

Minireview

Metabolic stress in insulin's target cells leads to ROS accumulation – A hypothetical common pathway causing insulin resistance

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Abstract The metabolic syndrome is a cluster of cardiovascular risk factors, and visceral adiposity is a central component that is also strongly associated with insulin resistance. Both visceral obesity and insulin resistance are important risk factors for the development of type 2 diabetes. It is likely that adipose tissue, particularly in the intra-abdominal depot, is part of a complex interplay involving several tissues and that dysregulated hormonal, metabolic and neural signalling within and between organs can trigger development of metabolic disease. One attractive hypothesis is that many factors leading to insulin resistance are mediated via the generation of abnormal amounts of reactive oxygen species (ROS). There is much evidence supporting that detrimental effects of glucose, fatty acids, hormones and cytokines leading to insulin resistance can be exerted via such a common pathway. This review paper mainly focuses on metabolic and other 'stress' factors that affect insulin's target cells, in particular adipocytes, and it will highlight oxidative stress as a potential unifying mechanism by which these stress factors promote insulin resistance and the development and progression of type 2 diabetes.

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1. Stress and insulin resistance in the metabolic syndrome and type 2 diabetes

Stress can be seen as a broad concept comprising various threats to the maintenance of the organism's homeostatic processes. There is currently much evidence that external and internal stress factors are of importance in the development of the metabolic syndrome and type 2 diabetes [1]. Such stress factors are found at a multitude of levels including the socio-economic, psychological, neuroendocrine, metabolic and

molecular environments. Oxidative stress, exerted by the intracellular accumulation of reactive oxygen species (ROS) has been implicated in atherosclerosis, microvascular complications of diabetes as well as in beta cell failure in type 2 diabetes [2,3]. Emerging evidence also support an important role of ROS in various forms of insulin resistance [4,5].

Type 2 diabetes is in most cases caused by a combination of beta cell dysfunction and insulin resistance. Physical inactivity, adiposity due to overeating, stress and smoking are risk factors that interact with susceptibility genes in the development of the disease. The metabolic syndrome is often used to define a cluster of risk markers that predict cardiovascular disease but also type 2 diabetes. Accumulating evidence suggest that abdominal obesity is a central component of this syndrome, that also include hypertension, dyslipidemia, and glucose dysregulation [6]. Insulin resistance is proposed as a major underlying cause of many of the components of the metabolic syndrome. However, there is no consensus in that respect, and, moreover, it is difficult to envisage visceral obesity as a direct consequence of insulin resistance, as insulin per se will promote lipid accumulation in adipose tissue.

Nonetheless, insulin resistance appears to be an important component of the complex pathophysiological processes that underlies the development of type 2 diabetes and potentially other associated conditions such as dyslipidemia, hypertension and atherosclerosis. Insulin resistance can be defined as an attenuated effect of insulin in target tissues, mainly muscle, fat and liver [7]. In muscle, insulin-stimulated transmembrane glucose uptake appears to be the major rate-limiting defect. In adipose tissue, insulin resistance is manifested as impaired glucose uptake and utilisation but in many cases also as an impaired suppression of lipolysis and release of free fatty acids (FFA) and, in addition, it can also lead to dysregulated production and secretion of adipokines and other adipose-derived biomolecules. In the liver, there is attenuated insulin action with respect to glucose uptake and storage as well as suppression of glucose and very low density-lipoprotein (VLDL) production. Interestingly, there can also be insulin resistance in the insulin-secreting beta cells of the endocrine pancreas and this can be of importance in type 2 diabetes, leading to an attenuation of proinsulin synthesis and hence capacity for insulin secretion. Stress mechanisms can be translated into risk of type 2 diabetes and metabolic syndrome partly via the insulin resistance pathway, but obviously there can also be other routes that directly affect the function of, for example, pancreatic beta cells and vascular endothelial cells [1]. The complex interplay

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Abbreviations: FFA, free fatty acids; IL, interleukin; IRS, insulin receptor substrate; JNK, c-Jun NH(2)-terminal kinase; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor kappa B; PKC, protein kinase C; ROS, reactive oxygen species; TNF, tumour necrosis factor

between different organs in the pathobiology of type 2 diabetes and the metabolic syndrome is schematically depicted in Fig. 1.

