

# Молекулярные механизмы развития резистентности к инсулину

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*Инсулиновая резистентность (ИР) — это феномен, связанный с нарушением способности инсулина стимулировать захват глюкозы клетками-мишенями и снизить уровень глюкозы в крови. Ответное усиление секреции инсулина поджелудочной железой и гиперинсулинемия являются компенсаторными реакциями организма. Развитие ИР ведет к неспособности клеток-мишеней реагировать на инсулин, в результате чего развиваются сахарный диабет 2 типа (СД2) и метаболический синдром. По этой причине метаболический синдром на практике определяется как сочетание ИР с одной или несколькими патологиями, такими как СД2, артериальная гипертензия, дислипидемия, абдоминальное ожирение, неалкогольная жировая болезнь печени и некоторые другие. Однако его физиологическим критерием всегда служит сочетание высокого уровня глюкозы и инсулина в крови.*

*ИР следует рассматривать как системный сбой эндокринной регуляции в организме. Физиологические причины ИР разнообразны. Основными являются пищевая перегрузка и накопление в клетках определенных липидов и их метаболитов, низкая физическая активность, хроническое воспаление и стресс различной природы, включая оксидативный и «стресс эндоплазматического ретикулума» (нарушение распада поврежденных белков в клетке). Как показывают исследования последних лет, эти физиологические механизмы, скорее всего, реализуются по единому внутриклеточному сценарию. Им служит нарушение передачи сигнала от рецептора инсулина к его мишеням по механизму отрицательной обратной связи во внутриклеточных инсулин-зависимых сигнальных каскадах.*

*В данном обзоре рассмотрены физиологические и внутриклеточные механизмы действия инсулина; основное внимание уделено их нарушениям при развитии ИР. В заключении обсуждаются возможные направления ранней молекулярной диагностики и терапии ИР.*

**Ключевые слова:** инсулиновая резистентность; сахарный диабет 2 типа; инсулин-зависимая внутриклеточная сигнализация; обратная связь; белок IRS, фосфорилирование

## Molecular Mechanisms of Insulin Resistance Development

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*Insulin resistance (IR) is a phenomenon associated with an impaired ability of insulin to stimulate glucose uptake by target cells and to reduce the blood glucose level. A response increase in insulin secretion by the pancreas and hyperinsulinemia are compensatory reactions of the body. The development of IR leads to the inability of target cells to respond to insulin that results in developing type 2 diabetes mellitus (T2DM) and metabolic syndrome. For this reason, the metabolic syndrome is defined in practice as a combination of IR with one or more pathologies such as T2DM, arterial hypertension, dyslipidemia, abdominal obesity, non-alcoholic fatty liver disease, and some others. However, a combination of high blood glucose and insulin levels always serves as its physiological criterion. IR should be considered as a systemic failure of the endocrine regulation in the body. Physiological causes of IR are diverse. The main ones are nutritional overload and accumulation of certain lipids and their metabolites in cells, low physical activity, chronic inflammation and stress of various nature, including oxidative and endoplasmic reticulum stress (impairment of damaged protein degradation in the cell). Recent studies have demonstrated that these physiological mechanisms likely act through a single intracellular scenario. This is the impairment of signal transduction from the insulin receptor to its targets via the negative feedback mechanism in intracellular insulin-dependent signaling cascades.*

*This review describes the physiological and intracellular mechanisms of insulin action and focuses on their abnormalities upon IR development. Finally, feasible trends in early molecular diagnosis and therapy of IR are discussed.*

**Keywords:** insulin resistance; type 2 diabetes mellitus; insulin-dependent intracellular signaling; feedback; IRS protein; phosphorylation

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## SYSTEMIC EFFECT AND TARGET ORGANS OF INSULIN

Upon food intake, the blood glucose concentration increases, stimulating insulin secretion by pancreatic  $\beta$ -cells. Insulin activates glucose uptake in skeletal muscle and adipose tissue cells. These cells contain substantial levels of glucose transporter type 4 (GLUT4). Insulin causes the exposure of GLUT4 on the cell surface, triggering glucose transport into cells. Glucose is stored in muscle cells (myocytes) as glycogen; in adipose tissue cells (adipocytes), it enters glycolysis, the products of which are used to synthesise fats. Insulin also affects hepatocytes, which are cells in the liver, a key metabolic organ. In them, it also stimulates glycogen synthesis and conversion of glucose to lipids. Unlike the adipose tissue, the liver is not a fat depot. It actively exports fats to other tissues, including the adipose tissue, via lipoprotein particles.

Hepatocytes do not have insulin-dependent transporter GLUT4, and therefore, insulin affects them via a mechanism that is different from that in myocytes and adipocytes [1]. The mechanism in hepatocytes is associated with a change in the activity of enzymes for three metabolic modules, but is not associated not with glucose transport into cells. First, insulin inhibits glycogen phosphorylase, which retards the degradation and increases the synthesis of glycogen in the liver and muscles. Second, insulin activates glycolytic enzymes and accelerates the decomposition of glucose to acetyl-coenzyme A, which is a substrate for fatty acid synthesis. Insulin simultaneously inactivates enzymes of gluconeogenesis, inhibiting the reverse synthesis of glucose. Third, insulin unblocks a key enzyme of fatty acid synthesis, acetyl-CoA carboxylase, stimulating the formation of malonyl-CoA. Additionally, insulin inhibits the activity of lipase, which cleaves triglycerides, enabling their formation from fatty acids. Therefore, the cumulative effect of insulin on all target tissues is directed at shifting the metabolic equilibrium towards the conversion of glucose to glycogen and lipids.

Between meals, insulin secretion is reduced and the insulin block of gluconeogenesis and glycogen breakdown in the liver is removed. The insulin-glucagon index decreases and the actions of glucagon and epinephrine are initiated. These hormones are functional antagonists of insulin. They enhance glycogen breakdown, and glucagon also stimulates gluconeogenesis and glucose release from hepatocytes.

When fasting, lipid synthesis in the liver is decreased and triglyceride hydrolysis in the adipose tissue is increased. Its products, free fatty acids, are transported by blood to the liver. The cell cannot convert fatty acids to glucose because of the irreversibility of the reactions catalysed by the pyruvate dehydrogenase complex. As an energy source, ketone bodies are predominantly synthesised from fatty acids and are transported by the blood to the peripheral organs. Some tissues, such as the myocardium, use ketone bodies as the main source of energy during fasting, but brain cells require

glucose. This required blood glucose level is maintained due to gluconeogenesis, which occurs in the liver; its sources are products of protein and amino acid catabolism. A metabolic shift towards ketone bodies increases the intensity of lipolysis in the adipose tissue and consumption of fat reserves during prolonged fasting.

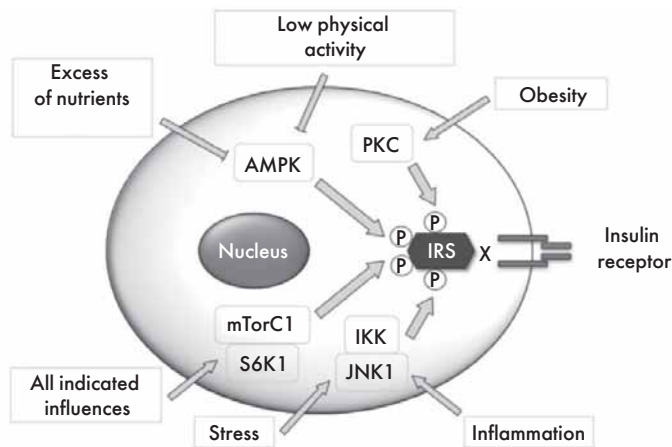
It is important for the body to maintain a balance between uptake, synthesis, and release of lipids from the liver. A shift in this balance towards lipid accumulation leads to a systemic reaction, affecting all insulin-dependent organs and resulting in the inevitable development of IR.

