

# Metabolic Syndrome: Psychosocial, Neuroendocrine and Classical Risk Factors in Type 2 Diabetes

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**ABSTRACT:** This paper summarizes some aspects of stress in the metabolic syndrome at the psychosocial, tissue and cellular levels. The metabolic syndrome is a valuable research concept for studying population health and social-biological translation. The cluster of cardiovascular risk factors labeled the metabolic syndrome is linked with low socioeconomic status. Systematic differences in diet and physical activity contribute to social patterning of the syndrome. In addition, psychosocial factors including chronic work stress are linked with its development. Psychosocial factors could lead to metabolic perturbations and increase cardiovascular risk via activation of neuroendocrine responses, e.g. in the autonomic nervous system and in several hormonal pathways. High glucocorticoid levels will promote lipid storage in visceral rather than subcutaneous adipose tissue. Adipocytes secrete several pro-inflammatory cytokines which considered major contributors to increase in oxidants and cell injury. Upregulation of heme-oxygenase 1 (HO-1) and peroxidase in the early development of diabetes produces a decrease in oxidative-mediated injury. Increased HO activity is associated with a significant decrease in superoxide, endothelial cell shedding and blood pressure. Finally, it is proposed that overexpression of glutathione peroxidase in beta cells may protect beta cell deterioration from oxidative stress during development of diabetes and hyperglycemia and this may result in attenuation of beta cell failure. If this proves to be the case, then the scene will be set to develop glutathione peroxidase mimetics for use in pre-clinical and clinical trials.

**KEYWORDS:** work stress; social epidemiology, neuroendocrine, adipokines, insulin resistance, antioxidants, adipocyte, glutathione, beta cell, glutathione

## INTRODUCTION

Accumulating epidemiological, biological and mechanistic evidence indicates that psychosocial, neuroendocrine, immunogenic and oxidative stress play critical roles in the development of metabolic syndrome, cardiovascular disease and type 2 diabetes. Each of these three 'stress-related' conditions are linked with low social status in developed economies.<sup>1,2</sup> This fact highlights the importance of a holistic approaches to preventing chronic disease as well as to understanding its etiology<sup>3,4</sup>. Brunner points out that prior to the industrial revolution the metabolic syndrome and type 2 diabetes were rare and that the use of the Whitehall model in present day populations can predict "at risk" individuals. Eriksson describes the role of neuroendocrine pathways in the development of insulin resistance in the progression of metabolic syndromes and type 2 diabetes<sup>5</sup>. Insulin resistance occurs mainly in muscle, fat and liver and the underlying mechanism(s) appears different in individual tissues<sup>6,7</sup>. The role of neuroendocrine pathways as possible mediators of the stress response in the brain is examined<sup>8</sup>. Stress leads to neuroendocrine responses that, over time, may be of significance in the development of insulin resistance and type 2 diabetes. This has led to the conclusion that both genetic and environmental factors are important and that humoral or neural mechanisms rather than intrinsic genetic defects on the target cells of insulin are the primary source of perturbations in stress related disease. Antioxidant gene, heme oxygenase-1 (HO-1) expression, is crucial in micro- and macrovascular disease in animal models of type 1 and 2 diabetes and that induction of HO-1, plays a critical role in preventing vascular endothelial cells death, and attenuating cardiovascular complications<sup>9,10</sup>. HO-1 induction results in increased levels of the heme degradation products carbon monoxide (CO) and bilirubin. These compounds are known to counteract the detrimental effects of oxidative stress in type I and 2 diabetes rendering endothelial cells resistant to diabetes-induced apoptosis by increasing the levels of antioxidant genes including EC-SOD and catalase. The experimental basis for chronic oxidative stress as an underlying mechanism for glucose toxicity in  $\beta$  cells is examined<sup>11,12</sup>. A role for the overexpression of glutathione peroxidase in  $\beta$  cells is proposed by Robertson's group as a cell destruction and to the normalization of a potential approach to limit blood glucose levels in diabetic patients. This concise report details the interactions of psychosocial and neuroendocrine factors, HO-1 and the underlying mechanisms involved in oxidative mediated injury in cardiovascular disease and diabetes.

### **Psychosocial Factors in the Metabolic Syndrome**

Life expectancy is at an all-time high in developed economies but health is not evenly distributed across social strata. Persistent health inequalities are observed in many causes of morbidity and mortality, and among the causes with major public-health importance, coronary heart disease (CHD) and type 2 diabetes mellitus stand out. Consistent with an underlying role in these health inequalities, there are inverse social gradients in prevalence of the metabolic syndrome in many populations, the lower the social position, the greater the risk of the syndrome.

