

Module 24.2

Insulin Resistance: from Pathophysiology to Clinical Assessment

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Learning Objectives

- Understand interactions between obesity, insulin resistance and metabolic syndrome;
- Understand the role of nutrients in the onset and modulation of insulin resistance;
- Understand the clinical impact of insulin resistance;
- Describe methods to measure insulin resistance in humans.

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Key Messages

- Insulin resistance can be defined as a lower than expected insulin effect on glucose metabolism and/or plasma concentration at a given insulin level;
- Causes of insulin resistance are complex and include: altered adipose tissue endocrine functions, altered lipid metabolism, inflammation and oxidative stress, altered nutrient sensing in gut and CNS. The gut microbiota are also increasingly being recognized as a major modulator of interactions between nutrients and whole-body metabolic responses;
- Insulin resistance is a very common alteration with profound implications for human disease, particularly in terms of metabolic and cardiovascular morbidity. Insulin resistance is a major underlying factor in the metabolic syndrome;
- Measurement of insulin resistance with reliable surrogates provides potentially important tools for effective management of metabolic and cardiovascular risk in metabolic syndrome patients.

1. Obesity, Insulin Resistance and Metabolic Syndrome

The ongoing, worldwide obesity epidemic is a major threat to patients and healthcare systems because of its related morbidity and costs (1). A major obesity-related burden is related to its metabolic and cardiovascular complications, with major roles for insulin resistance, type 2 diabetes and cardiovascular disease. The high prevalence of metabolic and cardiovascular complications does not imply that they are inevitable consequences of fat gain, and a sizable fraction of obese individuals remains metabolically healthy long after the onset of obesity (2). Identifying obese individuals at risk for life-threatening complications is a major clinical and epidemiological goal, since it would theoretically allow focusing treatment and resources on patients who would be likely to benefit the most.

Metabolic syndrome is a cluster of cardiovascular risk factors that occur simultaneously in the same individual (3). The clinical features that define metabolic syndrome may vary slightly by different classifications, but a strong consensus has built on the role of abdominal obesity and altered glucose metabolism, reflecting insulin resistance, as major pathogenetic factors (3). Understanding the causes, consequences and clinical markers of insulin resistance in metabolic syndrome patients is the object of this module.

2. Insulin Resistance Definition

Insulin is a key modulator of intermediate metabolism, involved in the regulation of a variety of fundamental cell and tissue functions. Important metabolic effects of insulin occur in the post-prandial state, when variable increments of plasma insulin concentration play a major role in nutrient utilization. Under these conditions, plasma glucose elevation stimulates insulin secretion leading to 1) stimulation of glucose uptake and utilization by insulin-sensitive skeletal muscle and adipose tissue; 2) inhibition of glucose production by gluconeogenesis and glycogenolysis in the liver. Major metabolic effects of insulin however also include maintenance of skeletal muscle mass by inhibition of protein breakdown and stimulation of synthesis of selected protein fractions (4,5), as well as stimulation of lipid deposition in adipose tissue (6). In addition, important insulin effects on endothelial function (7), regulation of cell cycle, redox state (8,9) and several other pathways have been clearly described.

Insulin resistance can be defined as the inability of insulin to exert its biological effects in target tissues. It should be however be kept in mind that the common clinical definition of insulin resistance refers to inadequate effects of insulin on glucose metabolism. In this context, **insulin resistance may be defined as a lower than normally expected insulin effect on glucose metabolism or plasma concentration at a given insulin level.** It is also important to consider that insulin resistance may occur in the same individual for glucose metabolism but not, or to a lesser extent, for other important insulin effects. As an example, insulin sensitivity of muscle protein turnover or adipocyte lipolysis in obese patients, who may be insulin resistant for glucose metabolism, is still under debate (6,10,11).

3. Insulin Resistance in Obesity

Obesity is a major risk factor for insulin resistance. Although not all obese individuals develop insulin resistance and its consequences, a majority of insulin resistant patients are overweight or obese. Understanding the causes of this strong association is a most important clinical goal. A major contribution of adipose tissue and altered lipid metabolism in orchestrating obesity-associated metabolic changes has been identified. An important role for oxidative stress and chronic inflammation has been also defined. In addition, new players in the regulation of insulin sensitivity have emerged with involvement of the gut and the central nervous system, and the gut microbiota have, in recent years, increasingly been recognized as major players in regulating host-nutrient interactions. These alterations will be outlined in the following section.