Although much effort has been made to elucidate the primary mechanisms responsible for the development of insulin resistance in the metabolic syndrome and type 2 diabetes, there is no consensus at present. Obviously, there is much evidence supporting the importance of genetic factors in human insulin resistance [8]. There are common polymorphisms that are associated with type 2 diabetes and/or insulin resistance, for example in the genes for the transcription factor TCF7L2, calpain-10, PGC-1 α (a coactivator of the peroxisome proliferator-activated receptor gamma, PPAR γ), PPAR γ itself, insulin receptor substrate 1 (IRS-1) and β -adrenergic receptors and glycogen synthase. However, the quantitative impact of indi-

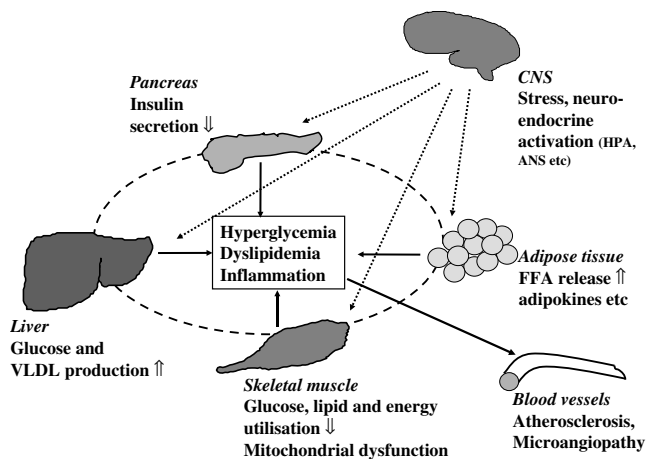


Fig. 1. Interplay between CNS and other tissues in the pathophysiology of type 2 diabetes and the metabolic syndrome. ANS, autonomic nervous system; FFA, free fatty acids; HPA, hypothalamo–pituitary–adrenal system; VLDL, very low density lipoprotein.

vidual gene variants with respect to risk for development of type 2 diabetes appears to be limited.

One obvious hypothesis on the cause of insulin resistance is that there would be a primary defect, inherited or acquired, occurring in the signalling or effector systems within insulin’s targets cells. A schematic summary of the normal signalling system that propagate insulin’s metabolic actions in target cells is presented in Fig. 2. Perturbations could occur anywhere along these signaling cascades as well as in alternate routes, e.g. via lipid raft/TC 10 and MAP-kinase pathways, that may be involved in metabolic as well as other effects of insulin [9]. Severe mutations affecting components of the cellular insulin-signalling machinery will certainly cause marked insulin resistance, but such defects are very rare and are not found in the common patients with the metabolic syndrome or type 2 diabetes. Instead, there is much evidence suggesting that cellular insulin resistance is not a critical primary phenomenon in the development of whole-body insulin resistance. For example, prediabetic subjects can display essentially normal cellular insulin sensitivity, and in insulin’s target cells obtained from type 2 diabetic patients, it seems that insulin resistance is largely reversible [10,11]. Thus, perturbations in the extracellular environment may appear first and they may lead to a secondary cellular insulin resistance in for example muscle and adipose tissue. Such ‘tissue environment’ factors may involve metabolic, neural and hormonal signals. With respect to ‘metabolic signalling’, it is well recognized that high levels of glucose and free fatty acids, that are hallmarks of type 2 diabetes, will have detrimental effects in some tissues, e.g. muscle and liver [12]. Moreover, hyperinsulinemia, occurring as a compensatory mechanisms during development of type 2 diabetes, can in itself contribute to insulin resistance. A popular concept in the etiology of type 2 diabetes and metabolic syndrome is that of intrauterine programming, suggesting that fetal malnutrition leads to a risk for adult metabolic disease. This can also be seen as an environmental stress factor,

