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REVIEW

Pathophysiology, Biochemistry, and Molecular Landscape of Insulin Resistance in Type 2 Diabetes

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Abstract—The pathophysiology of type 2 diabetes (T2D) remains poorly understood, largely because multiple early changes are obscure as they evolve during prolonged period of prediabetes. These changes are interconnected, involve feedback loops, and gradually develop in tissue-specific manner, ultimately leading to manifestation as overt diabetes. Insulin resistance (IR) and pancreatic β-cell dysfunction are regarded as central events driven by lipotoxicity and glucotoxicity. Understanding molecular mechanisms of their causes and consequences is essential for developing effective preventive and therapeutic strategies for T2D. This review describes the evolution of current perspectives on T2D pathophysiology, examines the mechanistic roles of lipotoxicity and glucotoxicity, and integrates current concepts on the molecular basis of IR. The hypotheses on the early events in prediabetes and potential role of IR in their progression toward overt T2D are discussed. A deeper understanding of T2D as a metabolic disease of biochemical origin may provide new insights into T2D prevention and major associated mortality risks, including cardiovascular complications and cancer.

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SECTION 1. INTRODUCTION: THE WALK OF LIFE

Diabetes is a major socially significant non-communicable disease with epidemic-level prevalence [1, 2]. Type 2 diabetes (T2D) accounts for 80-95% of all diabetes cases across various populations and exceeds 92% in the Russian Federation. The mortality rate in T2D is driven by comorbidities, such as cardiovascular diseases (~50%) and, to a lesser extent, cancer (~10%) [2]. As a metabolic disorder, T2D highlights the central role of metabolic dysregulation in the development of the most prevalent chronic diseases.

The pathophysiology of T2D, defined as a sequence of metabolic alterations occurring during its development, includes a prolonged period of prediabetes that ultimately progresses to the overt disease (Fig. 1). In its advanced stages, T2D is characterized by hyperglycemia and impaired insulin secretion, thus resembling type 1 diabetes (T1D). However, the critical metabolic disturbances arise much earlier, during prediabetes. In most cases, they are marked by dyslipidemia, including obesity, elevated triacylglycerol (TAG) levels in adipose tissue and the blood lipoproteins, increased plasma levels of free fatty acids (FFA), and ectopic lipid deposition in peripheral tissues. These changes are considered major predisposing factors, whereas insulin resistance (IR) is regarded as the principal predictor

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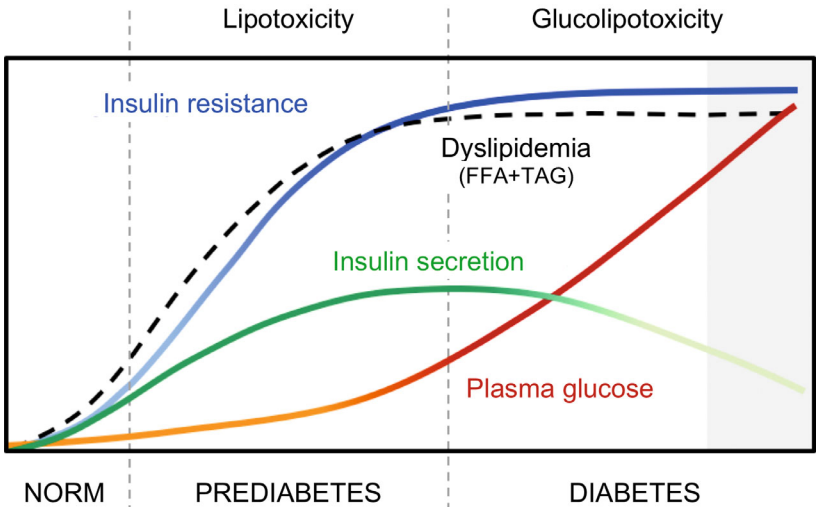

Fig. 1. Trajectory of metabolic alterations and IR during the pathophysiological progression of T2D. IR emerges and progresses in parallel with early hyperinsulinemia, preceding the onset of hyperglycemia. Dashed line indicates the approximate trajectory of total lipid burden (FFA and TAG) in tissues and plasma; shaded area on the right denotes the overt T2D stage.

and central pathophysiological feature of T2D [3-5]. IR emerges at the earliest stages of prediabetes, progressively worsens, and persists throughout advanced T2D. Although the temporal dynamics of metabolic parameters does not establish causality, it clearly defines two pathophysiological states: lipotoxicity, driven by excess lipid exposure, and glucolipotoxicity, reflecting a contribution of hyperglycemia (Fig. 1). Together, these trajectories support the notion that hyperglycemia is critical for the final β-cell defects that culminate in overt T2D.

IR is defined as a reduced responsiveness of insulin-dependent tissues to insulin. As IR develops, insulin progressively loses its ability to stimulate glucose uptake in skeletal muscle, suppress hepatic glucose production, and, as generally assumed, inhibit lipolysis in adipose tissue. Pancreatic β-cells are thought to compensate for the diminished insulin efficacy by increasing insulin secretion, resulting in compensatory hyperinsulinemia. With further progression of prediabetes, IR and associated metabolic disturbances lead to β-cell dysfunction, impaired insulin secretion, hyperglycemia, and, ultimately, manifestation of overt T2D (Fig. 1). However, the mechanistic interplay and temporal sequence of these events remain incompletely understood. Long-standing questions regarding which event arises first and what drives its emergence, continue to be debated. Answering these questions may help identify effective strategies for disease prevention and therapy.

A major challenge in understanding IR is its heterogeneous onset across different organs. According to the accepted paradigm, IR initially develops in skeletal muscle [3, 4], but the following sequence of events remains unclear. Clinically, hepatic steatosis accompanied by pronounced hepatic IR (non-alcoholic fatty liver disease, NAFLD) often precedes the onset of T2D and is thought to result from IR in adipose tissue, increased lipolysis, and elevated flux of FFA to the liver. Current concepts state that the excess lipid availability is the primary driver of IR in muscle cells, with FFA acting as the main inducers of IR [4]. In rodents, impaired carbohydrate metabolism in adipose tissue induces IR in both muscle and liver [6], suggesting a potential primary role for adipose IR. However, the underlying causes remain unclear, as lipid-induced IR pathways described in myocytes and hepatocytes are less applicable to adipocytes, which are inherently adapted for lipid storage and turnover. Chronic low-grade inflammation has been implicated as a major contributor to adipose IR [7, 8]; however, in rodents, high fat diet induces adipose IR before overt inflammation becomes evident [4]. In humans, the situation is even less clear due to the limitations of mechanistic interventions and reliance on observational metabolic data only. Interpretation is further complicated by substantial inter-individual variability, requiring large cohorts for robust conclusions. As a result, a key unresolved question remains: is IR a primary defect, or does it represent an adaptive cellular response to nutrient excess and hyperinsulinemia [9, 10]? Without a clear answer, clinical decisions are challenging, particularly regarding whether IR should be directly targeted therapeutically and whether insulin therapy is appropriate in T2D management [11, 12].

This review summarizes the historical milestones in understanding T2D pathophysiology, key biochemical and molecular alterations during disease progression, and the roles of lipo- and glucotoxicity

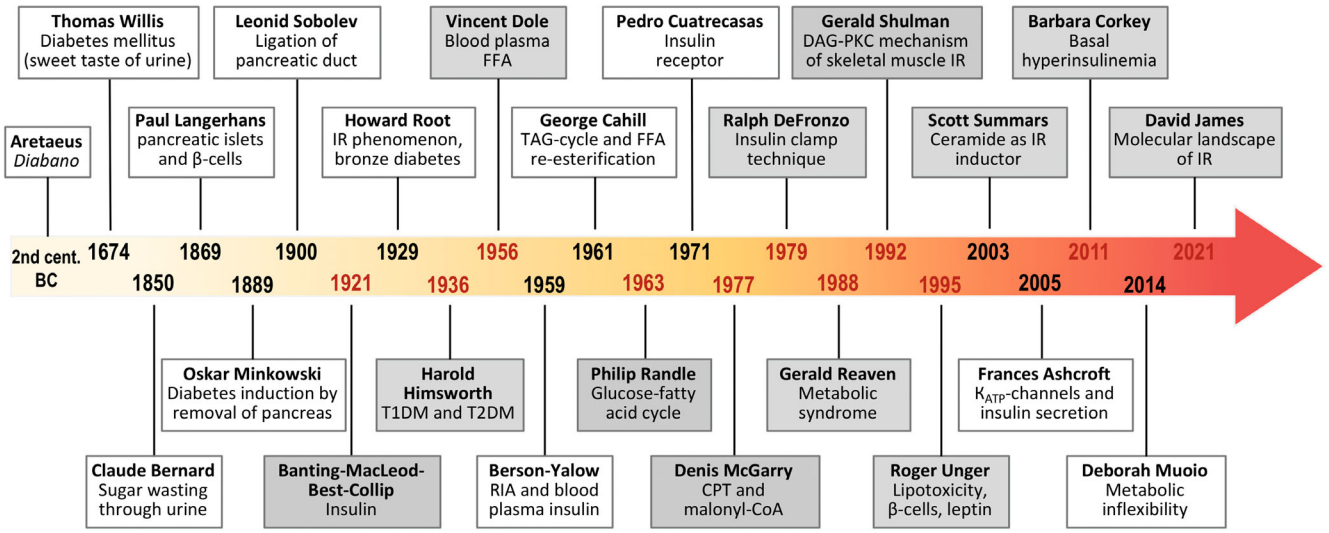

Fig. 2. The timeline of key discoveries in understanding T2D as a metabolic disease. Events highlighted in grey denote the major milestones also detailed in the text. Abbreviations not included in the general list: K_{ATP} channels, ATP-sensitive K^+ channels; RIA, insulin radioimmunoassay.

in prediabetes. It also examines the evidence on plasma FFA levels as markers of prediabetes, outlines cellular mechanisms of IR development in skeletal muscle, and discusses potential primary drivers and plausible scenarios of metabolic events leading to T2D development.

SECTION 2. NATURAL HISTORY OF TYPE 2 DIABETES: SHOW MUST GO ON

Disease history describes disease progression in an individual over time, whereas the *natural history* refers to its course in the absence of treatment. Unravelling the natural history of a disease provides insight into intrinsic mechanisms of its pathogenesis and helps identify the most relevant therapeutic targets. The multifactorial etiology of diabetes may explain its heterogeneity, including T1D, T2D, maturity-onset diabetes of the young (MODY), gestational diabetes, and other less common forms. In T2D, a prolonged latent period of prediabetes precedes the clinical onset of overt disease. The natural history of T2D remains poorly characterized due to systemic metabolic alterations that obscure individual contributing factors, their interactions, and the sequence of pathological events. The molecular mechanisms underlying the two hallmark defects, IR and β-cell dysfunction, are even less well defined. These defects characterize, respectively, the early prediabetes and the transition to overt diabetes and, therefore, have a primary focus in diabetes research for decades. Yet observational description of disease progression alone is insufficient to trace the development of these defects and to evaluate their contribution to the overall disease pathophysiology. Consequently, prevailing hypotheses must be validated experimentally in cellular and animal models, with careful extrapolation of findings to the human disease. The major milestones in the development and validation of these concepts are summarized in Fig. 2. They represent only a small fraction of the extensive literature; below, we address only the most essential aspects relevant to understanding IR mechanisms.

By the end of the 19th century, Oskar Minkowski and Joseph von Mering had already established that the pancreas produces a factor regulating carbohydrate metabolism. This factor was later named insulin, following the discoveries of Paul Langerhans, who identified the pancreatic islets, and Leonid Sobolev, who found that their function persists after pancreatic duct ligation. Building on these insights, Frederick Banting developed a procedure for insulin isolation. When working in the laboratory of John Macleod in Toronto, Banting and Charles Best extracted insulin, administered it to a pancreatectomized dog, and saved the animal [13]. In 1922, the first insulin injection was administered to a human patient, 14-year-old Leonard Thompson with diabetes. However, this extract was poorly purified and caused an allergic reaction. Macleod then recruited James Collip, who optimized the purification procedure, and the second injection was successful. The 1923 Nobel Prize in Physiology or Medicine followed immediately. It seemed that diabetes had been cured.

