Review

Pathogenesis of Type 2 (non-insulin-dependent) diabetes mellitus: candidates for a signal transmitter defect causing insulin resistance of the skeletal muscle

H. U. Häring and H. Mehnert

Institute for Diabetes Research and III Medical Department, Krankenhaus München-Schwabing, München, Germany

Summary. Insulin resistance of skeletal muscle, liver and fat combined with an abnormality of insulin secretion characterizes Type 2 (non-insulin-dependent) diabetes mellitus. There is increasing evidence that the insulin resistance of the skeletal muscle plays a key role early in the development of Type 2 diabetes. As a consequence recent research efforts have focussed on the characterization of insulin signal transduction elements in the muscle which are candidates for a localization of a defect causing insulin resistance i.e. the insulin receptor, phosphatases related to insulin action, glycogen synthase and the glucose transporters. In this review we at-

tempt to summarize present knowledge about abnormalities of these systems in skeletal muscle of Type 2 diabetic and pre-diabetic individuals. We try to classify abnormalities as secondary events or as candidates for putative primary molecular defects which might initiate the development of insulin resistance as early as in the “pre-diabetic” state.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, insulin resistance, insulin receptor, phosphatases, glycogen synthase, glucose transporter.

Clinically overt Type 2 (non-insulin-dependent) diabetes mellitus is characterized by insulin resistance of all major target tissues, i.e. skeletal muscle, liver and fat combined with an abnormality of insulin secretion [1]. Several studies in recent years have shown that the clinical onset of Type 2 diabetes in different high-risk populations is preceded by an undefined time period when insulin resistance may be detected [2–7]. The clinical features of the potential pre-diabetic state in high-risk groups include insulin resistance, increased abdominal fat, elevated very low density lipoproteins and decreased high density lipoproteins, as well as elevated fasting and postprandial insulin levels [2–7]. Furthermore, in some populations hypertension is associated [8–11] with this clinical syndrome which was recently named syndrome-X. It is also known as metabolic endocrine syndrome, hyperinsulinaemia-insulin resistance syndrome and what we had earlier designated [12] “Wohlstandssyndrom” (“affluence or post-war syndrome”).

As recently reviewed [13] a variety of different mechanisms which cause insulin resistance at different levels of the insulin signal transducing chain and at different target cells have been described in Type 2 diabetes. There is sufficient evidence to assume that insulin resistance found in Type 2 diabetes is the result of several different pathogenic mechanisms (Fig. 1). Most of these mechanisms might be the consequence of metabolic derangements such as

hyperglycaemia, elevated non-esterified fatty acids or alterations of the insulin level placing them in the category of secondary events in the development of the disease. On the other hand, the recently available data on the pre-diabetic populations have clearly shown that insulin resistance of the skeletal muscle is a very early event in the development of the disease which exists before metabolic derangements occur [2, 3, 14–19]. This strongly suggests the existence of a primary molecular defect of insulin action in the skeletal muscle. This putative primary defect might initiate a sequence of events leading to the secondary insulin resistance mechanisms which then in concert with the postulated primary defect cause the variable levels of insulin resistance which are observed in the different clinical stages of Type 2 diabetes (Fig. 1). Most of the data available on abnormalities of insulin signalling and action in Type 2 diabetes were obtained in late stages of the development of the disease. Only recently an increasing amount of information about the functional status of signal transduction elements in the skeletal muscle of pre-diabetic individuals has become available. In this review we attempt to give an update of the published data concerning the signal transduction elements which have been most extensively studied in recent years, i.e. the insulin receptor kinase, the phosphatases related to insulin action, glucose transporters and the glycogen synthase. We will also focus on whether abnormalities found in

Fig. 1. Stages in the development of Type 2 diabetes. The development of pre-diabetes and Type 2 diabetes could be initiated by a primary insulin signalling defect located in the skeletal muscle. Compensatory hyperinsulinaemia may cause secondary events increasing target tissue resistance

these systems may be candidates for the primary molecular defect initiating the pathogenesis of Type 2 diabetes. Criteria classifying these abnormalities are provided by data which concern reversibility of the "defects" and data which indicate a specific abnormality already seen in pre-diabetic populations. Furthermore, ongoing studies which are screening for mutations of the genes encoding these cellular signal transduction elements may provide evidence as to whether these abnormalities should be regarded as secondary or primary events.