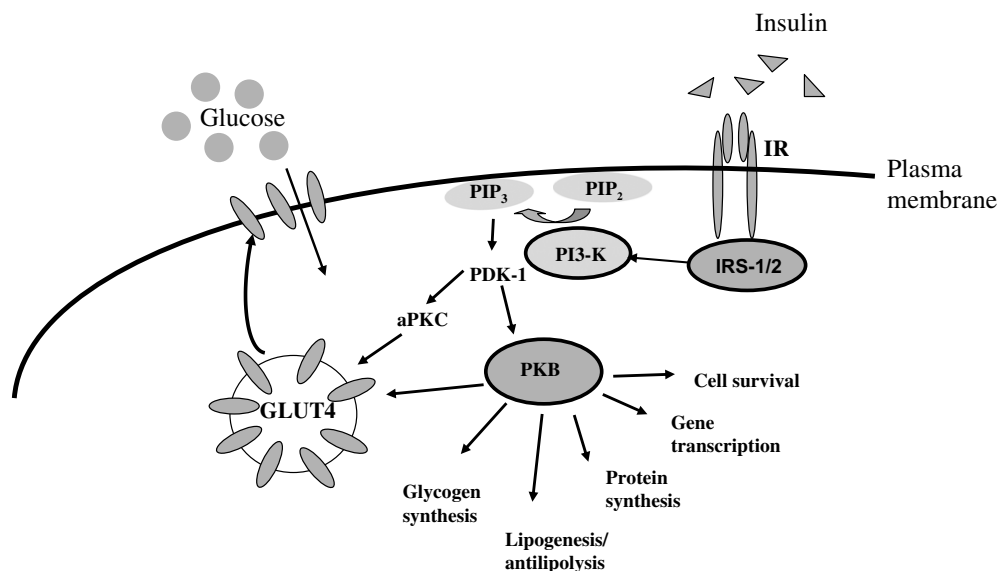


Fig. 2. Insulin signalling via the PI3K-dependent pathway in adipocytes and skeletal muscle. Insulin binding to its receptor will lead to glucose transport activation and other metabolic effects and this is exerted via a cascade of signaling proteins. GLUT4, glucose transporter 4; IR, insulin receptor; IRS1/2, insulin receptor substrates 1 and 2; PDK1, phosphatidylinositol dependent protein kinase 1, PI3K, phosphatidylinositol 3-kinase; PIP₂, phosphatidylinositol-3,4-phosphate; PIP₃, phosphatidylinositol-3,4,5-phosphate; aPKC, atypical protein kinase C; PKB, protein kinase B.

presumably leading to neuroendocrine overactivity via ‘imprinting’ in utero [13].

Although immense research efforts have been made in order to elucidate the mechanisms underlying insulin resistance, there is still no consensus on the exact defects at the cellular and molecular levels. There are several pathways that may contribute to the development of insulin resistance and type 2 diabetes. Such pathways include metabolic factors, e.g. glucose and fatty acids, that in elevated concentrations can exert detrimental effects. Neurohormonal mechanisms clearly can be involved, and glucocorticoids, growth hormone, sex steroids and catecholamines as well as insulin itself all have marked effects on insulin sensitivity in various tissues [14]. As visceral adiposity appears to be a very important component of the metabolic syndrome and also is a major risk factor for the development of type 2 diabetes and cardiovascular disease, adipose-related mechanisms are of interest. In adipose dysfunction, inflammatory mediators such as cytokines and chemokines as well as inflammatory cells, i.e. lymphocytes, neutrophils and macrophages may play important roles.

2. Metabolic stress – glucose, fatty acids and insulin

Regardless of the primary cause, when insulin resistance has developed, insulin levels will rise in order to compensate for the tendency of increase in circulating levels of glucose as well as FFA. Normally, there are fine-tuned feed-back loops to the insulin-secreting β -cells to keep not only glucose but also FFA levels within the physiological range. Once type 2 diabetes becomes established, the metabolic alterations will worsen and they will per se aggravate insulin resistance and thus create a vicious circle. In diabetes glucose levels are chronically elevated and high insulin levels are detected early in the disease but they then gradually fall as β -cell dysfunction progresses. Experimental evidence suggest that hyperinsulinemia can cause insulin resistance [15]. In addition, hyperglycaemia alone exerts detrimental effects on insulin secretion and insulin action [16], a phenomenon commonly referred to as glucotoxicity. In isolated human and rat adipocytes, long-term exposure (several hours) to high glucose, particularly in the presence of high insulin impairs insulin-activated glucose transport capacity [17,18]. A common denominator for an impaired insulin action on glucose uptake in our studies on rat, but not human, adipocytes seems to be a decrease in cellular IRS-1 content [17,18]. Interestingly, low cellular IRS-1 gene and protein expression has been reported to predict insulin resistance and risk for type 2 diabetes [19], and individuals with established type 2 diabetes have a marked reduction in adipocyte IRS-1 protein expression and function whereas the IRS-2 content is unaltered [19,20]. In both obesity and type 2 diabetes, there are high circulating FFA levels. They have negative effects on insulin sensitivity in muscle and liver, but not consistently in fat cells [21,22]. Chronic elevation of FFAs will also lead to an impairment of glucose-stimulated insulin secretion in β -cells [23].

Our recent data suggest that IRS function rather than amount, can be a critical mechanism for insulin resistance in ‘metabolic stress’ exerted by elevated insulin, glucose and possibly FFA levels. This is likely to be mediated via an altered phosphorylation status of IRS-1, specifically an enhanced Ser³¹² phosphorylation [24]. This impairs the interaction with

other signaling proteins such as phosphatidyl inositol 3-kinase, and it also appears to direct IRS-1 towards proteasome-mediated degradation [24]. Probably, activation of protein kinase C (PKC) isoforms that will increase IRS serine phosphorylation is a critical intermediate mechanism for insulin resistance elicited by glucotoxicity and lipotoxicity as well hyperinsulinemia [25].