The systemic coordination of insulin-dependent tissues is implemented by the central nervous system in conjunction with the hypothalamic-pituitary system, which plays a key role in this process [2]. These organs are also referred to as insulin-dependent tissues, which are insulin targets [3]. They receive afferent stimuli from the stomach (ghrelin), pancreas (insulin), and adipose tissue (leptin). Insulin and leptin act in the same direction, stimulating the storage of energy in the form of glycogen in the liver and muscles and triglycerides in the adipose tissue. Insulin causes short-term responses, while leptin induces long-term responses. Hypothalamic hormones (melanocortins, neuropeptide Y, agouti-like protein, etc.) affect the pituitary gland and convert afferent stimuli into efferent ones [4]. The sympathetic and parasympathetic nervous systems are responsible for the efferent regulation. The former controls the mobilisation of energy reserves, while the latter controls their accumulation due to the vagal regulation of the secretory activity of  $\beta$ -cells of the pancreas and peripheral effects of insulin. Other peripheral hormones are also involved in the systemic coordination of insulin-dependent tissues. The most significant of these are of adipocyte origin (adiponectin, resistin, and other adipokines) or are local proinflammatory cytokines (TNF- $\alpha$ , interleukins-1 and -6, etc.). The latter ones are usually associated with the role of inflammation as a risk factor and the physiological mechanism of IR [5].

## PHYSIOLOGICAL MECHANISMS FOR THE DEVELOPMENT OF IR

IR is heterogeneous by its nature. Its pathophysiological basis is the systemic impairment of the interaction between the four major insulin target organs. IR occurs in these organs consistently and, at first glance, independently, but it ultimately integrates all of them and becomes systemic [2]. The muscles, liver, and adipose tissue are the primary target organs. IR in adipocytes is the last to develop; the adipose tissue often retains sensitivity to insulin even after the liver and muscles are already resistant. The hypothalamus and pituitary gland play a coordinating role. Either directly or indirectly, they also act as IR targets. Thus, IR is a tissue-specific phenomenon, and its development can be affected by several hormonal systems. At the body level, the CNS may play a leading and even defining role in IR [6].

There are likely many causes for the pathogenesis of



**Figure 1.** Conceptual illustration of the article's main idea. Insulin resistance at the cellular level occurs due to the uncoupling of insulin signalling at the insulin receptor substrate (IRS) protein level. This phenomenon is based on a single mechanism of phosphorylation of IRS on serine residues. Inside of the cell, this effect is likely due to different protein kinases that are effectors of various physiological mechanisms, the main of which are depicted.

IR, and not all of them are known yet. At the moment, four basic physiological mechanisms are recognised. These are overnutrition and low physical activity, obesity, inflammation, and stress. However, all of them are apparently ultimately realised in cells through a single mechanism of the phosphorylation of an immediate substrate of the insulin receptor, the insulin receptor substrate (IRS) protein, and the impairment of its interaction with the receptor (Fig. 1). Therefore, the division of “physiological” versus “cell” mechanisms is rather formal and shows the level of the whole organism in the first case, while it does not go beyond the cell in the second one.

**Overnutrition and low physical activity** increase the ATP level and reduce its metabolite, AMP, in the cell. As a result, the AMP-activated protein kinase (AMPK) activity is decreased. This enzyme acts as a cell metabolic sensor, maintaining energy homeostasis [7]. The importance of AMPK function is evidenced by the fact that it (or its homologues) is present in all eukaryotic cells ranging from yeast to human, including plant cells [8]. AMPK controls the activity of mTorC1, the first protein complex based on mTOR (mammalian target of rapamycin) kinase. This complex is a master switch between catabolism and anabolism in the cell [9]. When activated, it stimulates anabolic processes that lead to protein synthesis, cell growth and division, fat synthesis (lipogenesis), and an increase in the body lipid depots due to the differentiation of preadipocytes into adipocytes (adipogenesis). Having received a signal from AMPK, mTorC1 loses its activity; the cell switches to catabolism and utilisation of the reserves or incoming energy sources. Apparently, this transition is somehow related to the reciprocal activation of the second protein complex, mTorC2. mTorC2 is responsible for translocation of the GLUT4 glucose transporter to the cell membrane and for insulin-dependent glucose transport into

adipocytes and myocytes [10].

Active ATP hydrolysis occurs in myocytes during exercise. The adenylate kinase enzyme regenerates ATP in the reaction  $2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$ , and the resultant AMP activates AMPK. The latter switches off mTORC1 and triggers the utilisation of energy reserves. Thus, the AMPK system is also a physical activity sensor. In the absence of the latter, the AMPK activity is decreased and that of mTORC1 is increased. Together, these events are the physiologically coordinated cell response to overnutrition and a lack of exercise.

AMPK is also the target of adiponectin, which is a hormone of the adipose tissue [11]. Adiponectin increases the sensitivity to insulin, but its secretion is reduced in obese people [12]. This leads to AMPK inhibition and mTOR is activation in obesity. In addition, adiponectin prevents the development of IR, activating the degradation of ceramides and the accumulation of an important signalling molecule, sphingosine-1-phosphate, in the cell [13]. Under conditions of obesity and adiponectin deficiency, ceramide degradation is impaired and the ceramide-dependent mechanism of IR induction is activated, which involves neither AMPK nor mTOR.

**Hyperlipidaemia (obesity)** is closely related to insulin resistance. Although it remains unclear which of these phenomena is the primary one, numerous clinical data suggest that obesity is a cause of IR [14]. In turn, IR accelerates further weight gain and promotes obesity [2], acting via the positive feedback mechanism whose details are still unknown. A restrictive diet and weight loss restore insulin sensitivity in people with a sedentary [15].

Morbid obesity associated with IR has a characteristic feature. The fat is in the visceral (abdominal) location, i.e., is deposited in the cells of the liver, muscles, heart, vascular wall, and other organs, concentrating in the peritoneal area. In contrast, fats in the normal case are deposited in adipocytes, and this fat layer is located subcutaneously and distributed much more uniformly throughout the body. As a result, pathologic fat depots are called ectopic, thereby indicating the abnormality of their location in the body [16]. The formation of ectopic, but not visceral, fat has led to the development of IR and metabolic changes [17].

The ectopic fat distribution should not be confused with its accumulation outside cells. The results obtained in animal models and patients have demonstrated that IR severity correlates with the accumulation of fats inside cells but not in the intercellular space [1]. These are intracellular lipids that impair signal transduction from the insulin receptor and reduce insulin-dependent glucose uptake in the cells of non-fatty tissues, causing the development of IR and T2DM [16]. This physiological mechanism is consistent with the concept that IR develops first in the liver and skeletal muscles [2], whereas the adipose tissue still remains insulin-sensitive for some time [18]. Most likely, IR arises first in the liver and only then develops in other organs with a different time delay [19]. Prolonged ectopic fat accumulation in the liver (obesity) leads to the development of nonalcoholic fatty liver disease [20].

Lipids constitute a broad range of molecules with different structures and functions. When entering the cell, free fatty acids are rapidly thiolated to form acyl-CoA. In the liver, acyl-CoA is subjected to  $\beta$ -oxidation, and it is used for the synthesis of triglycerides in adipocytes. Furthermore, it is used in all cells for the etherification of sphingosine to ceramides. Some lipid metabolites (e.g., diacylglycerides and ceramides) are regarded as second messengers in various signal cascades of the cell. They represent the main lipids, an increase in the level of which initiates IR.

In recent years, the mechanism of IR development in the liver has been elucidated in general [1]. It is associated with the activation of “new” isoforms of protein kinase C (PKC) by lipid metabolites, but not  $\text{Ca}^{2+}$  ions, which is additionally required by the typical PKC isoforms [21]. Either directly or indirectly, the “new” PKC isoforms impair signalling from the insulin receptor into the cell (see below). Ectopic lipid accumulation in hepatocytes increases the level of diacylglycerides that are activators of the “new” PKCs. The diacylglyceride content in lipid droplets in the hepatocyte cytoplasm of obese people is clearly correlated to the PKC $\epsilon$  activity and degree of IR [22].

The mechanism of the development of IR in myocytes is apparently similar. It also involves the “new” PKC isoforms and the disturbance of insulin signalling in cells [23, 24]. The IR dynamics in the muscle tissue coincides with the accumulation of diacylglycerol in myocytes, deterioration in signal transduction from insulin, and a decrease in the glucose uptake by muscle cells [25]. The ectopic lipid level in muscle is a more reliable predictor of IR than the level of circulating fatty acids [26].

It should be emphasised that the lipid mechanism involving PKC is the main, if not the only, mechanism that ensures the development of IR in the liver in humans. A special comparative analysis revealed virtually no contribution of alternative mechanisms, such as inflammation or endoplasmic reticulum stress [22]. However, an inverse correlation between IR in the liver and the adiponectin blood plasma level in patients was found [22], which suggests a possible role of adipose hormones in the initiation of IR. These data differ from the results of studies in animal models, according to which the contribution of the inflammation and endoplasmic reaction to IR is significant [1]. Moreover, experimental data indicate that the development of IR in rodent muscles is associated with mitochondrial dysfunction [23]. Although either the details of this mechanism, or its presence in humans, remains unclear, the involvement of free radicals may be assumed, which is often the case with mitochondrial dysfunction.