3.1 Adipose Tissue and Altered Lipid Metabolism

3.1.1 Adipose Tissue Endocrine Function and Adipokines

The traditional view of adipose tissue as an inert reservoir for passive fat storage has long been modified by the demonstration that adipocytes secrete a variety of hormones (adipocytokines or adipokines) involved in the regulation of intermediate metabolism, energy

metabolism, inflammation, and haemostasis (12-14). Among many secreted proteins, adiponectin, a 30-kDa adipocytokine, is uniquely associated with high insulin sensitivity and reduced cardiovascular risk (12,13). Adiponectin has also been reported to increase lean tissue, and particularly skeletal muscle lipid oxidation and mitochondrial function (15). This effect appears to be mediated by activation of the master metabolic regulator AMP-activated protein kinase (AMPK) (15,16). TNF-alpha and IL-6 are, on the other hand, known activators of the systemic inflammatory response that are also secreted by adipose tissue and related to low insulin sensitivity (17).

Opposite changes in plasma adipocytokine patterns are observed in obesity and following caloric restriction (17,18). The unfavourable obesity-related pattern of low adiponectin with parallel increment of proinflammatory cytokines including TNF-alpha is strongly associated with insulin resistance, and it can be at least in part reversed by calorie restriction and loss of body fat (17,18). Mechanisms underlying changes in adipose tissue endocrine function are not completely clear. Important roles have been proposed for oxidative stress and adipose tissue plasticity.

Adipose tissue oxidative stress - In vivo and in vitro evidence has connected excess production of adipose tissue reactive oxygen species, resulting in oxidative stress, to metabolically unfavourable patterns of adipocytokine expression and production (19). An elegant study demonstrated that plasma lipid peroxidation markers (TBARS) are associated negatively with circulating adiponectin and positively with BMI (19). Importantly, induction of oxidative stress in cultured adipocytes lowered adiponectin expression and increased the expression of IL-6, and these changes were reversible upon antioxidant supplementation (19).

Adipose tissue expandability - An increase in adipose tissue mass is the defining alteration in obesity. In recent years, evidence has however accumulated to show that the ability of adipose tissue to expand its mass in response to positive energy balance is in fact associated with less negative metabolic responses (20). This seemingly paradoxical observation could be explained by more effective and complete adipose lipid storage, thereby preventing ectopic lipid deposition in non-adipose tissues, which might indeed profoundly alter insulin action and cell metabolism (21). Adipose tissue expansion through initiation of adipocyte differentiation and enhanced proliferation is reported to be preferentially associated with metabolic benefits (20). On the other hand, expanded fat mass through cell hypertrophy without recruitment of newly differentiated cells may lead to adipocyte damage, cell death and ultimately enhanced inflammation through macrophage activation (22) (**Fig. 1**). Although methodological hurdles may limit clinical investigation in terms of measurement of adipocyte proliferation, number and size, several studies have provided proof of concept that these mechanisms may contribute to shape metabolic responses in human obesity and during modifications of body composition. As a relevant example, the effectiveness of thiazolidinediones in the treatment of type 2 diabetes appears to be at least in part mediated by reduced lipid accumulation in skeletal muscle and liver, with concomitantly enhanced fat mass through activation of the adipocyte differentiation stimulator PPARgamma (23). The regulation of fat mass expandability and adipocyte differentiation and proliferation are therefore very attractive recently-identified targets for basic and clinical research. The nature of the hormonal and nutritional modulation of this process remains largely unknown and deserves future studies both in the obesity field and, potentially, to limit fat-related nutritional and metabolic alterations in chronic disease patients with and without obesity.