However, it soon became evident that insulin was not universally effective. In 1936, H. Himsworth experimentally demonstrated that diabetes manifests in two different forms: one associated with impaired insulin secretion and another characterized by reduced

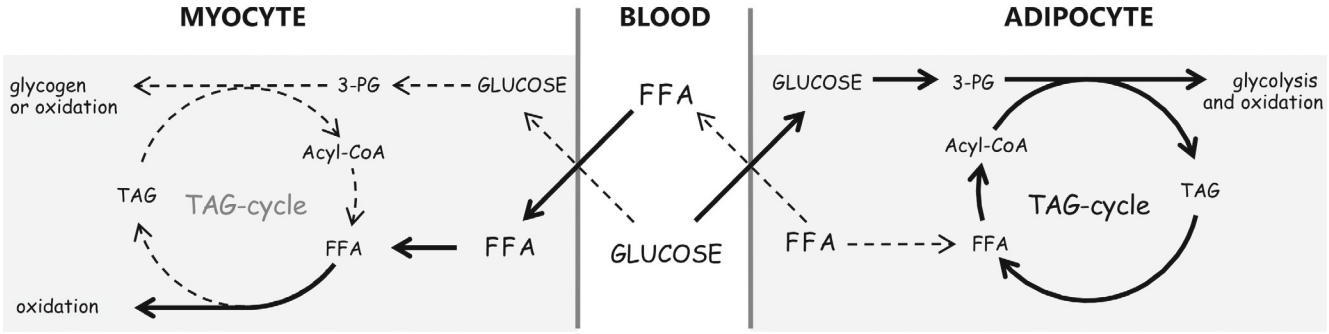

Fig. 3. Original hypothesis of the glucose–fatty acid cycle by P. Randle [22]. Intracellular lipid handling is illustrated through the TAG cycle, which relies on glucose to support FFA (re)esterification [18, 20]. When circulating FFA levels are high, such as during fasting, skeletal muscle predominantly utilizes FFA for oxidation, thereby reducing glucose uptake and metabolism (left). This increases glucose influx into adipose tissue and promotes FFA re-esterification into TAG in adipocytes (right). As plasma FFA levels decline, FFA flux into adipose tissue decreases, FFA oxidation in muscle diminishes, and glucose transport and metabolism are restored. Under the action of insulin (e.g., postprandially), the fluxes are reversed: FFA are directed primarily into adipocytes, whereas glucose uptake is enhanced in myocytes. 3-PG, glycerol 3-phosphate.

tissue responsiveness to insulin [14]. In his studies, patients received intravenous glucose along with insulin, followed by blood sampling. In one group, glucose levels fell and returned to baseline, whereas in the other, insulin had little effect and glucose levels continued to rise. In 1959, Solomon Berson and Rosalyn Yalow developed the radioimmunoassay for plasma insulin, providing definitive evidence for the existence of insulin-dependent (type 1) and insulin-independent (type 2) diabetes [15]. Since then, the concept of diminished biological response to insulin has been firmly established and now recognized as insulin resistance (IR).

It has long been known that individuals with T2D exhibit elevated plasma TAG levels and are frequently obese. In the early 1950s, V. Dole developed a quantitative assay for plasma FFA and showed that FFA levels vary inversely with glucose, rising during fasting and falling after glucose ingestion, and are elevated in obese individuals [16]. These findings indicated that adipose tissue is not merely a passive energy reservoir but plays an active metabolic role by temporarily storing energy as TAG and supplying other tissues with FFA via lipolysis. G. Reaven consistently emphasized the strong association between IR and lipid metabolism abnormalities, particularly elevated plasma TAG and FFA. Although initially met with skepticism, his idea that dysregulated lipid metabolism is central to T2D pathogenesis has been eventually accepted [17]. It was Reaven who coined the term “syndrome X” [5], now known as “metabolic syndrome”, which is widely recognized as a major risk factor for both T2D and cardiovascular disease. He believed IR to be the defining feature of metabolic syndrome, while the combination of IR and β-cell dysfunction marks the transition from prediabetes to T2D.

Studies using fragments of adipose tissue have revealed the TAG cycle in adipocytes, a continuous assembly-disassembly process in which a fraction of FFA is consistently re-esterified into TAG, even during intensive lipolysis [18, 19]. This cycle requires energy [19] and glucose to generate glycerol 3-phosphate, the glycerol backbone for TAG synthesis [20]. Current evidence confirms that glucose is the principal substrate in adipocytes [21], and that its uptake and utilization suppress lipolysis and FFA release.

Distinct oxidative pathways for glucose and fatty acids have been recognized since the early 20th century, both converging at their common end-product acetyl coenzyme A (CoA). The tricarboxylic acid (TCA) cycle was described in 1937 by Hans Krebs and Albert Szent-Györgyi. It was reasonable to hypothesize that the finite capacity of the TCA cycle limits the simultaneous maximal oxidation of both substrates. When both glucose and FFA are abundant, such as after a meal, they may compete for oxidative metabolism. This concept was formalized in 1963 by P. Randle [22], who integrated systemic glucose and FFA fluxes into the unified glucose-fatty acid cycle (Fig. 3).

The Randle hypothesis was transformative, offering a novel perspective on the T2D etiology. Randle proposed that disturbances in carbohydrate metabolism may arise as a consequence of altered lipid metabolism. This idea seemed counterintuitive, as the clinical paradigm of diabetes had been almost entirely glucose-centric and biochemists knew that lipids are synthesized from carbohydrates, not the reverse. The Randle’s seminal paper introduced this interplay as a “biochemical syndrome” in which “interactions between glucose and fatty-acid metabolism in muscle and adipose tissue take the form of a cycle (the glucose fatty-acid cycle), and are fundamental to the control of glucose and fatty acid concentrations in the

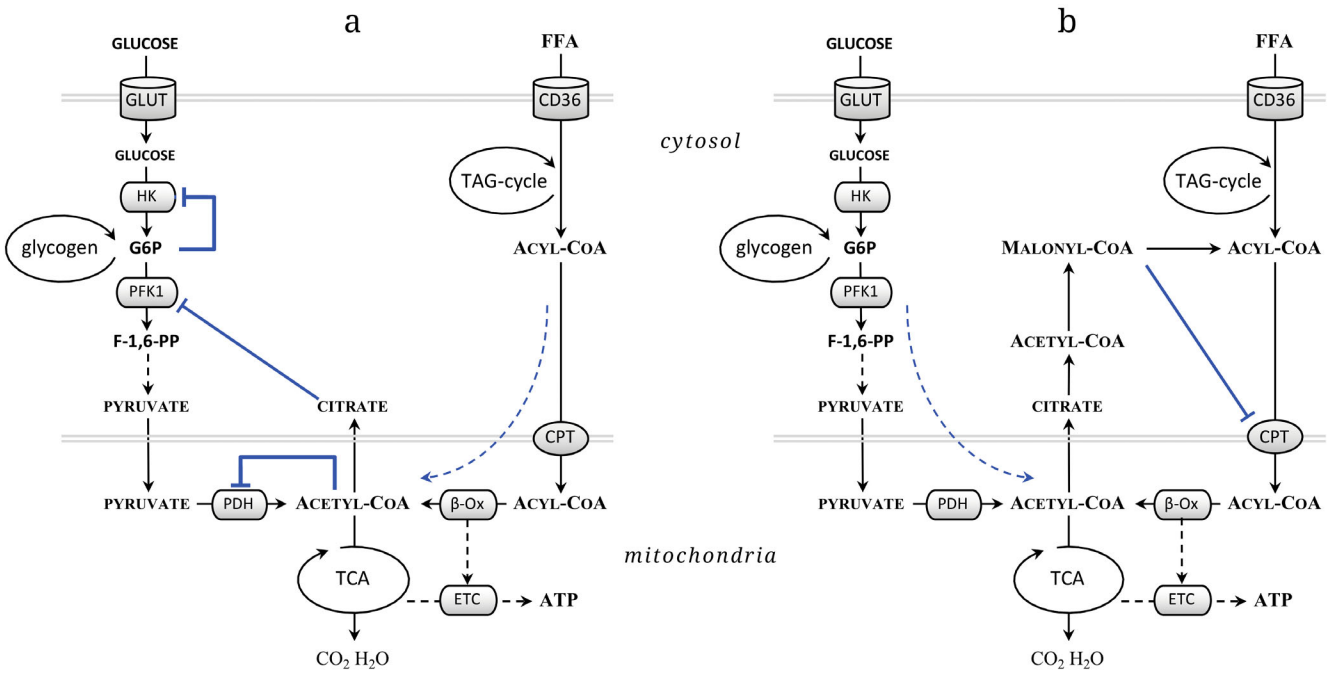

Fig. 4. Biochemical mechanisms underlying substrate competition between (a) FFA and glucose [22, 25] and (b) between glucose and FFA [26] as cellular energy substrates [26]. a) Excessive FFA availability drives mitochondrial accumulation of acetyl-CoA, which inhibits pyruvate dehydrogenase (PDH) within the pyruvate dehydrogenase complex. Acetyl-CoA is also exported to the cytosol via the citrate shuttle, where citrate inhibits phosphofructokinase 1 (PFK-1). This decreases the glycolytic flux and leads to glucose 6-phosphate (G6P) accumulation. Elevated G6P inhibits hexokinase (HK), reducing the transmembrane glucose gradient and limiting glucose uptake. b) Excess glucose availability enhances glycolytic flux and pyruvate oxidation, resulting in the mitochondrial acetyl-CoA accumulation. Cytosolic export of acetyl-CoA via the citrate shuttle fuels malonyl-CoA synthesis and *de novo* lipogenesis. Malonyl-CoA inhibits carnitine palmitoyltransferase (CPT) in the carnitine shuttle, suppressing the import of fatty acid into mitochondria and β-oxidation (β-Ox). Blue dashed arrows denote dominant fluxes; blue solid lines represent inhibitory interactions that maintain metabolic reciprocity; black dashed lines represent multistep glycolysis and electron transfer from β-oxidation and the TCA cycle via the electron transport chain (ETC).

blood, and of insulin sensitivity" [22]. This groundbreaking work has been cited more than 4000 times and still remains influential [23, 24].

The Randle hypothesis is founded on several key propositions: (1) glucose and fatty acid oxidation are interconnected yet independent; (2) catabolism of fatty acid, whether derived from circulating FFA or from intracellular TAG, suppresses glucose catabolism in muscle; (3) fatty acid oxidation suppresses insulin-stimulated glucose uptake in muscle; (4) hormonal regulation modulates this balance by influencing lipolysis, glucose transport, or TAG synthesis [22, 25]. Figure 3 illustrates the first three principles, showing that the increased availability of FFA from the bloodstream shifts muscle metabolism from glucose utilization toward fatty acid oxidation to support ATP generation. In adipocytes, higher glucose availability promotes re-esterification of fatty acids into TAG, thereby limiting FFA release into the circulation. Postprandial insulin reverses the metabolic fluxes by enhancing glucose transport into muscle and channeling FFA into adipocytes. Importantly, insulin does not suppress lipolysis in muscle cells, thereby promoting a shift in lipid balance toward the storage in adipocytes. In conditions such as obesity and diabetes, the glucose-fatty acid cycle becomes dysregulated due to altered availability of FFA and glucose.

Randle also proposed a biochemical mechanism to explain the substrate competition between FFA and glucose (Fig. 4a). This mechanism is particularly relevant to skeletal muscle, the major site of postprandial glucose disposal. Randle hypothesized that FFA suppress glucose oxidation by inhibiting pyruvate dehydrogenase (accounting for 40-60% of the effect) and phosphofructokinase 1, contributing additional 20-30%. Inhibition of the latter was predicted to cause an accumulation of glucose 6-phosphate, which inhibits hexokinase, thus increasing intracellular glucose concentrations, reducing the transmembrane gradient of glucose, and ultimately decreasing glucose uptake.

The reciprocal mechanism by which glucose suppresses fatty acid utilization had been unknown until 1977, when D. McGarry identified malonyl-CoA,

the first intermediate of the *de novo* synthesis of fatty acid from glucose, as an inhibitor of the carnitine shuttle that transports fatty acids into mitochondria [26] (Fig. 4b). McGarry proposed that excessive glucose, typical of individuals with IR and hyperglycemia, traps fatty acids in the cytosol, where they accumulate and become lipotoxic [27]. These findings laid the foundations for the concepts of lipo- and glucotoxicity (see Section 3) and their involvement in IR, impaired metabolic flexibility, and T2D pathogenesis. It was found later that plasma FFA levels change little in obesity, prompting the emergence of the concept of ectopic fat accumulation, which referred to inappropriate TAG deposition in non-adipose tissues (see Section 4). It also became evident that FFA can affect cells not only from the outside, but also internally, via metabolism.

A major methodological advance came with the development of clamp techniques by R. DeFronzo [28][1], which enabled quantitative and minimally invasive assessment of IR in humans and were instantly adopted. In the 1990s, G. Shulman applied these techniques to test the Randle hypothesis in humans (see Section 5). Individuals with T2D exhibited decreased insulin-stimulated glucose uptake in skeletal muscle, contributing to reduced whole-body glucose disposal [29]. This phenotype was reproduced in healthy subjects through lipid-heparin infusion, suggesting a causal role of FFA. Subsequent studies in skeletal muscle identified mechanisms involving accumulation of diacylglycerol (DAG), activation of Ca^{2+}-independent novel protein kinase C (nPKC) isoforms, and disruption of insulin signaling [4, 30].