The role of PKC in IR development in the adipose tissue has not been demonstrated. Physiological features of this tissue suggest that other mechanisms are implemented there. Unlike myocytes and hepatocytes, adipocytes normally contain high levels of triglycerides and dynamically change their level and composition. As a result, they constantly have a high level of diglycerides, which greatly exceeds that required for the complete activation of any PKCs. Therefore,

the mechanism of IR development in this tissue is still unclear. Perhaps, it involves inflammation, stress responses, or mitochondrial dysfunction. However, it should be noted that IR arises in this tissue as a consequence of pathological changes in the liver and muscles.

### Inflammation

Empirical observations of IR in patients with sepsis [27, 28] and the level of cytokines in people with obesity and diabetes [29, 30] have suggested that IR development may be caused by abnormal activation of innate immunity. Experiments in animals have demonstrated an elevated level of TNF $\alpha$  in the adipose tissue in obesity, and the neutralisation of TNF $\alpha$  recovered the glucose uptake by the peripheral tissues [31]. These results were also confirmed in people, where the TNF $\alpha$  level correlated with IR and dropped with a decrease in the body weight [32]. Finally, the mechanism was demonstrated by which inflammatory cytokines can interrupt signal transduction from the insulin receptor [33]. All of these data were the basis for developing a concept of the relationship between inflammation and IR [5].

Inflammation activates certain signalling cascades, which is a part of the normal physiological cell response in addition to present in pathologies. For example, both in healthy people and in obese patients without diabetes, exercise under aerobic conditions stimulates cytokine expression in skeletal muscles (MCP1 and IL-6) and activates the signalling cascade involving NF- $\kappa$ B. However, in patients with diabetes, the activity of this cascade is initially high and is not significantly increased with exercise [34].

Fasting or dysfunction in adipocytes abnormally enhances lipolysis and chemokine production [35]. Chemokines induce the movement of macrophages to the adipose tissue, where these cells become activated macrophages. They provide information regarding inflammation to other cells by increasing the secretion of cytokines, including TNF $\alpha$  and IL-6. Affecting adipocytes, these cytokines further stimulate lipolysis. For example, the infusion of IL-6 in healthy males enhances lipolysis and lipid oxidation within the femoral vascular bed [36]. The mechanism of cytokine action on lipid metabolism in adipocytes is partly related to the inhibition of the expression of proteins stabilising lipid droplets, perilipin [37] and FSP27 [38].

Transgenic mice with an increased production of the CCL2 chemokine in adipocytes have a significantly larger level of activated macrophages in the adipose tissue [39]. These animals develop peripheral and hepatic IR, the latter of which leads to steatosis. CCL2 blockade under the conditions of a high-calorie diet protects these mice from developing IR and decreases the lipid level in the liver [40]. In muscle cells, cytokines also stimulate lipid oxidation, and in extreme situations, they can trigger proteolysis and atrophy of the muscle tissue.

In contrast, cytokines exert the opposite effect in the liver, inhibiting lipid oxidation and enhancing lipogenesis [5]. This means that activated macrophages can affect the



inter-tissue energy balance, shifting lipid synthesis from the liver to the adipose tissue. It is unlikely that this effect has time to develop for short or local inflammation. However, macrophage activation has massive, long-term effects on systemic and chronic inflammation. Under these conditions, the probability of systemic changes in the energy balance is greatly increased, which may lead to a redistribution of lipids and their ectopic accumulation in the liver. At least in animal models, the experimental results are consistent with the participation of activated macrophages in the initiation of ectopic lipid accumulation and IR. It remains largely unclear to what extent this physiological mechanism is implemented in humans.

**The mechanism of damaged protein degradation** (unfolded protein response (UPR)) stimulates the adaptive responses of cells in the presence of limited nutrients [41]. This mechanism starts when the capacity of the intracellular system to fold newly synthesised proteins is deprived. This system is located on the endoplasmic reticulum (ER) of the cell in connection with which the UPR is often considered a cell response to stress associated with the endoplasmic reticulum. Because of this, the UPR is sometimes called “ER stress”, although it is more correct to consider the UPR a consequence of ER stress [42]. Chronic ER stress and UPR activation lead to oxidative stress and the formation of free radical molecules [43]. Thus, oxidative stress is a component of ER stress and these two mechanisms strongly overlap at the molecular level.

All intracellular systems of UPR activation are sensitive to the glucose level and are triggered when it starts to increase. This means that a high-calorie carbohydrate diet leads to UPR activation. The physiological significance of this response is protection against carbohydrate excess and the fast switching of the cellular metabolism to lipid synthesis (lipogenesis). In this sense, the UPR almost duplicates the insulin action on the cell metabolic balance without affecting glucose transport alone. The ability of UPR to cause IR may finally depend on whether the UPR shifts the balance towards lipogenesis to the point that there is ectopic lipid accumulation.

The UPR system is currently considered to be not only an activator of a number of metabolic responses in the cell, it is a vital integrator of the anabolic and catabolic processes occurring within cells [41]. The significance of the UPR system may be compared to that of master regulators of cellular metabolism, such as mTOR and AMPK complexes. It is no wonder that these three systems are closely interrelated in the cell, processing similar signals and affecting the activity of each other [44]. As a consequence, the UPR system not only responds to metabolic signals, it also provides the cell with an additional method for sensing systemic changes, including those typical of diabetes and metabolic syndrome. These include obesity, inflammation, and stress of different aetiologies. For this reason, the UPR uses the same signalling molecules in developing IR as those used by other physiological mechanisms that were mentioned previously [45].

At the first glance, it seems illogical that the UPR,

activated by glucose, disturbs signal transduction from the insulin receptor [45], and a reduction in the ER stress enhances the insulin signalling [46]. However, this phenomenon should be considered in the context of the reverse regulation, which is characteristic of metabolic control. IR develops as a defensive response of the cell and the body as a whole to nutrient excess, obesity, inflammation, and stress, and the UPR serves as one of the ways to implement it. Finally, UPR activation leads to the shutdown of insulin signalling at the same level of the IRS protein as it occurs in the case of mTOR-dependent, lipid-dependent, and inflammatory mechanisms.

## INSULIN SIGNALING

In most cells, the insulin receptor serves mainly to trigger the PI3-kinase cascade (Fig. 2) [47, 48]. Its main target in the cell is protein kinase B, which is better known as Akt [49]. Like all receptor tyrosine kinases, the insulin receptor also activates several other signalling systems. A feature of insulin signalling is the involvement of the IRS carcass protein, an insulin receptor substrate. As its name reflects, IRS acts as the main (if not the only) partner of the activated receptor. It has no enzymatic activity and serves as a binding site for a number of signalling target molecules for the insulin receptor. Thus, IRS literally performs a “transducing” function, and without it, a signal from the receptor does not pass into the cell.

Membrane interactions are the second feature of insulin signalling. They are provided by the presence of special structures (PH, PX, and FYVE domains) in participant proteins that bind phosphatidylinositols, membrane phospholipids that perform the alarm functions. IRS contains the PH (pleckstrin homology) domain at the N-terminus, which is anchored on the membrane near the receptor. Upon the binding of insulin, the receptor phosphorylates itself on tyrosine residues, and these residues are recognised by the phosphotyrosine binding (PTB) domain located at the C-terminus of the IRS protein. Furthermore, the receptor phosphorylates IRS on the C-terminal tyrosine residues, after which they bind to SH2 and PTB-domains of effector proteins from the cytosol. These proteins include PI3-kinase, phosphotyrosine phosphatase 2 (SHP-2), and adapter proteins Shc or Grb2. The latter activate small Ras GTPase and the Erk cascade of MAP kinases, which triggers cell division and differs from the stress-dependent cascade of JNK MAP kinases.

PI3-kinase, activated by binding to IRS, phosphorylates the third position of the inositol ring of phosphatidylinositol biphosphate (PIP<sub>2</sub>), forming phosphatidylinositol trisphosphate (hence, the enzyme name is phosphatidylinositol 3-kinase). This event is of fundamental importance because most PH domains recognise phosphate exactly at the 3-position of the inositol ring. Akt has this PH domain; through that domain, Akt is recruited onto the membrane. A similar domain is present in phosphoinositide-dependent protein kinase PDK1, which also binds to the membrane and phosphorylates Akt on the

Thr308 residue within the activation loop. This is how the receptor-dependent activation of Akt occurs [47].