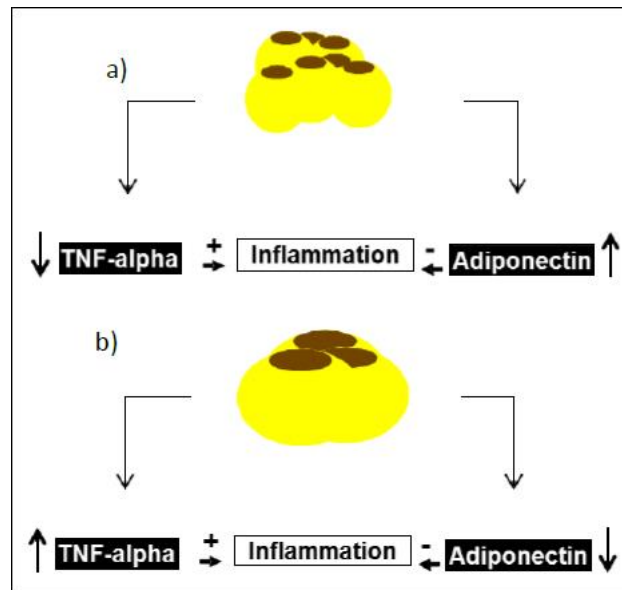


Fig. 1 Adipocyte number and size modulate adipokine secretion patterns in adipose tissue. a) adipocyte proliferation with small cell size is associated with favourable adipokine patterns and low inflammation; b) adipocyte hypertrophy with large cell size is associated with pro-inflammatory adipokine secretion pattern

3.1.2 Adipose Tissue Thermogenesis

Obvious potential advantages could be obtained in the treatment and prevention of obesity and metabolic syndrome by increasing body energy dissipation through heat production. To this aim, the “rediscovery” of human brown adipose tissue has been a major breakthrough in metabolic research. Brown adipocytes have higher thermogenic potential due to enhanced mitochondrial uncoupling that makes them less efficient ATP producers. Contrary to previous belief, convincing evidence has shown that brown adipose tissue is preserved after infancy and during adult life in humans (24,25). “Browning” of adipose tissue, i.e. interconversion of metabolically white into brown adipocytes, has been reported following metabolic stimuli such as cold exposure and physical exercise in experimental models. Browning may also be enhanced by thyroid hormone and limited by cortisol. Similar results have been reported following genetic manipulation of “browning” transcription factors such as PRDM16 in rodents, and these effects were importantly associated with improved glucose tolerance and metabolic phenotype (24). Additional research lines involve exercise-related myokines with potential “browning” effect such as irisin (24), as well as intriguing evidence of muscle cell interconversion into brown adipocytes (24,25). Translation of these findings into new clinical treatment strategies will likely require further investigation of potential differences in the regulation of adipose tissue thermogenesis in humans compared to experimental models, but it clearly represents an exciting research target for both obesity and nutritional complications of chronic diseases. Importantly, potential mechanisms for nutritional regulation of adipocyte browning and related changes in thermogenesis and energy metabolism remain virtually unknown.

3.2 Non-adipose Tissue and Systemic Inflammation and Oxidative Stress

In the last two decades, the role of low-grade inflammation in systemic, adipose and non-adipose tissues in the onset of obesity-associated insulin resistance has been convincingly demonstrated. While acute inflammation represents an adaptive mechanism contributing to limit specific infectious or traumatic insults, sustained activation of systemic inflammatory responses has a negative metabolic impact (26). In particular, pro-inflammatory cytokines including TNF-alpha activate the IKK-NFκB pathway that results in inactivation of IRS-1 and

insulin signalling blockade. Antiinflammatory drugs such as acetylsalicylate are, conversely, reported to prevent insulin resistance in rodent models of obesity (27,28).

Excess reactive oxygen species (ROS) generation and oxidative stress are major causes of low-grade inflammation in obese patients. It should be pointed out that moderate ROS production from incompletely reduced oxygen molecules is physiologically associated with oxidative substrate metabolism (8). Importantly, antioxidant defence systems dispose of excess ROS and maintain their concentrations within non-harmful levels. Indeed under physiological conditions ROS play an important role in maintenance of cell and tissue homeostasis. Excess ROS production as observed in the presence of excess lipid and glucose substrates in obese and diabetic individuals may however overcome antioxidant capacity, thereby leading to tissue oxidative damage and disease (29). Importantly, oxidative stress is reported to be involved in the onset of insulin resistance in experimental models of obesity (30). Amplification of pro-inflammatory changes in peripheral tissues through enhanced proinflammatory cytokine production and activation of NF- κ B nuclear translocation (31,32) is one potential mechanism involved (**Fig. 2**).