At the turn of the century, an alternative perspective emerged from the studies on β-cells by R. Unger [10, 31, 32] and on muscle and adipose cells by S. Summers [33]. By that time, it had become clear that saturated FFA, such as palmitate, are more lipotoxic than unsaturated FFA, such as oleate. Unlike oleate, palmitate is inefficiently incorporated into TAG and is instead diverted into ceramide synthesis, which induces IR and β-cell apoptosis [34]. Inhibitors of key enzymes of ceramide biosynthesis (serine palmitoyl transferase and ceramide synthase) suppressed IR in myocytes and adipocytes, and protected β-cells from apoptosis [35]. R. Unger studied Zucker rats, which have impaired leptin function and spontaneously develop obesity. He viewed these animals as a natural model of lipotoxicity and demonstrated that lipid overload initially stimulated β-cell proliferation and insulin secretion, but eventually led to the lipotoxic failure of β-cells [10]. Unger proposed that hyperinsulinemia as a primary event, while IR is a secondary adaptive response of peripheral tissues to avoid hypoglycemia, a concept that has been developed further in subsequent studies [12, 36, 37]. Mechanisms driving increased basal insulin secretion may involve FFA-induced generation of reactive oxygen species and altered β-cell redox status. Within this framework, primary hyperinsulinemia and IR are viewed as adaptive responses aimed to normalize circulating lipid and glucose levels under nutrient excess [9]. Further progression of adipose IR was thought to impair suppression of lipolysis while preserving lipogenic activity of insulin, thereby exacerbating obesity and lipotoxicity [10, 11]. In β-cells, increased lipid burden promotes ceramide accumulation, oxidative stress, and impaired insulin secretion, leading to hyperglycemia, glucolipotoxicity, and overt T2D [38].

Finally, the concept of impaired metabolic flexibility has emerged [39] that to a certain extent integrated prior hypotheses at both cellular and whole-body levels [40] by applying the same principles of reciprocal substrate and metabolite competition originally proposed by Randle and McGarry (Fig. 4). The dysregulation of these mechanisms, compounded by altered amino acid metabolism, leads to the mitochondrial overload and loss of ability to switch between glucose and fatty acids as cellular fuels [39]. Under these conditions, it is plausible that cells adapt to chronic FFA oversupply by downregulating insulin-stimulated glucose uptake as a protective response. Alternatively, this may represent an adaptation to chronic hyperinsulinemia. Recent studies suggest that the insulin metabolic signaling can be selectively attenuated, while its mitogenic signaling remains relatively preserved [41, 42] (see Section 6).

[1] *Hyperglycemic clamp*. Plasma glucose is rapidly raised by intravenous glucose infusion and maintained at a target level through continuous feedback-controlled adjustment of glucose infusion rate. Once the steady state is achieved, the glucose infusion rate reflects the whole-body glucose disposal and metabolism at intact endogenous insulin secretion. This clamp variant is used less frequently because it reflects the combined effects of IR and endogenous insulin release.

Hyperinsulinemic-euglycemic clamp. Plasma insulin is acutely elevated by insulin infusion and then maintained at ~100 μU/ml (~0.6 nM). Plasma glucose is monitored continuously, and the glucose infusion rate is adjusted in a feedback-controlled manner to maintain euglycemia at the individual's basal level. Under these conditions, the glucose infusion rate equals the whole-body glucose disposal and therefore provides a quantitative measure of sensitivity to exogenous insulin. This approach is more commonly used because it isolates IR without the confounding influence of endogenous insulin secretion. Combined use of both clamp procedures allows assessment of β-cell secretory dysfunction independently of IR.

Excessive FFA availability increases cellular demand for fatty acid disposal via lipid synthesis (phospholipids, sphingolipids, and ceramides), mitochondrial β-oxidation for ATP generation, or storage as TAG in lipid droplets. Overloading these pathways may induce endoplasmic reticulum (ER) stress, oxidative stress, or lipotoxicity, respectively, depending on the susceptibility of specific cell type. For example, when ATP consumption is low and ATP tends to accumulate, the FFA flux shifts toward lipid storage and synthesis, inducing formation of DAG and ceramides. The failure to adequately metabolize FFA may trigger lipotoxicity once the buffering capacity of FFA-binding proteins and/or pool of free CoA are exhausted. Depending on the cell type, impaired metabolic flexibility may reflect not only the IR itself, but also ER stress, mitochondrial overload, reduced electron transport chain activity, or increased oxidative stress [43, 44].

Despite significant progress, the mechanisms underlying metabolic dysfunction in IR and T2D remain poorly understood. As a result, a unified theory that conclusively delineates the sequence of pathogenic events in prediabetes or identifies definitive therapeutic targets has yet to emerge (see Section 7). Although disturbed lipid metabolism is widely recognized as a central contributor, understanding its complex consequences continues to be a major challenge.

SECTION 3. LIPOTOXICITY AND GLUCOTOXICITY: DOES THE TAIL WAG THE DOG?

The term *lipotoxicity* was introduced by R. Unger [31] in relation to the impaired insulin secretion by β-cells. The concept has been later expanded to other tissues, highlighting its broader systemic impact [45], including impaired leptin signaling [32]. Currently, lipotoxicity is understood as a series of interrelated events triggered by dysregulated lipid metabolism and excessive FFA action.

Dietary lipids enter the lymphatic system as chylomicrons and subsequently reach the systemic circulation through the superior vena cava. Lipoprotein lipase (LPL) located on the surface of vascular endothelial cells, hydrolyzes chylomicron TAG to release FFA. FFA cross the endothelial barrier with the assistance of albumin, which transiently binds them to mitigate acute cytotoxicity of unbound FFA [46]. High LPL activity markedly increases local FFA levels, leading to FFA "spillover," which may account for up to one third of circulating FFA [47]. Together with chylomicron remnants, these FFA eventually reach the liver, where they are re-esterified into TAG and packaged into very-low-density lipoproteins (VLDLs). In the postprandial state, the liver also receives FFA mobilized from adipose tissue and incorporates them into VLDL TAG. As VLDLs circulate, they gradually release FFA to peripheral tissues and are converted into intermediate- and low-density lipoproteins. In obesity, lipodystrophy, and conditions involving limited storage capacity or inflammation in adipose tissue, the plasma levels of circulating lipoproteins increase, reflecting increased lipid burden, enhanced lipid flux to peripheral tissues, and increased risk of FFA excess and lipotoxicity. Chronic expose to elevated FFA promotes ectopic lipid deposition in non-adipose organs. In the liver, this manifests as NAFDL, while in skeletal muscle, ectopic lipids serve as endogenous sources of FFA, which blunt insulin sensitivity, reduce glucose uptake [4, 27, 30], and suppress glucose catabolism [22, 25].

Lipotoxicity is defined as deleterious effects of FFA on cells. Circulating amphipathic FFA are inherently cytotoxic and are rapidly sequestered by serum albumin. Each albumin molecule can bind up to seven FFA, including three with high affinity (binding constants in the micromolar range) [48]. Although plasma FFA concentrations vary considerably, they rarely exceed 2 mM (see Section 4), while normal albumin levels (40-50 g/L) correspond to ~0.7 mM. Thus, under physiological conditions, albumin is in excess and effectively buffers FFA. However, albumin also transports other ligands, and its levels decline with aging and inflammation. Furthermore, glycation reduces albumin's binding capacity [49] and promotes expression of inflammatory cytokines in muscle cells [50]. Therefore, elevated levels of glycated albumin in hyperglycemia may increase susceptibility to lipotoxicity and could serve as a potential biomarker for T2D risk [51, 52].

Modeling lipotoxicity requires careful consideration because of the risk of direct FFA toxicity. Under such conditions, the membrane compartment, including ion channels, transporters, receptors, and their proximal targets, is expected to be particularly vulnerable. *In vitro*, FFA are typically delivered to cells in a complex with albumin, whereas *in vivo*, FFA levels are increased by infusing an emulsion of lipids (TAG) together with heparin to activate LPL. In both settings, the FFA-to-albumin ratio is critical [53]. Once it exceeds a threshold of ~3 mol FFA per mol of albumin, the concentration of unbound FFA approaches the solubility limit (10-20 nM), markedly increasing the risk of direct lipotoxicity. Although *in vivo* this ratio rarely exceeds 2 mol FFA per mol albumin, it may rise substantially in severe obesity, aging, hyperglycemia, or due to extensive albumin glycation.

Metabolic lipotoxicity arises from the intracellular accumulation of excessive FFA and typically occurs when one of the four major FFA-processing systems becomes limited: (1) the buffering capacity

of fatty acid-binding proteins (FABPs) that sequester intracellular FFA [54]; (2) the availability of free coenzyme A required for FFA activation, retention, and subsequent metabolic processing or carnitine [55]; (3) the capacity to synthesize and expand lipid droplets for extra TAG storage [56]; (4) mitochondrial β-oxidation capacity [39]. The availability of CoA is limited by its cytosolic concentration (~0.1-0.15 mM) [55]. The efficiency of lipid droplets in sequestering FFA into TAG depends on the cell type. For instance, skeletal muscle cells express low levels of fatty acid synthase and generate lipid droplets poorly, except in endurance-trained athletes, whose myocytes accumulate substantial TAG and droplets, a phenomenon known as the "athlete's paradox" [57]. Thus, mitochondrial oxidation serves as the primary route of FFA disposal in muscle cells. By contrast, adipocytes possess highly developed lipogenic machinery and active TAG cycling. White adipocytes oxidize FFA poorly due to low expression of carnitine palmitoyltransferase (CPT-I) [58], whereas beige and brown adipocytes express CPT-I and actively oxidize FFA to maintain lipid balance and support thermogenesis [59]. The term "toxicity" can be misleading, as it does not differentiate between acute physical damage and chronic metabolic reprogramming accompanied by adaptive cell responses. In essence, these metabolic shifts are reversible at both cellular and whole-body levels. A similar rationale applies to glucotoxicity, which refers to detrimental effects of sustained hyperglycemia [60].

The concept of glucotoxicity also originated in relation to β-cells [31]. Because the vascular endothelium in the pancreas is fenestrated, even modest elevations in plasma glucose rapidly stimulate insulin secretion. This insulin promotes glucose disposal primarily by skeletal muscles, which accounts for ~80% of glucose clearance from the circulation. Chronic hyperglycemia *in vivo* or prolonged exposure of isolated islets to glucose *in vitro* impair insulin secretion by β-cells, providing the basis for the glucotoxicity concept [60, 61]. Experimental diabetes induced by partial pancreatectomy in rats primarily resulted from impaired insulin secretion [62]. Treatment with phlorizin (an inhibitor of renal glucose reabsorption and a prototype of modern SGLT2 inhibitors) normalized blood glucose without affecting other blood parameters in these animals. These findings indicate that glycemic control is essential not only for reducing vascular risks, but also for reversing glucotoxic effects of hyperglycemia on β-cells and abating IR in skeletal muscle and liver [60].

Lipotoxicity is likely to precede glucotoxicity in T2D. However, this does not exclude the possibility that hyperglycemia can exacerbate the lipotoxic effects. As noted above, β-cells are particularly vulnerable to glucolipotoxicity [38]. Glycation reduces albumin's affinity for FFA [48], thereby increasing the risk of direct lipotoxicity. Because vascular endothelium in the liver and pancreas is fenestrated, hepatocytes and β-cells are directly exposed to albumin-bound FFA. They sense elevated FFA in complex with FABP4, originating from adipose tissue [63] or vascular endothelium [64], and respond by increasing glucose output (hepatocytes) or insulin secretion (β-cells). Platelets, frequently activated in metabolic disorders, increase phospholipid hydrolysis to release FFA that stimulate insulin secretion by β-cells [65]. This unexpected finding suggests another FFA-mediated mechanism of primary hyperinsulinemia induction occurring even before systemic FFA elevation or the onset of glucotoxicity.

The mechanism linking glucotoxicity and lipotoxicity remains poorly understood. It may involve accumulation of glucose-derived metabolic intermediates [21, 66, 67], some of which fuel *de novo* lipogenesis and TAG synthesis, thereby promoting ectopic fat accumulation and increasing susceptibility to lipotoxicity. Collectively, these changes converge into glucolipotoxicity. Alternatively, lipotoxicity may develop more insidiously, through the metabolic scenario, becoming clinically evident once glucotoxicity emerges. Perhaps, only in cases of severe obesity (body mass index, BMI > 35) lipotoxicity can occur independently, potentially as a result of direct FFA toxicity, when the buffering capacity of blood plasma proteins and hepatic mechanisms are exhausted, thereby increasing the mortality risk [68] (see below).

SECTION 4. OBESITY, FFA PLASMA LEVELS, AND ECTOPIC LIPIDS: OUTSIDE LOOKING INSIDE

Although T2D develops only in a subset of obese individuals, the population prevalence of obesity and T2D is comparable [69]. Obesity is strongly associated with IR and is considered a major risk factor for T2D [5, 17]. Early studies reported elevated plasma FFA in individuals with T2D [22, 70, 71]; several studies also found increased plasma FFA levels in people with obesity but without diabetes [16, 72, 73]. Cases of elevated FFA in the absence of obesity, yet accompanied by abnormalities in carbohydrate metabolism [71], may reflect genetic factors (e.g., MODY) or insufficient adipose storage capacity to remove FFA from the circulation, as observed in lipodystrophy [74]. These findings have raised questions about whether plasma FFA levels might be indicative of IR.

