Chapter III

Review Insulin Resistance: Intra-Uterine Growth Retardation, Life Style, Genetic Susceptibility, Prevention and Treatment

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Abstract

Overweight (BMI > 25) and obesity (BMI > 30), in our modern world of food abundance has become epidemic and is associated with a whole range of health problems like hypertension, type 2 diabetes, and an excess of cardiovascular and renal diseases. We will describe intra-uterine growth retardation due to maternal undernutrition, life-style and genetic susceptibility as causes for insulin resistance and β-cell dysfunction. The 'fetal' or 'early' origins of adult disease hypothesis provided evidence for the association between the perturbation of the early nutritional environment and the major risk factors (hypertension, insulin resistance, and obesity) for cardiovascular disease, diabetes, and the metabolic syndrome in adult life. In this review we give some epidemiological examples from the "Dutch Hunger Winter" a war induced famine between August 1944 and April 1946 in Amsterdam with less than 1,000 calories per day from government food rations. Epidemiological studies demonstrate that exposure to famine during gestation resulted in increases in impaired glucose tolerance, obesity, coronary heart diseases, atherogenic lipid profile, hypertension, microalbuminuria, schizophrenia, antisocial personality and affective disorders. Maternal undernutrition during gestation has important effects on health in later life, but it is hypothesized that the timing of the nutritional insult determines which organ system is affected. Maternal undernutrition can
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express itself in the second or third generation. Obesity due to diet and a sedentary lifestyle may result in excess adipose tissue which actively participates in the integration of whole-body energy and fuel metabolism by the secretion of many hormones like leptin, adiponectin and resistin. Life style features like a) disturbance in the autonomous nervous system (biological clock), b) gender, c) socioeconomic status, d) race and e) stress all may be contributing factors in the origin of metabolic syndrome and insulin resistance. Genetic factors influence the different components of the Metabolic Syndrome. Type 2 diabetes is a polygenic disorder. The "thrifty gene theory" states that it can be inherited from our ancestors living in an environment with unstable food supplies and famine. For them it could increase their probability of survival if they could maximize their storage of food surpluses. With the term "thrifty genes" one can think about underlying hormonal pathways and metabolic routes like growth hormone and insulin. Inflammation, a second characteristic of insulin resistance, can be caused by Interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α). Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) is a transcription factor involved in regulating genes in adipogenesis and, by implication, insulin action. Diet, exercise and preventing stress are the first-line strategies in the management of type 2 diabetes. Exciting results for medical glycaemic control are found in (thiazolidinediones (TZD eg. Pioglitazone). They are agonist for the PPARγ and also have an effect on the recently discovered hormone resistin which probably can link obesity to diabetes-2. However, recently a meta-analysis study and two clinical trials gave for thiazolidinedione-therapy with Rosiglitazone (Avandia) strongly evidence for increased cardiovascular risk and possible cardiotoxicity. Unless no new data are available of ongoing trials it is recommended to chose for other therapies to treat type 2 diabetes.

Keywords: insulin resistance, intra-uterine undernutrition, life style, genetics, PPAR, thiazolidinediones, Pioglitazone, Rosiglitazone.

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Metabolic Syndrome

Overweight (BMI > 25) and obesity (BMI > 30), especially visceral fat accumulation, are associated with a whole range of health problems like hypertension, type 2 diabetes and an excess of cardiovascular and renal diseases. The risks of obesity are known for more than 80 years, but since in our modern world of food abundance it has become epidemic, the economic impact of the obesity-related risks is becoming larger and larger (Egan and Julius, 2004).

Due to the excessive costs brought by obesity-induced health problems, there is great interest in the mechanisms by which obesity causes these problems. Hepatic steatosis which is often related to obesitas has become a research area of interest due to its close association with obesity and metabolic syndrome. In the USA nonalcoholic fatty liver disease (NAFLD) affects approximately 30 million Americans (Browning & Horton 2004), 20 million Americans have diabetes-2 and the number of diabetics is increasing by 5% per year (Feldman et al. 2001, Boulton et al. 2005). More in general, it is estimated that about 47 million US residents have the metabolic syndrome (Ford et al. 2002). In the United States, among adults, the estimated annual cost attributable to obesity-related diseases is about 100 billion dollar. Among children and adolescents annual hospital costs related to obesity were 127 million dollar during 1997-1999 (www.aaperd.org/thepulse/archives/Pulse-
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Vol3no4.pdf)). Because the USA has 281.4 million citizens and the European community (EU-12) 298.1 million citizens and there is nearly no difference in estimated prevalence of Metabolic Syndrome between the continents the annual societal costs in the EU may have similar figures. A lot of research has been extended on this subject. About 10 years ago, the concept of 'syndrome X,' also called ‘insulin resistance syndrome’, was introduced by Reaven (1993). In this syndrome the cluster of changes associated with resistance to insulin-mediated glucose uptake plays an important role in the etiology and clinical course of patients with non-insulin-dependent diabetes, high blood pressure, and coronary heart disease (Reaven, 1993). In 1998, a unifying definition was proposed by the WHO organisation and the insulin resistance syndrome was renamed the ‘metabolic syndrome’ instead of the ‘insulin resistance syndrome’ because it had not been established that insulin resistance is the cause of all components present in the syndrome (Heilbronn et al., 2004). The metabolic syndrome is a term that denotes a constellation of cardiovascular risk factors related to insulin resistance and obesity with a centripetal fat pattern. There are different definitions of the metabolic syndrome provided by the World Health Organisation (in 1999) and by the National Cholesterol Education Program (NCEP; in 2001) but any of them predict a significant risk of developing diabetes as well as an excess of coronary heart disease and total cardiovascular disease (Égan and Julius, 2004).
1. Intra-Uterine: Growth Retardation, Epigenesis

