellular proteins, including members of the insulin receptor substrate family (IRS) and SHC adaptor protein. Binding of IRS to the IR leads to activation the phosphatidylinositol 3-kinase (PI3K), which turns on the Akt pathway. Binding of Shc to the IR leads to activation of the extracellular signal-regulated kinase (ERK) cascade, one of the mitogen-activating protein kinase (MAPK) pathways [8]. Although the major role of insulin is metabolic, both the Akt and the MAPK pathways also have important roles in tumorigenesis. Indeed, insulin was found to stimulate cell cycle progression in MCF-7 breast cancer cells either by itself or synergistically with estradiol [9]. IRS-1 may also interact directly and activate the estrogen receptor (ER). Thus, activation of the insulin pathway may also affect the ER pathway [10].

The IR has a major role in the activation of the insulin pathway in breast cancer. The IR is expressed and can be stimulated by insulin in breast cancer cell lines, and overexpression of it can induce malignant transformation in breast epithelial cell lines. Stimulation by progestins, inactivation of p53 or activity of oncogenes such as Wnt-1, Neu and Ret can lead to overexpression of the IR in breast cancer [11].

Several clinical studies have investigated the role of the insulin pathway, and mainly the part played by the IR, in breast cancer. Papa et al. [12] measured IR content

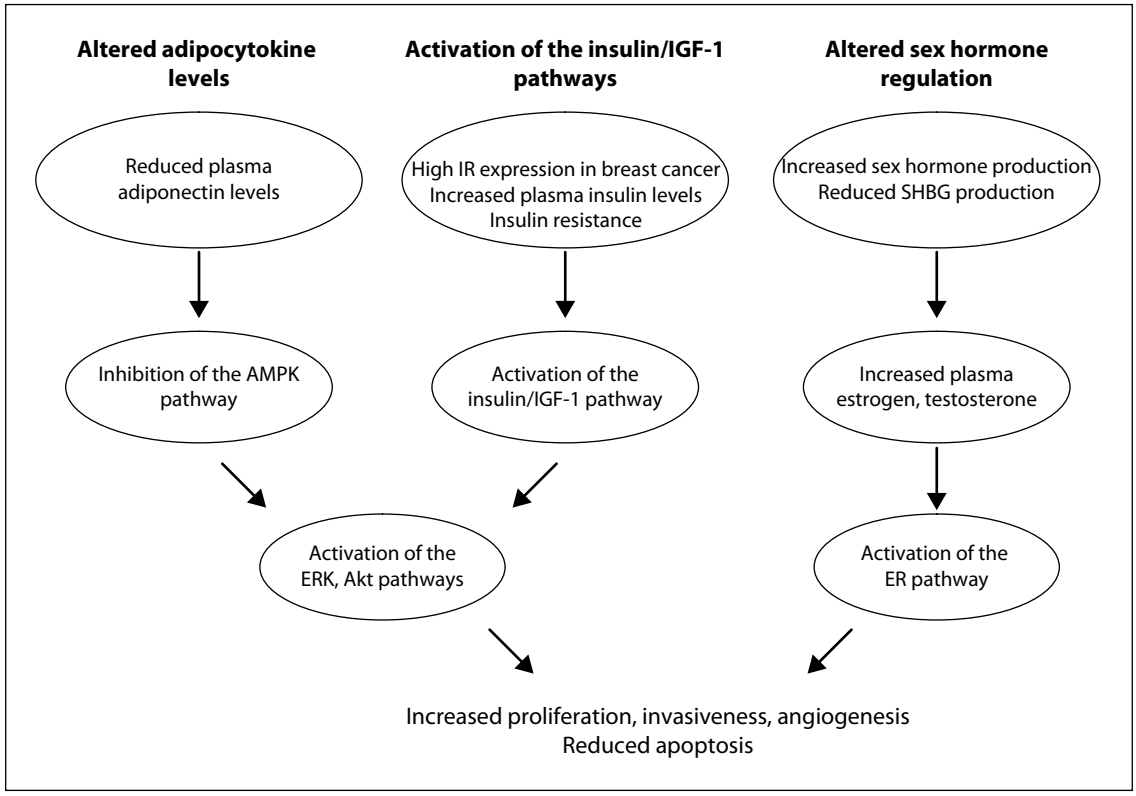

Fig. 2. Mechanisms associating type 2 diabetes and breast cancer. Insulin resistance leads to high plasma insulin concentrations, which activate the extracellular-related-kinase (ERK) and the AKT pathways through activation of the insulin receptor (IR) or the insulin-like-growth-factor-1 (IGF-1) receptor. High expression of the insulin receptor in breast cancer augments activation of these pathways. Diabetes is associated with reduced adiponectin plasma levels, which inhibits the AMP kinase (AMPK) and activates the ERK and Akt pathways in breast cancer cells. Diabetes increases production of sex hormones and deceases sex hormone binding globulin (SHBG) production, leading to high plasma-free estrogen concentrations, which in turn activate the estrogen receptor (ER). Activation of these pathways can lead to proliferation, invasiveness, angiogenesis and decreased apoptosis.

in 159 breast cancer specimens and found it to be sixfold higher than in 33 samples of normal breast tissues, and also higher than in other normal tissues, including the liver. High IR content correlated positively with tumor size, grade and ER content. Mathieu et al. [13] found detectable IR levels in 444 of 584 (76%) breast cancer specimens and found it to be a strong predictor of disease-free survival. Similarly, analysis of IR expression in a cohort of 191 early breast cancer patients revealed an association between high IR expression and favorable prognostic factors and improved disease-free and overall survival [14].

An important evidence for the adverse role of insulin on breast cancer comes from a recent report by Goodwin et al. [15] who found, in a prospective study of 512 early stage breast cancer patients, a direct association between fasting insulin levels and cancer recurrence and death (hazard ratio (HR) of 2.0 and 3.1 respectively for highest vs. lowest insulin quartile). The patients in the study were all non-diabetic and probably had lower insulin levels than diabetic patients. Whether the findings of this study are applicable to diabetic patients remains to be seen.

The IGF Pathway as a Possible Link between Diabetes and Breast Cancer

The IGF system comprises a network of ligands (IGF-1 and IGF-2), which are highly homologous to insulin; IGF-1 receptor (IGF-1R), which shares 55% homology with the IR; and IGF-binding proteins (IGF-BPs) [16]. The IR and the IGF-IR are capable of forming a hybrid receptor, which, like the IGF-IR, show high affinity to IGF-1 and lower affinity to insulin. Activation of the IGF-IR by its ligand results in activation of the same proteins and pathways activated by the insulin and IR, i.e. the IRS, SHC adaptor proteins, PI3K and MAPK. Thus, the specificity of the IGF pathway depends mainly on the ligand and its receptor, and not on the downstream parts of the cascade. The IGF system is considered to be a key regulatory pathway in breast cancer and is an attractive target for the development of novel breast cancer therapies. High circulating levels of IGF-1 and IGF-BP3 are associated with increased risk of premenopausal breast cancer, and increased IGF-1 is considered to be a link between obesity to increased risk of breast cancer [17]. However, type 2 diabetes usually affects postmenopausal women and, controlled for obesity, blood concentrations of IGF-1, IGF-2, and their binding proteins are usually not raised and may actually be reduced in both type 2 diabetes mellitus and the metabolic syndrome [18]. These findings suggest that the IGFs and the IGF-BPs may not play a major role in the association between diabetes and breast cancer. A high concentration of insulin could stimulate the IGF pathway in type 2 diabetes through the non-specific activation of the IGF-1R and the IGF-1R/IR hybrid receptor. However, the importance of this mechanism in the pathogenesis of breast cancer remains to be defined.

Altered Sex Hormone Regulation as a Possible Link between Diabetes and Breast Cancer

High endogenous plasma levels of estrogens and androgens and low plasma levels of sex hormone binding globulin (SHBG) are strongly associated with breast cancer risk in postmenopausal women. Obesity, a breast cancer risk factor, is characterized by increased production of sex hormones in the adipose tissue and decreased liver production of SHBG levels [6]. A meta-analysis of 43 prospective and cross-sectional studies, comprising 6,974 women, indicated lower levels of SHBG and higher levels of estrogen and testosterone among patients with type 2 diabetes, compared to controls, even after adjustment for obesity [19]. Thus, deregulation of sex hormones may be an important link between diabetes and breast cancer.

Adiponectin as a Possible Link between Diabetes and Breast Cancer

The adipocytokines are cytokine-like factors, which are exclusively produced by the adipose tissue and exert a wide array of endocrine, paracrine and autocrine activities. Two of the adipocytokines, adiponectin and leptin, have been implicated in breast cancer pathogenesis. While leptin has shown some growth stimulatory effect on breast cancer cells in vitro, clinical studies have yielded conflicting results. Thus, leptin role as a breast cancer risk factor is currently uncertain, and will not be discussed here. However, ample data, both clinical and laboratory, suggest a major role for low adiponectin levels as a breast cancer risk factor and as a possible mediator of breast cancer development in diabetic patients.

Adiponectin is a recently discovered adipocytokine which is produced and secreted mainly by adipose tissue [6, 20]. It is a 244-amino-acid protein that belongs to the soluble defense collagen superfamily. It exists in the circulation in a wide array of multimer complexes, mostly trimers, hexamers and high-molecular-weight complexes. Most of its biologic activity is currently attributed to the high-molecular-weight adiponectin complexes. Adiponectin serum concentrations are high, ranging from 3 to 30 μg/ml. Two adiponectin receptors are currently known, AdipoR1 and AdipoR2, which differ in their distribution and in their affinity to the various forms of adiponectin. The cell adhesion molecule T-cadherin, a member of the cadherin superfamily, may serve as an additional adiponectin receptor. Adiponectin activates the AMP kinase (AMPK) and the p38 MAPK pathways and upregulates peroxisome proliferator-activated receptor (PPAR) α, thus increasing insulin sensitivity. Adiponectin levels negatively correlate with body weight and are lower in obesity and higher in caloric restriction and weight reduction. Adiponectin levels inversely correlate with body weight and fasting insulin levels, and while reduced levels are found in insulin-resistant states, including obesity, type 2 diabetes mellitus and the metabolic syndrome, increased levels are strongly associated with reduced risk of diabetes.

Adiponectin may affect breast cancer either through modulation of insulin signaling, or directly. Several breast cancer cell lines express the adiponectin receptors, and treatment of these cells with adiponectin slows growth, activates the AMPK pathway and inhibits the ERK and Akt pathways [21]. Epidemiological data suggest an association between low adiponectin levels and increased risk of breast cancer, as well as other malignancies. Miyoshi et al. [22] found an association between increased breast cancer risk and lower adiponectin levels in 102 breast cancer patients, compared to 100 controls, even after adjustment for age, body mass index and menopausal status. Moreover, among the cancer patients, lower adiponectin levels were associated with unfavorable prognostic factors, including larger tumors, higher tumor grade and lower expression of hormone receptors. A case-control study of 174 postmenopausal breast cancer patients and matched controls by Mantzoros et al. [23] revealed a significant inverse association of adiponectin with breast cancer risk. Similarly, a prospective case-control study of more than 3,500 participants of the Nurses' Health Study revealed an association between low adiponectin levels and increased risk for

postmenopausal breast cancer [24]. These data suggest adiponectin as an important link between diabetes and breast cancer risk.

Diabetes Mellitus and Breast Cancer Risk

Different strategies were used in order to assess the association between diabetes mellitus and breast cancer risk. We divided these studies into three categories: (1) studies that evaluated the association between diabetes and breast cancer risk; (2) studies that evaluated the association between diabetes and breast cancer mortality, and (3) studies of the association between blood levels of insulin resistance markers, such as glucose or insulin and breast cancer risk.

Diabetes Mellitus and Breast Cancer Risk

Cohort Studies. To our knowledge, 16 cohort studies reported on the association between diabetes mellitus and breast cancer risk [reviewed in 25, 26]. These studies vary significantly in sample size (from 800 diabetic patients to more than 460,000), inclusion criteria (all diabetics or only type 2), population (hospitalized or ambulatory, population-based or sample) and years of the study (1965–1996). The major studies will be described in the following section.

Two studies included all hospitalized patients diagnosed as diabetics in Denmark from 1977 to 1989 (n = 55,010) [27] and in Sweden from 1965 to 1983 (n = 80,005) [28] and both reported on elevated risk of breast cancer among diabetic patients (standardized incidence ratios 1.2, 95% CI 1.1–1.2 and SIR 1.3, 95% CI 1.2–1.4 respectively). However, the results of these studies should be interpreted with some caution: use of hospitalization records and of former definitions of diabetes meant that both studies included patients with severe forms of type 2 diabetes compared to current definitions and did not exclude type 1 diabetes patients. In addition, both studies did not adjust properly for obesity. Michels et al. [29] reported on the association between type 2 diabetes and breast cancer in more than 6,000 participants of the Nurses' Health Study, which is a prospective population-based study. After adjustment of the HR for age, obesity, reproductive factors and benign breast disease, a modest but significant risk for breast cancer was found among postmenopausal diabetic women (HR 1.17, 95% CI 1.01–1.35). A study of 468,000 Korean women failed to demonstrate an association between fasting blood glucose levels and breast cancer, but identified a relatively strong association between overt diabetes and cancer risk (HR 1.51, 95% CI 1.26–1.8). Moreover, diabetes was associated with more than two-fold increase in breast cancer mortality (HR 2.23, 95% CI 1.49–3.33) [30]. A comparison of breast cancer incidence between Canadian women, aged 55–79 years, with newly diagnosed diabetes (n = 73,796) to women without diabetes (n = 391,714) revealed a significant increase in breast cancer among women with diabetes (HR 1.08, 95% CI 1.01–1.16). However, adjustment was conducted only for age and income and not for

obesity or other breast cancer risk factors [31]. Interestingly, the same database was used to assess prior breast cancers among newly diagnosed diabetic patients, and breast cancer was identified in 3.7% of diabetic women, compared to 3.1% of non-diabetic women (odds ratio (OR) 1.22, 95% CI 1.17–1.27) [32]. These results suggest that breast cancer risk may be increased already in the prediabetes phase.