Detailed analysis of diurnal FFA profiles revealed no significant differences between individuals with and without obesity [74, 75]. However, increased

lipoprotein-TAG and hyperinsulinemia in subjects with obesity indicated the presence of IR. In this study, small groups of participants (n = 10 each) were stratified by body mass index (BMI 27-32 vs. 19-25). Across all participants, the plasma levels of FFA varied substantially throughout the day, largely depending on the food intake and insulin levels, but were essentially independent on the obesity status. These findings prompted the authors to question whether FFA alone could account for IR and adverse metabolic consequences of obesity [74, 76]. Further support came from an extended analysis of blood samples from 1,591 individuals registered in the Oxford Biobank, which confirmed the absence of direct association between the BMI and fasting FFA levels [76]. By that time, results from the large Paris Prospective Study were also available (n = 5790; BMI < 33) [77]. Retrospective analysis of these data found no significant differences in FFA plasma levels across individuals without or with obesity of varying degrees [77].

The Stockholm study, which included ~4000 individuals provided further insights into relationship between obesity and circulating FFA [78]. Obesity was defined as BMI ≥ 30, although no upper BMI limit was specified. Obese individuals exhibited small but statistically significantly higher FFA plasma levels (0.71 ± 0.23 mM) compared to non-obese subjects (0.57 ± 0.23 mM). Similar differences were observed for plasma glycerol, a marker of adipose tissue lipolysis. Notably, no difference in FFA levels was found between individuals with or without IR.

A subsequent study examined healthy volunteers (n = 48), individuals with prediabetes (n = 20), and patients with either newly diagnosed or controlled T2D (n = 48 per group) [52]. Median plasma FFA levels in T2D patients were more than 3-fold higher than in healthy controls (~1.1 mM vs. ~0.3 mM, respectively), yet no definitive FFA threshold distinguishing healthy individuals from diabetic subjects was observed. A pilot prospective analysis revealed that in individuals with prediabetes who later progressed to T2D, FFA levels steadily increased over four years. These findings suggest that plasma FFA rise during prediabetes and become significantly elevated in T2D. Notably, the predictive value of FFA levels improved substantially when combined with the degree of albumin glycation [52]. Glycation-induced reduction in albumin's affinity for FFA may explain relatively weak lipotoxic effects in prediabetes, which later amplify as obesity, IR, hyperglycemia converge during T2D progression.

More recently, a large-scale Copenhagen population study was conducted that involved ~110,000 individuals. Metabolic profiling of blood samples was performed for nearly 30,000 participants, and mortality outcomes (all-cause, cancer-related, cardiovascular, and other causes) have been tracked over the following 10-11 years [68]. A distinctive aspect of this study was inclusion of individuals with BMI > 35, whereas earlier studies either rarely included participants with BMI exceeding 32-33 [76, 77], or did not clearly report the proportion of participants with BMI > 35 [78]. Thus, the Copenhagen study provided unique insights into metabolic features in individuals with severe obesity.

The study demonstrated that plasma glycerol levels increased proportionally to BMI and continued to rise in individuals with BMI > 35 [68]. Similar to FFA, glycerol served as a marker of adipose tissue lipolysis, indirectly reflecting FFA release, which was not directly measured. In contrast, plasma lipoprotein-TAG increased only up to BMI of 35-37, after which they plateaued, and declined at BMI > 40-42 [68]. This pattern suggests that lipoprotein buffering capacity becomes exhausted at BMI ~ 35, beyond which the liver can no longer sufficiently sequester FFA into lipoprotein TAG. Accordingly, 3-hydroxybutyrate plasma levels started to increase exponentially at BMI 33-35, indicating a metabolic shift toward channeling excess FFA into hepatic ketogenesis.

Both elevated glycerol and 3-hydroxybutyrate were strongly associated with mortality [68]. The hazard ratio surpassed the significance threshold and continued to rise with increasing 3-hydroxybutyrate levels at BMI ≥ 35. These findings suggest that FFA may become increasingly lipotoxic in severe obesity (BMI > 35). Moreover, the mortality risk correlated directly with plasma glycerol levels across their entire range, highlighting hepatic dysfunction as an important mortality risk, which manifests as impaired hepatic clearance of glycerol and FFA generated by high adipocyte lipolysis.

Collectively, the studies of plasma levels of FFA, metabolites of adipose-tissue lipolysis, and their hepatic products across different degrees of obesity suggest that the buffering capacity of circulating lipoproteins is generally sufficient to prevent overt lipotoxicity across a broad BMI range. However, this protective mechanism deteriorates when BMI exceeds ~35-37. This likely explains why earlier studies in individuals with BMI < 35 reported little or no association between plasma FFA levels and BMI [74-78]. The expected relationship is likely to emerge in severe obesity (BMI > 35-37), when the storage capacity of lipoproteins and, possibly, adipocytes, is exhausted, leading to marked FFA elevation that increases the risks of all-cause, cardiovascular, and cancer mortality [68].

In summary, individuals with T2D and IR exhibit substantially higher FFA plasma levels than healthy subjects. In contrast, those with mild-to-moderate obesity (up to BMI ~ 33-35) typically maintain plasma FFA within the normal range (< ~ 0.75 mM),

regardless of IR status. This raises an important question: how can FFA drive IR in prediabetes if their plasma levels remain normal? A plausible explanation is that chronic accumulation of FFA in peripheral tissues induces local IR [30, 74], effectively shifting the primary site of FFA action from extracellular (plasma-derived) to intracellular (ectopic fat).

SECTION 5. SKELETAL MUSCLE INSULIN RESISTANCE: WHO IS TO BLAME?

Tissue-specific mechanisms of IR have been recently reviewed in [4]. The studies of these mechanisms have shaped a concept of lipid-induced IR etiology across distinct tissues [79]. However, closer examination suggests that the mechanisms driving skeletal muscle IR may be more complex than initially assumed. A key challenge is that metabolic abnormalities observed in T2D individuals reflect an end-stage phenotype that develops gradually over a prolonged period of prediabetes. Because long-term interventional studies in healthy subjects are not feasible, short-term approaches have been used to infer causality. Few-hour hyperinsulinemic clamp protocols and lipid-heparin infusions have been employed in healthy volunteers to assess the acute effects of elevated plasma FFA. An inherent limitation of this approach is that acute FFA elevations may produce rapid direct effects on proximal targets, such as those near the plasma membrane, without fully recapitulating chronic metabolic remodeling. Nonetheless, the similarity between the findings from lipid infusion studies in healthy subjects and observations in T2D patients, who had elevated FFA without lipid infusion, suggested a common mechanism for lipid-induced IR in humans. Apparently, this rationale has guided the seminal studies by G. Shulman, G. Boden, and colleagues in the 1990s, which have shaped current understanding of lipid-induced IR.

G. Shulman employed ^{31}P and ^{13}C isotopes along with magnetic resonance spectroscopy (MRS) to quantify glucose 6-phosphate (G6P) in skeletal muscle in healthy individuals and patients with T2D. Contrary to the predictions of the Randle hypothesis, intracellular G6P levels were not elevated in T2D; instead, they were markedly reduced [80]. Moreover, insulin-stimulated glycogen synthesis, the primary route of glucose disposal in skeletal muscle [81], was noticeably impaired in T2D. These observations indicated that glucose uptake by myocytes is defective at a step preceding G6P formation, implicating abnormalities in either glucose transport or hexokinase activity (Fig. 4a).

To assess whether glucose transport is impaired, open microdialysis technique was used to quantify intracellular glucose and its transmembrane gradients in skeletal muscle *in vivo*. This technique had been developed to measure interstitial glucose concentrations in human adipose tissue during a clamp [82]. Later, it was combined with isotopic tracers and MRS, first validated in rats [83], and then applied in humans [84]. In rats, intracellular glucose amounted to <1 mM under hyperglycemia (20 mM) without insulin, when glucose uptake by cells was low, and to <0.1 mM under 10 mM glucose with hyperinsulinemia (1200 pM) [83]. Human data showed slightly different values, but followed the same the principle: intracellular glucose remained far below extracellular levels during hyperglycemic (10 mM) hyperinsulinemic (50-60 μU/mL, or 310-370 pM) clamp [84]. In healthy lean individuals (BMI ~ 22), intracellular glucose was ~0.1 mM, whereas in T2D patients (BMI ~ 31), it was ~0.24 mM, which was still ~25-fold lower than would be expected if hexokinase were inhibited. The whole-body glucose disposal in these patients was ~5-fold lower, accompanied by reduction in both glycogen synthesis and G6P levels.

These results demonstrate that intracellular glucose concentrations are consistently much lower than the extracellular levels, and that insulin further reduces intracellular glucose concentrations several-fold [83]. This implies that insulin stimulates intracellular glucose utilization. Although not explicitly discussed by the original authors, these data suggest that insulin regulates not only glucose transport but also intracellular glucose metabolism. That is, glucose influx into a cell may not be the primary rate-limiting step for metabolism. Instead, increased intracellular glucose consumption may itself enhance glucose influx, as intracellular utilization begins to outpace extracellular availability of glucose. This passive metabolic reinforcement by the “pull” mechanism may be complemented by the active “push” mechanism, whereby insulin drives the translocation of glucose transporters to the plasma membrane. To determine which mechanism predominates, it is necessary to find out which responds earlier to insulin stimulation. Recent metabolomic studies in cultured 3T3-L1 adipocytes [21], which share the canonical mechanism of insulin-dependent glucose uptake with skeletal muscle, have addressed this question. These studies showed that insulin activates intracellular glucose utilization before increasing glucose entry into the cells [21]. Specifically, insulin first rapidly stimulated glycolysis, lactate production, and the pentose phosphate pathway, while glycogen synthesis increased independently of glucose influx [85]. Although the mechanism of this rapid metabolic action of insulin remains unclear, it also involves triggering of synthesis of fatty acids and glycerol 3-phosphate required for TAG formation (Fig. 3), with increased glucose influx occurring only

subsequently [86]. These results are in line with observations that intracellular glucose concentration in skeletal muscle of T2D patients are elevated, rather than reduced compared to healthy controls [84], suggesting that the defects in T2D may involve not only glucose uptake, but also dysregulation of intracellular glucose metabolism. Notably, muscle glycogen content is reduced in T2D subjects [87], which rules out saturation of glycogen stores as the cause of increased glucose concentrations, but does not exclude the possibility that glucose catabolism is impaired in T2D.

This logic required examination of the effects of circulating FFA on glucose transport and metabolism in skeletal muscle of healthy humans *in vivo*. The earliest such studies were likely conducted by Boden and colleagues [88]. They showed that lipids rapidly (within an hour) replaced glucose as oxidation substrates in the *vastus lateralis* muscle, consistent with the Randle hypothesis. Acetyl-CoA levels increased more than 4-fold within 6 h of lipid infusion compared with the no-infusion control, supporting proposed inhibition of pyruvate dehydrogenase by excess FFA (Fig. 4a). In addition, following lipid infusion the glycogen synthase activity declined to basal levels, similar to those observed in the absence of insulin, indicating a loss of insulin-stimulated glycogen synthesis in response to FFA. The whole-body glucose disposal declined within 3-4 h of lipid infusion, although G6P and citrate levels in muscle remained unaltered at 6 hours. Notably, a 2-hour lipid infusion produced a comparable delay in the suppression of glucose uptake, which began to recover ~3 h after the end of infusion [87]. Together, these findings suggest that the classical Randle mechanism operates only transiently following increase in plasma FFA levels, after which additional mechanisms may take over.

Boden and colleagues then examined the dose-response relationship between circulating FFA and insulin action [89]. At low plasma FFA concentrations (0.05 mM), insulin-stimulated whole-body glucose disposal and glycogen synthesis in skeletal muscle were not affected. Physiological FFA levels (~0.55 mM, see [76 78]) caused detectable metabolic impairment: insulin increased glucose uptake only during the first 3 h of the clamp and failed to stimulate glycogen synthesis, whereas G6P levels declined. At higher FFA concentrations (0.75 mM), insulin no longer stimulated glucose uptake or glycogen synthesis, although intracellular G6P was transiently elevated between 4 and 6 h of the clamp. Taken together with the earlier findings [88], these results suggested that the Randle cycle may operate transiently and in a context-dependent manner following substantial elevation in plasma FFA. However, FFA also appear to act through additional mechanisms, inhibiting glucose utilization without accumulation of intracellular G6P.

Resolving these mechanisms required accurate *in vivo* quantification of intracellular G6P. Conventional biochemical assays performed in tissue homogenates are limited and can yield imprecise results [90]. To overcome these limitations and enable time-resolved quantification of G6P in human muscle *in vivo*, Shulman and colleagues combined ^{31}P- and ^{13}C-MRS and hyperinsulinemic euglycemic clamps [90]. Lipid infusion in healthy volunteers increased plasma FFA to ~2 mM and was shortly followed by a decline in glucose oxidation. After some delay, both glucose influx into muscle and glycogen synthesis decreased. Intracellular G6P concentrations increased only within the first hour and subsequently fell below the control values, consistent with a biphasic response: an initial transient Randle effect followed by impaired insulin-dependent glucose uptake and/or reduced G6P formation due to hexokinase inhibition.