Reduced Fetal Growth and Low Birth Weight

The 'fetal' or 'early' origins of adult disease hypothesis was originally put forward by David Barker and colleagues and stated that environmental factors, particularly nutrition, act in early life to program the risks for adverse health outcomes in adult life. This hypothesis has been supported by a worldwide series of epidemiological studies that have provided evidence for the association between the perturbation of the early nutritional environment and the major risk factors (hypertension, insulin resistance, and obesity) for cardiovascular disease, diabetes, and the metabolic syndrome in adult life (McMillen & Robinson 2005).
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A study of Valdez turned out that reduced fetal growth and a low birth weight have been associated with an increased risk of cardiovascular disease, type 2 diabetes and features of the metabolic syndrome developing later on (Valdez et al. 1994). In another study it turned out that children with a low birth weight showed significant more hyper-insulinemia and insulin resistance compared to controls with normal birth weight (Jaquet et al. 2000). A probable explanation could be the ‘thrifty phenotype’ theory (Hales et al. 1991). The theory suggests that low birth weight, due to intra-uterine undernutrition, might have the consequence of developmental adaptations in certain tissues (for example endocrine pancreas, adipose tissue and muscle fiber) predisposing individuals to cardiovascular and metabolic disturbances in adult life.

Not only low birth weight can induce insulin resistance, but also the other way round, in case of genetically determined insulin resistance. This means that genetically determined insulin resistance could result in low fetal growth and insulin resistance later on. This principle is known as the ‘fetal insulin hypothesis’ (Hattersley & Tooke 1999).

Several studies have been performed on the effects of maternal intrauterine undernutrition on offspring birth weights in a cohort of women born between August 1944 and April 1946 in Amsterdam, The Netherlands. This period included “the Dutch Hunger Winter”, a war-induced famine. Undernutrition was defined separately for each trimester of pregnancy as an average supply of less than 1,000 calories per day from government food rations (Lumey and Stein 1997).

Maternal birth weight itself was decreased after third trimester intrauterine exposure but not after first trimester exposure. The authors suggest that there may be long-term biological effects, even into the next generation, of maternal intrauterine undernutrition which do not correspond to the effects on the mothers’ own birth weights (Lumley and Stein 1997). This was later confirmed in studies on impaired insulin secretion by comparing the glucose tolerance in people who had been prenatally exposed to famine compared with people unexposed to famine (de Rooij et al. 2006). Kyle and Pichard (2006) stated in their review that exposure to famine during gestation resulted in increases in impaired glucose tolerance, obesity, coronary heart diseases, atherogenic lipid profile, hypertension, microalbuminuria, schizophrenia, antisocial personality and affective disorders. However in other studies no statistically significant differences were observed between participants exposed and unexposed to famine in utero in the mean profile of cortisol response to psychological stress protocol (de Rooij et al. 2006). Also greater prevalence of metabolic syndrome (de Rooij et al. 2007), the HPA axis activity at adult age (de Rooij et al. 2006) and increased arterial vessel wall stiffness (Painter et al. 2007) were unaffected. Differences between studies possibly can be ascribed to the period of maternal undernutrition during gestation. Painter et al. (2005) found more coronary heart disease, raised lipids, and altered clotting and more obesity after exposure to famine in early gestation compared to those not exposed to famine. Exposure in mid gestation was associated with obstructive airways disease and microalbuminuria. While decreased glucose tolerance was found in people exposed to famine in late gestation. This study shows that maternal undernutrition during gestation has important effects on health in later life, but that the timing of the nutritional insult determines which organ system is affected (Painter et al. 2005).

That the timing of undernutrition during the gestation period may be important for *homo sapiens* may be explained by a study which found differences in brain development between rodents and humans. In the normal adult rodent and primate, *arcuate nucleus* (ARH) neurons
function as conduits for transmitting metabolic hormonal signals into the hypothalamic circuitry that modulates feeding and energy expenditure. Grove et al. (2005) have shown that ARH projections do not fully develop until the 3rd postnatal week in the rodent. This is in stark contrast to the nonhuman primate (NHP) in which ARH projections develop during the 3\textsuperscript{rd} trimester of pregnancy. This species difference suggest that maternal diet and health are likely key factors for the development of ARH projections in the primate, whereas the postnatal environment (i.e., diet) would be more important in the rodent (Grove et al. 2005).

In contrast to the 'Dutch famine' studies were the effect of starvation was studied on the offspring, other authors investigated the effect of maternal adiposity on fetal development. Yanijik (2004) found that the body composition of Indian babies was influenced by maternal adiposity before pregnancy and by aspects of maternal nutritional intake and circulating nutrient concentrations during pregnancy. Increased nutrition in the face of a genetic predisposition increases maternal insulin resistance in late pregnancy and promotes fetal adiposity even in absence of marked hyperglycaemia (Yanyik 2004). In the study of Kaati et al. (2002), investigating historical data in families of an isolated parish village in North-Sweden the researchers found that seasonal overfeeding has transgenerational effects resulting in cardio-vascular disease and diabetes in the descendants of the second or third generation.

There are no strong paternal determinants of adiposity at birth (Yanyik 2004). In contrast, in the study of Pembrey et al. (2006) it was demonstrated that these aspects can also become transgenerational via the male line depending if food abundance occurs during the prepubertal period.

So undernutrition and overfeeding can result in an increased risk of cardiovascular disease, type 2 diabetes and features of the metabolic syndrome in the second or third generation. This implies a new insight in the interplay between inheritance and environment in health and development.