An additional level of complexity in the PI3-kinase cascade arises because one Thr308 phosphorylation is not sufficient for Akt to become active. Another phosphorylation on the Thr<sup>473</sup> residue is required for this (Fig. 2). For a long time, the significance of this process and the enzyme catalysing it were unknown. Only relatively recently did become clear that this enzyme is mTOR kinase within a second protein complex, mTORC2, and this very phosphorylation is a necessary, but not sufficient, condition for Akt activation. This second phosphorylation occurs in the background mode, but Thr<sup>308</sup> phosphorylation has no effect without it [48, 50]. In other words, the mTORC2 complex “primes” Akt to activation by insulin in the context of the PI3-kinase cascade. Given that Akt activates the first TORC1 complex and thus triggers protein synthesis, adipogenesis, and the mechanism of cell survival under adverse conditions [49], the regulatory control within the signalling module is very confusing and difficult to interpret [51]. At the moment, many questions remain open and the situation is further complicated by the presence of numerous feedback mechanisms and the central role of mTOR in the regulation of cell metabolism [52].

Activated Akt phosphorylates more than 100 substrates and regulates nearly all vital functions of the cell, including metabolism, growth, mobility, division, survival, and cell death [49]. Acting via mTORC1, Akt activates ribosomal S6 kinase 1 (S6K1) and suppresses the 4E-BP inhibitor of the eukaryotic translation initiation factor. Therefore, mTorC1 stimulates protein synthesis and cell growth and division (Fig. 2). mTorC1 is involved in both the regulation of lipid metabolism and cholesterol homeostasis, controlling phosphorylation of the transcription factors SREBP1 and PPAR $\gamma$  as well as lipin 1, which regulates triglyceride delivery to fat droplets of adipocytes [52].

Akt phosphorylates and activates AS160, a guanine nucleotide exchange factor of small GTPases in the Rab family (Fig. 2) [10]. These proteins regulate the intracellular transport of vesicles, and Rab10 is responsible for the fusion of GLUT4 containing vesicles with the cell membrane [53]. In this way, Akt triggers exposure of the glucose transporter and glucose transport into adipocytes and myocytes. In myocytes, glucose is phosphorylated and directed to glycogen synthesis, while it is used for lipogenesis to form triacylglycerides in adipocytes.

Akt also phosphorylates the transcription factor FOXO1, which activates PPAR- $\gamma$ , a master regulator of adipose differentiation (Fig. 2). In this way, Akt triggers adipogenesis and increases the number of adipocytes in the adipose tissue [54]. Thus, acting through Akt, insulin stimulates adipogenesis and lipogenesis, leading to lipid accumulation in cells. To avoid hyperactivation of the cascade and ectopic lipid accumulation, cells use the feedback mechanisms aimed at temporarily uncoupling the receptor and signal transducing chain. The next section discusses how the permanent activation of this feedback leads to IR development.

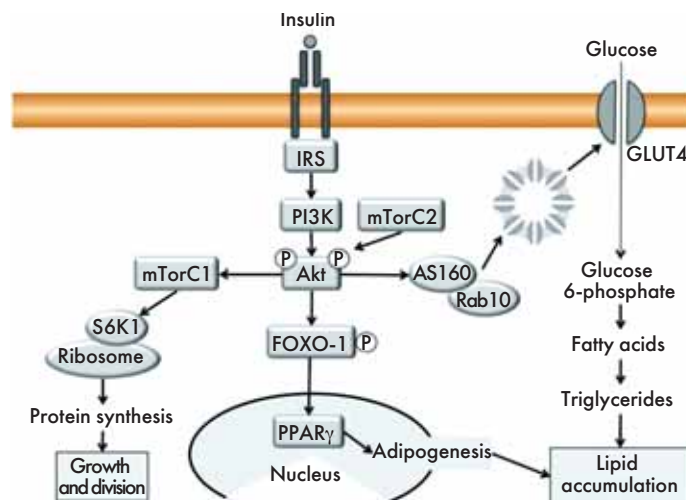


Figure 2. Signalling from the insulin receptor through the PI3-kinase cascade in adipose tissue and skeletal muscle cells. A general scheme is presented. Other target tissues of insulin lack the glucose transporter and the right signal branch via AS160/Rab10. Signalling through PPAR $\gamma$  is presented for adipocytes and may have its own features in other cells. See the main text for details.

## INTRACELLULAR MECHANISM OF INSULIN RESISTANCE DEVELOPMENT

The intracellular basis of IR is believed to be a disturbance in the signalling from the insulin receptor, which uncouples the insulin action and the corresponding cell response. It is now practically assured that this uncoupling occurs at the level of an insulin receptor substrate, the IRS protein. Insulin resistant cells are characterised by increased phosphorylation of IRS on serine residues, preventing the tyrosine phosphorylation of IRS that is required for signal transduction from the receptor into the cell. Thus, IR development is associated with the serine phosphorylation of the IRS protein.

As noted above, the physiological causes of IR are diverse; its target tissues (adipose tissue, muscles, and liver) have different physiology and times to IR development. For these reasons, in *different* cells and in *different* ways, the single mechanism of uncoupling insulin signalling is triggered by serine phosphorylation of the IRS protein, which has tissue-specific features [55]. First, the different physiological mechanisms activate the different signalling cascades in the cell, which similarly converge on the serine phosphorylation of IRS (see Fig. 1). Second, different protein kinases are responsible for this phosphorylation, but each of them phosphorylates one or more well-defined residues in IRS. Third, IRS has many serine residues that disturb insulin signal transduction. Fourth, each insulin target tissue has its own set of phosphorylated residues, which is specified by the physiological features and mechanisms of the IR development in a given tissue.

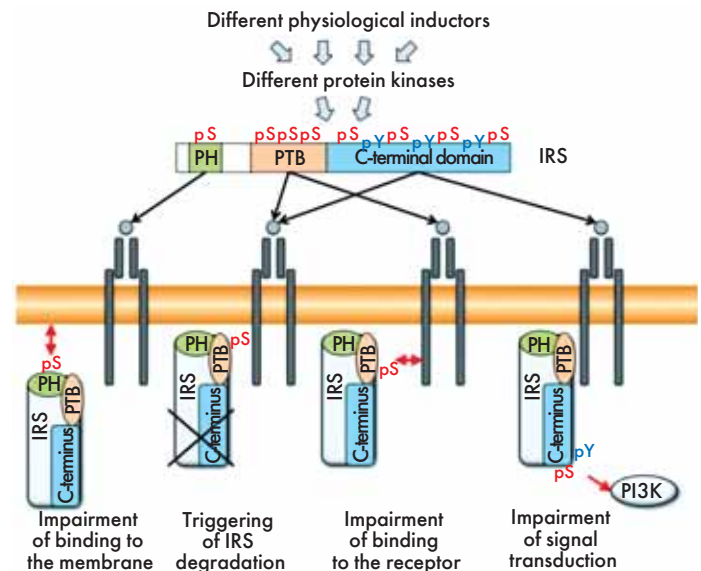
Theoretically, the distribution of phosphorylated residues in IRS and the involved protein kinases allow one to determine the intracellular cascades that mediate IR development. In turn, these cascades enable the establishment of a physiological mechanism of the

development and, in fact, the root cause of IR. For example, insulin and the insulin-like growth factor (IGF-1), as well as amino acids, act through the insulin-dependent signalling cascade with the involvement of Akt and the mTORC1 complex, causing phosphorylation of Ser<sup>302</sup> and Ser<sup>632/635</sup> in mouse IRS and Ser<sup>307</sup> and Ser<sup>636/639</sup> in human IRS [23]. At the same time, interleukins and TNF $\alpha$  mediate the inflammatory effects and cause phosphorylation of Ser<sup>307</sup> in mouse cells and Ser<sup>312</sup> in humans. The activation of different PKC isoforms ( $\delta$ ,  $\epsilon$ ,  $\theta$ , or  $\zeta$ ) occurs in dyslipidaemia and ectopic obesity even if the latter is not manifested visually. Each isoform of PKC causes phosphorylation of its own set of residues in IRS; however, Ser<sup>1101</sup> is preferably modified (in human IRS). This feature of the mechanisms of the IR pathogenesis enables determination of the aetiology of IR and its early diagnosis using the phosphorylation profile of a single protein (IRS). Recent studies have confirmed the key role of IRS phosphorylation in IR development as well as the considerably detailed the “molecular profile” of IRS [55].