Two meta-analyses of the above studies indicated an estimated risk for breast cancer among diabetic patients, as calculated from cohort studies, to be 1.25 (95% CI 1.19–1.31) and 1.2 (95% CI 1.11–1.3) [25, 26]. Thus, the results of the cohort studies suggest a modest increase of breast cancer risk among type 2 diabetic patients. However, as some of the studies did not properly controlled adjust for various confounding factors, including obesity, the possibility that diabetes is not a mutual exclusive risk factor for breast cancer cannot be ruled out.

Case-Control Studies. The association between diabetes and breast cancer has also been assessed in six case-control studies, which controlled for multiple confounding factors [25, 26]. Baron et al. [33] reported on the association between diabetes diagnosed after the age 35 and breast cancer in more than 5,500 breast cancer patients and matched controls (OR 1.2, 95% CI 1–1.4). Increased breast cancer risk among postmenopausal diabetic women (OR 1.5, 95% CI 1.1–2.0) was also reported by an Italian group [34]. An important feature of these case-control studies is the adjustment of all of them for multiple confounding factors, including body mass index. Two meta-analyses of case-control trials reported on an estimated risk of 1.13 (95% CI 0.99–1.28) and 1.18 (95% CI 1.05–1.32) [25, 26]. Thus, the results of the case-control studies support those of the cohort studies and suggest a modest association between breast cancer and diabetes.

Diabetes Mellitus and Breast Cancer Mortality

Another method to assess the effects of diabetes on breast cancer is through analysis of breast cancer mortality among diabetic and non-diabetic patients. Breast cancer mortality data reflect not only cancer incidence but also differences in natural history, diagnosis and treatment between diabetic and non-diabetic breast cancer patients. Thus, higher breast cancer mortality among diabetic patients may reflect a more aggressive disease or less-than optimal therapy, rather than higher cancer incidence. Four recent studies reported on breast cancer mortality among diabetic patients [25, 26]. Coughlin et al. [35] analyzed cancer mortality data from a large prospective cohort, the Cancer Prevention Study II, consisting of almost 590,000 women. In this study, 16 years after enrollment, diabetic patients had significantly elevated breast cancer mortality (relative risk (RR) 1.27, 95% CI 1.11–1.45). Jee et al. [30] in their analysis of 468,000 Korean women also noted an increased risk for breast cancer mortality among diabetic patients (RR 2.23, 95% CI 1.49–3.33). Two smaller studies yielded conflicting results: increased risk (RR 1.4, 95% CI 1.06–1.81) was reported in an Italian study of 7,148 patients, but no association was noted in a study of 5,066 patients from the UK. Thus, diabetes may be associated with

increased mortality among breast cancer patients. The causes of this association remain to be elucidated.

Insulin Resistance Markers and Breast Cancer Risk

Several studies investigated the association between breast cancer and blood levels of glucose, C-peptide and insulin, all markers of insulin resistance. No association between insulin or fasting glucose levels and breast cancer risk was found in three out of four cohort studies [25, 30] and only modest elevation with borderline statistical significance was found in another. Yet, four out of five case-control studies reported on a significant association between the different variables measured and breast cancer. Part of the differences between the cohort and the case-control studies may have resulted from bias in patient selection criteria in the case-control studies. In addition, the lack of more case-control studies reporting on negative results raise the possibility of a publication bias. To our view, the available data is currently insufficient to suggest any association between blood levels of insulin resistance markers and breast cancer risk.

Diabetes and Breast Cancer Risk in Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is an insulin resistance state, closely related to type 2 diabetes and women who suffer from GDM are at high risk for developing type 2 diabetes. A cohort study consisting of 917 patients found that higher plasma glucose levels during pregnancy were associated with up to 10.7 increase in the RR for breast cancer 18 years later [36]. The association between GDM and breast cancer was also assessed in 37,926 Israeli women who had one or more live births between 1964 and 1976 [37]. In this cohort, 1,626 cases of breast cancer and 410 cases of GDM were identified. The incidence of breast cancer was significantly increased among women 50 years and older who suffered from GDM (RR 1.5, 95% CI 1.0–2.1). Thus, GDM may be an early marker of breast cancer risk among postmenopausal women.

Diabetes Mellitus and Male Breast Cancer Risk

A significant association between male breast cancer and type 2 diabetes was reported in two Scandinavian studies [30]. A case-control study, consisting of 156 male breast cancer patients and 468 controls, identified an OR of 2.6 (95% CI 1.3–5.3). Weiderpass et al. [28] conducted a cohort study, consisting of nearly 64,000 patients without breast cancer and 13 male breast cancer patients and identified standardized incidence ratios of 2.0 (95% CI 1.0–3.4). The association between diabetes and breast cancer appears to be stronger in males than in female breast cancer and suggests a special importance of the insulin pathway in the pathogenesis of male breast cancer.

Type 1 Diabetes Mellitus and Breast Cancer Risk

As opposed to type 2 diabetes, which is associated with obesity, insulin resistance and high insulin levels, type 1 diabetes is a low insulin state. Two cohort studies investigated the association between type 1 diabetes and breast cancer risk, and both did not

identify any association between the two diseases [25]. The largest of these studies included more than 29,000 type 1 diabetic patients and reported on RR of 1.0 (95% CI 0.8–1.3).

Insulin treatment is administered in type 1 diabetes for many years and can result in very high plasma insulin levels, which theoretically might have pronounced mitogenic effects, yet breast cancer risk is not increased in these patients. Perhaps high insulin levels alone are insufficient to induce breast cancer and mechanisms associated with insulin resistance, such as low adiponectin levels and altered sex-hormone regulation, are needed to operate in concert with increased insulin levels in order to promote breast cancer tumorigenesis.

Diabetes Mellitus and Clinical Aspects of Breast Cancer

Diabetes Mellitus and Breast Cancer Screening

Screening mammography has been shown to reduce breast cancer mortality and is recommended by clinical practice guidelines for all women between the ages of 50 and 69 years. With current antidiabetic treatment, many patients with diabetes do not have additional comorbidity and thus may benefit from screening, yet in several countries diabetes may adversely affect attendance to screening mammography. Beckman et al. [38] found that American diabetic patients were less likely to undergo screening mammography, probably due to compromised attitude of their primary care physicians to preventive medicine and to high costs of mammography. Lipscombe et al. [39] investigated mammography rates in a large Canadian cohort, consisting of 69,168 women with diabetes and 663,519 women without diabetes. Although all patients were fully insured, diabetic patients had about one-third lower chances to perform screening mammography. On the other hand, a study from the UK revealed an attendance of more than 80% to screening mammography, and the high attendance rate was not affected by diabetes [40], and we did not find any differences in diagnosis method between diabetic and non-diabetic Israeli breast cancer patients [41]. In both countries the mammography screening program is free of charge and women are invited directly, irrespective of coexisting comorbidity. Thus, implementation of an affordable, invitation-based screening program for breast cancer has the potential to improve substantially the screening of patients with diabetes.

Mammographic breast density is a strong risk factor for breast cancer and is associated with obesity and various hormonal factors, yet an analysis of the Minnesota Breast Cancer Family Study cohort revealed no association between diabetes and increased breast density [42].

Diabetes Mellitus and Clinical and Pathological Characteristics of Breast Cancer

A prognostic factor is a measurement that correlates with disease-free or overall survival in the absence of therapy and therefore correlates with the natural history of the

disease. A predictive factor is a measurement associated with response to a given therapy. The most important prognostic factors in breast cancer are disease stage and pathological grade, and the most important predictive factors are the expression of hormone receptors, which predict response to hormonal therapy, and overexpression of HER2/*neu*, which predicts response to trastuzumab (Herceptin). The presence of prognostic and predictive factors can serve as an indirect indicator of outcome. We and others looked at the association between diabetes mellitus and various prognostic and predictive factors. In a retrospective analysis of 176 diabetic patients, Unterburger et al. [43] reported on a correlation between diabetes and the development of metastases. No association between diabetes and breast cancer stage or hormone receptor status was found by Guastamacchia et al. [44], however there was no adjustment to major confounding factors. We investigated the effects of type 2 diabetes on breast cancer characteristics at presentation among consecutive 79 diabetic breast patients compared to age-matched 158 non-diabetic patients [48]. Adjusted for obesity, tumor stage and size were significantly higher among diabetic patients. These data suggest an association between diabetes and poorer breast cancer outcome. Interestingly, diabetes was also associated with lower expression of hormone receptors. This finding suggests that hormonal mechanisms may play a less prominent role in the development of breast cancer in diabetes.

Diabetes Mellitus and Breast Cancer Outcome

Diabetes has been shown to be associated with inferior outcome of various malignancies, including colon, pancreatic and hepatocellular carcinoma. The direct biological effects of diabetes on breast cancer outcome are difficult to elucidate, mainly because of the presence of confounding factors such as obesity, older age, comorbidity, and differences in screening use or treatment allocation. These confounding factors are associated with undertreatment and decreased prognosis of breast cancer patients, yet most studies on the effects of diabetes on breast cancer outcome did not control for these factors. In a prospective study of 512 non-diabetic breast cancer patients, higher fasting insulin levels were associated with increased risk of distant recurrence and death [15]. In a cohort of 936 breast cancer patients, 12% of them were diabetic, higher comorbidity was associated with higher mortality, even after controlling for variables such as age, stage and grade [45]. Yancik et al. [4] investigated the role of age and comorbidity of 1,800 postmenopausal cases of breast cancer and reported on elevated all-cause mortality in diabetic patients. However, about half of the reported mortality causes were not cancer-associated and could be affected by diabetes (e.g. heart or cerebrovascular disease). Fleming et al. [46], who did a similar analysis in a cohort of 848 elderly breast cancer patients, did not find increased mortality among diabetic patients.

Diabetes may also be associated with local breast cancer recurrence. In an analysis of 246 women treated with postmastectomy radiotherapy, local chest wall recurrence was observed in 32% of the diabetic patients, compared to only 7% among the non-diabetics [47].

Several of the well-known complications of diabetes, including nephropathy, neuropathy, heart disease, impaired wound healing and tendency to infections can adversely affect all forms of cancer therapy: surgical, radiation, chemotherapy and even hormonal therapy.

Diabetes and Surgery for Breast Cancer

Diabetes is associated with increased risk of complications following breast cancer surgery. Analysis of 326 patients revealed a strong association between diabetes and wound infection following breast surgery: wound infection occurred in 12 of 264 (5%) non-diabetic patients but in 6 of 44 (14%) of the diabetic patients [48]. Diabetes is also associated with ipsilateral upper arm dysfunction 5 years following mastectomy [49].

Diabetes and Radiation Therapy for Breast Cancer

Data regarding efficacy and complications of radiotherapy for breast cancer are limited. However, diabetes is associated with increased risk of early and late complications of radiation. In patients treated for cervical cancer, diabetes was associated with formation of recto-vaginal fistula and bowel obstruction and, in elderly patients with prostate cancer given radiotherapy, with early and late gastrointestinal and genitourinary complications. Analysis of 828 patients treated with breast-conserving surgery and radiotherapy identified diabetes as an independent risk factor for post-treatment myocardial infarction [50]. An analysis of 246 women treated with postmastectomy radiotherapy did not identify diabetes as an independent risk factor for skin complications. However, only 19 diabetic patients were included in the analysis [47].

Diabetes and Chemotherapy for Breast Cancer

To our knowledge, no study has reported about the specific complications of chemotherapy in patients with breast cancer and diabetes. In a study of 33 patients with ovarian cancer and diabetes who were given cisplatin or paclitaxel, 21 (64%) had neurological symptoms and exacerbation of hyperglycemia; treatment changes were needed in 5 patients. Another study reported on the toxic effects of fluorouracil in 7 patients with diabetes that was poorly controlled; however, neither of these studies included a control group. Diabetes may accentuate other side effects and complications of cancer chemotherapy. For example, patients with diabetes who have peripheral neuropathy are more prone to complications of vinca alkaloids or taxanes, and those with diabetic cardiomyopathy are probably more susceptible to complications from cardiotoxic medications such as doxorubicin and trastuzumab (herceptin). The possibility of severe complications from infection should also be considered.