2. Life Style: Obesity, Sexe Differences, Race, Physical Activity and Psychosocial Stress

Life style (weight gain and sedentary lifestyle): Physical activity has an impact on many of the components of the metabolic syndrome. An increase of physical activity results usually in weight loss and reduction of body fat. Exercise training has been associated with favorable changes in serum triglyceride and HDL cholesterol concentrations and with improvement in insulin sensitivity (Knowler et al. 2002). The opposite is that low cardio respiratory fitness (little physical activity) is associated with an increased clustering of the metabolic abnormalities associated with the metabolic syndrome. A moderate grade of physical activity reduces the likelihood of the development of the metabolic syndrome (Laaksonen et al. 2002). A very important aspect is that of food intake because a surplus of fatty acids, and namely unsaturated fatty acids, can cause dyslipidemia (ref).

Obesity due to diet and a sedentary lifestyle may result in excess adipose tissue. Adipose tissue is not just ‘an organ’ designed for passive storage and release of triacylglycerols (TGs).
The abundant abdominal fat in most patients with fatty liver disease produces proinflammatory cytokines (Rudin & Barzilai, 2005). In addition, adipose tissue also actively participates in the integration of whole-body energy and fuel metabolism by the secretion of many hormones like leptin, adiponectin and resistin (den Boer et al. 2004).

Disturbance in the autonomous nervous system: The origin of the metabolic syndrome is probably in a disturbance in the balance between physical activity and food intake. The biological clock is a part of the autonomous nervous system and is located in the suprachiasmatic nucleus of the hypothalamus (SCN) (Kreier et al. 2007).

It has two functions a) a balance between sympathetic to parasympathetic activity in the body, b) regulation of the diurnal rhythm.

a) The biological clock regulates the rhythmic change from sympathetic to parasympathetic activity in the body and adapts the metabolism to this rhythm. In our lifestyle we constantly change between active and inactive periods, instead of one long active (day) and one long inactive period (night). The physical activity in general is reduced, so there is little fluctuation between activity and inactivity. This together results in conflicting information sent to the biological clock. The biological clock adapts metabolism to the sympathetic/parasympathetic rhythm. Because this rhythm is disturbed, the biological clock will also be disturbed and this results in an increased parasympathetic activity on the visceral adipose tissue and an increased sympathetic activity on the heart and skeletal muscles. The increased parasympathetic activity on the visceral adipose tissue results in an increased level of insulin secretion and an increase of fat. The increased sympathetic activity on the heart and skeletal muscles results in hypertension, and an induced glucose uptake in skeletal muscles. In normal situation the sympathetic and parasympathetic activity alternate, but in patients the sympathetic and parasympathetic activity works at the same time on different locations in the body (ref).

b) Furthermore in a study Kreier et al. (2007) provide the hypothesis that the ‘biological clock’, which normally prepares us each morning for the coming activity period, is altered due to a modern life style of low activity during the day and late-night food intake. So research indicated in aged and young patients with type 2 diabetes indications for a malfunctioning of the biological clock. While elderly patients have an impaired function of the SCN due to the degeneration of neurons, the researchers propose that in younger subjects the clock loses its ‘feeling’ for internal and external rhythms caused by the modern lifestyle. Sleeping late and less coupled with constant metabolic excess alter both internal and external environmental stimuli to the brain. In response to these alterations, the rhythm of the biological clock is disrupted which may lead to the metabolic syndrome and type 2 diabetes (Kreier et al. 2007).

Explanation for differences between sexes: For a long time, cardiovascular disease (CVD), one of the symptoms of metabolic syndrome, has been seen as a ‘male’ disease due to men’s higher absolute risk compared with women, but the relative risk in women of CVD
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morbidity and mortality is actually higher. In Europe CVD kills a higher percentage of women (55%) than men (43%) (Petersen et al. 2005).

Figure 2. The rise in morbid obesity in the UK (top) and the USA (bottom). % morbidity obesity UK (top) and the USA (bottom). Top figure: increasing prevalence of morbid obesity in England (BMI => 40 kg/m2). Bottom figure: faster rising morbid obesity in the USA.

Compared with men, CVD risk in women is increased to a greater extent by some traditional factors (e.g., diabetes, hypertension, hypercholesterolemia, obesity), and socioeconomic and psychosocial factors also seem to have a higher impact on CVD in women. Women with diabetes have 2.6 times the risk of dying from coronary heart disease than women without diabetes compared with a 1.8-fold risk among men with diabetes (Wengner 2003). Obesity is a huge problem, it has increased almost 3-fold over the past decades. Nevertheless there are no significant differences between girls and boys in the prevalence of overweight and obesity (Möller-Leimkühler 2007). However body composition and fat distribution are different between boys and girls across ages, and these differences may influence the relation between fatness and lipids (Möller-Leimkühler 2007). The prevalence of smoking is similar in girls and boys (Möller-Leimkühler 2007) but girls report less physical activity than boys, both before and during adolescence (Higgens, 2003). For biochemical compounds at menopause LDL is increasing. This effect is thought to be partly the result of advancing age and declining levels of estrogen (Möller-Leimkühler 2007). There is some evidence to suggest that high levels of triglycerides are a significant independent risk
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factor for CVD in both sexes, but more so in women than men (Castelli et al. 1992). There is a clear socioeconomic gradient in risk of CVD, with those in the lowest socioeconomic strata have the highest risk of cardiovascular events (Winkleby & Cubbin 2003).

Other factors between the sexes are women’s coronary vessels tend to be smaller than those of men which makes them more difficult to revascularise percutaneously as well as surgically (Jacobs 2003).

Socioeconomic status: is associated with obesity and components of the metabolic syndrome (even after multivariate analysis taking account of smoking, alcohol (ab)use and physical excersise (Brunner et al. 1997). Also a poor standard of education is negatively correlated with an elevated risk of the metabolic syndrome (in middle aged women) (Wamala et al. 1999). The fact is that mortality due to CVD is higher in people with low socioeconomic status. It is possible that the metabolic syndrome got its place in the causative pathway.