To determine whether FFA selectively impair glucose transport, the open microdialysis technique previously validated in animals [83] and in patients with T2D [84], was combined with MRS and euglycemic (5 mM) hyperinsulinemic (400 pM) clamp and muscle biopsies in healthy volunteers [91]. Lipid-heparin infusion raised FFA concentration to ~1.8 mM, whereas glycerol infusion (control) reduced FFA from ~0.5 mM to ~0.1 mM. In the lipid infusion group, glucose uptake, glucose oxidation, and glycogen synthesis were markedly reduced throughout the 6-hour clamp, while G6P levels remained largely unaltered. Overall, these responses resembled previous observations in patients with T2D [84], supporting the notion that these metabolic defects were driven by excess FFA. Intracellular glucose concentrations measured 2-4 h into the clamp were markedly lower in the lipid-infusion group (0.04 mM) than in the control group (0.25 mM) [91]. On the one hand, this pattern indicated that the Randle mechanism was not operative under these conditions, as intracellular glucose failed to accumulate. On the other hand, these values contrasted with earlier measurements in healthy subjects (~0.1 mM) with that in patients with T2D (~0.24 mM) [84]. This discrepancy suggests that chronic IR in T2D may involve additional defects in glucose catabolism that weaken the intracellular “pull” mechanism of glucose influx, similar to mechanisms described in adipocytes [21, 86]. Whether similar defects occur in human skeletal muscle and contribute to disease progression remains unresolved.

In summary, these complex *in vivo* studies demonstrated that skeletal muscle glucose transport is suppressed in a lipid-dependent manner and that the Randle cycle may operate only transiently at high FFA levels. Although these findings revealed acute, and possibly direct, lipotoxic effects of FFA, it is likely that additional metabolic mechanisms contribute

to impaired glucose utilization in skeletal muscle in response to chronic ectopic lipid accumulation in prediabetes. Many of these mechanisms are discussed by the authors themselves [4].

SECTION 6. MOLECULAR LANDSCAPE OF INSULIN RESISTANCE: WHAT IS TO BE DONE?

Because lipid infusion impairs insulin-dependent glucose uptake in skeletal muscle, it was logical to propose that the primary targets of FFA may reside in the plasma membrane compartment. Indeed, Western blot analysis of muscle biopsies from healthy volunteers demonstrated that lipid infusion blunted insulin-induced activation of phosphoinositide 3-kinase (PI3K), a principal intracellular effector of insulin action [91]. These observations led to the hypothesis, and subsequent demonstration, that FFA act by increasing intracellular levels of DAG, which in turn activates a subset of Ca^{2+}-independent nPKC isoforms (Fig. 5). Activated nPKCs phosphorylate insulin receptor substrate (IRS) proteins, the immediate downstream targets of the insulin receptor. This serine-directed phosphorylation of IRS proteins blocks insulin signaling via PI3K and Akt kinase (protein kinase B/AKT1-3). Subsequent studies identified similar IRS inactivation via serine phosphorylation in other cell types [8, 17]. This mechanism is now recognized as a part of a large regulatory network in which multiple serine/threonine kinases phosphorylate distinct serine residues in IRS proteins to attenuate various aspects of their function [42, 92, 93]. Collectively, these findings established the concept of lipid-induced IR, whereby elevated FFA suppress insulin signaling and glucose uptake in peripheral tissues [30, 79].

In addition to lipid-induced mechanisms that impair insulin signaling, several other pathways may contribute to FFA-induced metabolic dysfunction. Among these, ceramide-mediated mechanism has attracted particular attention [35]. Ceramides are synthesized from palmitate, the most abundant saturated FFA in humans [38]. Ceramides attenuate insulin signaling and exert independent metabolic effects [33-35]. With respect to insulin signaling, ceramides inhibit insulin action by activating Akt phosphatase or by preventing Akt translocation to the plasma membrane, thereby interrupting signaling downstream of the insulin receptor and IRS proteins. Independently of insulin signaling, ceramides promote FFA channeling into TAG in adipocytes and hepatocytes,

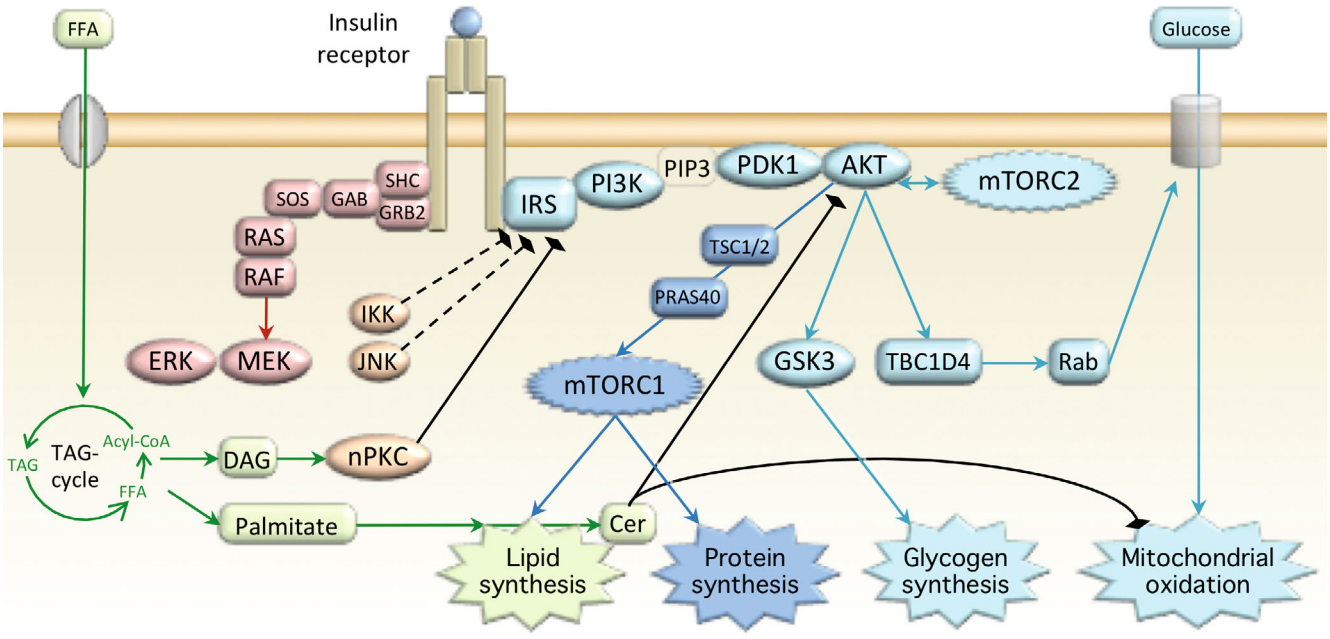

Fig. 5. Signaling mechanisms of insulin resistance. The scheme illustrates the major branches of insulin signaling, including the mitogenic arm (pink) and the metabolic arm (dark and light blue). The lower panel shows the major metabolic processes regulated by insulin: lipid synthesis in the ER, protein synthesis on the ribosomes of the rough ER, glycogen synthesis in the cytoplasm, and mitochondrial substrate oxidation for ATP production. Lipid metabolism and the TAG cycle are shown in green and include TAG, DAG, FFA, and ceramides (Cer). Black blunt-ended arrows indicate inhibitory signals that attenuate insulin signaling from insulin receptors and involve nPKCs, insulin-independent inflammatory protein kinases IKK (IkB kinase) and JNK (c-Jun N-terminal kinase), and ceramides. TSC1/2 (tuberous sclerosis proteins 1 and 2, GTPase-activating proteins for Rheb GTPase) and PRAS40 (proline-rich Akt substrate of 40 kDa, also known as AKT1S1) function as independent Akt effectors.

impair mitochondrial function, and suppress respiratory chain activity [44]. Importantly, IR mechanisms that do not directly involve canonical insulin signaling are receiving increasing attention. In addition to mitochondrial dysfunction, these include ER stress, accumulation of acyl-carnitines, and oxidative stress [4, 9]. Importantly, in endothelial cells, excessive FFA induce oxidative stress and cell death without affecting the IRS-Akt signaling, at least while cells remain viable for several days [94]. This observation highlights that FFA do not invariably act through direct lipotoxicity, but may also exert broader metabolic effects.

The insulin-signaling network coordinates a wide array of cellular processes that can be broadly grouped into mitogenic and metabolic branches [42] (Fig. 5). The metabolic arm is structurally heterogeneous and regulates carbohydrate metabolism as well as anabolic programs of lipid and protein synthesis via mTORC1 (mechanistic target of rapamycin complex 1). Accumulating evidence suggests that in IR and/or T2D, signaling defects arise in the metabolic branch, specifically at distal post-receptor nodes that are directly linked to the glucose transport control [41, 95-98]. One proposed explanation is that this may be due to an excess ("spareness") of the key signaling molecules, such as Akt, which may be unevenly distributed across downstream pathways [41]. Consequently, partial attenuation of Akt activity in IR or T2D may disproportionately impair the glucose-transport arm of insulin signaling. However, direct *in vivo* evidence has been lacking until recently, especially in the context of physiological postprandial response.

We addressed this gap by performing phosphoproteomic [99] and transcriptomic [100] profiling of skeletal muscle from healthy individuals and obese patients with T2D before and one hour after ingestion of a mixed meal adjusted for body weight. In healthy participants, plasma glucose and insulin peaked at ~30 min with glucose returning to baseline within an hour [101]. A weaker, prolonged second phase of insulin secretion was observed, that resolved by ~3 h, indicating that under physiological conditions, insulin action is largely completed within an hour, ensuring efficient clearance of excess glucose from the circulation. In contrast, obese T2D patients exhibited markedly higher glucose and insulin levels, which remained near maximum for at least 2 h postprandially and did not return to baseline even after 3 h. Fasting glucose and insulin were also elevated relative to healthy donors, consistent with chronic IR and uncompensated hyperglycemia.

Phosphoproteomic profiling of the *vastus lateralis* muscle revealed similar postprandial signaling patterns in healthy donors and obese patients with T2D. In healthy muscle, insulin activated virtually all signaling branches, including metabolic [Akt, TBC1D4 (GAP for Rab GTPase, also known as AS160), GSK3β (glycogen synthase kinase-3α/β), mTORC1, Cbl/TC10 (ubiquitin ligase Cbl/GTP-binding protein TC10)] and mitogenic (extracellular regulated MAP kinases Erk1/2) arms [99]. In T2D muscle, insulin signaling was attenuated, but not completely abolished. Consistently, transcriptomic analysis showed no major differences between the groups [100], suggesting at least partially preserved insulin responsiveness in T2D with obesity. Western blot analysis confirmed significantly increased postprandial phosphorylation of Akt in patients with T2D relative to fasting levels, whereas TBC1D4 phosphorylation remained unchanged. Notably, insulin failed to activate the Cbl/TC10 ubiquitin ligase pathway, which regulates trafficking of insulin-responsive GLUT4 storage vesicles to the plasma membrane [102]. These results indicate that insulin signaling in T2D with obesity is not globally impaired; rather, the metabolic branch governing glucose transport appears selectively affected. These findings support the emerging concept of selective IR, where discrete branches of metabolic signaling are differentially impaired in T2D [41, 96, 103]. Further studies with larger volunteer cohorts and detailed temporal analyses of postprandial signaling dynamics are needed to test this hypothesis and decipher specific defects in insulin signaling that emerge in obesity and prediabetes, ultimately shaping the molecular landscape of IR in overt T2D.

SECTION 7. HEPATIC LIPOTOXICITY AND B-CELL DYSFUNCTION: WHEN THE LEVEE BREAKS

The early coexistence of dyslipidemia, insulin hypersecretion, and IR in prediabetes makes it difficult to conclusively determine which of these features is primary (Fig. 1). The molecular mechanism underlying insulin secretion by β-cells and its dependence on plasma glucose levels have been well characterized [104]. Interestingly, in prediabetes, the arterial blood levels of FFA and glucose (both fasting and postprandial) typically remain within a normal range, while insulin levels are almost persistently elevated in obese individuals [74]. It is hard to imagine how and why IR would arise in skeletal muscle under these conditions, with compensatory hyperinsulinemia following. Consequently, some researchers believe that hyperinsulinemia is the primary abnormality, rather than the compensatory response, with IR developing subsequently [10, 36, 37, 95]. Primary hyperinsulinemia could be caused by increased lipid influx into β-cells, which stimulates insulin secretion [31], and/or by elevated production of reactive oxygen species in β-cells through mechanisms that are not yet fully

understood [9]. Regardless of the precise pathway, these observations underscore the central role of dyslipidemia and/or excess dietary fat.

Blood enters the liver from the pancreas. The liver and kidneys are the primary sites for insulin clearance from the blood. Incomplete hepatic degradation of insulin can therefore increase insulin levels in systemic circulation and sustain hyperinsulinemia. Normally, the liver removes up to 70% of insulin, but in the presence of fat infiltration, it degrades only 30-40% of insulin [105]. When combined with insulin hypersecretion by β-cells, this decreased hepatic degradation likely contributes to basal hyperinsulinemia in response to surplus food and chronic excess of lipids and carbohydrates in the venous outflow of the portal vein system.