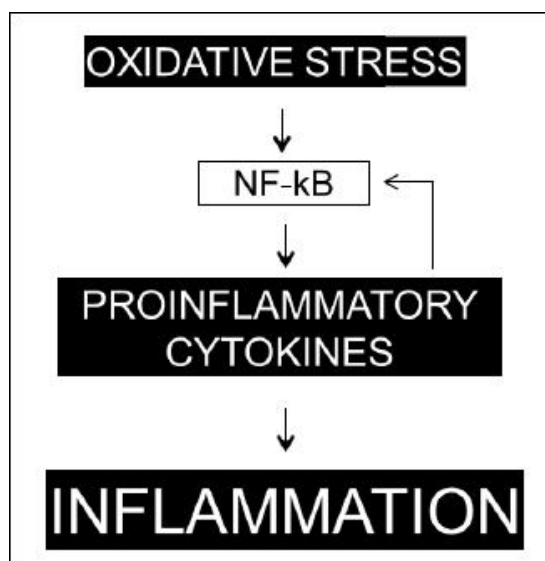


Fig. 2 Oxidative stress enhances tissue inflammation through NF- κ B activation and stimulation of proinflammatory cytokine gene expression

Obesity is often associated with elevation of clinical markers of inflammation such as plasma C-reactive protein or proinflammatory cytokines (33-35). Oxidative stress markers have been also reported to be elevated in obese individuals (19,36), and both alterations are commonly associated with insulin resistance in obese patients (33-36). Obesity-associated adipose tissue alterations may directly favour the onset of chronic systemic low-grade systemic inflammation by altering the balance between pro- and anti-inflammatory adipokines. Negative modifications in diet and physical activity may also directly contribute to inflammation and oxidative stress in the obese population.

Role of nutrients and diet – High-calorie, high-fat diets are reported to promote the onset of inflammation and oxidative stress (37-39), although these changes may vary in different tissues in terms of intensity and time-course (40). Studies *in vitro* also indicated direct proinflammatory and pro-oxidant effects of fatty acids (41-43), that are commonly elevated in obese individuals (44,45). High plasma fatty acids are *per se* strongly and causally associated with insulin resistance in humans, since fatty acid infusion induces insulin resistance of glucose metabolism (44-48). Proposed direct mechanisms involve induction of inflammation and oxidative stress, as well as endoplasmic reticulum stress (9,42,46-50). Importantly, negative metabolic effects of lipid substrates were mostly attributable to saturated fat, with palmitate commonly employed for *in vitro* studies (41-43) (**Fig. 3**). Some studies have

intriguingly suggested differential effects by unsaturated molecules (43,51,52), and potential protective effects of polyunsaturated fatty acids should be further investigated. One recent report however demonstrated that acute i.v. fatty acid elevation in the presence of physiological hyperinsulinaemia causes insulin resistance by inducing excess mitochondrial ROS production and by activating the proinflammatory I κ B-NF κ B pathway (9). High monounsaturated fatty acid content in the infusion mixture in this study indicated that deleterious metabolic effects may not be limited to saturated fatty acids *in vivo* (9). Most importantly, available human studies also link acute or chronic increase in total and saturated fat availability to oxidative stress, inflammation and insulin resistance (48-52), with potential protective effects from polyunsaturated n-3 fatty acids (51,52). Although the potential independent impact of glucose on inflammation and oxidative stress at systemic and tissue level has been less extensively studied, common observations in patients with diabetes mellitus and corresponding experimental models indicate pro-oxidant and inflammatory effects of hyperglycaemia (53), that can play a pivotal role in the onset of diabetic complications.

Role of physical inactivity - Acute exercise, particularly when performed in strenuous bouts, can cause elevation of oxidative stress and inflammation markers (54). Under trained conditions, these negative effects are however compensated by the stimulation of antioxidant and anti-inflammatory pathways, and the net effects result in protection against oxidative stress and systemic and tissue inflammation, with well-recognized robust health benefits (55-59). Aerobic training is also accordingly associated with improved insulin sensitivity (57-59). In recent years, elegant studies have also demonstrated that physical inactivity induces opposite metabolic derangements, with sustained proinflammatory and pro-oxidant changes as well as insulin resistance in otherwise healthy, young individuals undergoing voluntary bed-rest for periods of several weeks (60,61).