Obviously, neither high insulin, glucose or FFA levels are expected to be primary causes of insulin resistance, but as secondary phenomena they may certainly contribute to cellular as well as whole-body insulin resistance. One important mechanism whereby hyperglycemia can induce insulin resistance, is via cytokine release as has been shown in adipose cells [26]. Interestingly, high FFA levels, particularly in the portal vein, can lead to central activation of the HPA axis and, in addition, may elicit a sympathoadrenergic response [27]. Accordingly, we observed that poor metabolic control in type 2 diabetic patients is associated with both elevated cytokine and cortisol levels in the circulation [28].

3. Neuroendocrine stress

There are several insulin-antagonistic hormones that play an important role in situations of urgent need for extra fuel delivery to tissues, in prolonged fasting and in the defence against spontaneous or drug-induced hypoglycemia. Such hormones include glucagon, adrenaline, noradrenaline, growth hormone and cortisol [12]. The ability to increase blood glucose will, however, be a disadvantage when there is an inappropriate elevation of such hormones. In this context, the HPA axis controlling adrenal cortisol secretion and the sympathoadrenergic system have received most attention as pathways that can promote insulin resistance and development of type 2 diabetes. These two pathways originate from ‘stress centers’ in the hypothalamus where they also connect to and modulate each other [1]. Some previous results suggest elevated activities in both these systems in individuals with the metabolic syndrome, and this has been proposed to be linked to behavioral factors [29].

Cushing’s syndrome, i.e the clinical syndrome of glucocorticoid excess, is associated with insulin resistance, glucose intolerance, central obesity and hypertension. Pharmacological treatment with high doses of glucocorticoids also leads to an impairment of insulin sensitivity. In obesity the local conversion of cortisone to cortisol within adipose tissue, via the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD-1), appears to be elevated, and this can contribute to perturbations in glucose and lipid metabolism as well as to adiposity itself [30]. The insulin-antagonistic effects of glucocorticoids include both an impairment of insulin-dependent glucose uptake in peripheral tissues and a stimulation of gluconeogenesis in the liver. It seems that there are direct cellular effects leading to an impairment of glucose transport, and dexamethasone-induced insulin resistance in fat cells probably involves the glucose transporter 4 (GLUT4) translocation process [12]. Data from our research suggest that the impairment of glucose transport in human omental fat cells might be attributed to defects in the insulin-signaling pathway since cellular insulin receptor substrate-1 (IRS-1) and protein kinase B (PKB) were reduced by exposure to dexamethasone [31]. Part of the insulin-antagonistic effect of glucocorticoids appears to be second-

ary to a lipolytic effect leading to elevation of circulating FFA levels. In addition, glucocorticoids may also inhibit insulin secretion from pancreatic β -cells [32], and that may obviously contribute to their diabetogenic properties.

Sympathetic nerve activity and the mediators, circulating adrenaline and local tissue noradrenaline, exert profound interactions with the metabolic effects of insulin [12]. The autonomic nervous system can thus, mainly via release of catecholamines, directly lead to tissue insulin resistance and, hence, promote development of type 2 diabetes. In addition there are secondary effects since sympathoadrenergic activation will increase adipose tissue lipolysis and the resulting increase in circulating FFA levels will in turn oppose insulin action in muscle and liver. Studies in animal models of type 2 diabetes as well as patients with type 2 diabetes have revealed an altered sympathetic activity and, moreover, their carbohydrate metabolism seems abnormally sensitive to sympathetic stimulation [12]. In healthy subjects, insulin resistance appears to be associated with an altered balance in the autonomic nervous system with a relative increase in sympathetic vs parasympathetic activity following standardised stress [33] or following hyperinsulinemia [34]. Insulin-resistant subjects seem to have an attenuated response in the parasympathetic nervous system upon standardised stimuli [33]. Thus, dysregulation of the autonomic nervous system might be a potential mechanism leading to insulin resistance and promoting the development of metabolic syndrome and type 2 diabetes.