Why are we interested in the metabolic syndrome? A holistic approach to evaluating chronic disease risk is useful. While it is appropriate to consider serum cholesterol, blood pressure and other risk factor levels one at a time, whether in an individual or among populations, summary indexes of risk such as the Framingham score offer a global assessment of disease risk that single factors do not capture. The metabolic syndrome is, thus, a biological indicator of disease risk. Per Bjorntorp and others have proposed a specific causal link between chronic stress and the metabolic syndrome, but the evidence for such specificity has been elusive.

### **THE PSYCHOSOCIAL HYPOTHESIS**

As an internal response, stress is probably most often regarded as psychological, but the implication is that there may be (patho-) physiological consequences. Stress means different things in different situations: it usually refers to individuals, with secondary consideration of the wider context. In contrast, the psychosocial hypothesis builds on the observation that the social environment has strong psychological elements. The social hierarchy influences access to material resources. It also shapes the availability of psychological resources. Beliefs and emotions are socially patterned, and the regularity of such social differences across place and time suggests they may contribute to social differences in mental and physical health. The idea is intrinsic to a variety of psychosocial concepts including power, control (or autonomy), demands and reciprocity (effort and reward), justice and fairness as well as gender and racial discrimination.

### **METABOLIC SYNDROME DEFINITIONS**

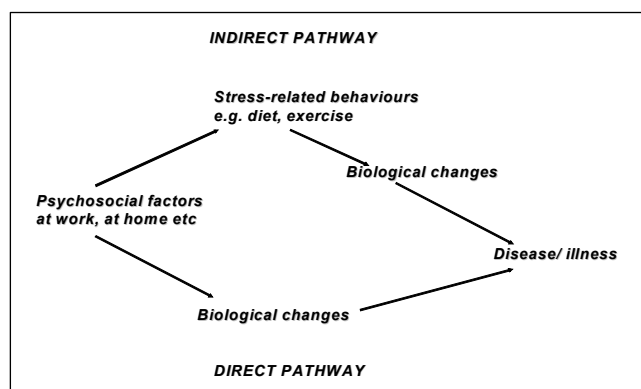
Various definitions of the metabolic syndrome exist. A scoring system identifies co-occurrence of several risk factors, typically 3 or more. Epidemiological studies have used cut-points based on observed distributions, for example, 3 or

more of 5 variables above the top quintile: serum triglycerides, HDL-cholesterol (bottom quintile), 2-hour post-load glucose, systolic BP and waist hip ratio, taking account of medication. Using this definition, prevalence of the syndrome in 1991-93 was 12% among healthy office workers aged 50 in the Whitehall II study.<sup>1</sup> NHANES III (1988-94) found that prevalence of the metabolic syndrome was 24% in US adults (32% among Mexican Americans).<sup>13</sup> Prevalence rose from 7% among 20-29 year olds to more than 40% over 60 years of age.

### RESEARCH MODELS

Selye's view was that repeated stress perceptions have cumulative physiological impact (the general adaptation syndrome). Risk of the metabolic syndrome and its component risk factors supplies a research model. Krieger's concept of embodiment provides an account of ways in which social position and racial discrimination gets under the skin to influence blood pressure levels. Building on Cannon's work showing that physiological systems maintain stability (homeostasis) via sensitive feedback and control mechanisms,<sup>14</sup> McEwen and Seeman's allostasis concept proposes that altered stable states are produced in response to repeated activation of neuroendocrine mechanisms, particularly the autonomic nervous system and hypothalamic pituitary adrenal axis.<sup>15</sup> Transition to some alternative state may be temporary or permanent.

The metabolic syndrome is a particularly common 'alternative state' today, but this was not the case in the past. In pre-industrial societies, metabolic syndrome and type 2 diabetes were rare if not unknown. Clues about its psychosocial origins come from the observation that it is linked to low social status.<sup>1</sup>



**FIGURE 1.** Direct and indirect pathways link psychosocial factors to disease.

Our research model connects psychosocial factors to disease development via two pathways, direct and indirect (Figure 1). There may be Interaction between adverse psychosocial circumstances and behavior-related factors such as obesity. In addition, pathophysiological states such as glucose intolerance are potentially reversible.

## EVIDENCE FROM WHITEHALL II

Findings from a nested case-control study within the Whitehall II cohort of office-based Civil Servants are consistent with a psychosocial component in the origins of the metabolic syndrome, showing that it is linked simultaneously with several indicators of adverse autonomic and neuroendocrine function.<sup>2</sup> A randomly chosen group of healthy men in civil service employment was studied. Nurses blind to the risk factor status of participants organized collection of a 24 hour urine sample over a working day. Compared with the control group, metabolic syndrome cases had higher urinary cortisol and normetanephrine (norepinephrine metabolites) outputs, higher heart rates and lower heart rate variability (HRV).