Diabetes and Hormonotherapy for Breast Cancer

Adverse interactions between hormone therapy and diabetes are probably uncommon. Tamoxifen is a selective ER modulator, which is commonly used for the treatment of breast cancer in the adjuvant, as well as metastatic setting. Tamoxifen use is associated with an up to 4 times increased risk of endometrial cancer, and several studies have also reported an up to 1.5 times increased risk of endometrial cancer in patients with diabetes. However, no evidence suggests an enhanced risk of endometrial cancer in patients with diabetes who were treated by tamoxifen compared with those without diabetes. Acute pancreatitis due to severe hypertriglyceridaemia after tamoxifen treatment in patients with diabetes has been described, but the frequency of this side effect is not known and is probably very low. Raloxifene is another selective ER modulator, which is used to treat osteoporosis and has also been shown to prevent breast cancer in high-risk populations. In a small randomized study of 39 postmenopausal diabetic women, raloxifene administration slowed progression of albuminuria [51]. It is currently unknown whether tamoxifen treatment has a similar beneficial effect. At present, there is no evidence for an interaction between aromatase inhibitors and diabetes.

Treatment for Diabetes Mellitus: A Potential Therapy for Breast Cancer?

Metformin. Metformin is a biguanide drug that is widely used for the treatment of type 2 diabetes. Some of the beneficial effects of metformin have been linked to activation of AMPK in muscle, adipose tissue, and liver. AMPK is a protein kinase which regulates energy metabolism and is activated by an increase in the intracellular ratio of AMP/ATP. Upon activation, AMPK phosphorylates a number of effector proteins leading to the activation of ATP-generating pathways, such as glycolysis, and the inhibition of ATP-consuming pathways. Activation of AMPK inhibits cancer cell proliferation through various mechanisms, including TSC2-dependent inhibition of mTOR and phosphorylation of p53. Treatment of breast cancer cells with metformin has been shown to inhibit their growth through the activation of AMPK [52]. In C3H/Sn mice, which are susceptible to breast cancer, long-term administration of phenformin reduced the incidence of mammary carcinoma from 80 to 21% [53]. In humans, a case-control study of nearly 1,000 diabetic patients and matched controls suggested that metformin may reduce the risk of cancer in patients with type 2 diabetes [54]. The efficacy of metformin as an adjuvant treatment for early breast cancer is currently being tested in a phase II trial.

Peroxisome Proliferator-Activated Receptor γ (PPARγ) Ligands. PPARγ is a member of the nuclear hormone receptor family that has been implicated in a wide array of biological processes including inflammation, tissue remodeling and atherosclerosis [55]. Natural ligands of it include prostanoids, prostaglandin D_2, 15 deoxy-D12, 14-prostaglandin J2 (15d-PGJ2), and synthetic ligands include

members of the thiazolidinedione family – rosiglitazone, pioglitazone, and troglitazone. These synthetic ligands decrease insulin resistance and thus serve as antidiabetic drugs, although the use of troglitazone has been discontinued because of liver toxicity. Upon binding of a ligand in the cytoplasm, PPARγ heterodimerizes with retinoid X receptors (RXR), and forms a complex that translocates to the nucleus and acts as a transcription factor. Most current data suggests a tumor-suppressor role for PPARγ in breast cancer. Breast cancer cells commonly express PPARγ, and use of various PPARγ ligands can inhibit proliferation and induce differentiation and apoptosis in both in vitro and in vivo models of breast cancer. These ligands also prevent carcinogen-induced transformation of breast tissue in mice. However, forced overexpression of PPARγ in the breasts of transgenic mice that are prone to mammary gland cancer accelerates the development of breast tumors compared with wild-type mice, suggesting a possible role for PPARγ in breast cancer tumorigenesis.

Measurement of PPARγ levels in 120 breast cancer tissues revealed lower expression compared to normal breast [56], as well as an association between lower levels of PPARγ and advanced disease, higher tumor grade and more aggressive histology. Use of troglitazone as treatment for breast cancer has been tested in a phase II study of 22 patients with refractory breast cancer, however none of these patients showed a response [57]. Whether PPARγ agonists may have a role in the treatment of earlier stages of breast cancer or as part of a combination therapy remains unknown.

Exendin-4. Glucagon-like peptide (GLP)-1 is a proglucagon-derived peptide which opposes several insulin actions and improves glycemic control through a combination of mechanisms, which include glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion, slowing of gastric emptying and reduced appetite [58]. These activities are mediated through activation of a GLP-1-specific G-protein-coupled receptor. GLP-1 is very rapidly degraded by dipeptidyl peptidase IV and cannot be effectively used as a medication. Exendin 4 (exenatide) is a GLP-1-receptor agonist, which is derived from the venom of the Gila monster lizard. It possesses similar activity to GLP-1 and has recently been approved for the treatment of type 2 diabetes in the USA. To our knowledge, antitumor activity of exendin-4 has not been elucidated yet. Moreover, GLP-1 has been shown to enhance proliferation and reduce apoptosis of pancreatic β-cells. We studied the activity of exendin-4 on breast cancer cells and our preliminary results indicate it as a potent inhibitor of proliferation [Rubinek and Wolf, submitted for publication]. Treatment of MCF-7, as well as other breast cancer cell lines, with exendin-4 significantly slowed growth and upregulated the tumor suppressors p53 and p21. Thus, exendin-4 may act in a cell-type-dependent manner: enhance proliferation of pancreatic β-cells but inhibit proliferation of breast cancer cells. Studies regarding in vivo activities of exendin-4 on breast cancer are ongoing.

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Nutrition, Diabetes, and Cancer

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Abstract

The progression of inflammatory signaling to the metabolomic complications of diabetes usually occurs slowly and creates cellular shifts in biochemistry. Individual nutrigenomics, chronic stress, environmental intoxication (such as exposure to chemical preservatives or heavy metals), poor dietary choices, gut health, sleep patterns, and other factors can trigger inflammation signaling and as a consequence alter blood sugar homeostasis, leading to insulin resistance and accumulation of visceral fat. Likewise, repeated dietary insult leads to blood glucose alterations, insulin resistance, and increase in visceral fat deposition and thus enhancing inflammatory pathways that trigger the downward spiral to chronic illness that eventually may lead to type 2 diabetes and its complications. A movement toward intracellular fermentative metabolism creates intracellular acidity and heightened risk for other chronic disease, including cancer. Proper nutrition and the use of targeted nutritional supplements can have a significant impact on slowing the progression of diabetes as well as the progression of cellular shifts toward the Warburg effect. Diet and dietary supplements targeted at glycemic regulation based on individual needs, including those improving insulin resistance, immunity, inflammatory responses, gut health, and chronic stress, are important in decreasing the metabolic spiral to type 2 diabetes and eventually cancer.

In all likelihood, the metabolic progression to cancer in a patient with type 2 diabetes is promoted by multiple vectors of inflammation. In the most basic construct, the physiology of those individuals with type 2 diabetes is more efficient at creating a cascade of inflammatory signaling that leads them to a complex milieu of comorbid symptoms, conditions, and pathologies including cancer. This is supported by the fact that in some type 2 diabetics, chronic conditions of autoimmunity and/or immune bystander effect are present.

What triggers inflammatory chemistry in the person with type 2 diabetes? Does diabetes trigger inflammatory chemistry or does the combination of genetics, environmental factors, and individual choices ignite the fires of inflammation, with diabetes simply being a more complete expression of the longstanding organic disruptions in metabolism? Several interesting developments are leading to a new level of understanding the dynamic aspects of diabetic chemistry. Individual nutrigenomic predisposition

and expression, chronic stress, environmental intoxication, poor dietary choices, sleep patterns, use of drug therapy, and poor or absent exercise habits among other factors can trigger inflammation signaling and as a consequence blood sugar and insulin homeostasis is altered. However, the converse can be true, where repeated dietary insults, such as excessive intake of refined carbohydrates, sugars, ω–6 oils, and partially hydrogenated oils, lead to blood glucose alterations, insulin resistance and visceral fat deposition, thus enhancing cytokines and other inflammatory pathways. The most intriguing model, and one that is the most diverse, is that the expression of symptoms and the complications of diabetes is being influenced by individual choices and environmental exposures that start from gestation and continue throughout one's lifetime. These choices and influences result in biochemical changes that lead individuals to illnesses, such as type 2 diabetes and cancer, and the resulting health consequences.

There is a growing realization that the effects of nutrition on health and diseases cannot be understood without a profound understanding of how nutrients work at the molecular level. The completion of several large genome projects early in this century has markedly altered the research agenda by drawing attention to the importance of genes in human nutrition, and has provided a wealth of new genetic information to be explored. There has been a growing recognition that micro- and macronutrients can be potent dietary signals that influence the genetic expressions of cells and play an important role in homeostasis. The fact that our diseases are actually less about our genes and more about the influences on the genes was one of the great surprises of the human genome project. Researchers have increasingly begun to recognize that genetic predisposition, environmental, and lifestyle choices are both important contributors to the main causes of mortality that are linked to diet, such as cardiovascular disease, type 2 diabetes, and cancer [1].

To date, the majority of research into disease progression in most conditions has not linked the multiple vectors or triggers of these disease progressions. Similarly, cancer may arise within the diabetic from a number of circumstances; however with cancer we know that inflammation is a primary component, which can both initiate and promote cancerous cell growth and division. In examining type 2 diabetes, there are multiple factors that assemble to create tremendous levels of inflammatory signaling – chronic imbalances in blood sugar, increases insulin growth factor-1, increases in cellular anaerobic metabolism, and alterations in immune vigilance occur. In this chapter we will discuss how nutritional support cannot only modulate those with phenotypes predisposed to biochemical imbalances that lead to type 2 diabetes and eventually cancer, but that can also redirect pathogenesis back to homeostatic function.

The Cascade of Inflammatory Signaling

Inflammation is the physiological response to biological, mechanical, or chemical stressors. In people with diabetes, increased risk of cardiovascular disease, kidney disease,

peripheral vascular disease, autoimmune disorders, obesity, and cancer as well as neurological disorders like Alzheimer's and Parkinson's disease are triggered at least in part from the inflammatory signaling that is chronically upregulated. The inflammatory state is closely related to obesity and insulin resistance, yet other vectors may lead to chronic inflammation, including chronic stress, gut-brain miscommunication, neuroendocrine-immune shifts, dietary choices (including artificial sweeteners), exercise frequency, drug use (prescription, non-prescription, and recreational), and environmental stressors (such as heavy metals and chemical preservatives in foods). Population-based studies have reported strong relationship between inflammatory markers and metabolic disturbances, obesity, atherosclerosis, and inflammation has been considered a 'common thread' between these conditions and type 2 diabetes [2, 3].

Cells are regularly exposed to stress, which mainly consists of inflammatory stress and metabolic stress. Inflammatory stress is exerted by cytokines that are released in large quantities by immune cells in response to invading microorganisms or other pathophysiological signals. The main cytokines involved in the pathogenesis of type 2 diabetes are interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), NF-κB, and IL-6, IL-18 and adipokines, which are considered as the main regulators of inflammation; leptin, more recently introduced, and several others, such as monocyte chemoattractant protein-1, suppressors of cytokine signaling proteins, resistin, angiotensinogen, and aromatase also are present with deleterious effects in diabetic pathogenesis. The characterization of these molecules helps to identify targeted diabetes treatment beyond the conventional interventions (lifestyle changes and pharmaceutical agents), and move toward the controlling of specific molecular pathways to greatly reduce inflammation.

Chronic stress has negative effects on inflammatory signaling, serum glucose, serum cortisol levels, serum thyroid hormone levels, and body weight. Chronic stress, through hyperexcitation of the hypothalamic-pituitary-adrenal axis and microglial cell activation of the immune system, directly affects fat storage and weight gain in stressed individuals. Elevated serum cortisol is associated with diabetes and its complications as well as being a known initiator of insulin resistance. This could be the clue to why so many type 2 diabetics have evidence of autoimmunity. Elevations of cortisol and depletion of dehydroepiandosterone (DHEA) pools are associated with memory loss and atrophy of the hippocampus combined with the known defect in glucose utilization in Alzheimer's disease could offer an important insight into neurodegeneration found in individuals with type 2 diabetes.

Oxidative stress in the diabetic, due to depleted levels of nitric oxide, can increase the pathways that lead to inflammatory signaling by upregulation of peroxyl nitrite free radicals. These processes accelerate pathological changes in endothelial tissues. Another vector associated with inflammatory signaling is obstructive sleep apnea syndrome. Sleep apnea produces more inflammatory signaling, which leads to more accumulation of visceral fat – cycles of the downward metabolic spiral. Surgical removal of visceral fat can reverse sleep apnea in a substantial number of patients,

demonstrating the role played by adipokines in this disorder. Even restricted sleep can induce insulin resistance and progression toward type 2 diabetes. Alterations in leptin, ghrelin, growth hormone, and body mass index have been found with sleep deprivation (approx. 4–5 h per night).

The quality of an individual's adaptive immune system can be evaluated through the balance of inflammatory cytokines it is producing. A healthy immune system is both balanced and dynamic – it should be balanced between Th1 and Th2 activity, switching back and forth between the two as needed. A failure of the Th1 arm of the immune system and an overactive Th2 arm is implicated in a wide variety of chronic illnesses, including autoimmune conditions, acquired immunodeficiency syndrome, chronic fatigue syndrome, candidiasis, allergies, multiple chemical sensitivities, blood sugar regulatory problems (including diabetes), and cancer. Likewise, overexpression of Th1- and cell-specific immunity can occur, leading to a subset of autoimmune disturbances.