At the molecular level, the phosphorylation of IRS on serine residues has four consequences that in different ways impair the transducing function of this protein (Fig. 3). In the first case, phosphorylation impairs binding of the PH domain of IRS to the membrane, diverting IRS from the receptor. In the second and third cases, phosphorylation within the PTB domain or near it causes either dissociation of IRS from the receptor or degradation of IRS. Finally, phosphorylation within the C-terminal domain disturbs the binding of IRS to effectors and signal transduction into the cell [53, 55]. However, in any case, different physiological inducers and mechanisms involve different kinases inside the cell, causing phosphorylation of IRS on different residues. These intracellular mechanisms are summarised in Fig. 4 and examined below in more detail.

**Overnutrition and low physical activity** reduce the activity of AMPK and, as a consequence, activate mTORC1. One of the main targets of mTORC1 is ribosomal kinase S6K1; its task includes the phosphorylation of the S6 component of the small ribosomal subunit and the initiation of protein synthesis (translation). However, apart from this, S6K1 phosphorylates IRS1 on several serine residues, interrupting its transduction functions [52, 56]. This intracellular feedback mechanism in insulin signalling (see Fig. 4) is believed to be key to the development of IR, obesity, and type 2 diabetes; it also plays an important role in tumour progression [57].

There are several options for the insulin-independent activation of Akt/mTORC1, such as when the feedback works intensively and the phosphorylation of IRS is maintained even upon impaired signal transduction from the receptor to Akt (Fig. 4). First, alternative Akt activation is possible due to phosphorylation of the Ser<sup>473</sup> residue in its hydrophobic domain. This reaction is catalysed by the same mTOR kinase, while belonging to the second complex (mTORC2) [48]. The mechanism of mTORC2 activation is not completely understood, but it is known to be triggered by growth factor receptors. They stimulate the production of



**Figure 3.** Four main consequences of IRS phosphorylation on serine residues (pS) leading to a decrease in the insulin sensitivity and to the development of IR. The IRS protein contains the pleckstrin homology (PH) domain at the N-terminus and phosphotyrosine binding (PTB) domain in the middle. The PH domain provides binding of IRS to the membrane near the receptor that is impaired in the case of serine phosphorylation within the PH domain. The PTB domain is responsible for the binding of IRS to the activated receptor that phosphorylates the C-terminal domain of IRS on tyrosine residues (pY), initiating signalling from the receptor into the cell. Serine phosphorylation of some residues within the PTB domain accelerates the degradation of IRS, and the phosphorylation of others impairs the interaction of the PTB domain with the receptor. Finally, the phosphorylation of serines within the C-terminal domain interrupts the signal transduction to PI3-kinase, the main target of IRS. Quoted from [55] (with some modifications).

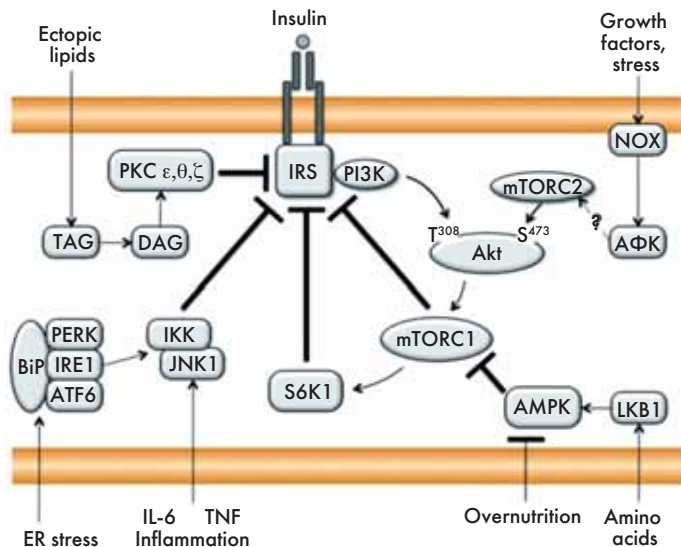
intracellular reactive oxygen species (ROS) that enhance the phosphorylation [58] and activation of Akt [59].

Another method for insulin-independent activation of mTORC1 is turning off its inhibitor, AMPK kinase. This occurs with overnutrition, accumulation of ATP in the cell, and a corresponding reduction in AMP [7]. mTORC1 activation also occurs during inflammation [41] and ER stress [44]. Thus, almost all physiological mechanisms of the IR development, to some extent, converge on the activation of mTORC1 and its immediate target, S6K1. mTORC1 and S6K1 directly phosphorylate a set of serine residues in the IRS protein (see Fig. 3), impairing insulin signal transduction.

S6K1 gene knockout mice have an increased sensitivity to insulin and are less prone to obesity associated with age or caused by a fat diet [56]. It remains unclear in which tissue this mechanism is realised in humans. Taking into account the data obtained from patients with the non-alcoholic liver disease [22], it is possible that this mechanism is primarily typical of the adipose tissue in humans because the lipid-dependent mechanism involving PKC is primarily active in the liver and muscles.

**A lipid-dependent mechanism** has received considerable





**Figure 4.** A general scheme of the intracellular mechanism of IR development due to turning the IRS protein off. Different ways for determining a single mechanism that correspond to the different physiological mechanisms of IR development are demonstrated. All pathways leading to serine phosphorylation and turning IRS off are summarised without details independent of the cell type. See the text for details.

experimental justification. It postulates that protein kinase C (PKC) is responsible for turning IRS off in liver and skeletal muscle cells. In this case, PKC itself does not phosphorylate IRS1/2; it only initiates a feedback cascade that leads to serine-threonine phosphorylation of IRS1/2 [1]. This function is performed by PKC- $\theta$  in muscles and by PKC- $\epsilon$  in the liver. Both isoforms belong to a group of “new” PKCs, which do not require  $\text{Ca}^{2+}$  ions for activation, but which are considerably more sensitive to diacylglycerol than classical PKC isoforms. These properties may, at least in part, explain the association of IR with obesity. The “new” PKC isoforms induce IR in the liver and muscles without involving insulin and its receptor because, independent of insulin, they are activated by ectopic lipid products (Fig. 4). There is also evidence that the same function is performed by PKC- $\zeta$  [60]. However, its relationship to obesity remains unclear because PKC- $\zeta$  refers to the “atypical” PKC isoforms and does not use  $\text{Ca}^{2+}$  and diacylglycerol as activators [21]. According to another viewpoint, PKC- $\zeta$  is involved in the ceramide-dependent mechanism of turning IRS off (see below).

The lipid-dependent mechanism has also been confirmed in animal models. Mice lacking PKC- $\theta$  did not develop fast IR with a massive infusion of lipids [61]. Similarly, PKC $\epsilon$  knockout mice did not develop IR during a high-calorie diet, although the lipid level in the liver was increased [62]. PK-C $\delta$  is another PKC isoform that is associated with IR development in the liver. Turning PK-C $\delta$  off leads to a reduction in the lipid level in the liver and an increase in the sensitivity to glucose, whereas PK-C $\delta$  overexpression causes the development of IR and hepatic steatosis [63, 64].

The ceramide-dependent mechanism is an exception to

the general rule because it is aimed not at IRS but at inhibiting Akt. Synthesis of ceramides is thought to be triggered by IKK kinase in the context of the NF- $\kappa$ B signalling cascade, which is activated by the Toll-like receptor 4 (TLR4) [65] or by the TNF $\alpha$  receptor. Thus, the formation of ceramides may be part of the cellular inflammatory response. Some researchers believe that saturated fatty acids are TLR4 ligands that cause the IR development during hyperlipidaemia [66]. Ceramides affect Akt2 activity in two ways. One is associated with the activation of PK-C $\zeta$  that then interacts with Akt and removes it from the insulin cascade [67]. In the other variant, ceramides activate PP2A phosphatase, which dephosphorylates and inactivates Akt [68]. In any case, ceramides reduce Akt activity, impairing signal transduction from the insulin receptor. It should be noted that the ceramide-dependent mechanism was discovered and studied in a cell model of IR, but it has not been confirmed yet in animal models.