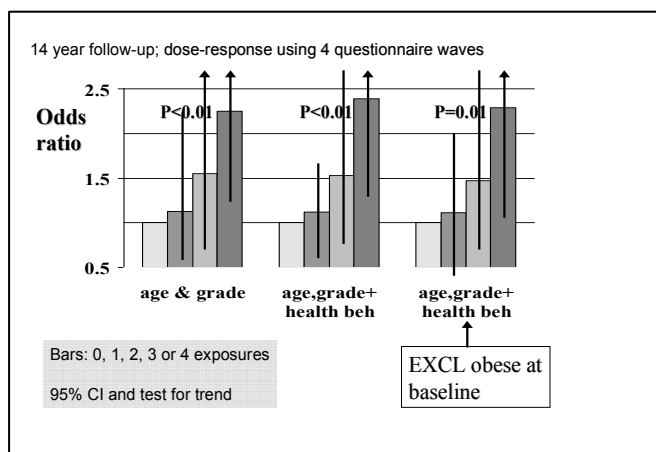
Having obtained such fascinating results, we conducted a larger study of in the Whitehall II cohort. We examined the inter-relationships of HRV and metabolic syndrome with employment grade and psychosocial factors. HRV measurement was made by means of a 5 minute electrocardiogram recording. Lower social position in the sample of 2197 men was associated with higher heart rate and lower HRV, indicating low vagal tone and sympathetic predominance. All five components and metabolic syndrome factor itself were associated with low HRV. In addition, the relationship between lower social position and higher risk of metabolic syndrome was mediated by HRV, behavioral factors and low job control. This study provided population-based evidence that disturbances of autonomic function are involved in mediating the excess coronary risk associated with low social position.

These two cross-sectional studies support other research suggesting psychosocial pathways are plausible.<sup>4</sup> Behavioral as well as psychosocial influences may account for the differences in autonomic and neuroendocrine activity. We show, for example, that level of physical activity is related both to the likelihood of having the metabolic syndrome and to heart rate variability in the Whitehall II study.<sup>16</sup>

Other cross-sectional studies have linked work stress with components of the metabolic syndrome in Whitehall II and other large samples.<sup>3,17</sup> Reviews show these associations are not consistent,<sup>18,19</sup> adding to the need for prospective studies. Accumulated data over 14 years of Whitehall II follow-up made a robust study possible. Repeated measurements of work characteristics over this period gave good characterization of psychosocial exposure. The association between four phases of measurement and ATPIII metabolic syndrome was

analyzed to test the hypothesis that there was a dose-response association.

The study proved to show such a dose-response relationship (Figure 2). Measurement of work 'stress' was based on the iso-strain model, using self-report questionnaires, which capture perceptions of the psychosocial work environment. Iso-strain is defined as high job demands, low job control, and low social support at work from coworkers and/or supervisors. The social gradient in metabolic syndrome is explained to a small degree by taking account of iso-strain (approximately 6%) compared to 50% when iso-strain, health behaviors and obesity were entered into the model. Nevertheless a causal inference can be drawn from this study. The prospective stress -- metabolic syndrome relation was robust to adjustment for social position and adverse health behaviors.



**FIGURE 2. Dose response effect of job stress (iso-strain) on incident metabolic syndrome. Whitehall II study.** X-axis labels show adjustment variables. Health beh = health behaviors over 4 exposure phases (current smoking, no daily fruit & vegetable consumption, heavy alcohol consumption, no exercise) Source: Chandola et al 2006.

A related 19 year prospective study showed that work stress was linked to development of obesity, including central obesity, during mid-life.<sup>20</sup> The study shows that in addition to the known effects of positive energy balance, there may be a psychosocial aspect to the secular trend in obesity prevalence.

### BEYOND WORK?

There are parallels between work hierarchies and wider social structure. Political philosophers such as John Rawls have argued that the distribution of autonomy (or control), reciprocity and justice are important qualities of the

social order, rooted in human preference. These qualities are partly psychosocial in nature, and provide a novel basis to consider why health and disease are unequally distributed across social strata. In the work context, Whitehall II shows that both perceived lack of reciprocity (from by the effort reward imbalance questionnaire) and a sense of injustice in the work place predict CHD.<sup>21</sup> Further follow-up of the cohort as participants move into retirement will enable us to examine health inequalities and the roles of psychosocial factors and the metabolic syndrome during the third age.

### **Role of Neuroendocrine Pathways in Insulin Resistance and Type 2 Diabetes**

There is now much support for psychosocial stress as a risk factor for metabolic syndrome and type 2 diabetes, and development of insulin resistance can be a common pathway in this context<sup>22,23</sup>. Insulin resistance can be defined as an attenuated effect of insulin in target tissues, mainly muscle, fat and liver<sup>24</sup>. 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<sup>5</sup>. Obviously, there is much evidence supporting the importance of genetic factors in human insulin resistance<sup>25</sup>, and there are common polymorphisms that are associated with type 2 diabetes and insulin resistance. However, there is evidence suggesting that insulin resistance is not a primary cellular defect and that there are factors in the surrounding 'tissue environment' that can have a causative role. These factors include metabolic, neural and hormonal signals, and for example 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.