The insulin receptor

The insulin receptor as the first element of cellular signal transduction is a strong candidate for the location of a defect in insulin signalling. A number of studies have shown that in syndromes of severe insulin resistance a number of different point mutations of the insulin receptor gene exist [20]. Some of these point mutations were functionally relevant and caused different forms of receptor defects with consequences for insulin binding characteristics, consequences for the signalling capacity of the tyrosine kinase of the insulin receptor or other abnormalities [20]. While in forms of extreme insulin resistance, structural and func-

tional defects at the receptor level have been clearly described, the situation in common Type 2 diabetes is less clear. The insulin receptor has been functionally characterized in all major target tissues – skeletal muscle, liver and fat [21–29]. In liver and skeletal muscle all data were obtained exclusively on isolated receptors after in vitro or in vivo stimulation [21–24, 29]. It is not known how these receptors behave in their natural cell environment. Only in fat cells from Type 2 diabetic patients are there data from intact cells [25, 26, 28] in addition to the information on isolated receptors. Binding capacity and affinity is generally found to be unaltered for the skeletal muscle [21–23, 27], and liver [24] while it was found to be decreased in fat [25]. Activation of the insulin receptor kinase was found to be decreased in receptors from all these tissues. In general, an approximate 50% decrease of the insulin response and an insensitivity towards low insulin concentrations were found.

Mechanisms of reduced insulin receptor kinase activity

Analysis of the defective mechanism on the protein level has revealed two interesting observations. For the insulin receptor kinase in fat it was shown that the total receptor population exists in active and inactive forms [28]. In Type 2 diabetes it appeared that the inactive form was increased [28]. In kinase isolated from skeletal muscle an altered autoactivation cascade was found suggesting that the partial inactivity might be a consequence of an incomplete autoactivation process [27]. The reason for the altered activation process is unknown.

For the kinase defect in fat a partial reversibility was demonstrated pointing towards a secondary mechanism at least in this tissue [26]. Analogous observations for muscle and liver are not available. Several studies have been performed which have investigated whether in analogy to the syndromes of severe insulin resistance a mutation of the insulin receptor gene causes the inactivity of the insulin receptor kinase. In the majority of cases relevant mutations of the insulin receptor gene have not been detected [30–34]. Using screening techniques such as denaturing gradient gel electrophoresis (DGGE) or single-stranded-conformation-polymorphism (SSCP) analysis more than 100 Type 2 diabetic patients were studied by different investigators [30–34]. Mutations were identified in only a few patients [33] and in most of these patients there is no proof that these mutations cause a reduced signalling capacity of the receptor. Taken together these results argue against a primary, genetically determined defect of the receptor and point rather to a regulatory, possibly secondary mechanism causing kinase inactivity in skeletal muscle from Type 2 diabetic patients. As reviewed recently [13] a number of mechanisms are known which can inhibit the activity of the insulin receptor kinase such as increased serine phosphorylation of the receptor, modulation by inhibitors such as G-proteins and inhibitory peptides, glucose induced inhibition through activation of protein kinase C or inhibition through chronic hyperinsulinaemia. There are interesting reports that peptide inhibitors of the receptor kinase are increased in fibroblasts of insulin resistant Type 2 diabetic patients [35,

36]. However, though potentially important, none of the above-mentioned mechanisms has proven to be relevant for Type 2 diabetic skeletal muscle.

Insulin receptor kinase activity in pre-diabetes

Independent of the underlying mechanism the question remains whether the kinase inactivity is an early event in the pathogenesis or whether it contributes later in the development of the disease, by increasing skeletal muscle insulin resistance in an additive way to the putative primary defect. Up to now two studies have addressed this question. One report on pre-diabetic Pima Indians showed that the tyrosine kinase of the skeletal muscle is normal in this population [37]. In contrast decreased basal and insulin-stimulated insulin receptor kinase activity were recently observed in a Danish pre-diabetic population [38] and thus the authors suggest that kinase inactivity is a candidate for the primary defect.

In summary, while there is sufficient evidence to conclude that insulin receptor kinase inactivity contributes to the insulin resistant state of the skeletal muscle in later stages of Type 2 diabetes, questions remain unanswered as to the underlying mechanism of kinase inactivity and to the status of the primary or secondary event.

Insulin receptor isoform pattern

Another aspect of the insulin receptor has recently attracted interest. As a consequence of tissue specific alternative splicing of mRNA of the proreceptor, the human insulin receptor (HIR) exists in two isoforms tentatively named HIR-A and HIR-B [39–41]. Most of the functional properties of the two receptor isoforms are identical [42–48] while some characteristics such as binding affinity [39, 42] internalization, recycling [48] and down-regulation appear to be different. However, at present specific differences in biological functions of the two isoforms are not understood. When the tissue specific expression of the isoforms in Type 2 diabetes and severe insulin resistance were investigated, conflicting results were reported. While we [49, 50] and others [51, 52] found evidence at both the protein and mRNA level for an altered isoform pattern, this finding was not confirmed by other investigators [53, 54]. The reason for the discordant results is unclear. Even though the biological significance of an altered insulin receptor isoform expression cannot be interpreted at present, it is interesting to note that in a pre-diabetic population the altered expression of receptor isoforms in the skeletal muscle was associated with skeletal muscle insulin resistance [55]. This suggests that an altered splicing of mRNA for the proreceptor is one of the candidates for very early molecular defects in the development of skeletal muscle insulin resistance.