Adipose tissue, which takes up dietary fats in response to insulin, protects the liver from fat infiltration. The levels of circulating FFA remain within the normal range throughout the day, even in individuals with moderate obesity [74, 75]. This suggests that in prediabetes, adipose tissue effectively accommodates increased dietary fat influx, thus protecting the liver from fat infiltration. Between meals, when plasma insulin is low, the liver adapts to the increased FFA influx from adipose tissue by redirecting FFA to lipoprotein TAG. The lack of such a protection in conditions associated with overload of adipocyte fat depots, such as lipodystrophy or morbid obesity, increases the risks of ectopic fat accumulation and T2D [74]. This again emphasizes the key role of adipose tissue in T2D pathophysiology and in neutralizing potential lipotoxicity of excess dietary fats.

Primary basal hyperinsulinemia may trigger a cascade of events, such as fat accumulation in adipocytes due to insulin lipogenic activity [10, 11]. The prolonged duration of prediabetes may reflect the slow progression of these events. Adipocyte hypertrophy is associated with macrophage infiltration and the development of latent inflammation [8], reduction in the proliferative and regeneration potential of progenitor cells [106], and emergence of IR in adipose tissue [7]. However, even in the absence of adipose IR, hypertrophied adipocytes can serve as a source of excessive postprandial FFA and leptin. Development of systemic leptin resistance may redirect FFA to other tissues, causing ectopic obesity and IR development in peripheral tissues [10, 32]. Determining whether, when, and to what extent IR develops in adipose tissue appears critical for understanding the pathophysiology of T2D.

The liver is the primary acceptor of FFA released from adipose tissue, placing a substantial burden on hepatic TAG synthetic machinery. Hepatocytes generate glycerol-3-phosphate for glyceroneogenesis, as part of gluconeogenic pathway, using glycerol and, potentially, lactate delivered from adipose tissue [107]. In adipocytes, glucose conversion to lactate is prioritized [85], and the lactate cycle operates in adipose tissue, similar to the Cori cycle in muscle [108]. Normally, insulin suppresses adipose lipolysis and hepatic gluconeogenesis, redirecting the FFA flux back to the adipose tissue within minutes. IR in adipose tissue is thought to remove the inhibition of lipolysis in adipocytes, thus increasing FFA export and its delivery to hepatocytes, whereas hepatic IR unrepresses glyceroneogenesis, promoting TAG synthesis and accumulation from incoming FFA [4]. The combination of adipose IR and hepatic IR disrupts coordinated regulation of the opposing lipid fluxes, thus shifting the balance toward TAG synthesis in the liver. The export of TAG in lipoproteins facilitates redistribution of the excessive fat to muscle and other tissues, thus triggering ectopic obesity, potentially, even in the absence of leptin resistance. This again underscores the metabolic connection between the liver and adipose tissue and the role of adipose IR in the development of hepatic IR.

According to this scenario, IR may develop last in skeletal muscle and represent a protective adaptation, reducing glucose influx into cells and lipogenesis in response to increased availability of circulating FFA [10]. Alternatively, IR may arise earlier, even first, in skeletal muscle if it results from excessive dietary fat. In this case, dietary lipids are transported from intestinal enterocytes by chylomicrons in the lymph into the *vena cava* and then into the arterial blood via the pulmonary circuit. In this scenario, skeletal muscle, like other organs, would be the first target of FFA, further exacerbated by "spillover" effects associated with high LPL activity [47]. The difference between these scenarios appears to lie in the source of plasma FFA: the chylomicron-derived FFA may first promote IR in skeletal muscle, whereas the liver lipoprotein-derived FFA may cause muscle IR at later stages.

The skeletal muscle response to plasma FFA likely also depends on the duration of FFA exposure [88, 89]. Whereas transient IR may develop through the Randle glucose-fatty acid cycle [22] during substantial intervals between the meals, such as overnight fasting, the risk of lipid-induced IR [4, 79] may increase with a continuous and/or abundant influx of chylomicrons into the circulation. Ectopic obesity is characterized by gradual accumulation and slow shifts in metabolic fluxes, the processes that may be accompanied by rewiring of intracellular insulin signaling network [42] and selective suppression of glucose uptake [41]. In contrast, the insulin signaling may be switched off completely probably only under extreme and/or long-term ectopic obesity [30], as observed in fat infusion and hyperinsulinemic clamp experiments

discussed in Sections 5 and 6. Regardless of the underlying mechanism, skeletal muscle IR reduces glucose clearance from the circulation, thereby causing hyperglycemia and increasing glucose flux back to the liver.

The clinical presentation of T2D suggests that fatty liver and β-cell dysfunction are key indicators of transition from prediabetes to T2D. Fatty liver likely becomes clinically evident once circulating and adipose fat depots are saturated with TAG. When hepatic export of excess TAG via lipoproteins is exceeded, ketone body production increases sharply and circulating FFA levels become elevated in people with severe obesity [68], as discussed in Section 4. It might be expected that under these conditions, the liver begins to accumulate excess fat and develop ectopic obesity while suppressing insulin signaling and control of glucose production. The resulting glucolipotoxicity would damage β-cells, impair insulin secretion, and lead to manifestation of overt T2D (Fig. 1). Thus, fatty liver may be regarded as an early warning sign of the metabolic levee break that culminates in systemic fat spillover and glucolipotoxicity.

SECTION 8. CONCLUSIONS: THE FINAL CUT

The network organization of systemic metabolism and its regulation by insulin, complicated by tissue-specific features, continues to hinder a full comprehension of IR etiology and sequence of events in T2D pathophysiology, which is essential for identifying effective therapeutic targets [4, 9, 12, 40, 42, 60, 95]. The framework outlined above integrates numerous findings, of which only the most representative are cited, and therefore inevitably constitutes a simplification. The progression from prediabetes to T2D is shaped by multiple factors, their combinations and interactions, resulting in substantial individual variability in disease histories and clinical phenotypes. Elucidation of molecular and (patho) physiological mechanisms that underlie this complexity poses a major challenge for both basic research and clinical medicine.

A central unresolved question is what triggers the metabolic dysfunction? The above analysis points to excessive nutrient availability and overnutrition, the hallmarks of modern society, as major contributors. Primary hyperinsulinemia appears to represent a critical early response; however, its underlying causes and downstream consequences remain poorly understood. Moreover, the tissue in which IR first arises is still unknown. Whether skeletal muscle IR constitutes an early initiating event or late adaptive response to sustained hyperinsulinemia or lipid overload needs further investigation and careful scrutiny. Hence, early diagnostics of IR and deep exploration of its molecular mechanisms across multiple tissues are highly important. In this respect, omics technologies are powerful tools for simultaneous monitoring of hundreds of molecules and their post-translational modifications [42, 99, 103], offering the possibility to identify specific molecular perturbations at different stages of prediabetes and in T2D.

Ectopic lipid accumulation and lipotoxicity appear to be central determinants in disease pathogenesis [30]; however, the underlying mechanisms remain incompletely understood and warrant further investigation. Adipose tissue plays a pivotal role at multiple steps of disease progression, yet molecular drivers of adipose IR remain poorly defined. Removal of adipose tissue to reduce fat mass, such as by liposuction, are problematic due to potential impairment of adipose endocrine function and/or regenerative capacity. In contrast, modulation of adipogenesis appears as a more promising method to upregulate the lipid-oxidative capacity of adipocytes. Combining these strategies with increased skeletal muscle activity to promote lipid utilization may offer the most realistic prospects for preventing T2D. Finally, hepatic steatosis becomes particularly critical during transition from prediabetes to overt T2D, potentially necessitating pharmacological interventions to prevent irreversible metabolic deterioration.

Insulin therapy for prevention or early treatment of T2D seems inappropriate [12] largely because IR represents, in essence, an adaptive response to increased availability of energy substrates. Exogenous insulin would not eliminate the underlying causes of IR and is likely to exacerbate metabolic stress by promoting additional energy influx. Instead, glucose-lowering therapies and dietary interventions that reduce fat mass are likely to be more effective. Achieving rapid and sustained metabolic improvements will require the development of new therapeutics directed at specific molecular targets. Identifying these targets remains a priority in metabolic research.

Abbreviations

Akt	protein kinase B/AKT1-3
BMI	body mass index (kg/m^2)
CoA	coenzyme A
Cbl/TC10	ubiquitin ligase Cbl/GTP-binding protein TC10
DAG	diacylglycerol
ER	endoplasmic reticulum
FFA	free fatty acids
G6P	glucose 6-phosphate
IR	insulin resistance
LPL	lipoprotein lipase
mTORC1 and mTORC2	mechanistic target of rapamycin (mTOR) complexes 1 and 2

IRS	insulin receptor substrate
PKC and nPKC	protein kinase C and its novel isoform
TAG	triacylglycerol
T1D and T2D	type 1 and 2 diabetes mellitus
VLDL	very-low-density lipoprotein

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Contributions
A.V.V. and M.V.Sh. developed the concept and supervised the study; A.V.V., N.V.P., and M.V.Sh. wrote and discussed the manuscript; A.V.V. and M.V.Sh. edited the manuscript.

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Ethics approval and consent to participate
This work does not contain any studies involving human or animal subjects.

Conflict of interest
The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., Cavan, D., Shaw, J. E., and Makaroff, L. E. (2017) IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040, *Diab. Res. Clin. Pract.*, **128**, 40-50, https://doi.org/10.1016/j.diabres.2017.03.024.
2. Dedov, I. I., Shestakova, M. V., Vikulova, O. K., Zheleznyakova, A. V., Isakov, M. A., Sazonova, D. V., and Mokrysheva, N. G. (2023) Diabetes mellitus in the Russian Federation: dynamics of epidemiological indicators according to the Federal Register of Diabetes Mellitus for the period 2010-2022, *Diabetes Mellitus*, **26**, 104-123, https://doi.org/10.14341/DM13035.
3. DeFronzo, R. A., and Tripathy, D. (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes, *Diabetes Care*, **32**, S157-S163, https://doi.org/10.2337/dc09-S302.
4. Petersen, M. C., and Shulman, G. I. (2018) Mechanisms of insulin action and insulin resistance, *Physiol. Rev.*, **98**, 2133-2223, https://doi.org/10.1152/physrev.00063.2017.
5. Reaven, G. M. (1988) Banting lecture 1988. Role of insulin resistance in human disease, *Diabetes*, **37**, 1595-1607, https://doi.org/10.2337/diabetes.37.12.1595.
6. Abel, E. D., Peroni, O., Kim, J. K., Kim, Y. B., Boss, O., Hadro, E., Minnemann, T., Shulman, G. I., and Kahn, B. B. (2001) Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver, *Nature*, **409**, 729-733, https://doi.org/10.1038/35055575.
7. Stafeev, I. S., Vorotnikov, A. V., Ratner, E. I., Menshikov, M. Y., and Parfyonova, Y. V. (2017) Latent inflammation and insulin resistance in adipose tissue, *Int. J. Endocrinol.*, **2017**, 5076732, https://doi.org/10.1155/2017/5076732.
8. Vorotnikov, A. V., Stafeev, I. S., Menshikov, M. Y., Shestakova, M. V., and Parfyonova, Y. V. (2019) Latent inflammation and defect in adipocyte renewal as a mechanism of obesity-associated insulin resistance, *Biochemistry (Moscow)*, **84**, 1329-1345, https://doi.org/10.1134/S0006297919110099.
9. Corkey, B. E., Deeney, J. T., and Merrins, M. J. (2021) What regulates basal insulin secretion and causes hyperinsulinemia? *Diabetes*, **70**, 2174-2182, https://doi.org/10.2337/dbi21-0009.
10. Unger, R. H. (2003) Lipid overload and overflow: metabolic trauma and the metabolic syndrome, *Trends Endocrinol. Metab.*, **14**, 398-403, https://doi.org/10.1016/j.tem.2003.09.008.
11. Pankov, Y. A. (2016) The adipogenic function and other biological effects of insulin, *Biochemistry (Moscow) Suppl. Series B*, **10**, 1-9, https://doi.org/10.1134/S199075081601011X.
12. Nolan, C. J., and Prentki, M. (2019) Insulin resistance and insulin hypersecretion in the metabolic syndrome and type 2 diabetes: Time for a conceptual framework shift, *Diab. Vasc. Dis. Res.*, **16**, 118-127, https://doi.org/10.1177/1479164119827611.
13. Banting, F. G., and Best, C. H. (1922) The internal secretion of the pancreas, *J. Lab. Clin. Med.*, **VII**, 251-266.
14. Himsworth, H. P. (1936) Diabetes mellitus: its differentiation into insulin-sensitive and insulin-insensitive types, *Lancet*, **227**, 127-130, https://doi.org/10.1016/S0140-6736(01)36134-2.