3.3 Lipotoxicity (Fig. 3)

Obesity-associated increments in plasma free fatty acids (44,45) may be caused by increased dietary intake as well as excess fatty acid release from adipose tissue (45). Besides their reported effects on ROS production and inflammation, excess fat substrates may accumulate in non-adipose cells and tissues, and these changes appear to have a direct negative impact on insulin action, a phenomenon referred to as lipotoxicity. Strong associations have been described between intracellular triglycerides in skeletal muscle and liver and insulin resistance (62). Liver fat accumulation defined as Non-Alcoholic Fatty Liver Disease (NAFLD) may progress to non-alcoholic steato-hepatitis (NASH), cirrhosis and cancer, and liver triglyceride accumulation may represent an independent risk factor for metabolic and cardiovascular complications (63-65). Studies in skeletal muscle also indicated that intermediate products of fatty acids and triglycerides such as diacyl-glycerol may be responsible for vicious cycles leading to enhanced insulin resistance (66). More recent studies opened new perspectives in our understanding of the interactions between tissue lipid content and insulin action, by showing an important role for fatty acid channelling towards sphingolipid synthesis and ceramide excess (67,68). Convincing evidence indicates that the above metabolic pathway may be exceedingly activated in the presence of saturated fatty acids and inflammation, both observed in obesity (67). Ceramides have in turn been demonstrated to induce negative changes in insulin signalling, mainly at the AKT level, with resulting inhibition of key steps of glucose uptake and utilization (67,68).

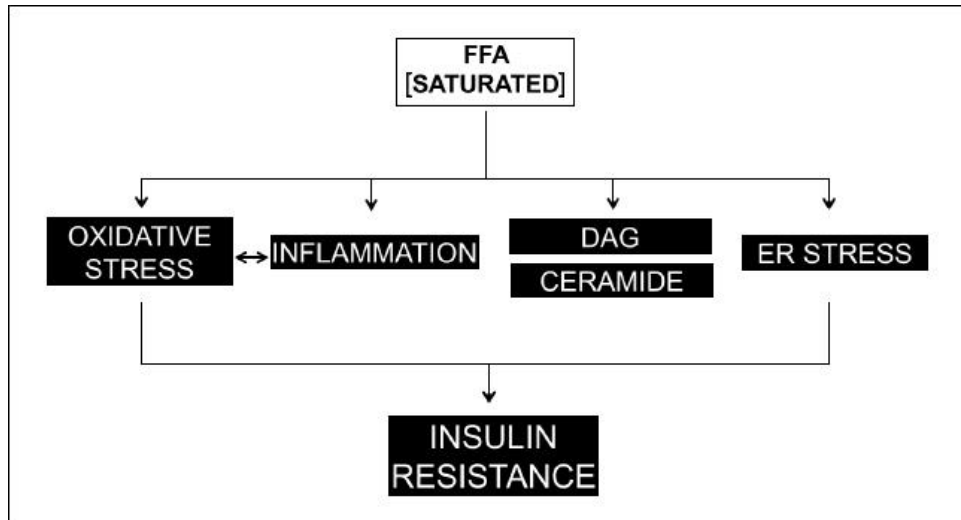


Fig. 3 Free Fatty Acids (FFA) may contribute to the onset of tissue and systemic insulin resistance through different mechanisms.
DAG: di-acyl glycerol; ER: endothelial reticulum.

3.4 Mitochondrial Dysfunction (Fig. 4)

An association between obesity, insulin resistance and skeletal muscle mitochondrial dysfunction in terms of reduced oxidative capacity and ATP production has emerged in the last 15 years (69). This association is notably less clear in the liver, where enhanced energy metabolism gene expression has been reported in some studies in obese insulin resistant patients (70,71). Reduced expression of regulators of muscle mitochondrial biogenesis such as PGC1alpha has been reported along with functional abnormalities in first degree relatives, including offspring, of insulin resistant, diabetic individuals (69,72). In addition, lipid-induced alterations of mitochondrial dynamics with excess mitochondrial fission and fragmentation have been reported in skeletal muscle, also potentially leading to reduced mitochondrial function (73). Dysfunctional mitochondria may cause both impaired lipid oxidation and increased reactive oxygen species generation, and they could therefore directly contribute to the onset of insulin resistance in skeletal muscle (69).

It should however be pointed out that insulin resistance and its changes have been also reported to occur independently of changes in muscle mitochondrial function (57,74-77). The onset of mitochondrial dysfunction could nonetheless cause a metabolic vicious cycle, with oxidative stress and impaired lipid utilization leading to worsened insulin action. Improvement of mitochondrial quality remains therefore an important potential target for insulin-sensitizing therapies. In recent years tissue autophagy, the process specifically removing damaged tissue organelles including mitochondria, has been reported to be impaired in experimental models of obesity, diabetes and insulin resistance (78,79). Enhancing autophagy might therefore be a novel potential target aimed at improving mitochondrial function and reducing oxidative stress in metabolic disease. Further studies are needed to confirm these concepts.