Post-kinase signal transducers

The mechanism of insulin signal transduction at the post-kinase level is still not well understood [13]. As a consequence no data are available concerning defects in the

post-kinase signal transmitting systems in Type 2 diabetic skeletal muscle. Phospholipase C activity, which might play a role in insulin signalling, has been reported to be increased in the liver of Type 2 diabetic patients [56]. A putative second messenger of insulin action, which might be released from the plasma membrane by phospholipase C activation, chiroinositol, is reduced in pre-diabetic Pima Indians [57]. The significance of these observations is not yet clear.

Phosphatases

Phosphatases are interesting candidates for a molecular defect causing insulin resistance as they modulate insulin signal transduction at different levels of the signal transmitting chain [13]. Tyrosine specific phosphatases in the cell are involved in the regulation of the autophosphorylation status of the insulin receptor controlling its signalling activity. Since at the post-kinase level tyrosine phosphorylation of substrate proteins may be involved in insulin signalling, an analogous role of tyrosine phosphatases for the regulation of post-kinase signalling mechanisms has to be assumed. Furthermore, it is believed that the insulin signal transmission mechanism switches at some point in the signalling chain from tyrosine phosphorylation to serine phosphorylation. At this level of signal transduction serine phosphatases play an important role.

Tyrosine phosphatase (PTPase) activity was studied in skeletal muscle of insulin-resistant Pima Indians [58]. In this study increased basal PTPase and insufficient insulin suppression of PTPase activity were found in resistant individuals. In another study in Type 2 diabetic patients, where PTPase activity against an insulin-receptor-like substrate was measured, a decreased PTPase activity was found in insulin-resistant skeletal muscle [59]. Thus, the significance of altered PTPase activity in the pathogenesis of skeletal muscle insulin resistance remains unknown.

Recently, interesting data concerning serine phosphatases were reported. Of major interest is how serine phosphatases control the activity of the enzyme glycogen synthase. A decreased basal and insulin-stimulated glycogen phosphatase activity and phosphorylase phosphatase activity (type-1 protein phosphatase, PP-1) in the skeletal muscle of insulin resistant individuals has been found [16, 19, 60]. Furthermore the effect of insulin on the gene expression of PP-1 is different between sensitive and resistant skeletal muscle [61]. These observations might be important for the understanding of the altered effects of insulin on glycogen synthase. It is important to note that these abnormalities of PP-1 are observed even in the pre-diabetic state.

Glycogen synthase

While in Type 2 diabetes both oxidative and non-oxidative glucose disposal in the skeletal muscle are impaired [1], only non-oxidative glucose disposal appears to be reduced in the pre-diabetic state [2, 3]. This observation indicates a pivotal role for glycogen synthase. The effect of

insulin on skeletal muscle glycogen synthase both in Type 2 diabetic patients as well as in pre-diabetic subjects has been well studied [1, 3, 14–16, 62–64]. Glycogen synthase activity is regulated by phosphorylation and dephosphorylation. A number of serine specific kinases phosphorylate glycogen synthase at different residues [65], and at least two phosphatases regulate the dephosphorylation [65]. Dephosphorylation converts the enzyme from a glucose 6-phosphate dependent form to a glucose 6-phosphate independent form. Insulin activates the enzyme by activating glycogen synthase phosphatase and inhibiting one of the glycogen synthase kinases, cAMP-dependent kinase [66]. The inhibition of cAMP-dependent kinase appears to lower the phosphorylation of glycogen synthase phosphatase-inhibitor-I leading to activation of glycogen synthase phosphatase [66]. It is generally found that the insulin effect on glycogen synthase from skeletal muscle of Type 2 diabetic patients is reduced [62–64]. Recently, it was shown by nuclear magnetic resonance technology that the decreased effect of insulin on the enzyme is relevant in vivo leading to a decreased rate of glycogen synthesis [67]. The mechanism of reduced glycogen synthase activity is not clear. It has been reported that the activation of the dependent enzyme form by glucose 6-phosphate is normal [62] suggesting that no defect of the enzyme itself exists. A recent study in skeletal muscle of Type 2 diabetic patients suggests that relevant mutations of glycogen synthase or the catalytic subunit of the glycogen synthase related protein phosphatase I are obviously not the cause of the reduced activity [68].