The renin–angiotensin system (RAS) can also be an important neuroendocrine pathway contributing to the development of insulin resistance [33]. Renin is an enzyme that catalyses the formation of angiotensin I from angiotensinogen, and then angiotensin II is formed by the action of angiotensin converting enzyme (ACE). Angiotensin II has profound vascular effects in itself and it also stimulates aldosterone secretion from the adrenal cortex. Overall effects of RAS include vasoconstriction, blood pressure elevation, renal effects such as fluid and sodium retention and potassium excretion. But there are also a multitude of other effects in the CNS, skeletal muscle, liver and adipose tissue. There appears to be effects on insulin sensitivity as antihypertensive medications that activate RAS seem to cause insulin resistance whereas those that reduce effects of RAS, e.g. ACE inhibitors and angiotensin receptor blockers (ARB), display a positive or neutral effect on insulin sensitivity [35]. In the adipose tissue it has been shown that a local RAS can impair adipocyte recruitment and differentiation, and that this can be prevented by ACE inhibitors or ARBs [36]. Moreover, there seems to be a link between RAS activity and inflammation within adipose tissue, and paracrine effects of angiotensin II are likely to be involved. The dominating regulation of circulating RAS activity is exerted via renin production in the kidneys, that in turn is mainly regulated by the sympathetic nervous system. Potentially, perturbations in endocrine and paracrine RAS action can contribute to the development of the metabolic syndrome, and they may also channel external stress factors into metabolic dysregulation.

4. Inflammatory stress

Previous studies have shown that elevated serum levels of C-reactive protein (CRP) and several cytokines are associated with obesity, insulin resistance and type 2 diabetes [14]. In the

adipose tissue there is local production of TNF- α and interleukin-6 both of which have potent insulin-antagonistic properties that are ascribed to a direct interference with insulin signalling [37]. These cytokines also have the potential to activate lipolysis, and moreover it has been indicated that TNF- α inhibits adiponectin production. Interestingly, an interaction between TNF- α levels and the cortisol system has also been implicated, and TNF- α may promote the conversion of cortisone to cortisol in adipocytes by activation of 11β -HSD-1 [38]. Both TNF- α and IL-6 can stimulate cortisol secretion, directly or via HPA activation. Our recent data indicate that high TNF- α , but not interleukin-6, levels are linked to insulin resistance in type 2 diabetic individuals and that high levels of both cytokines are associated with hyperglycemia [28]. Therefore, it might be suggested that in the hyperglycemic state, TNF- α is a factor that contributes to insulin resistance, and this can occur partly via increased cortisol levels. We also found that CRP levels were higher in poorly controlled than in well-controlled type 2 diabetic patients. CRP levels were strongly correlated to glycemic level, but surprisingly not to insulin resistance. These results suggest that hyperglycemia per se is an important factor that contributes to the previously reported increase in CRP in type 2 diabetes and prediabetes [28]. Thus, in type 2 diabetes, hyperglycemia could lead to insulin resistance partly via an inflammatory response in adipose tissue as reflected by release of cytokines into the circulation.

5. Adipose tissue as a target for stress factors

There are now several studies that link psychosocial factors to visceral adiposity, metabolic syndrome and type 2 diabetes. Stressful life events and also low educational level are associated with risk for type 2 diabetes in the general population. In women, work stress, low sense of coherence, and low emotional support and, in men, sleeping disorders are stress factors that have been associated with development of type 2 diabetes [12]. Stressful situations will lead to neuroendocrine responses, that in the long-term perspective might be important in the development of visceral adiposity and type 2 diabetes. The HPA axis together with the sympathetic nervous system can mediate effects of perceived stress in different organs. The downstream hormones of the sympathoadrenergic system and the HPA axis, i.e. adrenaline, noradrenaline and cortisol, respectively, are known to oppose the effects of insulin. However, there are not much data available that demonstrate altered levels of these hormones in individuals exposed to a high psychosocial stress level. Some studies suggest elevated cortisol levels in situations such as work stress and unemployment [39], and this is expected to lead to accumulation of abdominal fat. On the contrary, subjects with metabolic syndrome and abdominal obesity have been reported to have a decreased diurnal variability but not an overall change in cortisol levels [40]. Interestingly, in one study ACTH stimulation induced an attenuated adrenocortical response measured as peak serum cortisol in individuals with a family history of type 2 diabetes patients [41]. Björntorp and coworkers proposed that a hypothalamic arousal leads to insulin resistance and visceral obesity via excess cortisol production, but at a later stage there might be a shift to a “burn-out” phenomenon with low secretion of cortisol, growth hormone and sex steroids [1,42].

There is a clear relationship between central fat storage, i.e. visceral obesity, and features of the metabolic syndrome. The causal relationship is not established, but the association of visceral fat accumulation in the development of insulin resistance and type 2 diabetes has been generally accepted [40]. A link between central obesity and HPA axis dysregulation has also been suggested, and it is proposed that high glucocorticoid levels will promote lipid storage in visceral rather than subcutaneous adipose tissue. Moreover, an altered balance in the activity of the sympathetic and parasympathetic nervous systems, respectively, appears to be associated with visceral obesity [41]. Interestingly, in humans visceral adipose tissue seems to have a greater propensity for inflammation than subcutaneous adipose tissue, as evidenced by both higher levels of cytokines as well as more resident macrophages [43]. Visceral compared to subcutaneous adipocytes are more sensitive to the lipolytic effects of catecholamines, and to the insulin-antagonistic action of glucocorticoids, but they less sensitive to the antilipolytic and lipogenic effects of insulin [44]. In visceral adiposity, this could, in turn, result in diverting fatty acids to other tissues, e.g. muscle and liver.