15. Yalow, R. S., and Berson, S. A. (1960) Immunoassay of endogenous plasma insulin in man, *J. Clin. Invest.*, **39**, 1157-1175, https://doi.org/10.1172/JCI104130.
16. Dole, V. P. (1956) A relation between non-esterified fatty acids in plasma and the metabolism of glucose, *J. Clin. Invest.*, **35**, 150-154, https://doi.org/10.1172/JCI103259.
17. Reaven, G. M. (1995) The fourth Musketeer – from Alexandre Dumas to Claude Bernard, *Diabetologia*, **38**, 3-13, https://doi.org/10.1007/BF02369347.
18. Cahill, G. F., Leboeuf, B., and Renold, A. E. (1960) Factors concerned with the regulation of fatty acid metabolism by adipose tissue, *Am. J. Clin. Nutr.*, **8**, 733-739, https://doi.org/10.1093/ajcn/8.5.733.
19. Vaughan, M. (1961) The metabolism of adipose tissue *in vitro*, *J. Lipid Res.*, **2**, 293-316, https://doi.org/10.1016/S0022-2275(20)40471-7.
20. Cahill, G. F. (1961) Metabolic role of adipose tissue, *Trans. Am. Clin. Climatol. Assoc.*, **73**, 22-29.
21. Krycer, J. R., Yugi, K., Hirayama, A., Fazakerley, D. J., Quek, L. E., Scalzo, R., Ohno, S., Hodson, M. P., Ikeda, S., Shoji, F., Suzuki, K., Domanova, W., Parker, B. L., Nelson, M. E., Humphrey, S. J., Turner, N., Hoehn, K. L., Cooney, G. J., Soga, T., Kuroda, S., and James, D. E. (2017) Dynamic metabolomics reveals that insulin primes the adipocyte for glucose metabolism, *Cell Rep.*, **21**, 3536-3547, https://doi.org/10.1016/j.celrep.2017.11.085.
22. Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1963) The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus, *Lancet*, **281**, 785-789, https://doi.org/10.1016/S0140-6736(63)91500-9.
23. Hue, L., and Taegtmeyer, H. (2009) The Randle cycle revisited: a new head for an old hat, *Am. J. Physiol. Endocrinol. Metab.*, **297**, E578-E591, https://doi.org/10.1152/ajpendo.00093.2009.
24. Ashcroft, S. J. H., and Denton, R. M. (2021) Sir Philip John Randle, *Biogr. Mem. Fellows R. Soc.*, **70**, 359-385, https://doi.org/10.1098/rsbm.2020.0044.
25. Randle, P. J., Kerbey, A. L., and Espinal, J. (1988) Mechanisms decreasing glucose oxidation in diabetes and starvation: role of lipid fuels and hormones, *Diabetes Metab. Res. Rev.*, **4**, 623-638, https://doi.org/10.1002/dmr.5610040702.
26. McGarry, J. D., Mannaerts, G. P., and Foster, D. W. (1977) A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis, *J. Clin. Invest.*, **60**, 265-270, https://doi.org/10.1172/JCI108764.
27. McGarry, J. D. (2002) Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes, *Diabetes*, **51**, 7-18, https://doi.org/10.2337/diabetes.51.1.7.
28. DeFronzo, R. A., Tobin, J. D., and Andres, R. (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance, *Am. J. Physiol.*, **237**, E214-E223, https://doi.org/10.1152/ajpendo.1979.237.3.E214.
29. Shulman, G. I. (2000) Cellular mechanisms of insulin resistance, *J. Clin. Invest.*, **106**, 171-176, https://doi.org/10.1172/JCI10583.
30. Shulman, G. I. (2014) Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease, *N. Engl. J. Med.*, **371**, 2237-2238, https://doi.org/10.1056/NEJMc1412427.
31. Unger, R. H. (1995) Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications, *Diabetes*, **44**, 863-870, https://doi.org/10.2337/diabetes.44.8.863.
32. Unger, R. H., and Scherer, P. E. (2010) Gluttony, sloth and the metabolic syndrome: a roadmap to lipotoxicity, *Trends Endocrinol. Metab.*, **21**, 345-352, https://doi.org/10.1016/j.tem.2010.01.009.
33. Summers, S. A., Chaurasia, B., and Holland, W. L. (2019) Metabolic Messengers: ceramides, *Nat. Metab.*, **1**, 1051-1058, https://doi.org/10.1038/s42255-019-0134-8.
34. Chavez, J. A., Knotts, T. A., Wang, L. P., Li, G., Dobrowsky, R. T., Florant, G. L., and Summers, S. A. (2003) A role for ceramide, but not diacylglycerol, in the antagonism of insulin signal transduction by saturated fatty acids, *J. Biol. Chem.*, **278**, 10297-10303, https://doi.org/10.1074/jbc.M212307200.
35. Chaurasia, B., and Summers, S. A. (2021) Ceramides in metabolism: Key lipotoxic players, *Annu. Rev. Physiol.*, **83**, 303-330, https://doi.org/10.1146/annurev-physiol-031620-093815.
36. Corkey, B. E. (2012) Banting lecture 2011: Hyperinsulinemia: cause or consequence? *Diabetes*, **61**, 4-13, https://doi.org/10.2337/db11-1483.
37. Pories, W. J., and Dohm, G. L. (2012) Diabetes: have we got it all wrong? Hyperinsulinism as the culprit: surgery provides the evidence, *Diabetes Care*, **35**, 2438-2442, https://doi.org/10.2337/dc12-0684.
38. Lytrivi, M., Castell, A. L., Poitout, V., and Cnop, M. (2020) Recent insights into mechanisms of β-cell lipo- and glucolipotoxicity in type 2 diabetes, *J. Mol. Biol.*, **432**, 1514-1534, https://doi.org/10.1016/j.jmb.2019.09.016.
39. Muoio, D. M. (2014) Metabolic inflexibility: when mitochondrial indecision leads to metabolic gridlock, *Cell*, **159**, 1253-1262, https://doi.org/10.1016/j.cell.2014.11.034.
40. Goodpaster, B. H., and Sparks, L. M. (2017) Metabolic flexibility in health and disease, *Cell Metab.*, **25**, 1027-1036, https://doi.org/10.1016/j.cmet.2017.04.015.

41. James, D. E., Stöckli, J., and Birnbaum, M. J. (2021) The aetiology and molecular landscape of insulin resistance, *Nat. Rev. Mol. Cell Biol.*, **22**, 751-771, https://doi.org/10.1038/s41580-021-00390-6.
42. Burchfield, J. G., Diaz-Vegas, A., and James, D. E. (2025) The insulin signalling network, *Nat. Metab.*, **7**, 1745-1764, https://doi.org/10.1038/s42255-025-01349-z.
43. Diaz-Vegas, A., Sanchez-Aguilera, P., Krycer, J. R., Morales, P. E., Monsalves-Alvarez, M., Cifuentes, M., Rothermel, B. A., and Lavandero, S. (2020) Is mitochondrial dysfunction a common root of noncommunicable chronic diseases? *Endocr. Rev.*, **41**, bnaa005, https://doi.org/10.1210/endrev/bnaa005.
44. Diaz-Vegas, A., Madsen, S., Cooke, K. C., Carroll, L., Khor, J. X. Y., Turner, N., Lim, X. Y., Astore, M. A., Morris, J., Don, A., Garfield, A., Zarini, S., Zemski Berry, K. A., Ryan, A., Bergman, B. C., Brozinick, J. T., James, D. E., and Burchfield, J. G. (2023) Mitochondrial electron transport chain, ceramide and Coenzyme Q are linked in a pathway that drives insulin resistance in skeletal muscle, *Elife*, **12**, RP87340, https://doi.org/10.7554/eLife.|87340.3.
45. DeFronzo, R. A. (2010) Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009, *Diabetologia*, **53**, 1270-1287, https://doi.org/10.1007/s00125-010-1684-1.
46. Van der Vusse, G. J. (2009) Albumin as fatty acid transporter, *Drug Metab. Pharmacokinet.*, **24**, 300-307, https://doi.org/10.2133/dmpk.24.300.
47. Miles, J. M., and Nelson, R. H. (2007) Contribution of triglyceride-rich lipoproteins to plasma free fatty acids, *Horm. Metab. Res.*, **39**, 726-729, https://doi.org/10.1055/s-2007-990273.
48. Curry, S. (2004) Plasma albumin as a fatty acid carrier, *Adv. Mol. Cell Biol.*, **33**, 29-46, https://doi.org/10.1016/S1569-2558(03)33003-6.
49. Blache, D., Bourdon, E., Salloignon, P., Lucchi, G., Ducoroy, P., Petit, J. M., Verges, B., and Lagrost, L. (2015) Glycated albumin with loss of fatty acid binding capacity contributes to enhanced arachidonate oxygenation and platelet hyperactivity: relevance in patients with type 2 diabetes, *Diabetes*, **64**, 960-972, https://doi.org/10.2337/db14-0879.
50. Kravchenko, I. V., Furalyov, V. A., and Popov, V. O. (2020) Glycated albumin stimulates expression of inflammatory cytokines in muscle cells, *Cytokine*, **128**, 154991, https://doi.org/10.1016/j.cyto.2020.154991.
51. Freitas, P. A. C., Ehlert, L. R., and Camargo, J. L. (2017) Glycated albumin: a potential biomarker in diabetes, *Arch. Endocrinol. Metab.*, **61**, 296-304, https://doi.org/10.1590/2359-3997000000272.
52. Spiller, S., Blüher, M., and Hoffmann, R. (2018) Plasma levels of free fatty acids correlate with type 2 diabetes mellitus, *Diabetes Obes. Metab.*, **20**, 2661-2669, https://doi.org/10.1111/dom.13449.
53. Oliveira, A. F., Cunha, D. A., Ladriere, L., Igoillo-Esteve, M., Bugliani, M., Marchetti, P., and Cnop, M. (2015) In vitro use of free fatty acids bound to albumin: A comparison of protocols, *Biotechniques*, **58**, 228-233, https://doi.org/10.2144/000114285.
54. Smathers, R. L., and Petersen, D. R. (2011) The human fatty acid-binding protein family: evolutionary divergences and functions, *Hum. Genomics*, **5**, 170-191, https://doi.org/10.1186/1479-7364-5-3-170.
55. Naquet, P., Kerr, E. W., Vickers, S. D., and Leonardi, R. (2020) Regulation of coenzyme A levels by degradation: the 'Ins and Outs', *Prog. Lipid Res.*, **78**, 101028, https://doi.org/10.1016/j.plipres.2020.101028.
56. Zadoorian, A., Du, X., and Yang, H. (2023) Lipid droplet biogenesis and functions in health and disease, *Nat. Rev. Endocrinol.*, **19**, 443-459, https://doi.org/10.1038/s41574-023-00845-0.
57. Li, X., Li, Z., Zhao, M., Nie, Y., Liu, P., Zhu, Y., and Zhang, X. (2019) Skeletal muscle lipid droplets and the athlete's paradox, *Cells*, **8**, 249, https://doi.org/10.3390/cells8030249.
58. Warfel, J. D., Vandanmagsar, B., Dubuisson, O. S., Hodgeson, S. M., Elks, C. M., Ravussin, E., and Mynatt, R. L. (2017) Examination of carnitine palmitoyltransferase 1 abundance in white adipose tissue: implications in obesity research, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **312**, R816-R820, https://doi.org/10.1152/ajpregu.00520.2016.
59. Chouchani, E. T., Kazak, L., and Spiegelman, B. M. (2019) New advances in adaptive thermogenesis: UCP1 and beyond, *Cell Metab.*, **29**, 27-37, https://doi.org/10.1016/j.cmet.2018.11.002.
60. DeFronzo, R. A. (2009) Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus, *Diabetes*, **58**, 773-795, https://doi.org/10.2337/db09-9028.
61. Rossetti, L., Giaccari, A., and DeFronzo, R. A. (1990) Glucose toxicity, *Diabetes Care*, **13**, 610-630, https://doi.org/10.2337/diacare.13.6.610.
62. Rossetti, L., Shulman, G. I., Zawalich, W., and DeFronzo, R. A. (1987) Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats, *J. Clin. Invest.*, **80**, 1037-1044, https://doi.org/10.1172/JCI113157.
63. Hotamışlıgil, G. S., and Bernlohr, D. A. (2015) Metabolic functions of FABPs – mechanisms and therapeutic implications, *Nat. Rev. Endocrinol.*, **11**, 592-605, https://doi.org/10.1038/nrendo.2015.122.