Exposure to environmental chemicals, such as pesticides and heavy metals, may also disrupt the neuroendocrine-immune system, leading to upregulation of inflammatory signaling. Endocrine disruptors include dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and plasticizers such as phthalates and bisphenol A (BPA). Endocrine disruptors may be found in many everyday products – including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides and have been linked to insulin resistance. Exposure to BPA has been found to cause biological effects, and its mode of action appears to mimic that of the female hormone, estrogen. Studies have found that BPA does increase the risk of developing cancer [4]. Phthalates are another compound commonly found in cosmetics and personal care items such as shampoos. Phthalates are reported to also affect neuroendocrine-immune balance. A recent study reported that approximately 75% of the US population has measurable levels of phthalates in their bodies [5]. Chronic heavy metal exposure, including lead, mercury, aluminum, and cadmium, may lead to the upregulation of inflammatory signaling pathways. Mercury, commonly found in dental amalgam fillings, can cause microglial activation and lead to localized flora disturbances and immune activation in the gut. Mercury, commonly found in dental amalgam fillings, may leak into the gut, causing imbalances in the natural gut flora. This may lead to and increase in inflammatory signaling and neuroendocrine-immune imbalances. Similarly, chronic lead exposure can damage the neuroendocrine-immune system, leading to inflammatory signaling.

A disturbed gastrointestinal terrain can serve as an unseen 'motor' of inflammation, leading to a cycle of inflammatory signaling. There is a complex balance that exists between the indigenous flora and the adjacent immune system of the gut mucosa and liver. Evidence supports that impairment of normal gut barrier function, through environmental stressors (such as heavy metals and chemical preservatives), poor dietary habits (such as high in refined sugars and fructose), food allergies, various drug therapies, and chronic stress, results in the loss of the counterinflammatory flora balance and

leads to the expression of uncontrolled inflammation [6]. Food and bacterial proteins, such as dairy lectins, can act together to damage the gut and allow toxic protein complexes to get through the tight junction glycoprotein and toll receptor network that is normally supposed to be resistant to such a breach – termed 'leaky gut'. When there is an imbalance in the natural flora of the gut, bacterial lipopolysaccharide released by increasing populations of pathogenic bacteria, such as *Escherichia coli* and *Candida* sp., causing oxidation that leads to gut ischemia. Downregulation of immunologic activation is an active, energy-requiring process, therefore gut ischemia may impair this normal anti-inflammatory function, and promote a state of systemic inflammation.

Symptoms such as gas and bloating, abdominal pain and diarrhea, can occur with imbalances in gut flora. As these imbalances continue they can progress to more profound symptoms such as headaches, nerve pain, skin rashes, and joint pain. The disorders that result or could be aggravated by an unhealthy gut include are celiac disease, Crohn's disease and irritable bowel syndrome, multiple sclerosis, migraines, attention deficit, autism, depression, eczema, acne, rheumatoid arthritis, fibromyalgia, diabetes, chronic fatigue, and others. Many people are being diagnosed with multiple conditions, including type 2 diabetes, without the obvious connection of the overload in inflammatory signaling of the gut with certain foods that may be a driving force in the disease process in many of these illnesses.

Insulin Resistance and Cancer

The cycle of inflammatory chemistry that is activated through chronic stress and cortisol release, leaky gut, environmental stressors (such as chemical preservatives, plastics, and heavy metals), obesity, thyroid dysfunction, and immune system imbalances causes chronic imbalances in blood glucose homeostatis, eventually lead to type 2 diabetes. This progression to diabetes and its metabolic consequences has also been linked in clinical studies with the development of cancer [7, 8]. Researchers have known for decades that cancer cells consume more glucose than normal cells. All cells use both oxidative phosphorylation and glycolysis pathways for energy (ATP) but rely overwhelmingly on oxidative phosphorylation, switching to glycolysis at times of oxygen deprivation. Cancer cells, however, have been reported to exhibit increased glycolysis due in part to mitochondrial respiration injury and hypoxia. A shift in energy production from oxidative phosphorylation to glycolysis – the so-called 'Warburg effect' – is a fundamental property of cancer cells, not just a by-product of the cell's transformation into cancer. Warburg [9] reported that many tumors relied on glycolysis even in the presence of oxygen. Certain nutrients are now focused on decreasing this intracellular shift to glycolysis (the Warburg effect) through improving insulin regulation and decreasing cellular oxidation. Elevated intra- and extracellular glucose concentrations also result in oxidative stress, leading to an increase in inflammatory signaling.

Studies have also reported that high levels of insulin decrease the production of insulin-like growth factor-1 (IGF-1) binding proteins and hence increase levels of free IGF-1 [10]. It is well established that bioactivity of free IGF-1 increases tumor turnover rate and can lead to various types of cancer.

Drug-Nutrient Depletion

Many of the side effects from drug therapies may not be directly due to the drug itself, but rather the result of nutritional deficiencies caused by the drug when taken over time. Drugs given to treat conditions such as type 2 diabetes or cardiovascular disease, such as diuretics for hypertension, statins for hypercholesterolemia, or metformin for blood sugar regulation, may actually be causing a cascade of biochemical changes in the body due to drug/nutrient depletion, further complicating the metabolomic chemistry of the individual. These biochemical changes can imbalance the homeostatic body system, leading to the cascade of inflammatory signaling.

Sulfonylurea medications, including glipizide (Glucotrol®), tolazamide (Tolinase®), chlorpropamide (Diabenese®), and glyburide (Diabeta®, Micronase®) have been reported in the literature to deplete coenzyme Q10 from the body [11]. A deficiency of CoQ10 may be associated with long-term conditions including heart disease and high blood pressure. Symptoms of deficiency include gingivitis, muscle weakness, reduction in neuroprotective functions and mitochondrial energetics, decreased insulin production, memory loss, loss in stamina, and weakened immune function.

Biguanide medications, including metformin (Glucophage®) have been reported to deplete folic acid and vitamin B_{12} from the body [12]. Studies indicate that long-term metformin therapy significantly decreases serum vitamin B_{12} levels. Additional studies suggest that short-term treatment with metformin increases homocysteine levels, and supplementation with B vitamins or folic acid can moderate this response [13]. More specifically, serum folic acid levels have been reported to decrease 7% and vitamin B_{12} levels decrease by 14% while using metformin therapy in type 2 diabetic individuals [14]. Homocysteine is implicated as a risk factor for development of cardiovascular disease, kidney disease, and Alzheimer's disease.

Nutrient Intervention

So what can be done for the individual that is metabolically spiraling toward type 2 diabetes and cancer? Today the molecular profile of the diabetic is becoming clearer, which leads us to an opportunity to target the various biomarkers through novel approaches. Replenishing nutrients that may be insufficient due to genetic variances or poor dietary choices is of upmost importance for an individual's health. Controlling inflammatory signaling by factors such as decreasing stress, controlling

visceral fat weight accumulation, improving sleep patterns, eating a proper diet, decreasing environmental stressors, balancing immunity, decreasing *Candida* overgrowth, balancing adrenal, thyroid and sex hormones, and exercising regularly can reduce and even reverse the metabolic spiral to a state of chronic blood sugar imbalances. Although the nutrigemonics and nutrigenetics of each individual can vary, this chapter has included some of the most common nutrients that have been reported to help decrease inflammatory signaling, decrease oxidation, balance neuroendocrine-immune signaling pathways, and help maintain blood sugar homeostasis and reduce the Warburg effect in cancer metabolism.

ω–3 Essential Fatty Acids

ω–3 fatty acids are a group of polyunsaturated fatty acids (including α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) that come from food sources or dietary supplements. Food sources include fish and fish oils (including salmon, rainbow trout, mackerel, krill, anchovy, and sardines), flaxseed oil, berries (such as lingonberry and black raspberry), walnuts, and wheat germ. However, ω–3 sources that are not from fish require conversion in the body and are therefore not a preferred source.

A high ω–6/ω–3 ratio, as is found in today's Western diets, promotes the pathogenesis of many chronic diseases, including cardiovascular disease, diabetes, asthma, and possibly cancer. Increased dietary intake of linoleic acid leads to oxidation of low-density lipoprotein (LDL), platelet aggregation, and interferes with the incorporation of essential fatty acids (EFA) in cell membrane phospholipids. Both ω–6 and ω–3 fatty acids influence gene expression. ω–3 fatty acids have strong anti-inflammatory effects via the suppression of inflammatory cytokines IL-1, TNF-α, and IL-6. ω–6 fatty acids tend to be proinflammatory. Because inflammation is at the base of many chronic diseases, including coronary heart disease, imbalances in the ω–6/ω–3 ratio plays an important role in the manifestation of disease, particularly in persons with genetic variation, as for example in individuals with genetic variants at the 5-lipoxygenase genes. Increased dietary arachidonic acid significantly enhances the apparent atherogenic effect of the genotype, whereas increased dietary intake of ω–3 fatty acids EPA and DHA blunts this effect. The diet-gene interaction further suggests that dietary ω–6 fatty acids promote, whereas marine ω–3 fatty acids EPA and DHA inhibit leukotriene-mediated inflammation that leads to atherosclerosis.

Carotenoids

Carotenoids are the pigments that give fruits and vegetables such as carrots, cantaloupe, sweet potato, and kale their vibrant orange, yellow, and green colors. β-Carotene,

lycopene, and lutein are varieties of carotenoids. Epidemiologic evidence suggests that serum carotenoids are potent antioxidants and may play a protective role in the development of chronic diseases including cancers, cardiovascular disease, and chronic inflammatory signaling [15]. Variations in insulin-mediated glucose disposal in healthy individuals have been found to be significantly related to plasma concentrations of lipid hydroperoxides and liposoluble antioxidant vitamins. Research has found that synthetic β-carotene may increase the risk of lung cancer, prostate cancer, intracerebral hemorrhage, and cardiovascular and total mortality in people who smoke cigarettes or have a history of high-level exposure to asbestos [16]. Natural carotenoids from foods or supplementation have not been reported to have this effect.

Chromium

Absorption of chromium from the intestinal tract is low, ranging from <0.4 to 2.5% of the amount consumed, and the remainder is excreted in the feces. Enhancing the mineral's absorption are vitamin C (found in fruits and vegetables and their juices) and the B vitamin niacin (found in meats, poultry, fish, and grain products). Absorbed chromium is stored in the liver, spleen, soft tissue, and bone.

The body's chromium content may be reduced under several conditions. Diets high in simple sugars (comprising more than 35% of calories) can increase chromium excretion in the urine. Infection, intense exercise, pregnancy and lactation, and stressful states (such as physical trauma) increase chromium losses and can lead to deficiency, especially if chromium intakes are already low. There are also reports of significant age-related decreases in the chromium concentrations of hair, sweat, and blood [17].

Chromium depletion can lead to symptoms including: increased blood cholesterol, problems with sugar metabolism, fatigue, an increased accumulation of plaque in the aorta, increased blood pressure, anxiety, impaired physical growth in the young, slower healing time after surgery or injury, atherosclerosis, decreased glucose tolerance, reduced conversion of thyroxine to triiodothyronine in the periphery, and possibly decreased fertility and longevity.

Chromium supplementation has been reported to improve insulin regulation and glucose tolerance in people with glucose intolerance and type 1, type 2, gestational, and steroid-induced diabetes. Chromium supplements improve glucose tolerance in people with diabetes through improving insulin sensitivity. Chromium may also lower total cholesterol, LDL cholesterol, and triglycerides.

A few trials have reported no beneficial effects from chromium supplementation, usually due to low dosages and poorly absorbable forms of chromium [18]. Until recently, glucose tolerance factor was considered the biologically active form of chromium and is the preferred form of chromium as a dietary supplement. Recently

chromium histidinate has become available and is known to be the most bioavailable, followed by chromium nicotinate, chromium picolinate and chromium chloride. Chromium is found in some foods, such as meats, animal fats, fish, brown sugar, coffee, tea, some spices, whole-wheat and rye breads, and brewer's yeast, but is not available in significant enough quantities in the diet to replete tissue stores or to induce an improvement in blood glucose control.

Vitamin D

Vitamin D is needed to maintain adequate blood levels of insulin. Vitamin D deficiency has been shown to impair insulin synthesis and secretion in humans and in animal models of diabetes. Vitamin D deficiency predisposes individuals to type 1 and type 2 diabetes, and receptors for its activated form – 1α,25-dihydroxyvitamin D_3 – have been identified in both β-cells and immune cells. Serum 25-hydroxyvitamin D levels have been reported to be inversely related to body mass index and body fat content, and correlated directly with hypertension, degree of insulin resistance and progression to type 2 diabetes [19]. 1,25-Dihydroxyvitamin D_3 directly regulates adipocyte 11β-HSD-1 expression and, consequently, local cortisol levels and that this may contribute to the preferential loss of visceral adiposity by high-calcium diets. Furthermore, epidemiological studies suggest a link between vitamin D deficiency in early life and the later onset of type 1 diabetes. In some populations, type 1 diabetes is associated with certain polymorphisms within the vitamin D receptor gene.

Alterations in vitamin D levels have also been reported to be a causative factor in certain cancers. The actions of 1,25-dihydroxyvitamin D_3 are mediated via the vitamin D receptor (VDR), and a number of polymorphisms in the *VDR* gene have been identified. *VDR* polymorphisms are associated with breast cancer risk and may be associated with disease progression.