Overweight among Racial/Ethnic Groups, Differences between Races

Boys: In figure 3 the top panel gives the Prevalence of overweight (Sex and aged specific BMI) for boys from three racial/ethnic groups. Overweight has increased for all children and adolescents over time. NHANES data indicate disparities among racial/ethnic groups. The most recent NHANES data (2003-2004) showed that for boys, aged 12-19 years the prevalence rate of overweight was slightly higher among adolescent non-Hispanic white boys (19.1%) than among non-Hispanic black boys (18.5%) and Mexican American boys (18.3%) (Hedley et al. 2004, Ogden et al. 2006).

Data from NHANES (1988-1994) through NHANES (2003-2004) showed that adolescent non-Hispanic white and black boys experienced larger increases in the prevalence of overweight (7.5% and 7.8% respectively) compared to the increase among Mexican American boys (4.2%) (Hedley et al. 2004, Ogden et al. 2006).
Figure 3. Differences between ethnical groups for obesity in the USA for the period 1988-1994 versus 2003-2004 split up to gender.

Girls: In figure 3 the bottom panel give the Prevalence of overweight (Sex and aged specific BMI) for girls from three racial/ethnic groups. Overweight has increased for all children and adolescents over time. NHANES data indicate disparities among racial/ethnic groups. The most recent NHANES data (2003-2004) showed that for girls, aged 12-19 years the Non-Hispanic black girls had the highest prevalence of overweight (25.4%) compared to that of non-Hispanic white (15.4%) and Mexican American (14.1%) girls. (Hedley et al. 2004, Ogden et al. 2006). Data from NHANES (1988-1994) through NHANES (2003-2004) showed that non-Hispanic black adolescent girls experienced the largest increases in the prevalence of overweight (12.2%) compared to non-Hispanic white adolescent (8.0%) and Mexican American adolescent (4.9%) girls (Hedley et al. 2004, Ogden et al. 2006).

Stress activates the sympathoadrenal system and the hypothalamic-pituitary-adrenocortical (HPA) axis. Increased sympathetic activity might contribute to several aspects of the metabolic syndrome, such as glucose uptake in skeletal muscle, lipolysis and development of hypertension (Hjemdahl 2002). A complex relationship is proved between the HPA axis function, obesity and metabolic parameters suggesting an etiological role for these factors in the development of the metabolic syndrome (Bjorntorp & Rosmond 2000). People suffering of the metabolic syndrome had an increased urinary excretion of cortisol metabolites and normetanephrine and lower heart rate variability (Brunner et al. 2002). This means that chronic stress results in elevated glucocorticoid levels (cortisol), and this is a strong indication that psychosocial factors, like stress, can contribute or even cause the metabolic syndrome (Boulogne & Vantyghem 2004).
Glucocorticoids are interesting hormones to study in relation to metabolic syndrome because ‘chronic stress’, as a consequence of our ‘modern’ lifestyle results in elevated glucocorticoid levels. These may be a contributing or even causative factor for metabolic syndrome (Boulogne & Vantyghem 2004). Evidence for this hypothesis comes from two different studies. Cortisol replacement by diet indicated it was a necessary condition for the development of diet-induced obesity, alterations in insulin sensitivity and TG lipoprotein metabolism (Mantha et al. 1999). In another study with transgenic mice with overexpression of 11-β hydroxysteroid dehydrogenase (11-β HSD) in adipose tissue (resulting in increased glucocorticoid production), Metabolic Syndrome (with concomitant features: visceral obesity, hyperlipidaemia and insulin resistance) could be induced (Masuzaki et al. 2001). Recently an interaction between corticosterone and insulin has been demonstrated. Glucocorticosteroids provoke a dose related increase in total caloric intake in combination with increasing insulin concentrations resulting in obesity (La Fleur 2004). Still the interactions between hepatic steatosis, elevated corticosteroids and insulin levels (pancreas) are not understood.

3. Genetic Susceptibility: "Thrifty Genes and Inflammation"

Genetic factors influence the different components of the Metabolic Syndrome, for example insulin resistance. Type 2 diabetes is a polygenic disorder (Gloyn et al. 2006). Insulin resistance clusters within families, 45% of first degree relatives of patients with type two diabetes are insulin resistant compared to 20% of individuals without a family history of diabetes (Groop et al. 1996). The estimation of heritability in obesity has varied (20-90%) depending on whether the results are based on twin, adoption or family studies (Maes et al. 1996). Another aspect is the combination diabetes mellitus and waist to hip ratio: first degree relatives of patient with type 2 diabetes have an increased waist to hip ratio compared to subjects without family history type 2 diabetes (Groop et al. 1996). Heritability also influences hypertension (Levy et al. 2000) triacylglycerols and HDL cholesterol (Snieder et al. 1999) and micro-albuminuria (Forsblom et al. 1999).

Obesity, type 2 diabetes and the Metabolic Syndrome seem to develop in societies with a fast transition from rural to urban lifestyle. In 1962 Neel (Neel 1962) proposed the ‘thrifty gene’ theory. He suggested that our ancestors living in an environment with unstable food supplies and famine would increase their probability of survival if they could maximize their storage of food surpluses (Neel 1962). So genetic selection would favor energy storing genes in an environment like in a hunter-gatherer society. If this ‘storing energy’ genotype which was advantageous in the past, is expressed in the western lifestyle with abundant food supply, it becomes disadvantageous, by causing obesity and diabetes. In this theory thrifty genes could predispose to the metabolic syndrome. It is not clear which genes exactly belong to this genotype, because not all genes are already detected and/or analyzed. But the genes that regulate fat storage, body weight, fat distribution, lipolysis, FFA metabolism, fuel oxidation, insulin resistance and skeletal muscle glucose metabolism are definitely belonging to this genotype (ref).
obese British children showed that 10 percent has a defect in this gene. The underlying mechanism is still unknown (Saad et al. 2002). In another research it was shown that the concentration of the hormone PYY (peptide YY3-36), which reduces the release of ghrelin, is lower in obese people than in non-obese people (Baterham et al. 2003).