An interesting hypothesis is that of 'adipose tissue overflow'. In situations of excess energy intake, excess calories will be stored primarily in adipose tissue. The best place for this is the subcutaneous adipose depot, where fat accumulation does not do much harm. In a situation of chronic calorie overload, however, subcutaneous adipose tissue eventually reaches its upper limit for further triglyceride storage, and this may trigger adipose inflammation as well as lipid 'spill-over'. This means that energy storage will be partitioned towards the visceral fat depot and subsequently into ectopic fat depots, i.e. intrahepatocellular and intramyocellular lipids (IHCL and IMCL), both of which will have direct negative effects on insulin action in these tissues [45]. The capacity for fat storage in subcutaneous adipose tissue could be determined by genetic programming of recruitment of new adipocytes, but also by neuroendocrine interactions as well as adipose inflammation. A dysfunctional subcutaneous adipose tissue in this respect may be an important cause of faulty deposition of lipids in the intraabdominal and intramyocellular compartments. Inappropriate activity in the WNT-signalling system could have a pivotal role in evoking this detrimental adipose phenotype, e.g. by preventing adipogenesis as recently suggested by work in the laboratory of U. Smith [46]. Interestingly, enlargement of human fat cells can be taken as a sign of filled adipose energy stores, and it is independently associated with insulin resistance both *in vitro* and *in vivo* [47].

It has been shown that an increased adipocyte size is associated with insulin resistance [47]. Furthermore, enlarged subcutaneous abdominal adipocytes can predict the development of type 2 diabetes and this is partly independent of insulin resistance [48]. Leptin and adiponectin are two adipocyte-specific products that are secreted and eventually appear in the circulation. Hyperleptinemia seems to be an independent marker of fat cell hypertrophy, and leptin levels are also correlated to the amount of body fat [47]. High leptin levels can thus be seen as a reflection of the overfilled subcutaneous lipid stores. Leptin has hypothalamic effects leading to inhibition of food intake and increased energy expenditure [49]. However, in peripheral tissues there are also direct insulin-antagonistic effects that seem to be mediated via interactions with the insulin signalling cascade, e.g. enhanced serine phosphory-

lation of IRS-1 [50]. In contrast to leptin, the circulating levels of adiponectin display inverse associations with insulin resistance and body fat, and experimental data suggest that adiponectin can reduce hyperglycemia, insulin resistance, inflammation, atherosclerosis and potentially adiposity [51,52].

Increased secretion of adipokines, i.e. cytokine-like molecules derived from adipose tissue, can partly explain why adipocyte enlargement promotes insulin resistance [48]. Taken together, substantial evidence support that fat cell size as well as potential adipocyte-derived factors such as TNF- α , IL-6, serum amyloid A, cortisol and FFAs are associated with insulin resistance [47,53]. However, it should be appreciated that circulating TNF- α and IL-6 do not mainly originate from adipocyte tissue, and moreover, the main source of TNF- α within adipose tissue is non-adipose cells [53].

6. Oxidative stress – a common final pathway in insulin resistance?

Several types of insulin resistance appear to be linked to cellular oxidative stress [5,54]. Oxidative stress indicates the intracellular accumulation of reactive oxygen and nitrogen compounds, mainly the so-called reactive oxygen species (ROS). The major ROS variants are hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl), superoxide ($\cdot\text{O}_2^-$), hydroxyl ($\cdot\text{OH}$), peroxy ($\cdot\text{RO}_2^-$) and hydroperoxyl ($\cdot\text{HO}_2^-$) [5]. In mitochondrial respiration, ROS are generated in the electron transport chain, as a by-product in the ATP generating process [3]. This occurs in situations of enhanced oxidation of energy substrates such as glucose and FFA, unless uncoupling compensates and prevents ROS formation. The enzyme NAD(P)H oxidase plays a key role to stimulate ROS formation and it can be activated by various cytokines. The mitochondrial mechanisms involve increased levels of electron donors, mainly FADH₂ and NADH that are generated by the tricarboxylic acid cycle, and they will push electrons into the protein complexes of mitochondrial respiratory chain. This will lead to electrons piling up in the mitochondrial electron transport chain, and eventually they will be donated to molecular oxygen generating superoxide, that subsequently can be degraded into hydrogen peroxide by manganese superoxide dismutase [3]. Interestingly, excessive amounts of the ubiquitous biological messenger nitrogen oxide (NO) appear to have detrimental effects that are similar to those of ROS. It has been reported that NO production via inducible NO synthase (iNOS) is increased in animal models of obesity and that deletion of the iNOS gene (*Nos2*) can prevent diet-induced insulin resistance in skeletal muscle [55].