Most foods contain little or no vitamin D. As a result, adequate sunshine is often the deciding factor in whether vitamin D deficiency occurs, but it is no guarantee. Vitamin D is depleted by certain medications, include corticosteroids (prednisone/Deltasone), aluminum-containing antacids (Gaviscon, Alternagel), butalbital (Fiorinal, Fioricet), carbamazepine (Tegretol), cimetidine (Tagamet), orlistat (Xenical, Alli), and ranitidine (Zantac).

Magnesium

Magnesium plays a critical role in carbohydrate metabolism and preventing metabolic syndrome. It may influence the release and activity of insulin and help balance glucose levels. Low magnesium levels are associated with the development of metabolic syndrome and type 2 diabetes. Hypomagnesemia may worsen insulin resistance,

a condition that often precedes type 2 diabetes, or may be a consequence of insulin resistance. In older adults, correcting magnesium depletion may improve insulin response and action. Several clinical studies have reported that magnesium supplementation is beneficial in reducing blood glucose levels, improving insulin control, and stabilizing the metabolic syndrome cascade [20, 21]. Low magnesium intake is also associated with an increase in inflammatory markers, such as C-reactive protein and E-selectin. Symptoms of low magnesium status can include restless legs, muscle tightness and cramps, palpitations, arrhythmias, headaches, muscle weakness, and fatigue. Drugs that deplete magnesium include hyrochlorthiazide, oral contraceptives, and corticosteroids. The major dietary sources of magnesium intake include whole grains, legumes, nuts, and green leafy vegetables.

Fermented Wheat Germ Extract

A good example of the application of modern science as applied to nutritional therapies is a proprietary fermented wheat germ extract (Avemar). Avemar has been studied clinically to have a wide array of immunomodulating and inflammatory mediating activity specifically in patients with cancer, with its hallmark trait being the ability to block the Warburg effect in cancer cell chemistry [22, 23]. Wheat germ is also a valuable nutritional source. In addition to proteins of high nutritional importance, tocopherol and B vitamins, wheat germ contains quinone compounds in a methoxy-substituted glycolysated form. Concentration allows the immunomodulatory effects of the substituted benzoquinones to be obtained without the consumption of impractically large amounts of wheat germ.

Natural anticancer immune response is based on the activity of the 'cellular' immune system. Primary anticancer cellular immune response depends on the activity of the natural killer (NK) cells. A low level of major histocompatibility complex (MHC-I) antigens enhances the activity of NK cells. Cancer cells enhance the synthesis of MHC-I and can effectively camouflage themselves from NK cells. Treatment with Avemar decreases the concentration of MHC-I on the surface of cancer cells by up to 90%, thus making them primary targets for NK cells. On the other hand, Avemar does not interfere with the MHC-I levels of healthy cells.

Avemar inhibits the enzyme glucose-6-phosphate dehydrogenase (G6PDH). Enzymes are proteins that help specific chemical reactions to occur. Avemar restricts the activity of the enzyme G6PDH that is essential to cancer cells' ability to grow and divide to make new cancer cells. All body cells go through an orderly process of resting, growing, and then dividing, called the cell cycle. If the cell cycle is interrupted and cannot go forward to the next step, it is forced to self-destruct through a process known as apoptosis, also called cell suicide or programmed cell death. In a 2002 study, Avemar completely inhibited the activity of G6PDH after 72 h of administration, forcing the cancerous cells under study into apoptosis [24]. Avemar did not

affect normal cells in the same way. This is one way in which Avemar has anticancer effects without harming normal cells.

The enzyme PARP-1 (poly-(ADP-ribose)-polymerase) functions is to repair damage at the genome level in cancer cells. Avemar has been reported to inactivate the enzyme PARP through proteolytic cleavage [25]. Cancer cells use between 10 and 50 times more glucose than normal cells through an enzyme called transketolase (TK). TK allows cancer cells to divide much more rapidly than normal cells. Avemar inhibits TK activity in vitro, decreasing cancer cell growth. Avemar decreases the glucose uptake of tumor cells (thereby diminishing their proliferative potential) in a dose-dependent manner and, at the same time, alters the intracellular transformation of glucose – decreasing the 'Warburg effect' seen in cancer cells. Its inhibition of ribose and deoxyribose synthesis also inhibits the production of messenger and ribosomal RNA, DNA replication and, at the same time, increases fatty acid synthesis.

The inhibitory effects of Avemar on the ribose synthesis pathway supportive of cancer cell proliferation plays an important role in the overall survival rate seen in colon cancer patients. When used in a study of 170 postsurgical colorectal cancer patients also receiving standard of care therapy such as chemotherapy, and/or radiation, addition of Avemar reduced new recurrences by 82%, metastases by 67%, and deaths by 62%, compared to use of radiation and chemotherapy alone [26]. It also lengthened the time it took for cancer to become measurably active again after primary therapy (surgery) and adjuvant therapy (chemotherapy and/or radiation treatment).

TNF-α plays an important role in local inflammatory and adhesion processes. TNF-α can destroy tumor cells by directly inducing apoptosis or by producing free radicals, and indirectly by inhibiting tumor angiogenesis or by enhancing other cellular antitumor processes. In order to activate their own antitumor capabilities, macrophages need to reach and then penetrate into the tumor, aided by the protein ICAM-I, an intracellular adhesion molecule (CD54). ICAM-I helps TNF-α to cross the vessel wall and also to transport them to the target. Avemar is capable of increasing the level of ICAM-I molecules, an effect synergistic with its TNF-α-like effect. In this way, Avemar enhances ICAM-I production in two distinct ways – on its own and through the enhancement of TNF-α production in macrophages – thereby helping the leukocytes to reach tumor cells. Cancerous tumors that have undergone angiogenesis are characterized by an almost complete lack of ICAM-I.

IL-10 levels typically rise with age, suppressing immunity against tumor cells. Avemar has been reported to reduce levels of IL-10 and help rebalance lymphocyte the subclasses Th1 and Th2. The inhibition of humoral immune response by Avemar may be beneficial in the treatment of autoimmune diseases, while the enhancement of the cellular immune response helps in destroying malignant tumors. Inflammation caused by the inflammatory cyclooxygenase enzymes (COX-1 and COX-2) also plays a major role in developing autoimmune diseases, and is believed to be a risk factor for developing cancer. Avemar has been reported to reduce production of COX-1 and COX-2 in human colorectal cell lines [27]. The anti-inflammatory effects of Avemar

have been recently reported to be beneficial as adjunctive therapy for rheumatoid arthritis [28].

Bitter Melon (*Momordica charantia*)

Bitter melon (*M. charantia*) is a green, bitter vegetable from the gourd family, grown in tropical and subtropical regions throughout the Amazon, East Africa, Asia, the Caribbean, and South America. The slender, climbing vine produces a warty, oblong gourd resembling a small cucumber. Bitter melon is known by many names which include wild cucumber, bitter gourd, balsam apple, balsam pear, ampalaya (Philippines), and Kerala (India). Clinical conditions for which bitter melon extracts (primarily from the fruit) are currently being used include diabetes, dyslipidemia, microbial infections, and potentially as a cytotoxic agent for certain types of cancer.

Laboratory studies have found that bitter melon extracts may enhance insulin secretion by the islets of Langerhans, reduces glycogenesis in liver tissue, enhances peripheral glucose utilization, and increases serum protein levels [29, 30]. In an animal study, alcohol-extracted charantin from bitter melon consists of mixed steroids and was found to be more potent than the oral hypoglycemic agent tolbutamide [31]. Bitter melon also contains an insulin-like polypeptide, polypeptide-P, similar in structure to bovine insulin. It was found to decrease blood sugar levels when injected subcutaneously into type 1 diabetic patients [32]. Bitter melon extracts may restore the altered histological architecture of the islets of Langerhans. Also, laboratory studies support the cholesterol-lowering properties of bitter melon, attributed, in part, to decreasing lipid peroxidation [33]. Bitter melon supplements used for type 2 diabetes should be a proprietary standardized to contain 10% charantins (Glukokine™, Vinco Inc., Evans City, Pa., USA).

α-Lipoic Acid

α-Lipoic acid, also known as lipoic acid (LA) or thioctic acid, and its reduced form, dihydrolipoic acid, are powerful antioxidants. LA scavenges hydroxyl radicals, hypochlorous acid, peroxynitrite, and singlet oxygen. Dihydrolipoic acid also scavenges superoxide and peroxyl radicals and can regenerate thioredoxin, vitamin C, and glutathione, which in turn can recycle vitamin E. There are several possible sources of oxidative stress in diabetes including glycation reactions, decompartmentalization of transition metals, and a shift in the reduced-oxygen status of the diabetic cells. Diabetics have increased levels of lipid hydroperoxides, DNA adducts, and protein carbonyls. Available data strongly suggest that LA, because of its antioxidant properties, is particularly suited to the prevention and/or treatment of diabetic complications that arise from an overproduction of reactive oxygen and nitrogen species, such as neuropathy [34, 35]. In addition to its antioxidant properties, LA increases

glucose uptake through recruitment of the glucose transporter-4 to plasma membranes, a mechanism that is shared with insulin-stimulated glucose uptake. Further, recent trials have demonstrated that LA improves glucose utilization and insulin sensitivity in patients with type 2 diabetes [36, 37]. Lastly, α-lipoic acid has a nephroprotective effect reducing the oxidative burden on glutathione stores of the kidneys.

Dehydroepiandrosterone

The body naturally produces the hormone dehydroepiandrosterone (DHEA) in the adrenal glands. The body uses DHEA to produce a number of other hormones, including the male and female hormones testosterone and estrogen. The levels of DHEA in the body peak in the 20s and then slowly begin falling as an individual ages.

DHEA has been reported to prevent oxidative stress in several in vivo and in vitro models [38]. Research indicates that DHEA treatment helps balance the HPA axis, ameliorates the oxidative imbalance induced by hyperglycemia, downregulates the TNF-α/TNF-α receptor system, and prevents advanced glycation end product formation, suggesting a beneficial effect on the onset and/or progression of chronic complications in type 2 diabetic patients [39].

Dietary Modifications

Alterations in neuroendocrine-immune balance and movement toward intracellular fermentative metabolism create intracellular acidity and heightened risk for other chronic disease, including cancer. Dietary changes can reduce the progression to these shifts and are imperative in controlling inflammatory signaling and its complex milieu of comorbid symptoms, conditions, and pathologies.

The focus of treating a patient with type 2 diabetes, obesity, cancer, or any other condition should be on the impact of multiple nutritional imbalances (both excess and deficiency) in the individual. Various nutrients specific for an individual's biochemistry may help to modulate factors associated with disease. Using dietary changes and nutritional supplements to help bring homeostasis back to the individual's biochemical makeup is of upmost importance. Dietary factors have effects on inflammatory signaling, independent of smoking, hypercholesterolemia, and hypertension.

The first priority for an individual with insulin resistance is to control carbohydrate intake whether they have progressed to type 2 diabetes or not. Food choices must perform a more comprehensive job in controlling insulin and glucose levels so the spiral to metabolic chaos does not occur. Studies report that a diet low in carbohydrates improves the metabolic profile of an individual when compared with a corresponding high glycemic index diet [40].

Diets low in glycemic index/load that use whole grain products versus refined flours have been associated with decreased concentrations of inflammatory markers and increased adiponectin levels among diabetic patients. These associations appear to be independent of body weight, glycemic control, and other cardiovascular risk factors. The protective effects of low glycemic load and whole grains on systemic inflammation may be explained, in part, by reduction in hyperglycemia-induced oxidative stress and by amelioration in insulin resistance, adiposity, dyslipidemia, and hypertension. There are a number of studies that suggest that a lower carbohydrate, along with a higher fat and protein diet, is performing better not only for weight loss, but for waist-to-hip ratio and lipid profiles, triglycerides, insulin, and glucose levels [41].

Chronic consumption of high glycemic index foods, such as white bread and refined sugars, may also lead to chronically high oxidative stress and release of stress hormones (such as cortisol), which initiates the inflammatory signaling pathways. The consumption of high glycemic index foods results in higher and more rapid increases in blood glucose levels than the consumption of low glycemic index foods. Rapid increases in blood glucose are potent signals to the β-cells of the pancreas to increase insulin secretion. Over the next few hours after eating high glycemic foods, the high insulin levels induced by consumption of high glycemic index foods may lead to hypoglycemia. On the other hand, the consumption of low glycemic index foods results in lower but more sustained increases in blood glucose and lower insulin demands on pancreatic β-cells. The release of stress hormones, such as epinephrine and cortisol, are produced with dramatic increases and decreases in blood sugar levels. Repeated insults with high glycemic meals can lead to chronic upregulation of inflammatory signaling.

The dietary sources of high glycemic load foods – refined grains, fruits, and starchy vegetables, such as potatoes and corn – must be decreased until the insulin and glucose are regulated. If individuals have normal fasting glucose and insulin, they should have postprandial glucose and insulin levels checked to see how high the levels rise in response to 60–70 gram carbohydrate load, especially if the normal glucose is borderline high, and tryglycerides are elevated. Usually this means switching to a higher protein diet – consuming more lean red meats, fish, chicken, turkey, and bison. Diets consisting of 20% calories from carbohydrate have been reported to control insulin response rates and reduce diabetic complications.