With the term “thrifty genes” one can think about underlying hormonal pathways and metabolic routes. We will discuss growth hormone and insulin. Also adipose tissue should be considered as endocrine organ because adipocytes are known to secrete hormones. The list of proteins and factors is growing rapidly and would fall beyond the scope of this review. For an excellent review see (Heilbronn et al. 2004). In this review we will discuss Interleukine-6 (IL-6) and Tumor necrosis factor-α (TNF-α) as inflammatory agents. Furthermore new medicines “the Thiazoladinediones” of which the most important is “Pioglitazone Hydrochloride” have an impact on resistin, a recently discovered hormone.

Metabolic hormones. Insulin and growth hormone, are the major hormones involved with glucose metabolism. The association of chronic liver disease (CLD) with impaired glucose metabolism has been known since 1906 (Naunyn 1906). Acquired GH resistance can be observed from the very early stages of CLD (Picardi et al. 2006) GH resistance even worsens in parallel with the progression of liver disease (Picardi et al. 2003). Knowledge about the effect of alterations of the GH-IGF-1 axis on the blood glucose control is a prerequisite in understanding the role of an impaired liver on GH and glucose regulation.

Also, impaired insulin signaling due to a certain gene (Foxo1) has emerged as a possible unifying mechanism for various common abnormalities of type 2 diabetes. It can be hypothesized that there are two critical contributors to type 2 diabetes: a) increased glucose production, b) decreased insulin production. Previously it was thought that these processes were independent abnormalities caused by another problem in diabetes, the resistance of new fat cells to insulin. But it seems that these processes are regulated through a gene Foxo1 in the liver (Nakai et al. 2002; Langhans 2003). In a recent study it was demonstrated that when the production of the gene Foxo 1 was lowered, the level of glucose in diabetic mice declined to normal levels via two mechanisms: firstly, the liver reduced glucose production, and secondly cells in the pancreas regained their ability to make insulin, the hormone which is important in removal of glucose from the bloodstream (Nakai et al. 2002).

Interleukin-6 (IL-6). Both obesity and insulin resistance are considered chronic inflammatory states (Arkan et al., 2005). Thus in both conditions, just like in reaction to an infection or an injury, cytokines (IL-6 and TNF-α) are released from the site of tissue injury (Tilg & Hotamisligil 2006). These cytokines promote an acute-phase response. This response is characterised by the production of a range of proteins, primarily from hepatocytes (liver cells), but also from other cells such as monocytes (precursors to macrophages), fibroblasts (connective tissue cells) and adipocytes (fat cells) (Arkan et al., 2005). Production of cytokines is one of the earliest events in many types of liver injury, triggering the production of other cytokines that together recruit inflammatory cells and initiate a healing process in the liver that includes fibrogenesis (Tilg & Hotamisligil 2006).

Tumor Necrosis Factor α (TNF-α). The cytokine which is accepted widely as a cytokine involved in the inflammation reaction due to fat accumulation in the liver is tumour necrosis factor α. (TNF-α). TNF-α is increased in obese subjects. It impairs insulin signalling by serine phosphorylation of insulin receptor substrates (Bays et al., 2004). From different studies the conclusion has been made that TNF-α is an important mediator of insulin resistance through
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its ability to influence the tyrosine kinase activity of the insulin receptor. TNF-α is being over expressed in humans with obesity and correlates with the body mass index. TNF-α is able to impair insulin release by inducing insulin resistance in pancreatic β cells and by stimulating the release of mediators that are toxic for these cells. Thus, TNF-α exerts local pancreatic effects, and the induction of insulin resistance may be more related to the local tissue TNF-α concentration than to the levels in plasma (Bays et al., 2004).

For example, Miyazaki et al. (2003) found the following correlations with TNF-α, insulin resistance, obesity and T2DM. At first, an increase in circulating TNF-α concentration is associated with peripheral insulin resistance and increased plasma glucose and insulin levels prior to the onset of type 2 diabetes. Secondly, serum TNF-α levels are elevated in type 2 diabetic patients and are unrelated to the degree of obesity (BMI). And finally, the increased serum TNF-α concentration in type 2 diabetic individuals is not correlated with the severity of insulin resistance. This lack of correlation may be explained by the presence of hyperglycemia, hyperinsulinemia or other factors which have a stronger impact on peripheral insulin sensitivity in type 2 diabetes mellitus.

In addition, TNF-α polymorphisms have been reported to influence the susceptibility to different hepatic diseases, including alcoholic steatohepatitis, and increasing evidence suggests that TNF-α is involved in the pathogenesis and progression of liver diseases of different etiology. The rise of TNF-α may be related to liver fibrosis, and might promote liver fibrosis (Browning, 2003).

Oxidative stress. The pathological changes that develop in e.g. hepatocytes are often ascribed to oxidative stress due to fatty-acid oxidation (Fernandez-Checa & Kaplowitz 2005). It has been proved that the transition from steatosis to steatohepatitis is established by the role of reactive oxygen species (ROS) (Sanyal et al. 2001). This oxidative stress may result in a mitochondrial dysfunction (Pessayre, 2001), but increased oxidation of long chain fatty acids is reportedly a major source of ROS in NASH; free fatty acids are also toxic (Day, 2002; Sanyal et al. 2001). The distinction between steatosis and steatohepatitis, and the assessment of the severity of the disease rely on liver histology alone (Ratziu et al, 2005).