### **Neuroendocrine Mechanisms in Metabolic Regulation**

Neuroendocrine pathways have received much attention as possible mediators of stress response of the brain. In general, stress can be seen as a threat of the organism's homeostasis, and reactions are elicited at the central nervous, neurohormonal, cellular and molecular levels that aim to disarm the stressor and restore the equilibrium<sup>22</sup>. However, when overactive or prolonged these defense mechanisms will have detrimental effects on brain function, the cardiovascular system as well as regulation of nutrient

metabolism. For rapid signalling from the CNS to peripheral tissues, the autonomic nervous system is utilized. Essentially all metabolically active tissues, e.g. skeletal muscle, heart, adipose and liver, have autonomic innervations with both sympathetic and parasympathetic nerves. Catecholamine release from the adrenal medulla is also regulated mainly by autonomic nerve activity. For more long-term communication the brain can use the hypothalamo-pituitary hormonal systems and other neuroendocrine pathways. In this context, prolonged elevation of insulin-antagonistic hormones like cortisol<sup>26</sup> and growth hormone can contribute to insulin resistance in various tissues. Moreover, dysregulation of the autonomic nervous system might be a potential mechanism for early insulin resistance in the development of type 2 diabetes<sup>27</sup>.

Clinical conditions with endogenous or exogenous glucocorticoid excess are associated with insulin resistance, glucose intolerance, central obesity and hypertension, i.e. features of the metabolic syndrome. In clinical obesity, there are alterations in cortisol metabolism, and an enhanced local conversion of cortisone to cortisol via 11 $\beta$ -hydroxysteroid dehydrogenase type 1 in the adipose tissue may contribute to the development of the metabolic syndrome. The insulin-antagonistic effects of cortisol include impairment of insulin signalling and glucose uptake as well as enhanced hepatic glucose output and an increased adipose tissue lipolysis<sup>22</sup>. Moreover, there are data suggesting that there are detrimental effects on insulin secretion and  $\beta$ -cell survival<sup>28</sup>. Besides glucocorticoids, gonadal steroid hormones, mainly estrogen and testosterone, are of interest. Altered levels of these hormones have been implicated in abdominal obesity and insulin resistance, obviously with different patterns in men and women<sup>29</sup>.

The autonomic nervous system can also be involved in insulin resistance and development of type 2 diabetes<sup>27</sup>. 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 standardized stress<sup>8</sup> or following hyperinsulinemia<sup>30</sup>.

### **A Paragon of Adipokines in Inflammation and Insulin Resistance**

The role of hormones and other molecules secreted by the adipocyte and other cell types, e.g. macrophages, in adipose tissue has been extensively investigated during recent years. Cytokine-like factors secreted by adipose tissue are often designated "adipokines". Some of these, e.g. TNF- $\alpha$  and interleukin (IL)-6, have been indicated as culprits in the development of insulin resistance. IL-6 levels display a strong association with insulin resistance and type 2 diabetes<sup>31</sup>, and this cytokine can directly inhibit insulin receptor signal transduction in hepatocytes as well as adipocytes<sup>32</sup>. However, the metabolic effects of IL-6 are complex, and in the brain, there is both an

activation of the HPA axis and also effects that prevent obesity, as demonstrated in animal experiments. Leptin is a crucial signal from the adipose tissue to the brain that induces satiety and promotes CNS-mediated increase in energy expenditure<sup>33</sup>. These effects of leptin are exerted in the hypothalamus via inhibition of NPY neurons and activation of POMC neurons in the arcuate nucleus, hence leading to MC4 receptor activation in the paraventricular nucleus. Leptin levels are associated with insulin resistance and obesity, whereas there is an inverse relationship for adiponectin<sup>34</sup>. There are probably also several other known and unknown factors secreted by adipose tissue, such as retinol-binding protein 4, MCP-1 etc, that are linked to insulin resistance. High FFA levels directly and indirectly will contribute to insulin resistance. FFAs in the portal vein can lead to activation of the HPA axis, but in addition may elicit a sympathoadrenergic response<sup>35</sup>. In type 2 diabetic patients, we observed that hyperglycemia is associated with both elevated cytokine and cortisol levels in the circulation<sup>36</sup>. Taken together, there are data that support a vicious circle connecting visceral adiposity, neuroendocrine overactivity, insulin resistance/hyperglycemia and proinflammatory cytokines (Fig 3).