In a study of 190 healthy men and women with an average age of 48 years, it was found that high intakes of various B vitamins – riboflavin, pantothenic acid, and biotin – actually increased micronucleus frequency in lymphocytes, a standard measure of genome damage [42]. The study also found that increasing one's calcium intake further enhanced the genome-protective effect of a high-folate diet whereas a high riboflavin intake further exacerbated genome damage associated with a low-folate diet. This is consistent with epidemiologic studies showing that cancer rates tend to be higher among populations that consume more red meats, which are very high in riboflavin, more alcohol (which depletes folate), and fewer vegetables (a rich

source of folate) [43]. Processed meats have health risks of their own, including increasing the risk of type 2 diabetes and colorectal cancer, but seem to be much more dangerous to health when consumed along with low amounts of vegetables [44]. Because lean, red meat is an important contributor to nutrition, it is important to eat plenty of fresh vegetables to balance the negative effects of consuming red meats high in riboflavin. Flavonoids found in vegetables also help bind excess iron in the body found with dietary meat consumption – a high intake of iron is a powerful trigger for reactive oxygen species (ROS) and cancer induction and growth [45]. Flavonoids found in vegetables also help bind excess iron in the body found with dietary meat consumption – a high intake of iron is a powerful trigger for ROS and cancer induction and growth.

Diets with a high glycemic response may also increase the ability of *Candida* to grow, thereby increasing the likelihood of developing dysbiosis and the resulting inflammatory cascade. Reducing foods high in sugars and starch limits a primary source of fuel for the gastrointestinal yeast, *Candida albicans*, which is known to release toxins, such as oxylipins, that damage intestinal cells and compromise the integrity of intestinal linings.

Gut flora imbalance and the subsequent compromised integrity of protective mucosal linings is the primary reason dietary lectins can become a problem. Of the food lectins, grain/cereal lectins (especially wheat), dairy lectins, and legume lectins (especially peanut lectin and soybean lectin) are the most common ones associated with reports of aggravation of inflammatory signaling in the gut and in the body. In individuals who are displaying any of the signs and symptoms of gut flora imbalance as discussed above, it is critical to eliminate intake of refined sugars and starches while also reducing one's intake of other dietary sugars and starches, and can be very helpful to limit one's consumption of high dietary lectin foods.

Another important but not well-appreciated dietary change has been the substantial increase in fructose intake (in the form high fructose corn syrup), which appears to be an important causative factor in the metabolic syndrome. Soft drink consumption is, for most people, the largest source of dietary fructose. Fructose is a powerful reducing sugar, and can react with proteins through the Maillard reaction (glycation), which may account for several complications of diabetes mellitus and accelerating aging. Fructose may contribute to development of insulin resistance, weight gain, accelerated LDL oxidation, triglyceride elevation, hypertension, protein glycation.

A study in the *American Journal of Clinical Nutrition* [46] investigated the relationship between a dietary pattern associated with biomarkers of inflammation and the incidence of type 2 diabetes. The nested case-control study was performed of 656 cases of type 2 diabetes and 694 controls among women in the Nurses' Health Study and two prospective cohort studies of 35,340 women in the Nurses' Health Study and 89,311 women in the Nurses' Health Study II, who were followed for incident type 2 diabetes. The dietary pattern that was strongly related to inflammatory markers and an increased risk of type 2 diabetes was high in fructose-sweetened soft drinks,

refined grains, diet soft drinks, and processed meat and low in red wine, coffee, cruciferous vegetables, and yellow vegetables.

Trans-fatty acids (TFAs) increase the risk of heart disease and diabetes. High consumption of *trans*-fat has been associated with high oxidative stress in humans, which could increase the risk of the development or acceleration of diabetes and cancer. TFAs are found in small amounts in various animal products such as beef, pork, lamb and the butterfat in butter and milk; however, these foods are not likely to be found to be problematic since the naturally occurring *trans*-fat in them is conjugated linoleic acid, which has health benefits. The problematic TFAs are those formed during the process of hydrogenation in making margarine, shortening, and some cooking oils. Any of these or the foods made from them is a major source of TFA in the diet. Partially hydrogenated vegetable oils provide about three-fourths of the TFAs in the US diet. The *trans*-fat content of foods is printed on the package of the nutrition facts label. It is important to keep *trans*-fat intake to less than 1% of the total daily calories, or eliminate them all together.

A significant body of epidemiologic evidence associates certain dietary patterns with an increased risk of cancer [47]. In addition to eating for maximum regulation of glycemic response and for gut health, and eliminating other inflammation-promoting foods like high fructose corn syrup and *trans*-fats, it is prudent for someone with existing diabetes to follow the dietary patterns associated with decreased cancer risk. A diet rich in fat and meat, and low in fiber, fruits, and vegetables, is associated with an increased risk of colorectal, mammary, and other common forms of cancer. Links between high glycemic index and glycemic load to the development of various cancers have been reported. Obesity, nutrient sparse foods such as concentrated sugars and refined flour products that contribute to impaired glucose metabolism (which leads to diabetes), low fiber intake, consumption of red meat, and imbalances of ω–3 and ω–6 fats all contribute to excess cancer risk. Intake of EFA and abundant portions of fruits and vegetables may help lower cancer risk. Antioxidant and anticancer phytochemicals found in cruciferous vegetables (such as broccoli, cabbage, and cauliflower), provide protection against certain cancers including breast, colon, and bladder cancers. These phytochemicals include β-carotene, sulforaphane, and indole-3-carbinol.

Studies have also found that nitrite or nitrate-treated meats such as bacon, sausage, smoked foods, and lunch meats increase the risk of cancer [48]. Studies have also found that increased levels of vitamin C in the diet may decrease the risk of developing gastric cancer due to the harmful effects of nitrites/nitrates in the diet [49]. Animal proteins that are cooked to the point of burning may also cause cancer. Studies have also reported that foods that are grilled or fried at high enough temperatures to blacken and char the meat have increased amounts of heterocyclic amines – known carcinogens [50]. Many of the problems in studies that found links between the consumption of saturated fats from meats and the incidence of cancer may be related to chemical contaminants, such as growth hormones, nitrates/nitrites, and heterocyclic amines. The EPIC study showed that intake of vegetables and fruit negated cancer effects from nitrates.

Growth hormones used in animal husbandry may also be implicated in cancer. In many countries including the USA, growth hormones are used in dairy cows and cattle raised for beef and milk consumption. The changes in hormones that occur in the animals may also disrupt hormonal balance in humans who eat the meat or drink the milk. Estimates in the USA show that up to 80% of beef is raised using growth hormones. Many of these hormones are estrogenic, and while some experts believe the slight increases in estrogens that would be consumed when eating the meat do not cause problems, others disagree.

A European committee was appointed to study whether eating hormone-treated meats increases risk of cancer and they concluded that they are a cancer risk. The European Union banned imports of US-raised beef because of this problem. Some experts believe that eating growth hormone-treated beef and milk may be largely responsible for the earlier sexual maturation being seen in many US children, and may be related to increases in hormonal-sensitive cancers, such as breast and prostate cancer. This seems to corroborate with the studies that link increased dairy consumption to increased prostate cancer risk. These issues may never be conclusively resolved, but since they are a potential problem, it may be well advised to err on the side of caution and eliminate growth hormone-treated meats and cow's milk dairy products from one's diet.

And finally, the quality and quantity of certain beverages should be considered in dietary intake. As noted above, high fructose containing soft drinks and other beverages like fruit juices/drinks, and sports drinks should be avoided. In addition, one should be moderate if alcohol is consumed as excess alcohol consumption leads to a variety of health problems, including increasing the risks of developing type 2 diabetes and cancer. Drinking green tea may also be beneficial for health. The quality of water should also be considered since many tap waters may contain cancer-promoting chemicals such as perchlorate (jet fuel) or toxic heavy metals such as lead.

Conclusion

Controlling the diverse molecular metabolic shifts seen with the upregulation of inflammatory signaling compounds is necessary in the prevention and management of type 2 diabetes. Preventing the spiral of metabolic disruption that leads to immune signaling alteration and cellular shifts in fermentative metabolism (the Warburg effect) can help decrease the conditions that are favorable for cancer development and the ability of the cancer to flourish.

Type 2 diabetes and cancer can be greatly affected by the overarching processes of obesity, inflammation, neuroendocrine-immune balance, poor nutrient status, and oxidative and psychological stress. Nutrigenomics represents a major effort to improve our understanding of the role of nutrition and genomic interactions in these areas.

Good nutrition status and dietary selections should be an essential component to an overall lifestyle, which also includes regular exercise, not smoking or drinking alcohol in excess, limiting environmental exposures and stress management. Keeping blood sugar levels balanced through aggressive management can help slow the onset and progression of type 2 diabetes and blood sugar regulatory complications. Imbalances in blood sugar homeostasis, causing and caused by obesity, poor diet, inflammation, unhealthy bowel terrain including *Candida* overgrowth, chronic stress, toxicity and nutrient depletion can lead to chronic health conditions including type 2 diabetes and cancer. Proper nutrition and repletion of nutrients using a healthy diet rich in nutrients and antioxidants and dietary supplements that support important molecular deficiencies can have a significant impact on slowing the progression of type 2 diabetes as well as the progression of the cellular shift toward the Warburg effect in cancer biology. It is important to understand that the patient is in a dynamic metabolic process that is moving them either to complications of diabetes and progression to heart disease, cancer and other pathologies, or through intervention a redirection to a more robust and resilient metabolism. By understanding all the vectors of metabolic influence that affects the individual, we can more thoroughly comprehend all the strategies needed to intervene on there behalf.

Nutrition in the 21st century is poised to be an exciting and highly relevant field of research, as each new day is accompanied by advances in our understanding of how the interactions between lifestyle and genotype contribute to health and disease, taking us one step closer to achieving the highly desirable goal of personalized nutrition.

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Diabetes and Cancer: The Road Ahead

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Abstract

Otto Warburg recognized that cancer cells generate excessive lactate in the presence of oxygen (aerobic glycolysis). There is a renewed interest in the fundamental role of the switch from oxidative phosphorylation to glycolysis of cancer cells. It is a key hallmark of many rapidly proliferating cancers that they metabolize glucose and other fuels at an elevated rate concomitantly with an increase of lactate production and decrease of mitochondrial respiration. Several signaling pathways, including PI_3K/Akt/mTOR, HIF-1 α and Myc, contribute to this reprogramming of glucose and energy metabolism. Cancer and diabetes mellitus type 2 have common epidemiological molecular denominators, e.g. an aberrant growth glucose utilization, increased ROS formation, augmented growth factor stimulation of PI_3K/Akt/mTOR signaling, and mitochondrial dysfunction, which may foster survival of cancer cells and fatal comorbidities of diabetes, such as neuro- and nephropathies or blindness. According to the rising number of afflicted people by both diseases, physicians have to be aware of the interdependency of these two diseases of Western lifestyle and they should educate their patients to better control the daily nutritional use of glucose and other hyperglycemia-inducing foodstuff.

General Energetic and Molecular Consideration

In many cancer cells, the chemical energy (ATP) necessary for survival and proliferation is derived from glycolysis rather than from oxidative phosphorylation, even when oxygen supply would be adequate to sustain them. Increased conversion of glucose to lactic acid associated with decreased mitochondrial respiration is a unique feature of many tumors and was first described in the 1920s by the Nobel Prize laureate Otto Warburg.

The mystery behind the molecular mechanisms of the phenomenon, known as the Warburg effect, is now being unraveled. The reduced mitochondrial respiration rate

and the increased glucose uptake associated with lactic acid production, and acidosis of the microenvironment, may be due to the activation of cellular mediators of signal transduction and gene expression, including the phosphatidylinositol-3-kinase (PI_3K)/Akt/mTOR (mammalian target of rapamycin) system, hypoxia-inducible factor 1 (HIF-1), and Myc.

Unregulated cellular proliferation leads to formation of cellular masses that extend beyond the resting vasculature, resulting in an increased consumption and requirement of oxygen and the extracellular fuels glucose, amino acids and fatty acids in order to produce ATP (chemical energy) and macromolecules that are necessary to support biosynthesis of cellular components.

The PI_3K/Akt/mTOR pathway, HIF-1α, and Myc participate in the various facets of this metabolic phenotype. The binding of a growth factor to its surface receptor brings about activation of PI_3K and the serine/threonine kinases Akt and mTOR. Constitutive activation of the pathway can occur in tumors due to mutations of inhibitory regulators of these pathways, such as the tumor suppressors PTEN, TSC1, and TSC2. Metabolic effects of the PI_3K/Akt/mTOR pathway include enhanced uptake of glucose and essential amino acids and protein translation. The transcription factor HIF-1α is involved in determining the manner in which cells utilize glucose carbon atoms. Translation of HIF-1α is enhanced during growth factor stimulation of the PI_3K/Akt/mTOR pathway. In the presence of oxygen, HIF-1α is modified by prolyl hydroxylases, which target it to an ubiquitin ligase complex that includes the tumor suppressor von Hippel-Lindau (VHL) protein. This association results in a constitutive normoxic degradation of the HIF-1α protein.

Hypoxia, mutation of VHL, or accumulation of reactive oxygen species (ROS) impair HIF-1α degradation, allowing it to enter the nucleus and engage in transcriptional activity. Transcriptional targets include genes encoding glucose transporter 1 (GLUT-1), lactate dehydrogenase-A (LDH)-A, and pyruvate dehydrogenase kinase 1 (PDK1). The combined effect on glucose metabolism is to increase both glucose utilization and lactate production. The transcription factor myc increases expression of many metabolic enzymes, including glycolytic enzymes, LDH-A, and several enzymes required for nucleotide biosynthesis. All in all, these processes may induce a reprogramming of glucose, glycolysis, and lactate production by a reciprocal decrease in mitochondrial respiration. These findings delineate for the first time the molecular mechanisms underlying the Warburg effect in a human cancer (for review, see DeBerardinis et al. [1]). In particular, it has been shown by Semenza [2] that HIF-1α mediates the Warburg effect in clear cell renal carcinoma.