Key Players in Insulin Resistance: PPAR-Gamma

In the pathogenesis of insulin resistance, some key players, essential molecules in the process can be introduced like Peroxisome proliferator-activated receptor-gamma (PPAR-gamma). This is a molecule of recent investigation leading to a new generation of medicines the Thiazoladinediones of which the most important is Pioglitazone Hydrochloride. Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) is a transcription factor involved in regulating genes involved in adipogenesis and, by implication, insulin action. (LeRoith and Zick, 2001)

In recent years great efforts have been directed toward understanding the molecular mechanisms that govern adipogenesis and, therefore, body fat mass. PPAR-gamma, the third member of a subset of nuclear receptors, which also includes PPAR-alpha and PPAR-delta, is now recognised to be central to this process (ref. in Gurnell et al., 2003). Human PPAR-gamma plays a role in the induction of adipose tissue differentiation. Loss of function
mutation in PPAR-gamma results in lipodystrophy and administration of PPAR-gamma antagonists promotes adipogenesis (Gurnell et al., 2003).

PPARs possess a ligand-binding domain and a DNA-binding domain. PPARs bind to cognate DNA elements called PPAR response elements (PPREs) in the 5'-flanking region of target genes. PPARs, like other nuclear hormone receptors, form protein-protein interactions with a variety of nuclear proteins known as coactivators and corepressors, which mediate contact between the PPAR-RXR heterodimer, chromatin, and the basal transcriptional machinery and which promote activation and repression of gene expression, respectively. Although it has been possible to generate several high affinity synthetic PPAR-gamma ligands, e.g. thiazolidinedione (TZD), true endogenous ligands in vivo are still to be found (Rosen and Spiegelman, 2001).

Also, PPAR-gamma enhances insulin sensitivity in both muscle and liver (LeRoith and Zick, 2001). In a review Gurnell et al. (2003) estimate it to be likely that the dramatic diminution in peripheral limb and gluteal fat found in individuals with PPAR-gamma mutations contributes to their insulin-resistant phenotype. They suggest that even the residual adipose tissue depots in these individuals are dysfunctional, perhaps resulting in exposure of skeletal muscle and liver to unregulated fatty acid fluxes and thereby impairing insulin action in these sites. Thiazoladinediones (TZD), a synthetic PPAR-gamma ligand is used clinically as an insulin sensitizer in patients with type 2 diabetes (Rosen and Spiegelman, 2001).

At last, PPAR-gamma activation also reduces IL-6 and TNF-alpha levels, cytokines that are involved in the pathogenesis of insulin resistance. There is evidence that TZDs might repress a fat cell-produced factor called resistin, which induces insulin resistance (Rosen and Spiegelman, 2001).

Resistin

Resistin is a recently discovered hormone secreted by white and brown adipose tissue, monocytes, bone marrow and other tissues (Tejero et al. 2005). Resistin received its name from the original observation (by Steppan in 2001) that it induced insulin resistance in mice, and could potentially link obesitas to diabetes (Steppan et al. 2001). Resistin belongs to the family of resistin-like molecules (RELMs), also known as “found in inflammatory zone” (FIZZ). The FIZZ/RELM family consists of 4 members, each of which has a conserved 11-cysteine pattern at the C terminus. (X11-C-X8-C-X3-C-X10-C-X-C-X9-CC-X3-6-END.) Each of these FIZZ proteins has unique tissue distribution, and both resistin (FIZZ-3) and FIZZ-1 are expressed in adipose tissue. FIZZ was initially discovered in mice, in which it is predominantly expressed by adipocytes. In contrast, macrophages, rather than adipocytes, appear to be the most important source of resistin in human subjects (Rea et al. 2004).

Resistin thought to a possible link between adiposity and insulin resistance by having an possible metabolic effect on insulin sensitivity in murine models (Steppan et al. 2001). In mice, elevation of resistin levels leads to insulin resistance and glucose intolerance and symptoms of type 2 diabetes (Tejero et al. 2005). But levels of resistin have been reported to be either increased, unchanged, or decreased in murine and human obesity and type 2 diabetes (Fantuzzi 2005).
While some studies have reported that resistin expression is almost undetectable in human adipose tissue other studies have demonstrated that resistin expression is increased in abdominal compared to thigh fat depots in obese humans (Tejero et al. 2005).

However, recent data indicate that stimulation of macrophages in vitro with endotoxin or proinflammatory cytokines leads to a marked increase in resistin production (Lehrke et al. 2004).

And administration of endotoxin to human volunteers is associated with dramatically increased circulating resistin levels (Lehrke et al. 2004). Thus in human subjects resistin seems to act as a critical mediator of the insulin resistance associated with sepsis and possibly other inflammatory conditions. Only a few reports have investigated the effects of resistin in the modulation of inflammatory responses, showing that resistin upregulates expression of MCP-1, as well as vascular cell adhesion molecule 1 and ICAM-1, in endothelial cells. As mentioned above, the adhesion molecule-upregulating effects of resistin are antagonized by adiponectin (Fantuzzi 2005).

Figure 4. The target tissues of resistin. (ook aangeven waar je dit plaatje vandaan hebt!)

The human resistin gene is located on chromosome 19p13.3. Several polymorphisms of this gene have been identified in humans, and associated with traits related to obesity and insulin sensitivity (Cao and Hegele 2001).
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The complex nature of obesity and its related disorders indicates that the single candidate gene approach may not be the most appropriate for the study of these conditions. Thus, the genome scan approach has been applied to investigate the genetic component in obesity in a variety of populations (Clement et al. 2002).