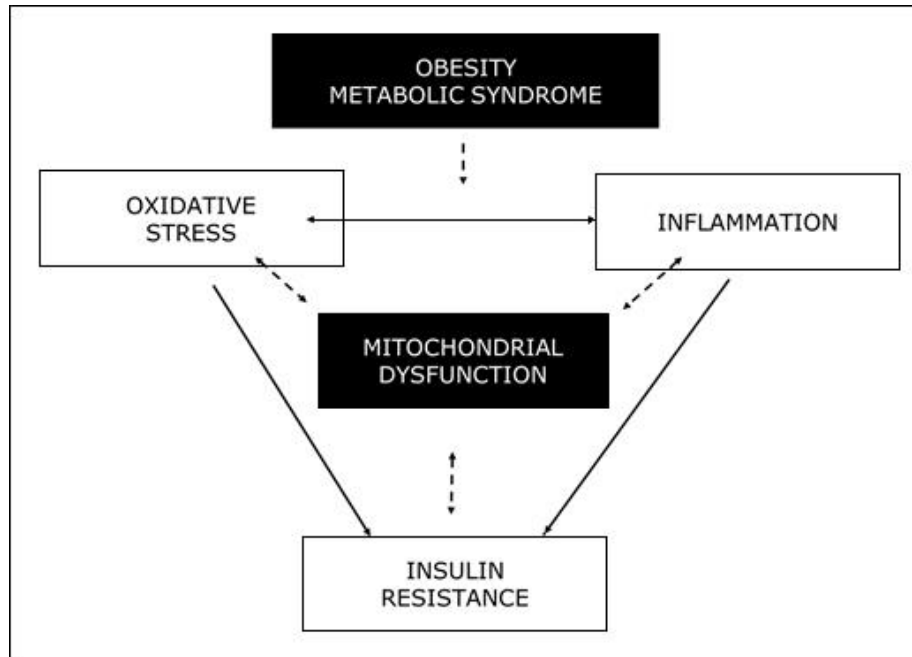


Fig. 4 Obesity-associated clusters of oxidative stress, inflammation and insulin resistance may create vicious cycles leading to mitochondrial dysfunction and worsened metabolic abnormalities

3.5 Gut and Host-nutrient Interactions

3.5.1 Gut microbiota (Fig. 5)

The gut microbiota have increasingly been recognized as a major contributor to the modulation of host-nutrient interactions, with particular regard to metabolic responses to food intake. Although the detailed description of gut bacteria is beyond the scope of this module, changes in gut microbiota are clearly associated with profoundly different responses to nutrient intake in terms of energy balance and intermediate metabolism. The negative metabolic role of selected bacterial species is demonstrated by lack of diet-induced obesity and metabolic alterations in germ-free animals and after antibiotic treatment leading to gut microbiota depletion (80,81). On the other hand, the highest diversity and variability of gut bacterial species has been associated with a lower risk of obesity and its complications in humans, individuals with less abundant bacterial species conversely having a higher risk of being obese (82). Also interestingly, dietary restriction has been recently shown to modulate gut microbiota as expressed by gene cluster analysis (86), leading to an increased richness of bacterial species that was largely but not completely reversed upon return to a weight-maintaining diet (83). Although mechanisms underlying microbiota-induced changes in energy balance remain only partly defined, it is increasingly clear that ability to produce short-chain fatty acids plays a relevant role (81,84). Moreover, in animal models host responses to short-chain fatty acids appear to modulate susceptibility to obesity further, and specific variants of SCFA receptors are associated with differential adiposity patterns (84).

Most interestingly, the gut microbiota have been shown to contribute directly to diet-induced metabolic alterations that play a direct role in the onset of insulin resistance, also independently of the onset of obesity (85). In particular, convincing evidence from experimental models has shown that combined overproduction of proinflammatory mediators, including lipopolysaccharide (LPS), and enhanced gut permeability are associated with deleterious bacterial patterns as well as with consumption of diets rich in saturated fat (85). This process has been defined as metabolic endotoxaemia and it may play a major role in the onset of metabolic derangements including insulin resistance, fatty liver and adipose tissue dysfunction in experimental models (85).