Paradoxically, certain ROS may have insulin-mimicking effects, and for example hydrogen peroxide can increase transmembrane glucose transport and inhibit lipolysis [5]. Nonetheless, ROS accumulation in general is associated with cellular insulin resistance, and this can occur through activation of stress kinases, injury to cellular membranes, the endoplasmic reticulum and nuclear DNA. Oxidative modification of cellular proteins and lipids may have functional consequences that contribute to insulin resistance [5]. The detrimental cellular effects of ROS are mediated by downstream effector mechanisms, but the details are poorly understood, and there are probably different mechanisms involved in different cell types and cellular functions. However, with respect to insulin

desensitisation, it is believed that various so-called stress kinases, e.g. JNK, p38-MAPK, NF κ B and certain PKC isoforms are involved. A common denominator for these kinases seems to be their ability to directly or indirectly increase serine/threonine phosphorylation of IRS proteins, thus attenuation insulin signalling. In addition, some of these kinases can increase cytokine production, that also can impair insulin action.

There is now much emerging evidence that suggest that several factors that cause insulin resistance have a common pathway in the excessive formation of ROS [4,5,54]. This seems to be true for inflammation, glucotoxicity, lipotoxicity and some endocrine mediators. Fig. 3 summarises factors that may promote insulin resistance via ROS accumulation.

Inflammatory cytokines can increase oxidative stress, and there are results suggesting that this can be mediated via ceramide formation that stimulates mitochondrial ROS production. In adipose cells, TNF- α can thus increase ROS levels that will activate the stress kinase JNK that in turn may increase IRS-1 serine phosphorylation [5,54]. Antioxidant agents given in the presence of TNF- α in vitro can prevent both insulin resistance and oxidative stress. Antioxidant treatment of insulin-resistant mice in vivo also leads to improvement of glycemic control and insulin resistance [54]. Thus, there is strong support for a pivotal role of ROS in cytokine-induced insulin resistance [54].

Increased flux of energy substrates into oxidative pathways will lead to elevated activity of mitochondrial respiratory chain and ROS generation. ROS accumulation could be seen as an mitochondrial sensor of energy overflow into cells, and it may act as a safeguard preventing further inflow and storage of substrate. Thus, both high FFA and glucose levels will lead to increased substrate oxidation and a secondary increase in mitochondrial ROS formation [56]. In 'overloaded' adipocytes, it is possible that an increase in intra-adipocyte fatty acid levels will be accompanied by oxidative stress. This may partly explain the strong link between fat cell enlargement and insulin resistance [47]. Clinical data also suggest that overall oxidative stress is increased in subjects with visceral obesity [57].

Hyperglycemia-induced oxidative stress is likely to be of great importance in diabetes, particularly in patients whose glucose is poorly controlled. Importantly, further support is provided by a study showing that hyperglycemia-induced insulin resistance

can be prevented by antioxidant treatment [58]. In addition, there are data indicating that postprandial glucose peaks in type 2 diabetes, have a particular propensity to increase ROS formation in the vessel walls [59]. This can be of major importance for the development of micro- and macrovascular diabetic complications. Taken together, there are data supporting that oxidative stress is a critical mechanism both in glucotoxicity that contributes to cellular insulin resistance as well as β -cell failure and in the development of organ injuries that are associated with chronic hyperglycemia and vascular dysfunction [3,26,59]. In fairness, there are also data that suggest no increase in oxidative stress in type 2 diabetes, e.g. in skeletal muscle [60]. In insulin's target organs, such as adipose tissue and skeletal muscle, glucotoxicity is thought to be mediated via the so-called hexosamine pathway [11]. Together with oxidative stress it could serve as a compensatory mechanism signalling that no more fuel is needed and consequently attempting to downregulate insulin-mediated glucose uptake. On the other hand, in other tissues such as nerves and blood vessels, where glucose flux is not insulin-dependent, there will be continuing glucose overload and subsequently irreversible damage, i.e. chronic diabetic complications affecting nerves, kidneys and the retina.

Interestingly, some neuroendocrine pathways leading to insulin resistance also involves ROS formation in target cells. It was recently reported that dexamethasone-induced insulin resistance in adipose cells is associated with ROS formation [54]. Interestingly, insulin resistance was avoided upon concomitant treatment with antioxidants as well as in cells with overexpression of ROS-metabolizing enzymes. With respect to RAS, there is support for ROS involvement in some of the effects of angiotensin II that lead to inflammation and insulin resistance, and ARBs seem to attenuate oxidative stress [61,62].