**Inflammation** is almost always associated with activation of two intracellular signalling cascades; these cascades mediate the inflammatory response and phosphorylation of IRS1 (Fig. 4). The NF- $\kappa$ B cascade is associated with the activation of IKK kinase, which directly phosphorylates IRS or acts indirectly by triggering the synthesis of ceramides (see above). Another cascade initiates stress-dependent MAP kinase JNK1, which directly phosphorylates IRS [33].

IKK $\epsilon$  knockout mice have an increased catabolic status and a significantly reduced volume of the adipose tissue, and they do not develop IR [69]. Mice deficient in JNK1 have a similar phenotype [70]. Knockout of the *JNK1* gene in the adipose tissue protects mice from the development of steatosis and the hepatic form of IR, although they normally gain weight and their volume of adipose tissue increases [71]. These mice had inflammation in the adipose tissue and moderate steatosis, although the insulin sensitivity in the liver and adipose tissue was unchanged.

**ER stress (the UPR mechanism)** is triggered when the ER is overloaded with synthesised or misfolded proteins. ER stress is mediated by three signalling modules (Fig. 4) that are physically associated with the ER membrane [41, 42]. First, is PERK kinase, which phosphorylates and inhibits the eukaryotic translation initiation factor 2a, suppressing protein synthesis. Second, is inositol-dependent kinase IRE1, which possesses endoribonuclease activity. It activates unconventional mRNA splicing of the XBP1 transcription factor and its translation [44]; in turn, XBP1 triggers the expression of chaperones and proteasomal proteins that are required to overcome ER stress. Third, is the ATF6 transcription factor; it is activated by partial proteolysis, and its cytosolic fragment is translocated to the nucleus, where it initiates expression of ER chaperones. PERK, IRE1, and ATF6 form an inactive complex with the BiP/GRP78 (HSP70 or HSPA5) chaperone. BiP acts as a metabolic sensor of the UPR system; its expression is enhanced by glucose starvation. When the UPR is activated, BiP leaves the complex with PERK, IRE1, and ATF6, which leads to the activation of these signalling modules. The terminal target of at least one of them (IRE1) is MAP kinase JNK1



phosphorylating IRS [45, 72]. Thus, the stress-dependent and inflammatory pathways use a single agent, JNK1, for IR development.

**Oxidative stress** and mitochondrial dysfunction lead to an increased production of free radical molecules and, in particular, reactive oxygen species (ROS). On one hand, ROS activate ER stress [44] in an apparently PERK-dependent manner [73]. As noted above, JNK1 is activated as a result, serine phosphorylation of IRS occurs, and the insulin cascade is impaired. On the other hand, ROS support the insulin signalling and improve insulin sensitivity in adipocytes [59]. These conflicting data suggested the so-called “redox paradox” in the insulin signalling [74].

The redox paradox is resolved if different sources and a significantly higher intracellular ROS level upon oxidative stress are taken into account. In the case of stress, a ROS excess triggers the JNK1-dependent mechanism characteristic of inflammation and ER stress. This leads to the serine phosphorylation of IRS, uncoupling insulin signalling, and a reduction in Akt activation. In contrast, in the absence of stress, physiological activation of growth factor receptors stimulates the assembly of NADPH oxidase complexes (NOX) as well as controlled ROS production (Fig. 4). These ROS act locally in small quantities. Acting as second messengers, they enhance the signal transduction from receptors into the cell [75]. In this way, they provide complete phosphorylation and activation of Akt in adipocytes [58, 59]. In the near future, studies should demonstrate whether the ROS action is related to the redox-dependent activation of mTORC2 or not (see Fig. 4).

## PERSPECTIVES OF THE MOLECULAR DIAGNOSIS AND THERAPY OF INSULIN RESISTANCE

In recent years, significant progress has been achieved in understanding the mechanisms of insulin resistance in target tissues. The liver is most likely to be the first target of IR. Ectopic lipid accumulation in hepatocytes promotes the rapid development of the pathology in the muscles, and the adipose tissue gradually changes. The hypothalamic-pituitary system coordinates these processes and can participate in them starting at the earliest stages. The high-calorie diet and lack of physical activity as well as the presence of stress and inflammation significantly exacerbate the pathogenesis of IR, eventually leading to the development of metabolic syndrome, type 2 diabetes, hepatocellular and cardiovascular pathologies.

The single molecular mechanism of insulin resistance development associated with the serine phosphorylation of IRS has become a crucial piece of knowledge in recent years. This means that an increase in the IRS phosphorylation level may serve as an indicator and method of diagnosing IR early. Further, the localisation of phosphorylated residues in the IRS molecule may indicate physiological causes of the IR development and enable the selection of appropriate therapy for the patient. Further progress in this area will undoubtedly be associated with the development of new drugs that target the activity of intracellular enzymes, providing, either directly or indirectly, phosphorylation of IRS. This is a completely new field of pharmacology that investigates at a deeper cellular level than the current approaches that are traditionally aimed at the hormone-receptor interactions outside of cells. It certainly requires a thorough solution of many problems, both applied and basic, including targeted delivery and the specificity of pharmacological agents.

The opportunity of IRS “molecular profiling” in tissue biopsies is promising as a part of personalised medicine. It is furnished with the modern means of high-performance analysis, such as mass spectrometry, and enables the identification of a set of residues that are phosphorylated in IRS in people with metabolic syndrome or diabetes. This knowledge provides information on the availability and aetiology of IR in a given patient as well as allows for the differential diagnosis of comorbidities and permanent correction of our current ideas about the molecular mechanisms of IR development.