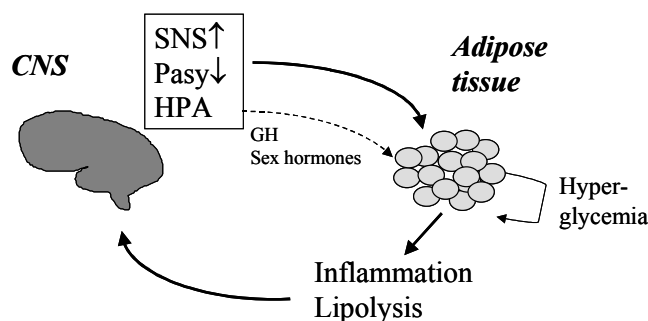
### **Stress, Visceral Adiposity and Diabetes**

Stressful situations will lead to neuroendocrine responses, that in the long-term perspective might be important in the development of insulin resistance 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 HPA axis, and the sympathoadrenergic system, i.e. cortisol and adrenaline and noradrenaline, respectively, are known to oppose the effects of insulin. Some studies suggest elevated cortisol levels in situations such as work stress and unemployment<sup>37,38</sup>. A chronically stressed HPA axis appears to display a decreased diurnal variability<sup>22</sup>. Bjorntorp and coworkers proposed that a hypothalamic arousal may contribute to insulin resistance via excess cortisol production<sup>23</sup>, but then there might be a shift to a “burn-out” phenomenon with low secretion of cortisol, growth hormone and sex steroids.

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<sup>39</sup>. A link between central obesity and HPA axis dysregulation has also been suggested<sup>40</sup>. Moreover, an altered activity in the sympathetic and parasympathetic nervous systems may be associated with visceral obesity<sup>41</sup>. One attractive hypothesis is that in a situation of calorie overload, subcutaneous adipose tissue eventually reaches its upper limit for further triglyceride storage, and that this triggers adipose inflammation and lipid

‘spill-over’ that is diverted to visceral fat and with time also to ectopic locations, i.e. in liver and muscle.

As mentioned before in this chapter, there are now several studies that link psychosocial factors to the metabolic syndrome. Stressful life events, low educational level, low sense of coherence, work stress, low emotional support as well sleeping disorders have all been associated with development of type 2 diabetes<sup>42,43</sup>. Taken together, much evidence support that psychosocial stress that leads to long-term neuroendocrine dysregulation is likely to increase the risk for type 2 diabetes.



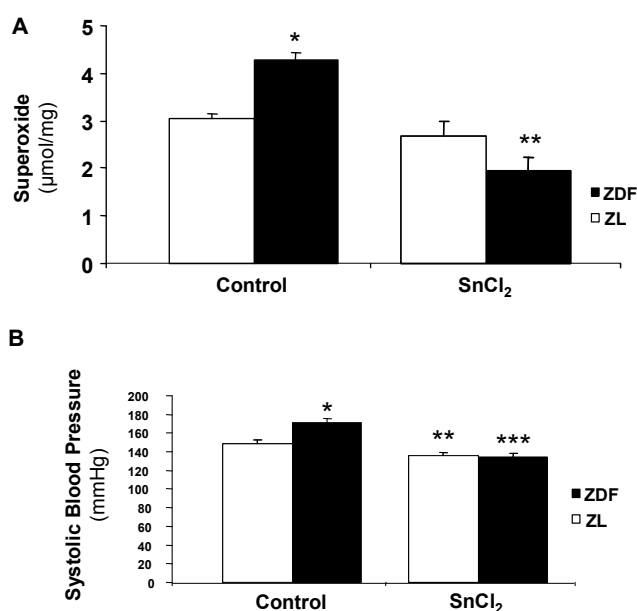
**Figure 3.** A hypothetical vicious circle. Neuroendocrine stress response leads to adipose tissue insulin resistance and lipolysis. This promotes hyperglycemia, via interactions with pancreas, muscle and liver, that in turn can elicit release of proinflammatory cytokines from adipose tissue. Such cytokines can together with elevated FFA levels further activate the neuroendocrine pathways in the CNS. GH, growth hormone; HPA, hypothalamo-pituitary-adrenal system; Pasy, parasympathetic nervous system; SNS, sympathetic nervous system.

### Paragon of Antioxidants Gene, HO-1 in Type 2 Diabetes

Heme oxygenase-1 (HO-1) is the rate limiting enzymatic step that catalyzes the breakdown of heme into equimolar amounts of biliverdin, an antioxidant rapidly converted to bilirubin, and carbon monoxide (CO), an anti-apoptotic vasodilator, with the release of its iron moiety<sup>9</sup>. Oxidant stress strongly induces heme oxygenase-1 (the inducible form of HO), which guards against oxidative insult. Upregulation of HO-1 decreases oxidative stress, attenuates endothelial cell sloughing and fragmentation, and restores endothelial cell function in experimental diabetes.