Furthermore, the resulting hypoxia triggers a number of critical adaptations that enable cancer cell survival, e.g. activation of genes integral to angiogenesis, apoptosis suppression and cellular locomotion induction, processes intimately involved with metastatic tumor spread.

Reactive Oxygen Species: More for Tumor Cell Initiation, Less for Metastasis Formation?

ROS and RNS are closely linked to degenerative diseases such as Alzheimer's disease, Parkinson, neuronal death including ischemic and hemorrhagic stroke, acute and chronic degenerative cardiac myocyte death, diabetes mellitus type 2 and cancer. As a by-product of oxidative phosphorylation (mitochondrial respiration), a steady stream of reactive species emerges from our cellular energy plant, the mitochondria. ROS and RNS potentially cause damage to all cellular components. Structure alterations, biomolecule fragmentation, and oxidation of side chains are trade-offs of the cellular energy production. ROS and RNS production results in the activation of cytosolic stress pathways, DNA damage, and the upregulation of JNK, p38, and p53. Incomplete scavenging of ROS and RNS, e.g. by the enzymes superoxide dismutase and catalase particularly, affects the release of mitochondria cytochrome c with subsequent activation of caspase 9 and 3 and ultimately induces the intrinsic death pathway.

However, if normal protective cell growth mechanisms are defective due to (i) increased and uncontrolled ROS and RNS production, e.g. caused by increased glucose consumption, and (ii) the resulting glycolytic state does not lead to an elimination of affected cells (apoptosis), then ROS and RNS may cause mutagenesis of nuclear proto-oncogenes (initiation phase of carcinogenesis) and drive nuclear replication (promotion phase of carcinogenesis), resulting in the presence of a tumor promoter in a cancer cell.

It is widely accepted that increased levels of ROS contribute to carcinogenesis. However, this claim has been scarcely confirmed by experiments and may be only plausible for the initiation of a normal cell, which become a cancer cell during the process of dedifferentiation. In contrast, it has been clearly demonstrated that ROS are normal cellular signals and defense mechanisms that induce cell differentiation and apoptosis, the opposite process to cancer [3].

Otto Warburg recognized that cancer cells (not normal cells!) generate excessive lactate in the presence of oxygen (aerobic glycolysis) which is a return to the more glycolytic metabolism of embryonic tissue. In this context, Gillies and Gatenby [4] describe a more profound teleological understanding of the need for altered metabolism of invasive cancer cells. They used mathematical models and empirical observations to define the adaptive advantage of aerobic glycolysis during the somatic evolution of invasive cancer and explain its remarkable prevalence in human cancers. Increased consumption of glucose in metastatic lesions is not used for substantial energy production via Embden-Meyerhoff glycolysis, but rather for production of lactic acid, which gives the cancer cells a competitive advantage for invasion by causing an unfavorable environment for normal cells; furthermore, glucose is used for generation of reducing equivalents (NADPH) or anabolic precursors (ribose).

The Need to Understand Glucose Metabolism in Normal and Aberrant Tissues for Diagnostic Considerations

A key hallmark of many cancers, at least the most aggressive ones, is the capacity to metabolize glucose at an elevated rate – a phenotype detected clinically using positron emission tomography (PET). This phenotype provides cancer cells that are invasive/or participate in metastasis formation a distinct competitive edge over normal cells. Adaptation to hypoxia and acidosis of tumor cells causes an upregulation of glucose transporter 1 and 3 (GLUT-1 and GLUT-3), which gives the cells an advantage of higher per-cell glucose consumption and higher per-cell lactate production compared to non-cancerous finite lifespan cells of the microenvironment. Concomitantly, this feature allows a more accurate diagnostic discrimination of non-cancerous and cancerous tissue using the highly sophisticated PET technique with the appropriate glucose markers.

Gatenby et al. [5] and Smallbone et al. [6] successfully established models which nicely demonstrate that adaptation of initiated cancer cells to hypoxia and acidosis represents key events in transition from in situ to invasive cancer. The models suggest three phases of somatic evolution from self-limited premalignant growth to invasive growth. The evolutionary modeling of cancer cell metabolism calls for novel strategies directed towards novel diagnostic tools and towards interrupting the hypoxia-glycolysis-acidosis cycle and thus delaying or preventing metastatic spread.

In good accordance with this modeling is the observation by Yeh et al. [7] that the glycolytic pathway and glycolysis-related genes my play an important role in the tumorigenesis/progression of disease of human colorectal cancers.

The metabolomics of cancer cells with respect to glucose utilization are represented by the same key enzymes, such as hexokinase II [8], and increased expression of a variety of enzymes involved in glycolysis, tricarboxylic acid (TCA) cycle, and oxidative phosphorylation, as shown for human breast cancer cells forming brain metastases [9]. Most interestingly, although these cells do not only increase the enzymes for conversion of glucose to lactic acid, but also activate enzymes involved in the Embden-Meyerhoff pathway, these cells concomitantly show enhanced activation of the pentose phosphate pathway (PPP) and the glutathione system, which can minimize production of ROS from enhanced oxidation metabolism without losing the capacity to produce enough macromolecules and chemical energy for rapid self-renewal. Within this context it can be hypothesized – as put forward in part by Lu [10] – that cancer cells developing an invasive phenotype show decreased levels of ROS.

The bioenergetic phenotype of two human cancer cell lines, H460 and A549, was investigated and both lines showed increased glycolysis and reduced mitochondrial respiration [11]. The swift from the Embden-Meyerhoff pathway to glycolysis and lactic acid production is mandatory for a decreased ROS production. Glycolytic breakdown of glucose is preceded by the transport of glucose across the cell membrane, a rate-limiting process mediated by glucose transporter proteins belonging to the facilitative glucose transporter/solute carrier GLUT/SLC2A family [also see A. Schürmann, this

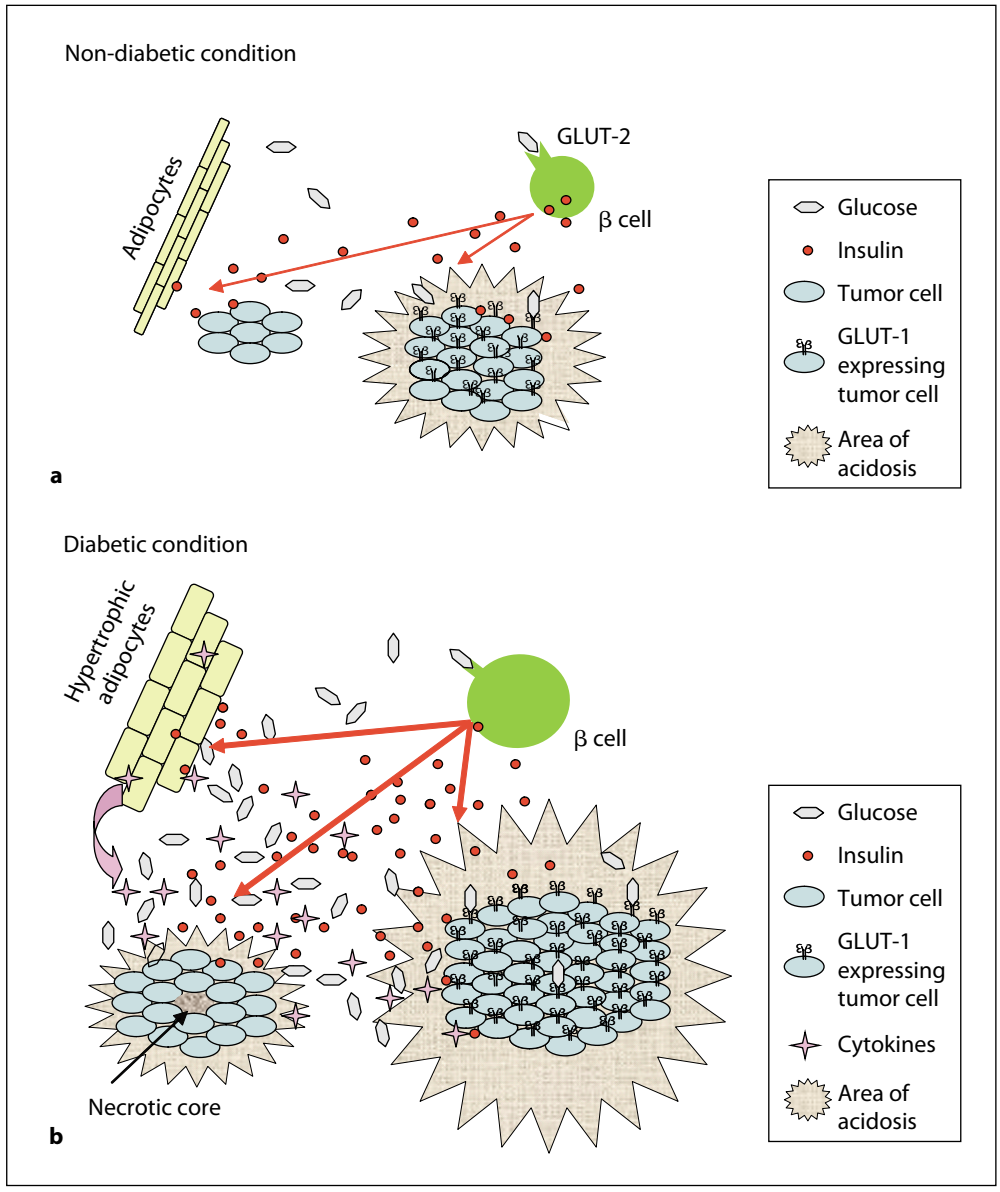

Fig. 1. **a**, **b** Consequences of alterations in carbohydrate metabolism at non-diabetic versus diabetic conditions in malignant cells – a network between tissues. The scheme compares the glucose metabolism of tumor cells growing under either non-diabetic or diabetic conditions – comparing in addition the influence of the expression of GLUT-1. In general there is the observation that malignant cells show an increased flux of glucose, which will be further stimulated by insulin. Additional stimuli are the cytokines, secreted from the adipose tissue. Adipocytes growing under hyperglycemic/diabetic conditions display an increased proliferation – and over time will become hypertrophic. These adipocytes will secrete large amounts of various cytokines, which will lead to an increased proliferation of tumor cells, in addition to the insulin stimulus. Under non-diabetic conditions a tumor will proliferate during times shortly after the uptake of nutrition, when blood glucose as well as insulin levels are increased. Under non-diabetic conditions this elevated level of glucose and insulin will appear several times per day for approximately 2 h. Therefore the stimulus for the proliferation of

volume, pp. 71–83]. Tumors frequently overexpress GLUTs, especially the hypoxia-responsive GLUT-1 and GLUT-3 proteins [12]. GLUT-1 antibodies alone or in combination with chemotherapy induce growth arrest and apoptosis in human cell lines [13].

On the road to discovering cancer's Achilles heel, the emergence of the metabolic switch from oxidative phosphorylation to glycolysis is not only of therapeutic interest but also of diagnostic importance. PET imaging with ^{18}F-fluorodeoxyglucose (FDG), which reflects tumor glucose metabolism, provides relevant information regarding treatment response and assessment of novel drug distribution [14]. FDG uptake of a tumor correlates to the histopathological findings and the variable appearance of tracer uptake on the PET scan depends on distribution of different tissue components with different utilization of glucose in the tumor, thus reflecting intratumoral heterogeneity [15].

Cancer cells possess a unique phenotype which is characterized by high glucose uptake, increased glycolytic activity and lactic acid production, decreased mitochondrial activity, low bioenergetic expenditure and increased phospholipid turnover. These features, when properly addressed, are a challenge to induce metabolic catastrophe as therapeutic approach to kill the ineradicable cells.

Undoubtedly there are existing molecular links and common denominators between cancer and diabetes mellitus type 2. Some are obvious. It appears evident that the abundance of glucose in extracellular fluids associated with diabetes mellitus type 2 will support cell proliferation and represent a selective advantage for growth of cancer cells. Moreover, during the course of diabetes mellitus type 2 relatively high concentrations of insulin will be found – either because of insulin resistance or due to iatrogenic application of insulin for the control of blood sugar – which will trigger PI_3K/Akt/mTOR signaling [16, 17] and promote the metabolic reprogramming that is characteristic of proliferating cancer cells (see figs. 1, 2).