Resistin gene expression is induced during adipocyte differentiation, and the resistin polypeptide is specifically expressed and secreted by adipocytes. Resistin circulates in mouse serum, and its level is increased markedly in both genetic and diet-induced obesity. Immuno neutralization improves blood glucose and insulin action in this model of type 2 diabetes. By contrast, administration of resistin impairs glucose tolerance and insulin action in normal mice. In mouse, a single mRNA of roughly 750 residues is robustly expressed in white adipose tissue but not in several other mouse tissues. Resistin expression is greater in white adipose tissue than in brown adipose tissue, where resistin mRNA is barely detectable. Resistin mRNA levels varied as a function of white adipose depot and gender, with the highest level of expression in female gonadal fat (Fantuzzi 2005).

4. Prevention and Treatment

The key factor for understanding the metabolic syndrome is insulin resistance and β-cell dysfunction (Campell 2004) and particularly the relative failure of insulin to exert its multiple biological effects on carbohydrate and lipid metabolism. Hulbert et al. (2005) propose two mechanisms to explain the initiation of the metabolic syndrome: a) decreased membrane polysaturation may act to decrease the activity of the major energy-consuming processes of the cell such as reducing the flux of ions and protons and subsequently the energy needed to maintain ionic homeostasis, b) membrane fatty acid composition influences insulin action via alteration of membrane proteins specifically associated with the action of insulin. Such mechanism would include modulation both of receptor affinities and translocation to the membrane of nutrient transporters (Hulbert et al 2005).

Taking away the underlying mechanism, can (partly) prevent the metabolic syndrome.

Diet, exercise and preventing stress are the first-line strategies in the management of type 2 diabetes; however, few patients maintain glycemic control using this regime alone.

A good start could possibly be treatment with statins or ACE-inhibitors (to lower cholesterol and blood pressure (Faggiotto & Paoletti 1999). Other correlated features of the metabolic syndrome will then decrease, for example hepatic steatosis and insulin resistance. Of course there is a range of medicines to treat metabolic syndrome, from ones with blood lipid decreasing effect until ones that improve glycemic control.

Currently available drug treatments, used alone or in combination, include oral glucose-lowering agents:

a) Sulphonylureas and glinides: which increase insulin secretion. They are the first-line oral antihyperglycaemic medications (OAMs) for treating hyperglycaemia in patients with type 2 diabetes who have not responded to appropriate dietary and exercise modifications. Sulphonylureas improve glycemic control with a high rate of success as primary agents (60-70%). As a class, sulphonylureas have no
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consistent effects on serum lipids in people with type 2 diabetes (Tan et al. 2004). Examples of sulphonylureas are Glimepiride (Deroase et al. 2004)
b) α-glucosidase inhibitors: which retard digestion of complex carbohydrates (Tan et al. 2004).
c) metformin: which reduces endogenous hepatic glucose production (Einhorn et al. 2000).
d) Insulin has multiple effects. In addition to its primary effects on glucose homeostasis, insulin also promotes a number of other cellular events including regulation of ion and amino acid transport, lipid metabolism, glycogen synthesis, gene transcription and mRNA turnover, protein synthesis and degradation, and DNA synthesis. The major target tissues of insulin are skeletal muscle, adipose tissue, and liver. The anabolic hormone insulin plays a key role in the storage of ingested fuel and in normal cellular growth and differentiation so a tight control of this hormone is important for the homeostasis of the organism (Cheatham and Kahn, 1995) and a suppletion in case of a shortage is an important drug treatment.

A new group of pharmacological oral agents used for the treatment of type 2 diabetes mellitus are the thiazolidinediones (TZDs, such as is pioglitazone. (They are agonist for the peroxisome proliferator activated receptor-γ (PPARγ), a nuclear receptor involved in the transcription of genes that modulate carbohydrate and lipid metabolism and insulin regulation (Tan et al. 2004, Campell 2004). For the understanding of PPAR-gamma in insulin resistance the reader should see the paragraph genetic factors (see above). TZDs appear to address the dual defects of insulin resistance and β-cell dysfunction. The glucose-lowering effect of pioglitazone in patients with type 2 diabetes is related to its ability to reduce insulin resistance in tissues in which the majority of glucose uptake occurs (skeletal muscle, adipose tissue and liver) (Campell 2004). By reducing insulin resistance, pioglitazone lowers fasting and postprandial blood glucose concentrations, circulating free fatty acids (FFAs) and insulin levels and also hepatic glucose production may decline (Campell 2004). Additionally, data suggest that TZDs may preserve pancreatic β-cell function by a mechanism that is independent of the correction of glucose toxicity (Campell 2004). Furthermore pioglitazone reduces the composite of all-cause mortality, non-fatal myocardial infarction, and stroke in patients with type 2 diabetes who have a high risk of macrovascular events (Dormandy et al. 2005). Pioglitazone can be given as monotherapy, double therapy (with metformin or a sulphonylureum), or as triple therapy (pioglitazone + metformin + sulphonylureum).

Finally, Pioglitazone can be given in combination with insulin in patients. The combination of Pioglitazone in combination with metformin significantly improved glycated hemoglobin (HbA1c) and fasting plasma glucose (FPG), with positive effects on serum lipid levels and no evidence of drug-induced hepatotoxicity. These effects were maintained for > 1.5 years (Einhorn et al. 2000). The combination of pioglitazone in combination with glimepride (from the latest second generation sulfonylurea (SU)), resulted in a significant improvement in lipid and lipoproteins variables in patients who did not respond previously adequately on diet intervention or a sulfonylurea or metformin as medicine solely (Derosa et al. 2004). The combination of pioglitazone with sulfonylurea therapy improved glycated hemoglobin (HbA1c) and fasting plasma glucose levels with beneficial effects on serum triglyceride and
HDL-cholesterol levels (Kipnes et al. 2001). Finally, in a group of patients with type 2 DM whose disease was inadequately controlled with insulin monotherapy, the combination of Pioglitazone with insulin further improved their glycemic control (Mattoo et al. 2005).