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## REFERENCES

- Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *The Lancet*. 2010;375(9733):2267–2277. DOI: [http://dx.doi.org/10.1016/S0140-6736\(10\)60408-4](http://dx.doi.org/10.1016/S0140-6736(10)60408-4)
- Isganaitis E, Lustig RH. Fast Food, Central Nervous System Insulin Resistance, and Obesity. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2005;25(12):2451–2462. DOI: <http://dx.doi.org/10.1161/01.ATV.0000186208.06964.91>
- Grayson BE, Seeley RJ, Sandoval DA. Wired on sugar: the role of the CNS in the regulation of glucose homeostasis. *Nat Rev Neurosci*. 2013;14(1):24–37. DOI: <http://dx.doi.org/10.1038/nrn3409>
- Flier J. Obesity Wars Molecular Progress Confronts an Expanding Epidemic. *Cell*. 2004;116(2):337–350. DOI: [http://dx.doi.org/10.1016/S0092-8674\(03\)01081-X](http://dx.doi.org/10.1016/S0092-8674(03)01081-X)
- Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444(7121):860–867. DOI: <http://dx.doi.org/10.1038/nature05485>
- Schwartz MW, Seeley RJ, Tschöp MH, Woods SC, Morton GJ, Myers MG, et al. Cooperation between brain and islet in glucose homeostasis and diabetes. *Nature*. 2013;503(7474):59–66. DOI: <http://dx.doi.org/10.1038/nature12709>
- Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev Mol Cell Biol*. 2012;13(4):251–262. DOI: <http://dx.doi.org/10.1038/nrm3311>
- Ghillebert R, Swinnen E, Wen J, Vandesteene L, Ramon M, Norga K, et al. The AMPK/SNF1/SnRK1 fuel gauge and energy regulator: structure, function and regulation. *FEBS Journal*. 2011;278(21):3978–3990. DOI: <http://dx.doi.org/10.1111/j.1742-4658.2011.08315.x>
- Wullschlegel S, Loewith R, Hall MN. TOR Signaling in Growth and Metabolism. *Cell*. 2006;124(3):471–484. DOI: <http://dx.doi.org/10.1016/j.cell.2006.01.016>
- Watson RT, Pessin JE. Bridging the GAP between insulin signaling and GLUT4 translocation. *Trends in Biochemical Sciences*. 2006;31(4):215–222. DOI: <http://dx.doi.org/10.1016/j.tibs.2006.02.007>
- Kadowaki T, Yamauchi T. Adiponectin Receptor Signaling: A New Layer to the Current Model. *Cell Metabolism*. 2011;13(2):123–124. DOI: <http://dx.doi.org/10.1016/j.cmet.2011.01.012>
- Goldfine AB, Kahn CR. Adiponectin: linking the fat cell to insulin sensitivity. *The Lancet*. 2003;362(9394):1431–1432. DOI: [http://dx.doi.org/10.1016/S0140-6736\(03\)14727-7](http://dx.doi.org/10.1016/S0140-6736(03)14727-7)
- Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med*. 2010;17(1):55–63. DOI: <http://dx.doi.org/10.1038/nm.2277>
- Oppert J, Nadeau A, Tremblay A, Després J, Thériault G, Dériaz O, et al. Plasma glucose, insulin, and glucagon before and after long-term overfeeding in identical twins. *Metabolism*. 1995;44(1):96–105. DOI: [http://dx.doi.org/10.1016/0026-0495\(95\)90295-3](http://dx.doi.org/10.1016/0026-0495(95)90295-3)
- Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes*. 1999;48(4):839–847. DOI: <http://dx.doi.org/10.2337/diabetes.48.4.839>
- Snel M, Jonker JT, Schoones J, Lamb H, Roos A, de Pijl H, et al. Ectopic Fat and Insulin Resistance: Pathophysiology and Effect of Diet and Lifestyle Interventions. *International Journal of Endocrinology*. 2012;2012:1–18. DOI: <http://dx.doi.org/10.1155/2012/983814>
- Fabbrini E, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proceedings of the National Academy of Sciences*. 2009;106(36):15430–15435. DOI: <http://dx.doi.org/10.1073/pnas.0904944106>
- Mizuno TM, Funabashi T, Kleopoulos SP, Mobbs CV. Specific preservation of biosynthetic responses to insulin in adipose tissue may contribute to hyperleptinemia in insulin-resistant obese mice. *J Nutr*. 2004;134(5):1045–1050.
- Kitamura T, Kahn CR, Accili D. Insulin receptor knockout mice. *Annu. Rev. Physiol*. 2003;65(1):313–332. DOI: <http://dx.doi.org/10.1146/annurev.physiol.65.092101.142540>
- McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis*. 2004;8(3):521–533. DOI: <http://dx.doi.org/10.1016/j.cld.2004.04.004>
- Steinberg SF. Structural Basis of Protein Kinase C Isoform Function. *Physiological Reviews*. 2008;88(4):1341–1378. DOI: <http://dx.doi.org/10.1152/physrev.00034.2007>
- Kumashiro N, Erion DM, Zhang D, Kahn M, Beddow SA, Chu X, et al. Cellular mechanism of insulin resistance in non-alcoholic fatty liver disease. *Proceedings of the National Academy of Sciences*. 2011;108(39):16381–16385. DOI: <http://dx.doi.org/10.1073/pnas.1113359108>
- Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*. 2006;55(Suppl 2):9–15. DOI: <http://dx.doi.org/10.2337/db06-S002>
- Yu C, Chen Y, Cline GW, Zhang D, Zong H, Wang Y, et al. Mechanism by Which Fatty Acids Inhibit Insulin Activation of Insulin Receptor Substrate-1 (IRS-1)-associated Phosphatidylinositol 3-Kinase Activity in Muscle. *Journal of Biological Chemistry*. 2002;277(52):50230–50236. DOI: <http://dx.doi.org/10.1074/jbc.M200958200>
- Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, et al. Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J. Clin. Invest*. 1999;103(2):253–259. DOI: <http://dx.doi.org/10.1172/JCI5001>
- Krassak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a <sup>1</sup>H NMR spectroscopy study. *Diabetologia*. 1999;42(1):113–116. DOI: <http://dx.doi.org/10.1007/s001250051123>
- Iochida LC, Tominaga M, Matsumoto M, Sekikawa A, Sasaki H. Insulin resistance in septic rats – a study by the euglycemic clamp technique. *Life Sciences*. 1989;45(17):1567–1573. DOI: [http://dx.doi.org/10.1016/0024-3205\(89\)90423-2](http://dx.doi.org/10.1016/0024-3205(89)90423-2)
- Shangraw RE, Jahoor F, Miyoshi H, Neff WA, Stuart CA, Herndon DN, et al. Differentiation between septic and post-burn insulin resistance. *Metabolism*. 1989;38(10):983–989. DOI: [http://dx.doi.org/10.1016/0026-0495\(89\)90010-3](http://dx.doi.org/10.1016/0026-0495(89)90010-3)
- Pickup JC, Crook MA. Is Type II diabetes mellitus a disease of the innate immune system. *Diabetologia*. 1998;41(10):1241–1248. DOI: <http://dx.doi.org/10.1007/s001250051058>
- Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is inter-

- leukin-6 the link. *Atherosclerosis*. 2000;148(2):209–214. DOI: [http://dx.doi.org/10.1016/S0021-9150\(99\)00463-3](http://dx.doi.org/10.1016/S0021-9150(99)00463-3)
31. Hotamisligil G, Shargill N, Spiegelman B. Adipose expression of tumor necrosis factor- $\alpha$ : direct role in obesity-linked insulin resistance. *Science*. 1993;259(5091):87–91. DOI: <http://dx.doi.org/10.1126/science.7678183>
  32. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor- $\alpha$  in human obesity and insulin resistance. *J. Clin. Invest.* 1995;95(5):2409–2415. DOI: <http://dx.doi.org/10.1172/JCI117936>
  33. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH2-terminal Kinase Promotes Insulin Resistance during Association with Insulin Receptor Substrate-1 and Phosphorylation of Ser307. *Journal of Biological Chemistry*. 2000;275(12):9047–9054. DOI: <http://dx.doi.org/10.1074/jbc.275.12.9047>
  34. Tantiwong P, Shanmugasundaram K, Monroy A, Ghosh S, Li M, DeFronzo RA, et al. NF- $\kappa$ B activity in muscle from obese and type 2 diabetic subjects under basal and exercise-stimulated conditions. *Am J Physiol Endocrinol Metab*. 2010;299(5):794–801. DOI: <http://dx.doi.org/10.1152/ajpendo.00776.2009>
  35. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 2003;112(12):1796–1808. DOI: <http://dx.doi.org/10.1172/JCI19246>
  36. Wolsk E, Mygind H, Grondahl TS, Pedersen BK, van Hall G. IL-6 selectively stimulates fat metabolism in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2010;299(5):832–840. DOI: <http://dx.doi.org/10.1152/ajpendo.00328.2010>
  37. Bézaire V, Langin D. Regulation of adipose tissue lipolysis revisited. *Proc. Nutr. Soc.* 2009;68(04):350–360. DOI: <http://dx.doi.org/10.1017/S0029665109990279>
  38. Ranjit S, Boutet E, Gandhi P, Prot M, Tamori Y, Chawla A, et al. Regulation of fat specific protein 27 by isoproterenol and TNF- $\alpha$  to control lipolysis in murine adipocytes. *The Journal of Lipid Research*. 2011;52(2):221–236. DOI: <http://dx.doi.org/10.1194/jlr.M008771>
  39. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *Journal of Clinical Investigation*. 2006;116(6):1494–1505. DOI: <http://dx.doi.org/10.1172/JCI26498>
  40. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J. Clin. Invest.* 2006;116(1):115–124. DOI: <http://dx.doi.org/10.1172/JCI24335>
  41. Hotamisligil GS. Endoplasmic Reticulum Stress and the Inflammatory Basis of Metabolic Disease. *Cell*. 2010;140(6):900–917. DOI: <http://dx.doi.org/10.1016/j.cell.2010.02.034>
  42. Rutkowski DT, Hegde RS. Regulation of basal cellular physiology by the homeostatic unfolded protein response. *The Journal of Cell Biology*. 2010;189(5):783–794. DOI: <http://dx.doi.org/10.1083/jcb.201003138>
  43. Cullinan SB, Diehl JA. Coordination of ER and oxidative stress signaling: The PERK/Nrf2 signaling pathway. *The International Journal of Biochemistry & Cell Biology*. 2006;38(3):317–332. DOI: <http://dx.doi.org/10.1016/j.biocel.2005.09.018>
  44. Appenzeller-Herzog C, Hall MN. Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. *Trends in Cell Biology*. 2012;22(5):274–282. DOI: <http://dx.doi.org/10.1016/j.tcb.2012.02.006>
  45. Ozcan U, Cao Q, Yilmaz E, Lee A, Iwakoshi NN, Ozdelen E, et al. Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes. *Science*. 2004;306(5695):457–461. DOI: <http://dx.doi.org/10.1126/science.1103160>
  46. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO. Chemical Chaperones Reduce ER Stress and Restore Glucose Homeostasis in a Mouse Model of Type 2 Diabetes. *Science*. 2006;313(5790):1137–1140. DOI: <http://dx.doi.org/10.1126/science.1128294>
  47. Hawkins PT, Anderson KE, Davidson K, Stephens LR. Signalling through Class I PI3Ks in mammalian cells. *Biochem Soc Trans.* 2006;34(Pt 5):647–662. DOI: <http://dx.doi.org/10.1042/BST0340647>
  48. Pearce LR, Komander D, Alessi DR. The nuts and bolts of AGC protein kinases. *Nat Rev Mol Cell Biol*. 2010;11(1):9–22. DOI: <http://dx.doi.org/10.1038/nrm2822>
  49. Manning BD, Cantley LC. AKT/PKB Signaling: Navigating Downstream. *Cell*. 2007;129(7):1261–1274. DOI: <http://dx.doi.org/10.1016/j.cell.2007.06.009>
  50. Alessi DR, Pearce LR, Garcia-Martinez JM. New insights into mTOR signaling: mTORC2 and beyond. *Sci Signal* 2009;2(67):27–10. DOI: <http://dx.doi.org/10.1126/scisignal.267pe27>
  51. Bhaskar PT, Hay N. The Two TORCs and Akt. *Developmental Cell*. 2007;12(4):487–502. DOI: <http://dx.doi.org/10.1016/j.devcel.2007.03.020>
  52. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol*. 2010;12(1):21–35. DOI: <http://dx.doi.org/10.1038/nrm3025>
  53. Leto D, Saltiel AR. Regulation of glucose transport by insulin: traffic control of GLUT4. *Nat Rev Mol Cell Biol*. 2012;13(6):383–396. DOI: <http://dx.doi.org/10.1038/nrm3351>
  54. Penkov DN, Egorov AD, Mozgovaya MN, Tkachuk VA. Insulin resistance and adipogenesis: Role of transcription and secreted factors. *Biochemistry Moscow*. 2013;78(1):8–18. DOI: <http://dx.doi.org/10.1134/S0006297913010021>
  55. Boura-Halfon S, Zick Y. Phosphorylation of IRS proteins, insulin action, and insulin resistance. *Am J Physiol Endocrinol Metab*. 2009;296(4):581–591. DOI: <http://dx.doi.org/10.1152/ajpendo.90437.2008>
  56. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature*. 2004;431(7005):200–205. DOI: <http://dx.doi.org/10.1038/nature02866>
  57. Dann SG, Selvaraj A, Thomas G. mTOR Complex 1–S6K1 signaling: at the crossroads of obesity, diabetes and cancer. *Trends in Molecular Medicine*. 2007;13(6):252–259. DOI: <http://dx.doi.org/10.1016/j.molmed.2007.04.002>
  58. Tyurin-Kuzmin PA, Morozov YI, Sukhova AA, Sagaradze GD, Zdanovskaya ND, Agaronian KM, et al. Differences in the effects of PDGF and EGF on migration and mitotic activity of NIH-3T3 fibroblasts are due to redox dependent phosphorylation of PKB/Akt, but not Erk1/2. 2014 (in press).
  59. Mahadev K, Wu X, Zilbering A, Zhu L, Lawrence JT, Goldstein BJ. Hydrogen Peroxide Generated during Cellular Insulin Stimulation Is Integral to Activation of the Distal Insulin Signaling Cascade in 3T3-L1 Adipocytes. *Journal of Biological Chemistry*. 2001;276(52):48662–48669. DOI: <http://dx.doi.org/10.1074/jbc.M105061200>
  60. Ravichandran LV, Esposito DL, Chen J, Quon MJ. Pro-