Yet, novel therapeutics of type 2 diabetes mellitus addresses the secretion of insulin via the incretin hormone glucagon-like peptide-1 (GLP-1) [see B. Gallwitz, this volume, pp. 30–43]. GLP-1 will be secreted into the bloodstream from intestinal L-cells stimulated by the uptake of nutrition. However, GLP-1 displays an extremely short biological half-life of less than 5 min, due to degradation by the dipeptidyl peptidase IV (DPPIV). Therefore, DPPIV-stable GLP-1 analogues as well as inhibitors of DPPIV have been developed within antidiabetic strategies. But especially the DPPIV inhibitors, because of their possible adverse side effects, are coming increasingly into the focus of molecular and clinical research. DPPIV exhibit a broad spectrum of substrates, not only directed to the incretin hormones (GLP-1, GIP) but also to peptides (e.g. SDF-1, RANTES, eotaxin)

GLUT-1-negative tumors will be reserved only for postprandial periods. A GLUT-1-bearing tumor will proliferate mainly all over time. Because of the high affinity for glucose of the GLUT-1 transporter, those malignant cells will have a permanent supply of energy. Even under diabetic conditions the expression of GLUT-1 will be an advantage, since GLUT-1-expressing cancer cells will be able to proliferate longer than GLUT-1-negative tumors. Thereby the diffusion of glucose is the limiting factor for tumor growth. Therefore, GLUT-1-expressing tumors are able to proliferate even under hypoglycemic conditions, while GLUT-1-negative cells will face necrosis under the same conditions.

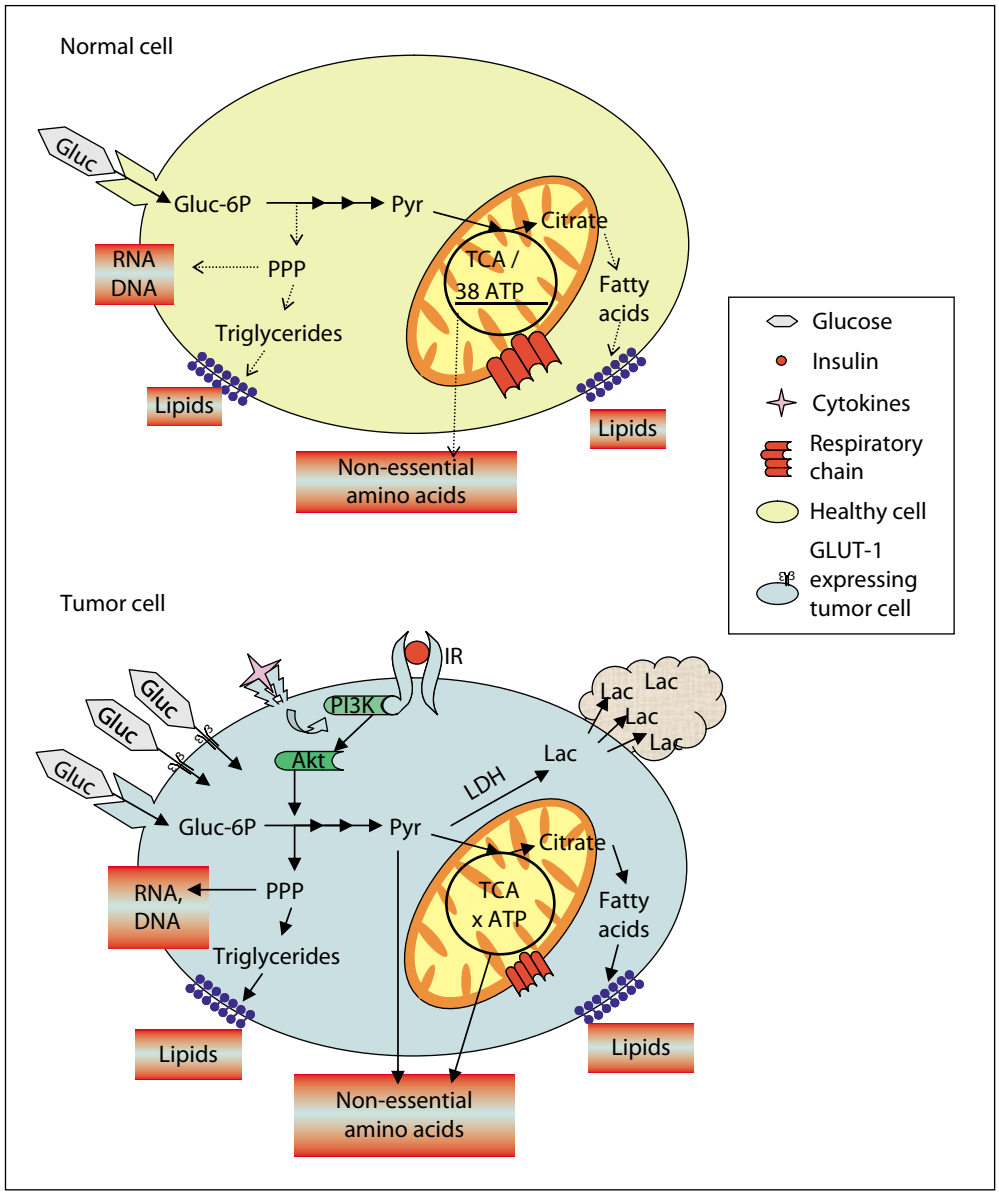

Fig. 2. Normal cells versus high-proliferating tumor cells concerning the utilization of glucose. The high flux of glucose in malignant cells can be explained by the effect of aerobe glycolysis – known as the 'Warburg effect'. At first sight, the energy yield of aerobe glycolysis in tumor cells seems to be ineffective when compared to non-proliferating healthy cells. The cells of normal tissues will use glucose mainly for ATP production with a maximal (theoretical) yield of 38 mol ATP/mol glucose. But besides the production of ATP, the intermediate products of glycolysis are the main components for many biosynthetic processes to support proliferation. Especially the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway (PPP) will maintain proliferation by the generation of amino acids, lipids as well as nucleotides. Some of the malignant cells acquired various (additional) mutations responsible for an increase in glucose uptake (GLUT-1) or glycolysis (LDH), resulting in dramatically elevated levels of glucose conversion to pyruvate and finally lactate. This surplus of lactate will be secreted into the surrounding tissue and thereby significantly decreasing the pH, making the direct neighborhood

with immunologically functions [18]. All these substrates display a very short half-life due to DPPIV-mediated cleavage, the result of which is the deactivation of these signal molecules. Moreover, there are no known naturally occurring inhibitors of DPPIV, implying the importance of a rapid turn-off of those signals. This broad spectrum of DPPIV substrates suggests a certain likelihood that inhibition of DPPIV also affects other biological systems and organ functions like the immune system.

Most interestingly, Boonacker and van Noorden [18] reviewed in 2003 that the level of soluble CD26 equals serum DPPIV activity in respect to various physiological and pathophysiological processes (e.g. aging, colon cancer, diabetes, hypertension and immunosuppression). Within the above conditions, serum CD26 levels are already downregulated. This is an indication that low DPPIV activity is associated with different diseases. Therefore, a further inhibition of DPPIV activity seems to be doubtful or even contraindicative when e.g. diabetes is associated with cancer or immunological disorders. In addition, a prolonged GLP-1 activity induced by the treatment with DPPIV inhibitors for the treatment of diabetes will result in increased insulin secretion, a hormone which promotes the metabolic reprogramming of proliferation and enhanced glucose utilization of cancer cells.

Increased ROS formation has also been found associated with diabetes mellitus type 2 [19], which may contribute to its pathogenesis [20, 21] and/or to progression of the disease: 'glucolipotoxicity of diabetes mellitus type 2' [22].

Another hallmark of type 2 diabetes mellitus is the formation of advanced glycation end products (AGEs), which are the result of a chain of chemical reactions after an initial glycation reaction. The receptor for AGEs (RAGE) is a multifunctional receptor with multiple ligands that is known to play a key role in several diseases, including diabetes, arthritis, and Alzheimer's disease. Recent evidence indicates that this receptor also has an important role in cancer. RAGE ligands, which include the S100/calgranulins and high-mobility group box 1 (HMGB1) ligands, are expressed and secreted by

of the tumor inheritable for normal cells without an increased H+ transporter activity (e.g. N+/H+ exchanger), so that healthy tissue will be eliminated by acidosis. Because GLUT-1's high affinity for glucose a GLUT-1-positive tumor will be able to grow for longer periods, since the supply with the main source of energy and biosynthetic processes will not diminish. The worst case scenario will be a tumor growing under diabetic conditions – comparable to the metabolic syndrome. Glucose concentrations have been elevated on a 24-hour basis over a long period of time. As a consequence of the increased blood glucose levels, pancreatic β cells permanently secrete insulin. Caused by this plethora of glucose and insulin, the adipose tissue stored the energy in the form of fat, thereby an increase in visceral fat accumulation occurs. Mainly these visceral adipocytes will secret high amounts of cytokines – further stimulating tumor growth and additionally promoting a low systemic inflammation. Therefore, cytokines together with insulin as well as other growth hormones activate tumor cells via the 'growth hormone receptor', followed by PI_3K activating the Akt pathway. Especially Akt promotes the increased glucose consumption and therefore supports aerobic glycolysis. Thus, even a GLUT-1-negative tumor will grow under diabetic conditions more extensively compared to non-diabetic circumstances. At very late stages such tumors will develop areas of necrosis in the center of the cluster. Because of the high affinity of GLUT-1 these tumors will have a further advantage since glucose diffusion rates may be high enough for a tumor growth without a necrotic core.

cancer cells and are associated with increased metastasis and poorer outcomes in a wide variety of tumors. These ligands can interact in an autocrine manner to directly activate cancer cells and stimulate proliferation, invasion, chemoresistance, and metastasis [23].

As described above, ROS impair HIF-1α degradation [24, 25], allowing it to transactivate an array of genes that increase both glucose utilization and lactate production. In addition, lack-of-function mutations and polymorphisms in genes that regulate mitochondrial oxidative phosphorylation have also been linked to development of diabetes mellitus type 2 [26]. ROS may also trigger mitochondrial dysfunction resulting in decreased oxidative phosphorylation [27]. Hence the metabolic condition encountered in diabetes mellitus type 2 associated with mitochondrial dysfunction could provide an adequate basis to favor the metabolic reprogramming that is characteristic of the Warburg effect. Therefore, comorbidity of cancer and diabetes mellitus type 2 may produce additive or even potentiating effects on the molecular processes underlying both diseases as well as their pathology.

There are lines of evidence suggesting that cancer is a largely preventable disease, as is diabetes mellitus type 2. Patterns of cancer and diabetes mellitus type 2 are altered by environmental factors that show a genetic predisposition but are not necessarily genetically determined. The pattern of food and drinks, of physical activity, and of body composition has changed remarkably throughout human history. With urbanization and industrialization, food supplies usually become more secure, and more food is available for consumption. In general, diets become more energy-rich, as they contain fewer starchy foods but more fats and oils, sugars and additives. Populations become increasingly sedentary, their drain of energy from food drops, and the incidence of overweight and obesity increase, which are major risk factors for cancer and diabetes mellitus type 2. The spectrum of liver diseases in diabetes mellitus type 2 ranges from non-alcoholic fatty liver disease to cirrhosis and hepatocellular carcinoma (HCC). The incidence of HCC is increasing in the Western world, but the temporal changes of risk factors remain unclear. A significant proportion of HCC develops in cryptogenic cirrhosis, and may represent the most worrisome complication of non-alcoholic steatohepatitis. Non-alcoholic steatohepatitis is tightly related to insulin resistance and several features of the metabolic syndrome. HCC is the result of a multifactorial process in which insulin resistance seems to play a major role in the initial accumulation of fat in the liver, whereas multiple causes of mitochondria dysfunction and oxidative stress can induce the secondary occurrence of necroinflammatory lesions and fibrosis [28]. A case of lipid-rich clear cell HCC arising in non-alcoholic steatohepatitis in a patient with diabetes mellitus type 2 was described immunohistochemically and ultrastructurally [29]. Tumor cells had lipid droplets, glycogen, swollen mitochondria, rough endoplasmatic reticulum, Mallory bodies, small bile canaliculi, desmosomes and gap junction. Surrounding non-tumoral hepatocytes had a largely normal ultrastructure with prominent glycogen and lipid droplets. Similar histological features to the patients of human non-alcoholic steatohepatitis were found in hepatocyte-specific tumor suppressor gene PTEN-deficient mice [29]. These hepatocytes showed

enhanced lipid accumulation, inflammatory change, and hyperoxidation; moreover, they developed into HCC. Thus, impairment of the PI_3K/PTEN signaling may possibly be involved in a part of non-alcoholic steatohepatitis/HCC cases in human.

What You Can Take to Ma'!

Every year, 10.7 million new patients are diagnosed for cancer worldwide; 6.7 million people die because of the disease. It has been estimated that during the last 5 years, 24.6 million patients were alive with cancer burden. It is also roughly estimated that 250 million people are afflicted by diabetes mellitus type 2. As outlined in this book, a fatal glucose metabolism is a unique feature of both diseases.

Every physician treating cancer patients and patients suffering from diabetes mellitus type 2 should be aware of common mechanisms underlying the observed epidemiological and molecular links between diabetes and cancer. Above all, nutrient limitation of glucose, monitoring the glucose status and diagnosis of glycolytic metabolism of cancer suspicious tissue, e.g. by the most advanced PET technique, should be within the focus of physicians for their patients to prevent or delay the onset of both diseases.

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Note Added in Proofs

After receiving the galley proofs, an article by Ishikawa et al. was published in *Science* [2008; 320:661–664], which reports ROS-generated mutations of the mitochondrial genome that possibly might contribute to a metastatic phenotype of cancer cells. This is somewhat in contrast to what was hypothesized by F. Lu [10, p. 137], whereby cancer cells with an invasive phenotype might show decreased levels of ROS.

Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, Nakada K, Honma Y, Hayashi J: ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 2008;320:661–664.

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