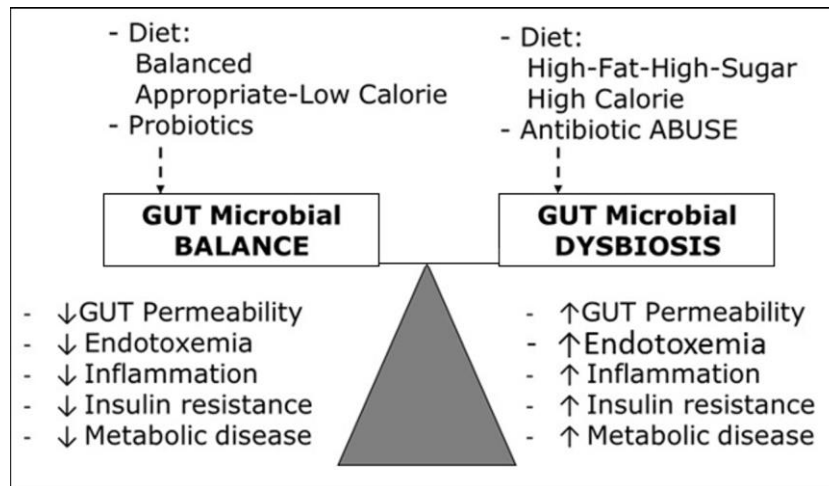


Fig. 5 Gut microbial dysbiosis may be enhanced by poor nutrition and may favour the onset of systemic inflammation, insulin resistance and obesity by enhancing gut permeability

3.5.2 Altered Nutrient Sensing

Nutrient-sensing pathways and feedback signalling mechanisms involving both the gut and the central nervous system (CNS) have been extensively studied in the last few decades. Importantly, gut hormones involved in nutrient sensing have emerged with pleiotropic effects on appetite, insulin secretion and substrate utilization. Glucagon-Like Peptide 1 (GLP1) is one clinically relevant example, whose negative effects on appetite and positive effects on insulin secretion and activity may be impaired in insulin-resistant type 2 diabetes and obesity (86). These observations have strongly supported the use of GLP1 analogues in the treatment of type 2 diabetic patients (86), with emerging, very promising results. The CNS has also been extensively studied in experimental models, leading to identification of nutrient-sensing areas whose direct effects on nutrient intake and metabolism could exert potential relevant roles in the regulation of insulin sensitivity. CNS studies remain however difficult to reproduce in humans and further methodological developments will likely be needed before more robust clinical data become available.

4. Clinical Burden of Insulin Resistance: Metabolic and Cardiovascular Disease

Insulin resistance is directly associated with metabolic and cardiovascular disease (87). Most importantly for this Module, insulin resistance also represents a major candidate in the role of “common soil” for the onset of the clustered metabolic abnormalities in metabolic syndrome patients. These associations have been well established in epidemiological studies and will be discussed below.

4.1 Insulin Resistance and Metabolic Syndrome

Most sets of diagnostic criteria for metabolic syndrome do not directly include insulin resistance (see: Module 24.1), but they commonly include elevated plasma glucose as a surrogate marker of altered glucose metabolism. Despite the close association between obesity and insulin resistance, whose causal mechanisms have been discussed above, BMI is also not directly included among diagnostic criteria for metabolic syndrome. On the other hand, elevated waist circumference, reflecting visceral fat content, is a better predictor of insulin resistance, metabolic disease and cardiovascular risk than BMI itself and is included in all sets of diagnostic criteria for metabolic syndrome (3,88-91). A number of epidemiological studies has indeed strongly established the very close link between visceral fat accumulation, insulin resistance and their metabolic and cardiovascular complications (89-91). In prospective studies, increased visceral fat is an independent risk factor for coronary artery disease, stroke,