### Effect of HO-1 on Vascular $O_2^-$ Levels and Blood Pressure in Diabetes

Levels of both  $O_2^-$  (Figure 4A) and blood pressure (4B) 22-week-old ZDF were significantly elevated compared to ZL and were reduced by the induction of HO.  $O_2^-$  levels were  $4.26 \pm 0.16$   $\mu\text{mol}/\text{mg}$  in ZDF compared with  $3.05 \pm 0.09$   $\mu\text{mol}/\text{mg}$  in ZL ( $P < 0.005$ ). HO induction significantly reduced levels of  $O_2^-$  in ZDF to  $1.96 \pm 0.27$   $\mu\text{mol}/\text{mg}$  ( $P < 0.05$ ) while an observed decrease in ZL ( $2.676 \pm 0.31$   $\mu\text{mol}/\text{mg}$ ) was not statistically significant. ZDF treated with  $\text{SnCl}_2$  demonstrated  $O_2^-$  levels equivalent to 8-week old control ZL while heme levels were reduced to levels significantly below 8-week-old ZL ( $P < 0.05$ ) and ZDF ( $P < 0.005$ ) controls.



**Figure 4.** Superoxide levels in aortic tissue from 22 week old ZL and ZDF (mean  $\pm$ SE;  $n=4$ ), \* $P < 0.005$  vs. ZL control; \*\* $P < 0.05$  vs. ZDF control. **B** Systolic Blood pressure measurements from 22 week old ZL and ZDF, \* $P < 0.001$  vs. ZL control, \*\* $P < 0.05$  vs. ZL control; \*\*\* $P < 0.000001$  vs. ZDF control and  $P < 0.05$  vs. ZL control.

As an assessment of the potential clinical benefits of HO-1 induction, we measured systolic blood pressure (SBP) and circulating endothelial cells (CEC) following treatment with  $\text{SnCl}_2$ . SBP was significantly lower in control ZL than in ZDF, demonstrating the link between T2DM and hypertension (Figure 4 B). Following HO induction with  $\text{SnCl}_2$ , SBP was decreased in ZL and in ZDF.

### **Type 2 Diabetes, Hyperglycemia, and Chronic Oxidative Stress**

The pathogenesis of type 2 diabetes, also known as adult-onset diabetes, is usually attributed to a combination of pancreatic islet beta cell dysfunction and resistance to the action of insulin in important targets, such as liver, muscle, and fat tissue. The cause of beta cell failure is polygenic in nature, whereas the cause of insulin resistance is at least partially explained by associated obesity. It is important to note, however, that many type 2 diabetic people are not obese, and that the majority of obese individuals do not develop type 2 diabetes. Thus, it appears that type 2 diabetes is primarily a genetic disease that can be made worse, but is not caused, by excessive body fat.

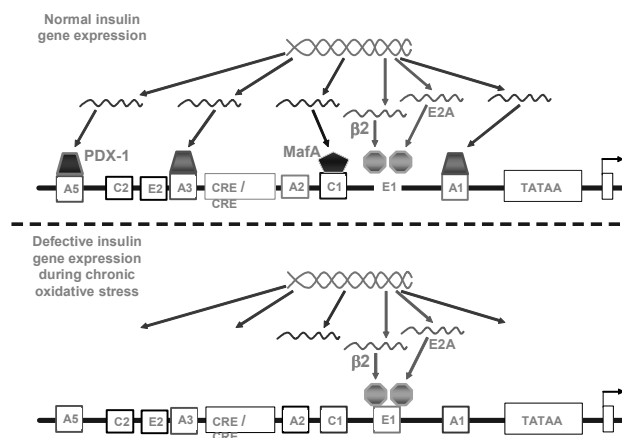
A major issue in the field of type 2 diabetes research is why a continual and inexorable decline in glucose control occurs despite optimal drug treatment. This decline in beta cell function is associated with chronically elevated blood glucose levels leading to the notion of beta cell exhaustion because of either continual stimulation by glucose, or that high concentrations of glucose are chemically toxic to the beta cell. There is an intrinsic paradox at play here. Since glucose is considered to be a physiologic compound and supportive of beta cell function at many levels from insulin gene transcription through insulin secretion.

One concept that has emerged is that of glucose toxicity, i.e. chronically high glucose levels form metabolites that can be harmful. This has led to the idea that glucose toxicity of the beta cell might be attributable to formation of excess levels of reactive oxygen species. One can envision that the normal route of glycolysis and oxidative phosphorylation might become oversaturated with glucose. This in turn might lead to shunting excess traffic of glucose molecules along any of several alternative routes, including methylglyoxal formation and glycation; enediol and  $\alpha$ -ketoaldehyde formation (glucosylation); diacylglycerol formation and protein kinase C activation; glucosamine formation and hexosamine metabolism; and sorbitol metabolism<sup>11</sup>. One needs only to imagine a flooding of all these pathways by glucose as a mechanism for excessively high concentrations of ROS in many tissues, including pancreatic beta cells.

This pathophysiologic construction suggests that ROS, which like glucose function as positive chemical mediators in physiologic processes, become negative forces in excess concentrations. Both ROS and glucose have good and evil sides, depending on whether their levels are normal or excessive leading to the term glucose toxicity with a major mechanism of action being chronic oxidative stress.