- tein Kinase C-zeta Phosphorylates Insulin Receptor Substrate-1 and Impairs Its Ability to Activate Phosphatidylinositol 3-Kinase in Response to Insulin. *Journal of Biological Chemistry*. 2001;276(5):3543–3549. DOI: <http://dx.doi.org/10.1074/jbc.M007231200>
61. Kim JK, Fillmore JJ, Sunshine MJ, Albrecht B, Higashimori T, Kim D, et al. PKC- $\theta$  knockout mice are protected from fat-induced insulin resistance. *J. Clin. Invest.* 2004;114(6):823–827. DOI: <http://dx.doi.org/10.1172/JCI200422230>
  62. Raddatz K, Turner N, Frangioudakis G, Liao BM, Pedersen DJ, Cantley J, et al. Time-dependent effects of Prkce deletion on glucose homeostasis and hepatic lipid metabolism on dietary lipid oversupply in mice. *Diabetologia*. 2011;54(6):1447–1456. DOI: <http://dx.doi.org/10.1007/s00125-011-2073-0>
  63. Bezy O, Tran TT, Pihlajamäki J, Suzuki R, Emanuelli B, Winnay J, et al. PKC $\delta$  regulates hepatic insulin sensitivity and hepatosteatosis in mice and humans. *J. Clin. Invest.* 2011;121(6):2504–2517. DOI: <http://dx.doi.org/10.1172/JCI46045>
  64. Frangioudakis G, Burchfield JG, Narasimhan S, Cooney GJ, Leitges M, Biden TJ, et al. Diverse roles for protein kinase C delta and protein kinase C epsilon in the generation of high-fat-diet-induced glucose intolerance in mice: regulation of lipogenesis by protein kinase C delta. *Diabetologia*. 2009;52(12):2616–2620. DOI: <http://dx.doi.org/10.1007/s00125-009-1543-0>
  65. Holland WL, Bikman BT, Wang L, Yuguang G, Sargent KM, Bulchand S, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J. Clin. Invest.* 2011;121(5):1858–1870. DOI: <http://dx.doi.org/10.1172/JCI43378>
  66. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J. Clin. Invest.* 2006;116(11):3015–3025. DOI: <http://dx.doi.org/10.1172/JCI28898>
  67. Powell DJ, Hajduch E, Kular G, Hundal HS. Ceramide disables 3-phosphoinositide binding to the pleckstrin homology domain of protein kinase B (PKB)/Akt by a PKCzeta-dependent mechanism. *Molecular and Cellular Biology*. 2003;23(21):7794–7808. DOI: <http://dx.doi.org/10.1128/MCB.23.21.7794-7808.2003>
  68. Teruel T, Hernandez R, Lorenzo M. Ceramide mediates insulin resistance by tumor necrosis factor-alpha in brown adipocytes by maintaining Akt in an inactive dephosphorylated state. *Diabetes*. 2001;50(11):2563–2571. DOI: <http://dx.doi.org/10.2337/diabetes.50.11.2563>
  69. Chiang S, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, et al. The protein kinase IKKepsilon regulates energy balance in obese mice. *Cell*. 2009;138(5):961–975. DOI: <http://dx.doi.org/10.1016/j.cell.2009.06.046>
  70. Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, et al. A central role for JNK in obesity and insulin resistance. *Nature*. 2002;420(6913):333–336. DOI: <http://dx.doi.org/10.1038/nature01137>
  71. Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ, et al. A Stress Signaling Pathway in Adipose Tissue Regulates Hepatic Insulin Resistance. *Science*. 2008;322(5907):1539–1543. DOI: <http://dx.doi.org/10.1126/science.1160794>
  72. Tuncman G, Hirosumi J, Solinas G, Chang L, Karin M, Hotamisligil GS. Functional in vivo interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. *Proceedings of the National Academy of Sciences*. 2006;103(28):10741–10746. DOI: <http://dx.doi.org/10.1073/pnas.0603509103>
  73. Liu L, Wise DR, Diehl JA, Simon MC. Hypoxic Reactive Oxygen Species Regulate the Integrated Stress Response and Cell Survival. *Journal of Biological Chemistry*. 2008;283(45):31153–31162. DOI: <http://dx.doi.org/10.1074/jbc.M805056200>
  74. Goldstein BJ, Mahadev K, Wu X. Redox Paradox: Insulin Action Is Facilitated by Insulin-Stimulated Reactive Oxygen Species With Multiple Potential Signaling Targets. *Diabetes*. 2005;54(2):311–321. DOI: <http://dx.doi.org/10.2337/diabetes.54.a2.311>
  75. Ткачук ВА, Тюрин-Кузьмин ПА, Белоусов ВВ, Воротников АВ. Пероксид водорода как новый вторичный посредник. Биологические мембраны: журнал мембранной и клеточной биологии. (2012); 29(1–2): 21. [Tkachuk VA, Tyurin-Kuz'min PA, Belousov VV, Vorotnikov AV. Peroksid vodoroda kak novyy vtorichnyy posrednik. *Biochemistry (Moscow) Supplement. Series A, Membrane and cell biology*. 2012;29(1–2):21]

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