Smoking is another factor that is known to cause insulin resistance and it is associated with risk for type 2 diabetes development. The mechanisms involved are not defined, but they may include activation of the sympathetic nervous system, altered levels of cytokines and endothelial dysfunction seem to be of importance. Interestingly, smoking is also reported to increase oxidative stress in different cell types, and this can be an important mechanism for insulin resistance as well as for atherosclerosis in smokers [63].

There are some clinical studies addressing potential treatments aiming at reduction of oxidative stress, and they have mainly focused on treatment or prevention of cardiovascular disease. In general, such programs have primarily tested antioxidant vitamins and other potential ROS scavengers, and the overall results have so far been disappointing with respect to cardiovascular outcome. This is also true for the few trials addressing metabolic control in type 2 diabetes. Clinical biomarkers of oxidative stress have been applied in some studies, and such markers include variants of lipids, lipoproteins, prostaglandin derivatives and amino acids that are modified by ROS. However, such measures need more validation before they can be generally recommended in large-scale studies or in clinical practice.

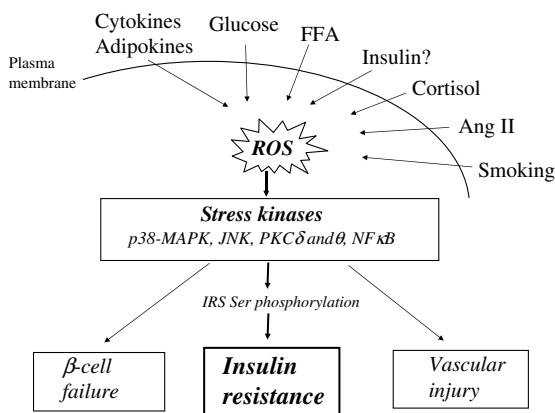


Fig. 3. ROS accumulation as a unifying pathway leading to and insulin resistance. Several factors exerting cellular stress are proposed to cause insulin resistance via increased ROS production and oxidative stress.

7. Conclusion

The mechanisms causing insulin resistance in type 2 diabetes and the metabolic syndrome, are yet not fully understood. It is generally accepted that a complex interplay between genetic

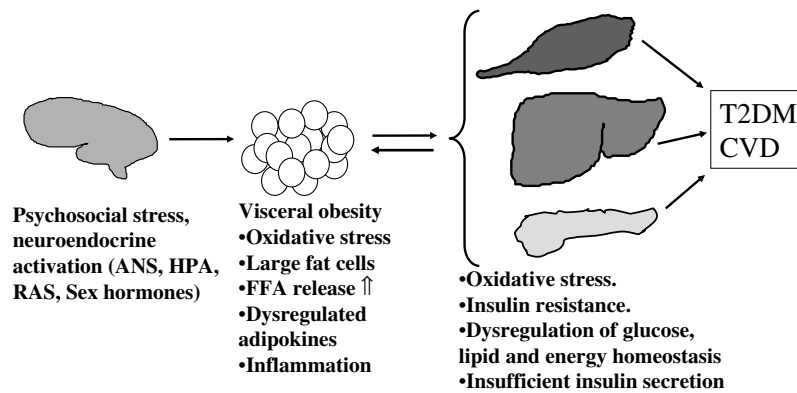


Fig. 4. A hypothesis on the development of insulin resistance and type 2 diabetes over time. Various stress mechanisms challenge the organism's metabolic homeostasis. In a decompensated situation, where the total stressor load exceeds the capacity of compensatory mechanisms, oxidative stress occurs at the cellular level and this leads to insulin resistance and eventually beta cell failure and vascular damage.

and acquired factors is key in the pathobiology of these disorders. It is likely that stressors in various forms hit the organism at many different levels and that this adds up to a pathogenic process moving towards insulin resistance and diabetes. Such stressors, i.e. social, psychological, neural, endocrine, metabolic, inflammatory factors, may merge into a final common pathway namely oxidative stress at the cellular level. It is proposed that this is a critical pathway for the development of insulin resistance in insulin's target tissues, but also for beta cell dysfunction and macro- and microvascular damage in diabetes. A schematic summary of this hypothesis is depicted in Fig. 4. Future research in the field of metabolic diseases should include elucidation of potential new mechanisms, biomarkers and pharmacological targets related to ROS production as well as downstream signals activated by ROS accumulation. This would improve our understanding of the complex disease mechanisms and facilitate development of new therapeutic approaches.

Conflict of interest statement

The author is an adviser at AstraZeneca R&D.

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