### Experimental evidence for chronic oxidative stress as a mechanism for glucose toxicity of the beta cell

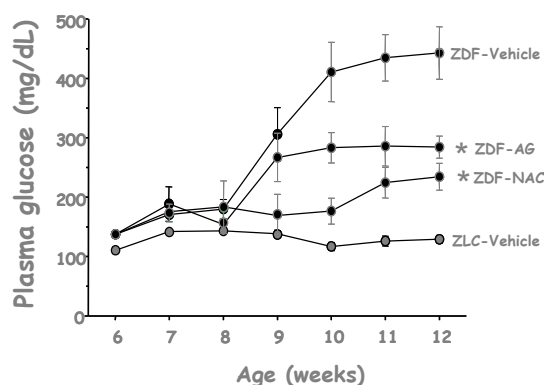
The concept of glucose toxicity was first proposed in 1985<sup>44</sup> and the first biochemical and molecular evidence to support this hypothesis at the level of the beta cell was reported in the early 1990s. In studies using the beta cell line HIT-T15, serial observations were made over many passages that demonstrated chronic exposure of beta cells led to decreased insulin gene expression, insulin stores, and glucose-induced insulin secretion<sup>12</sup>. Protein levels of two critically important transcription factors, PDX-1 and MafA, were low to non-detectable after prolonged culturing of HIT-T15 cells in media containing supraphysiologic concentrations of glucose<sup>45,46</sup>. It was determined that the molecular mechanism for decreased PDX-1 levels was post-transcriptional while the mechanism for decreased MafA was post-translational<sup>47</sup>. The transcriptional machinery needed for insulin gene expression was not abnormal<sup>45</sup>, so attention was focused on the insulin promoter region through a series of studies involving mutation of the PDX-1 and MafA DNA binding sites and reconstitution of glucotoxic beta cells with these two proteins<sup>45-47</sup>. Mutation of the insulin promoter binding sites for either protein in non-glucose toxic beta cells led to marked decreased in promoter activity. Reconstitution of glucotoxic beta cells by transient transfection of either PDX-1 or MafA lead to improved promoter activity. It was recently shown that adenoviral reconstitution of HIT-T15 cells with both PDX-1 and MafA fully restored insulin promoter reporter activity and partially normalized levels of insulin mRNA<sup>47</sup> and Figure 5.



**Figure 5:** Molecular mechanisms of actions leading to defective insulin gene expression in glucotoxic beta cells. In this model, insulin gene transcription

is intrinsically normal, but gene expression of two critical transcription factors, PDX-1 and MafA, is not. Normally, PDX-1 binds to the insulin promoter at three sites, A1, A3, and A5. MafA binds to C1 only. During the development of glucose toxicity, PDX-1 fails to become expressed because of a post-transcriptional defect, and MafA fails to become expressed because of a post-translational defect. Consequently, insulin gene expression at the mRNA level decreases, as does insulin stores and glucose-induced insulin secretion. Taken from ref. <sup>11</sup>.

It became evident from the concepts put forward by Wolff and colleagues <sup>48</sup> that the mechanism of glucose toxicity might involve generation of reactive oxygen species (ROS), specifically from glucose autoxidation. This led to early work assessing whether the defects in insulin gene expression and abnormal insulin secretion associated with exposure to high glucose concentrations could be ameliorated by antioxidants. Treatment of db/db mice and ZDF rats with NAC preserved insulin gene expression and beta cell function <sup>49,50</sup>. Inclusion of NAC or aminoguanidine in media containing supraphysiologic concentrations of glucose protected HIT-T15 cells against the loss of PDX-1 and insulin gene expression <sup>51</sup> and Figure 6.



**Figure 6:** Plasma glucose levels in Zucker Diabetic Fatty (ZDF) rats, a genetic model of type 2 diabetes, who were treated with placebo, n-acetylcysteine, or aminoguanidine beginning at 6 weeks of age. Both drugs are antioxidants and both drugs ameliorated the degree of hyperglycemia developed by the animals. Zucker lean controls (ZLC) that do not develop hyperglycemia are shown for comparison. Taken from ref. <sup>49</sup>.