64. Inouye, K. E., Prentice, K. J., Lee, A., Wang, Z. B., Dominguez-Gonzalez, C., Chen, M. X., Riveros, J. K., Burak, M. F., Lee, G. Y., and Hotamışlıgil, G. S. (2023) Endothelial-derived FABP4 constitutes the majority of basal circulating hormone and regulates lipolysis-driven insulin secretion, *JCI Insight*, **8**, e164642, https://doi.org/10.1172/jci.insight.164642.
65. Kolczyńska-Matysiak, K., Kassouf, T., Stegner, D., and Sumara, G. (2025) Platelets in the regulation of glucose and lipid metabolism-functions beyond the maintenance of vascular integrity are being revealed, *FEBS J.*, **292**, 4992-5007, https://doi.org/10.1111/febs.70198.
66. Rabbani, N., and Thornalley, P. J. (2024) Hexokinase-linked glycolytic overload and unscheduled glycolysis in hyperglycemia-induced pathogenesis of insulin resistance, beta-cell glucotoxicity, and diabetic vascular complications, *Front. Endocrinol.*, **14**, 1268308, https://doi.org/10.3389/fendo.2023.1268308.
67. Shah, M. S., and Brownlee, M. (2016) Molecular and cellular mechanisms of cardiovascular disorders in diabetes, *Circ. Res.*, **118**, 1808-1829, https://doi.org/10.1161/CIRCRESAHA.116.306923.
68. Johansen, M., Afzal, S., Vedel-Krogh, S., Nielsen, S. F., Smith, G. D., and Nordestgaard, B. G. (2023) From plasma triglycerides to triglyceride metabolism: effects on mortality in the Copenhagen General Population Study, *Eur. Heart J.*, **44**, 4174-4182, https://doi.org/10.1093/eurheartj/ehad330.
69. Kahn, S. E., Hull, R. L., and Utzschneider, K. M. (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes, *Nature*, **444**, 840-846, https://doi.org/10.1038/nature05482.
70. Golay, A., Chen, Y. D., and Reaven, G. M. (1986) Effect of differences in glucose tolerance on insulin's ability to regulate carbohydrate and free fatty acid metabolism in obese individuals, *J. Clin. Endocrinol. Metab.*, **62**, 1081-1088, https://doi.org/10.1210/jcem-62-6-1081.
71. Fraze, E., Donner, C. C., Swislocki, A. L., Chiou, Y. A., Chen, Y. D., and Reaven, G. M. (1985) Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance, *J. Clin. Endocrinol. Metab.*, **61**, 807-811, https://doi.org/10.1210/jcem-61-5-807.
72. Bierman, E. L., Dole, V. P., and Roberts, T. N. (1957) An abnormality of nonesterified fatty acid metabolism in diabetes mellitus, *Diabetes*, **6**, 475-479, https://doi.org/10.2337/diab.6.6.475.
73. Opie, L. H., and Walfish, P. G. (1963) Plasma free fatty acid concentrations in obesity, *N. Engl. J. Med.*, **268**, 757-760, https://doi.org/10.1056/NEJM196304042681404.
74. Frayn, K. N. (2019) Turning over our fat stores: the key to metabolic health. Blaxter Award Lecture 2018, *Proc. Nutr. Soc.*, **78**, 398-406, https://doi.org/10.1017/S0029665118002598.
75. McQuaid, S. E., Hodson, L., Neville, M. J., Dennis, A. L., Cheeseman, J., Humphreys, S. M., Ruge, T., Gilbert, M., Fielding, B. A., Frayn, K. N., and Karpe, F. (2011) Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes*, **60**, 47-55, https://doi.org/10.2337/db10-0867.
76. Karpe, F., Dickmann, J. R., and Frayn, K. N. (2011) Fatty acids, obesity, and insulin resistance: time for a reevaluation, *Diabetes*, **60**, 2441-2449, https://doi.org/10.2337/db11-0425.
77. Charles, M. A., Eschwège, E., Thibult, N., Claude, J. R., Warnet, J. M., Rosselin, G. E., Girard, J., and Balkau, B. (1997) The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study, *Diabetologia*, **40**, 1101-1106, https://doi.org/10.1007/s001250050793.
78. Arner, P., and Rydén, M. (2015) Fatty acids, obesity and insulin resistance, *Obes. Facts*, **8**, 147-155, https://doi.org/10.1159/000381224.
79. Samuel, V. T., Petersen, K. F., and Shulman, G. I. (2010) Lipid-induced insulin resistance: unravelling the mechanism, *Lancet*, **375**, 2267-2277, https://doi.org/10.1016/S0140-6736(10)60408-4.
80. Rothman, D. L., Shulman, R. G., and Shulman, G. I. (1992) 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus, *J. Clin. Invest.*, **89**, 1069-1075, https://doi.org/10.1172/JCI115686.
81. Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A., and Shulman, R. G. (1990) Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy, *N. Engl. J. Med.*, **322**, 223-228, https://doi.org/10.1056/NEJM199001253220403.
82. Trajanoski, Z., Brunner, G. A., Schaupp, L., Ellmerer, M., Wach, P., Pieber, T. R., Kotanko, P., and Skrabal, F. (1997) Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous ex vivo measurement of glucose concentration, *Diabetes Care*, **20**, 1114-1121, https://doi.org/10.2337/diacare.20.7.1114.
83. Cline, G. W., Jucker, B. M., Trajanoski, Z., Rennings, A. J., and Shulman, G. I. (1998) A novel 13C NMR method to assess intracellular glucose concentration in muscle, *in vivo*, *Am. J. Physiol.*, **274**, E381-E389, https://doi.org/10.1152/ajpendo.1998.274.2.E381.
84. Cline, G. W., Petersen, K. F., Krssak, M., Shen, J., Hundal, R. S., Trajanoski, Z., Inzucchi, S., Dresner, A., Rothman, D. L., and Shulman, G. I. (1999) Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes, *N. Engl. J. Med.*, **341**, 240-246, https://doi.org/10.1056/NEJM199907223410404.

85. Krycer, J. R., Quek, L. E., Francis, D., Fazakerley, D. J., Elkington, S. D., Diaz-Vegas, A., Cooke, K. C., Weiss, F. C., Duan, X., Kurdyukov, S., Zhou, P. X., Tambar, U. K., Hirayama, A., Ikeda, S., Kamei, Y., Soga, T., Cooney, G. J., and James, D. E. (2020) Lactate production is a prioritized feature of adipocyte metabolism, *J. Biol. Chem.*, **295**, 83-98, https://doi.org/10.1074/jbc.RA119.011178.
86. Krycer, J. R., Quek, L. E., Francis, D., Zadoorian, A., Weiss, F. C., Cooke, K. C., Nelson, M. E., Diaz-Vegas, A., Humphrey, S. J., Scalzo, R., Hirayama, A., Ikeda, S., Shoji, F., Suzuki, K., Huynh, K., Giles, C., Varney, B., Nagarajan, S. R., Hoy, A. J., Soga, T., Meikle, P. J., Cooney, G. J., Fazakerley, D. J., and James, D. E. (2020) Insulin signaling requires glucose to promote lipid anabolism in adipocytes, *J. Biol. Chem.*, **295**, 13250-13266, https://doi.org/10.1074/jbc.RA120.014907.
87. He, J., and Kelley, D. E. (2004) Muscle glycogen content in type 2 diabetes mellitus, *Am. J. Physiol. Endocrinol. Metab.*, **287**, E1002-E1007, https://doi.org/10.1152/ajpendo.00015.2004.
88. Boden, G., Jadali, F., White, J., Liang, Y., Mozzoli, M., Chen, X., Coleman, E., and Smith, C. (1991) Effects of fat on insulin-stimulated carbohydrate metabolism in normal men, *J. Clin. Invest.*, **88**, 960-966, https://doi.org/10.1172/JCI115399.
89. Boden, G., Chen, X., Ruiz, J., White, J. V., and Rossetti, L. (1994) Mechanisms of fatty acid-induced inhibition of glucose uptake, *J. Clin. Invest.*, **93**, 2438-2446, https://doi.org/10.1172/JCI117252.
90. Roden, M., Price, T. B., Perseghin, G., Petersen, K. F., Rothman, D. L., Cline, G. W., and Shulman, G. I. (1996) Mechanism of free fatty acid-induced insulin resistance in humans, *J. Clin. Invest.*, **97**, 2859-2865, https://doi.org/10.1172/JCI118742.
91. Dresner, A., Laurent, D., Marcucci, M., Griffin, M. E., Dufour, S., Cline, G. W., Slezak, L. A., Andersen, D. K., Hundal, R. S., Rothman, D. L., Petersen, K. F., and Shulman, G. I. (1999) Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity, *J. Clin. Invest.*, **103**, 253-259, https://doi.org/10.1172/JCI5001.
92. Tkachuk, V. A., and Vorotnikov, A. V. (2014) Molecular mechanisms of insulin resistance development, *Diabetes Mellitus*, **2014**, 29-40, https://doi.org/10.14341/DM2014229-40.
93. Boura-Halfon, S., and Zick, Y. (2009) Phosphorylation of IRS proteins, insulin action, and insulin resistance, *Am. J. Physiol. Endocrinol. Metab.*, **296**, E581-E591, https://doi.org/10.1152/ajpendo.90437.2008.
94. Samsonov, M. V., Podkuychenko, N. V., Khapchaev, A. Y., Efremov, E. E., Yanushevskaya, E. V., Vlasik, T. N., Lankin, V. Z., Stafeev, I. S., Skulachev, M. V., Shestakova, M. V., Vorotnikov, A. V., and Shirinsky, V. P. (2022) AICAR protects vascular endothelial cells from oxidative injury induced by the long-term palmitate excess, *Int. J. Mol. Sci.*, **23**, 211, https://doi.org/10.3390/ijms23010211.
95. Czech, M. P. (2017) Insulin action and resistance in obesity and type 2 diabetes, *Nat. Med.*, **23**, 804-814, https://doi.org/10.1038/nm.4350.
96. Fazakerley, D. J., Krycer, J. R., Kearney, A. L., Hocking, S. L., and James, D. E. (2019) Muscle and adipose tissue insulin resistance: malady without mechanism? *J. Lipid Res.*, **60**, 1720-1732, https://doi.org/10.1194/jlr.R087510.
97. Sylow, L., Tokarz, V. L., Richter, E. A., and Klip, A. (2021) The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia, *Cell Metab.*, **33**, 758-780, https://doi.org/10.1016/j.cmet.2021.03.020.
98. Van Gerwen, J., Shun-Shion, A. S., and Fazakerley, D. J. (2024) Insulin signalling and GLUT4 trafficking in insulin resistance, *Biochem. Soc. Trans.*, **51**, 1057-1069, https://doi.org/10.1042/BST20221066.
99. Yakupova, E. I., Podkuychenko, N. V., Smirnov, I. P., Zgoda, V. G., Vavilov, N. E., Lednev, E. M., Vepkhvadze, T. F., Tomilova, A. O., Shestakova, M. V., Vorotnikov, A. V., and Popov, D. V. (2025) Early phosphoproteomic response of skeletal muscle to a meal in type 2 diabetes is comparable to that in healthy individuals, *Biochemistry (Moscow)*, Article submitted.
100. Makhnovskii, P. A., Lednev, E. M., Gavrilova, A. O., Kurochkina, N. S., Vepkhvadze, T. F., Shestakova, M. V., and Popov, D. V. (2023) Dysregulation of early gene response to a mixed meal in skeletal muscle in obesity and type 2 diabetes, *Physiol. Genomics*, **55**, 468-477, https://doi.org/10.1152/physiolgenomics.00046.2023.
101. Lednev, E. M., Gavrilova, A. O., Vepkhvadze, T. F., Makhnovskii, P. A., Shestakova, M. V., and Popov, D. V. (2023) Disturbances in dynamics of glucose, insulin, and C-peptide in blood after a normalized intake of a mixed meal in obesity and type 2 diabetes mellitus, *Hum. Physiol.*, **49**, 668-674, https://doi.org/10.1134/S0362119723600297.
102. Bogan, J. S. (2012) Regulation of glucose transporter translocation in health and diabetes, *Annu. Rev. Biochem.*, **81**, 507-532, https://doi.org/10.1146/annurev-biochem-060109-094246.
103. Vorotnikov, A. V., Popov, D. V., and Makhnovskii, P. A. (2022) Signaling and gene expression in skeletal muscles in type 2 diabetes: current results and OMICS perspectives, *Biochemistry (Moscow)*, **87**, 1021-1034, https://doi.org/10.1134/S0006297922090139.

104. Ashcroft, F. M. (2023) K_{ATP} channels and the metabolic regulation of insulin secretion in health and disease: the 2022 Banting medal for scientific achievement award lecture, *Diabetes*, **72**, 693-702, https://doi.org/10.2337/dbi22-0030.
105. Pivovarova, O., Bernigau, W., Bobbert, T., Isken, F., Möhlig, M., Spranger, J., Weickert, M. O., Osterhoff, M., Pfeiffer, A. F. H., and Rudovich, N. (2013) Hepatic insulin clearance is closely related to metabolic syndrome components, *Diabetes Care*, **36**, 3779-3785, https://doi.org/10.2337/dc12-1203.
106. Stafeev, I., Podkuychenko, N., Michurina, S., Sklyanik, I., Panevina, A., Shestakova, E., Yah'yaev, K., Fedenko, V., Ratner, E., Vorotnikov, A., Menshikov, M., Yashkov, Y., Parfyonova, Y., and Shestakova, M. (2019) Low proliferative potential of adipose-derived stromal cells associates with hypertrophy and inflammation in subcutaneous and omental adipose tissue of patients with type 2 diabetes mellitus, *J. Diabetes Compl.*, **33**, 148-159, https://doi.org/10.1016/j.jdiacomp.2018.10.011.
107. Beale, E. G., Hammer, R. E., Antoine, B., and Forest, C. (2002) Glyceroneogenesis comes of age, *FASEB J.*, **16**, 1695-1696, https://doi.org/10.1096/fj.02-0407rev.
108. Brooks, G. A. (2018) The science and translation of lactate shuttle theory, *Cell Metab.*, **27**, 757-785, https://doi.org/10.1016/j.cmet.2018.03.008.