In conclusion: we demonstrated that the intra-uterine environment, lifestyle and genetic susceptibility all three can contribute to type 2 diabetes. Diet, exercise and preventing stress are the first-line strategies in the management of type 2 diabetes; however, few patients maintain glycemic control using this regime alone. Therefore new medicines are developed for which the thiazolidinediones (TZD), the most important “Pioglitazone hydrochloride”, a synthetic PPAR-gamma ligand, is used clinically as an insulin sensitizer in patients with type 2 diabetes and gives exciting results. Note however that after the draft of this manuscript it has become clear that another thiazolidinedione (Rosiglitazone) possibly gave an increased Cardiovascular risk.

**Thiazolidinedione therapy (especially with Rosiglitazone) and its Cardiovascular risk and possible Cardiotoxicity**

After the draft of this manuscript was prepared some critical notes and new information reached the authors of this article. Because diabetes type 2 is considered an epidemic in the United States (Waksman 2008) the use of medication is also increasing and the Thiazolidinediones represent an interesting and potentially important class of drugs (Psaty & Furberg 2007). During 2000-2001, the use of thiazolidinedione use has grown to 16.1% (Masoudi et al. 2003). However, in this critical note we will review the published literature of another thiazolidinedione than the earlier mentioned Pioglitazone which is Rosiglitazone. Marketing approval in the United States in 1999 after some small, short-term trials (Avandia, GlaxoSmithKline, Inc.) (Psaty & Furberg 2007, Nathan 2007a, Waksman 2008) was recently associated with cardio-vascular risk and cardiotoxicity (vide: reviews Nathan 2007a, Psaty & Furberg 2007, Waksman 2008).

In the editorial of NJEM, Psaty & Furberg (2007) summarizes some of the characteristics of Rosiglitazone: i). It is a thiazolidinedione and an antagonist of the PPARs (see above this book chapter §3: "Key Players in Insulin Resistance: PPAR-Gamma"), primarily $\gamma$-receptors, in the cell nucleus; ii). Characteristic for these kind of PPAR-$\gamma$ agonists are their polygenic action, only some of which are currently known; iii). These ligand-activated nuclear transcription factors activate the transcription of genes that affect glucose and lipid metabolism; iv). Rosiglitazone increases hepatic and peripheral insulin sensitivity and reverses insulin resistance; v). In some small, short-term trials it proved to reduce levels of fasting glucose and glycated hemoglobin (reviewed: Psaty & Furberg, 2007).

The meta-analysis study by Nissen & Wolski (2007) suggested a significant increase in the risk of myocardial infarction associated with treatment with Rosiglitazone (Avandia) and an increase of similar magnitude, albeit non-significant, in the risk of death from cardiovascular causes (reviewed: Psaty & Furberg 2007, Nathan 2007a, Waksman 2008). Waksman (2008) reviewed two other randomized trials, which were published after the meta-analysis study of Nissen & Wolski (2007). The first clinical trial of Dargie et al. (2007) demonstrated that Rosiglitazone significantly improved glycemic control without affecting left ventricular ejection fraction; however, it was associated with greater incidence of peripheral edema and worsening heart failure. The second clinical trial was a specifically designed trial to assess the cardiovascular safety of Rosiglitazone (Home et al. 2005), of which we shall discuss the outcomes more detailed in the following part of this critical note. Home and colleagues (2005) designed a study (including employees of the drug's manufacturer of Rosiglitazone, GlaxoSmith-Kline), the so called: “Rosiglitazone Evaluated..."
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for Cardiac Outcomes and Regulation of Glycemia in Diabetes”; or "RECORD", trial (NCT00379769), which was specifically designed to study the effects on cardiovascular outcomes on treatment of: i). Rosiglitazone combined with metformin or sulfonylurea, as compared with ii). Treatment of metformin combined with sulfonylurea (Home et al. 2005). The latter treatment was not a good choice for Control-treatment because a sub-study of the UK Prospective Diabetes Study (UKPDS) Group (UKPDS, 1998) indicated that this treatment (ii); was associated with a 96% increase in diabetes-related mortality (reviewed: Nathan 2007a).

In the 'Editorials' of the NEJM, Nathan (2007a) critically evaluated the interim analysis of this RECORD study published by Home et al. (2007a) and concluded that: i). As mentioned earlier that the choice of the "Control-group" (only treated with metformin combined with sulfonylurea) was due to the mortality caused by this treatment alone under diabetes 2 patients 'problematic'; ii). The RECORD study lasted only 3.75 years instead of the planned 6-years of duration; iii). The risk of congestive heart failure, which more than doubled, was significantly more frequent with Rosiglitazone.

The in these note-summarized studies of the potential cardiovascular risks: i). A drug that might have an 8% excess risk of severe cardiovascular disease or death from cardiovascular causes (Nathan 2007a); ii). The reported absence of other health advantages like glycemic control during like long term studies (Home et al. 2007b), makes the rationale for prescribing Rosiglitazone to diabetes 2 patients at this moment unclear (Pasty & Furberg 2007). The situation may be reevaluated if new ongoing trials using Rosiglitazone may provide new important data and insights that this compound is not cardiotoxic. Other medication regimes as an alternative are still available like: i). Pioglitazone (which for completeness of this critical note has until now limited clinical trial but suggest a protective cardiovascular effect (Dormandy et al. 2005); ii). In this book chapter in § 4: 'Prevention and Treatment' several therapies are mentioned but Nathan (2007a,b) also mentions in his manuscript (Nathan 2007b) that at this moment nine classes of antidiabetic medications are available, including several older medications that are relatively efficacious in lowering glycated hemoglobin levels and are less expensive than the thiazolidinediones.

However when in practice by physicians thiazolidinediones are described, careful consideration should be given to patients with known heart failure. In addition, all patients should be educated regarding heart failure and monitored for signs and symptoms (Hartung et al. 2005).

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