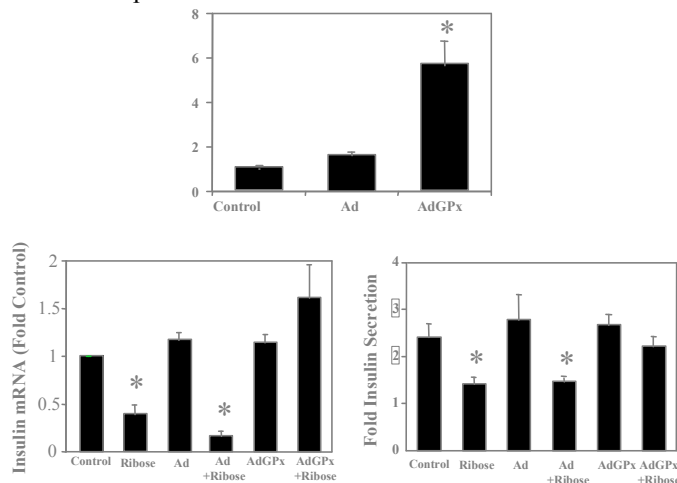
### A Central Role for Glutathione Peroxidase in the Beta Cell

The status of antioxidant enzyme expression in pancreatic beta cells is of major importance to the thesis that chronic oxidative stress may cause beta

cell dysfunction. The pancreatic islet is unusual in that it contains the lowest complement of antioxidant enzymes of any other tissue. Isolated islets from rodents contain very low levels of SOD-1, SOD-2, catalase, and glutathione peroxidase mRNA, protein, and activity levels<sup>52</sup>. Similar observations have been made using human isolated islets raising the question whether the islet is designed so that low levels of ROS are purposely encouraged to facilitate processes such as gene transcription and exocytosis. These low levels of antioxidant enzymes become, however, a handicap when excess levels of ROS are formed in the beta cell or surrounding tissue.

This paradoxical situation wherein low levels of ROS might be good for the beta cell, but higher levels might not be, led to a series of experiments in which rodent and human islets exposed to high concentrations of glucose formed greater ROS concentrations than islets exposed to normal glucose concentrations<sup>51</sup>. Therefore, the potential protective effect of glutathione peroxidase overexpression was tested in islets exposed to high concentrations of glucose. Glutathione peroxidase was chosen because it metabolizes both hydrogen peroxide and lipid peroxides. SOD-1 and SOD-2 were excluded because their catabolization of superoxide forms hydrogen peroxide, an ROS. Catalase was not chosen because it catabolizes hydrogen peroxide, but not lipid peroxides. Glutathione peroxidase overexpression increased activity of the enzyme 6-fold, roughly equivalent to the activity present in liver, thus preventing the deleterious effects of ribose on insulin gene expression, insulin content, and glucose-induced insulin secretion<sup>51</sup>.

This led the Robertson lab to assess the potential benefits of transgenic experiments in which glutathione peroxidase is overexpressed in the beta cells of animal models of type 2 diabetes. Overexpression of this enzyme was examined in beta cells in db/db mice to determine if it would protect them from beta cell deterioration as they begin to develop hyperglycemia and, if so, whether this would result in attenuation of beta cell failure (Figure 7), as seen in the case of NAC and aminoguanidine treatment of Zucker Diabetic Fatty rats. If this proves to be the case, it will encourage the development of glutathione peroxidase mimetics for use in pre-clinical and clinical trials. This approach will determine whether such drugs will provide a novel, ancillary layer of protection to patients being treated with conventional anti-diabetic drugs, which usually do not completely normalize blood glucose concentrations in patients with diabetes.



**Figure 7:** Preventive effects of adenoviral infection of GPx cDNA in isolated islets against the oxidative effects of ribose. Ribose in non-infected cells decreases insulin gene expression and glucose-induced insulin secretion from rat islets in vitro (two lower panels). Adenoviral infection of islets with GPx cDNA increase intrinsic GPx activity in islets 6-fold (top panel) and prevents the adverse effects of ribose on islets (bottom panels). Infection with virus not containing GPx (Ad) has no effects on islets under control conditions or on the adverse effects of ribose on islet function (bottom panels). Taken from Ref. <sup>51</sup>

## CONCLUSION

There are now several studies that link psychosocial factors to the metabolic syndrome and type 2 diabetes. Much effort has been made to find the mechanisms of insulin resistance in type 2 diabetes and the metabolic syndrome, there is however still a need for better understanding. Obviously, both genetic and acquired factors are of importance. It is likely that either humoral or neural mechanisms rather than intrinsic defects in insulin's target cells are primary perturbations. An enhanced neuroendocrine 'vulnerability' upon stressful environmental stimuli could be of importance, and this could be defined by inherited as well as acquired factors. The intrinsically low level of antioxidant gene expression in beta cells puts them at particular risk for oxidative damage from environmental oxidants. Furthermore, uncontrolled hyperglycemia generates reactive oxygen species, which further damage already compromised beta cells in type 2 diabetes. Therapy directed towards increasing antioxidant protection may facilitate the management of type 2 diabetes.

## ACKNOWLEDGEMENTS

This work was supported by NIH grants DK068134, HL55601 and HL34300 (N.G.A), financial support was given by the Swedish Research Council (Medicine 14287) and the Swedish Diabetes Association, and the scientific contributions by Jonas Burén, Stina Lindmark, Magdalena Lundgren, Frida Renström and Maria Svensson are gratefully acknowledged (J.W. E) and NIH grant DK-38325 (RPR)

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