Glycogen synthase activity in pre-diabetes

An abnormality of glycogen synthase activation is likely to be a very early event in the development of Type 2 diabetes as this abnormality has been reported in the pre-diabetic state [14–16, 69]. The level of expression of the glycogen synthase protein in skeletal muscle in pre-diabetic individuals and Type 2 diabetic patients after insulin stimulation is not decreased. On the contrary it seems to be somewhat increased which might reflect a compensatory mechanism [69]. In summary, concerning the underlying mechanism, the above-mentioned data suggest that glycogen synthase itself is not defective. It appears rather that some step in the signal transduction between the insulin receptor and glycogen synthase, possibly at the level of signal transduction to the phosphatases, may be altered. However, the observation that in all pre-diabetic populations the abnormality of glycogen synthase and related phosphatases is already present makes this system a strong candidate for the initiating event of muscle resistance.

Glucose transporter

Nuclear magnetic resonance studies suggest that in Type 2 diabetic muscle the transport of glucose is decreased [70]. The molecular mechanism is unclear. No analogous data have been reported for the pre-diabetic state. The glucose transporter consists of a family of isoforms with different

tissue specific expression named GLUT 1–5 [71]. The effect of insulin on glucose transport involves a translocation of glucose transporters from intracellular membranes to the plasma membrane [72], but the second step probably involves the activation of transporters in the plasma membrane [73]. The question of whether an abnormality of the glucose transport system contributes to the insulin resistance in Type 2 diabetes was first addressed in adipocytes from Type 2 diabetic patients. In these studies both a decreased expression of the relevant glucose transporter isoform GLUT 4 as well as a decreased translocation and activation of glucose carriers to the plasma membrane was found [74]. A number of studies have since addressed the question of whether alterations of GLUT 4 expression can be detected at the level of the skeletal muscle. The majority of the data suggest that the expression of GLUT 4 in skeletal muscle is not significantly decreased. The functional properties of the glucose transporters are not known as no studies on translocation or activation of GLUT 4 from Type 2 diabetic skeletal muscle have been published. In skeletal muscle biopsies from musculus vastus lateralis, normal expression of GLUT 4 both at the mRNA and protein level was described [75, 76]. Other data obtained in musculus rectus abdominis suggested a decreased level of GLUT 4 [77] which is in agreement with our own studies in musculus gastrocnemius [78]. On the other hand this group showed in an elegant study in musculus rectus abdominis from insulin-resistant patients that the state of insulin resistance does not correlate with the total level of GLUT 4 protein [79]. It will be important to know whether GLUT 4 levels in plasma membranes and transverse tubules after insulin stimulation are altered. At least in the basal state we observed an altered subcellular distribution of GLUT 4 in gastrocnemius muscle [78]. The discrepancies among the results might be the consequence of the different muscle types which were studied or more likely a consequence of different patient characteristics. In our study patients were clearly older and had a longer duration of Type 2 diabetes than in the other studies where no abnormality could be detected. This suggests that an alteration of GLUT 4 expression might be a very late phenomenon in the development of skeletal muscle insulin resistance and may not be considered as a primary defect. This conclusion is in agreement with a recent report where in pre-diabetes no altered expression of GLUT 4 could be detected [80]. Furthermore no mutation of the glucose transporter gene could be detected in Type 2 diabetic patients [32, 81]. These data do not exclude that an abnormality of GLUT 4 activation or translocation might occur at an earlier stage of the pathogenesis of Type 2 diabetes, as published studies have not addressed this important question.

Speculation on the primary defect

Available data suggest that neither the normal function of the insulin receptor kinase nor the glucose transporter GLUT 4 nor glycogen synthase itself are altered due to a mutation in these proteins. The reduced insulin stimulated activity of the insulin receptor and the glycogen syn-

Fig. 2. Synopsis of published data on abnormalities of signal transduction in the skeletal muscle in clinically overt Type 2 diabetes and in pre-diabetes. The figure shows the chain of signal transducing events at the target cells. The sites of defects in the signal flow are marked in bold letters

these both seem to be a consequence of altered regulatory mechanisms. Whether the intrinsic activity or the translocation of GLUT 4 is altered is unknown. Studies in pre-diabetic populations have uniformly shown that the impaired glycogen synthase activity is a very early event in the development of Type 2 diabetes while the data concerning the insulin receptor kinase inactivity are controversial. Other early events might be altered receptor isoform patterns and altered functions of PP-1. Thus the synopsis of the available data (Fig. 2) does not allow the initiating event in the development of skeletal muscle resistance to be defined. Hopefully a better understanding of the post-kinase signal transmitting steps will allow future progress in this field. Recently, the first putative substrate of the insulin receptor kinase (IRS1) was cloned [82]. This will allow the question of whether the expression level of this substrate or its functional properties are altered in Type 2 diabetic patients and pre-diabetic populations to be specifically addressed.

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Dr. H. U. Häring
 Institut für Diabetesforschung
 Kölner Platz 1
 W-8000 München 40
 Germany