and death (3, 89-91). Surgical removal of visceral fat in rodent models accordingly restores insulin sensitivity and improves metabolic and cardiovascular risk profiles, resulting in prolonged lifespan (92). The reasons for the negative metabolic impact of visceral fat appear to include biologically distinct profiles of gene expression and secretion of proinflammatory and pro-thrombotic cytokines, including TNF- α , IL-6 and plasminogen activation inhibitor-1 (PAI-1) (93). Elevated release of free fatty acids that directly reach the liver and impair hepatic insulin action is also a relevant metabolic complication of visceral fat accumulation. In addition, it has been hypothesized that excess visceral fat accumulation could result from low expandability of subcutaneous adipose tissue, which could therefore also display less favourable metabolic characteristics (94). Taken together, the above observations suggest a pivotal role for central obesity and related metabolic alterations in the pathogenesis of metabolic syndrome. Central obesity-associated insulin resistance is likely to play a key role in this association (**Fig. 6**). This view is fully supported by the potential pathogenetic role of insulin resistance in the onset of hypertension (through impaired nitric oxide production and altered endothelium-dependent vasorelaxation) (95) and hypertriglyceridaemia (through enhanced VLDL production), i.e. the other components of the metabolic syndrome. In full agreement with this view, surrogate markers of insulin resistance such as fasting plasma insulin are good biomarkers of future incidence of metabolic syndrome (96).

4.2 Insulin Resistance and Type 2 Diabetes

In insulin resistant patients, plasma glucose can be maintained within the normal range through enhanced beta-cell secretion (**Fig. 8**). Type 2 diabetes is therefore preceded by a variable but usually long period - often up to 10 years - of insulin resistance but normal plasma glucose. The inability to enhance insulin secretion indefinitely and rather a decline in beta-cell function may then lead to pre-diabetic alterations that include Impaired Fasting Glucose (IFG: 100-125 mg/dl fasting glucose (5.6-6.9mmol/l)) and Impaired Glucose Tolerance (IGT: 140-199 mg/dl (7.8-11.1) 2-hour glucose following Oral Glucose Tolerance Test). These conditions represent high-risk factors for the development of diabetes and are collectively defined as "prediabetes". The diagnosis of type 2 diabetes finally requires fasting plasma glucose above 126 mg/dl (7.0 mmol/l), 2-hour OGTT glucose above 200 mg/dl (11.2 mmol/l) or HbA1c above 6.5%. The natural history of type 2 diabetes can be accelerated by worsening of insulin resistance due to further weight gain, changes in lifestyle or intercurrent disease that may enhance insulin resistance. Importantly, insulin resistant people with prediabetes already have higher risk of developing cardiovascular disease compared to metabolically healthy individuals (97).

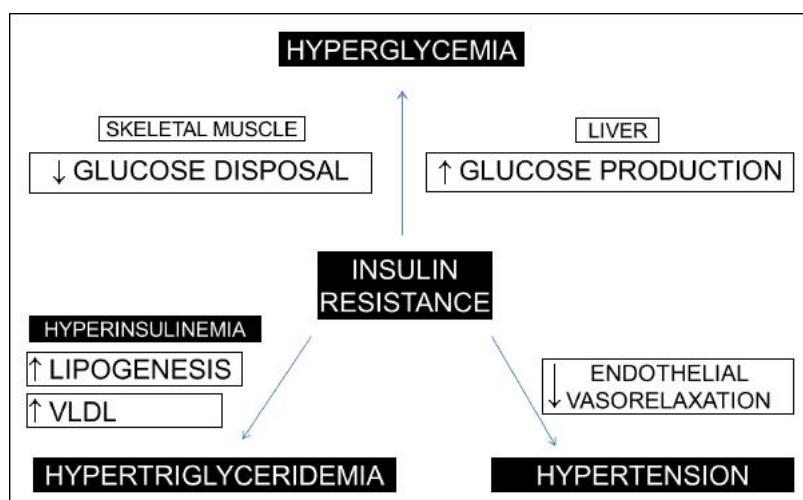


Fig. 6 Insulin resistance contributes to the onset of metabolic abnormalities defining the metabolic syndrome

4.3 Insulin Resistance and Cardiovascular Disease

There is no doubt that insulin resistance is a strong risk factor for cardiovascular disease, and surrogate markers of insulin resistance such as oral glucose tolerance test or HOMA index are accordingly good predictors of incident cardiovascular disease (98,99) (**Fig. 7**). As discussed above and throughout this module, a cluster of separate, independent risk factors may also contribute to enhance the risk for cardiovascular events in insulin-resistant individuals. These additional factors include those combined in the metabolic syndrome, as well as low-grade systemic inflammation, elevated proinflammatory cytokines, prothrombotic alterations, altered adipokine patterns. It is important to point out that several studies, although not all, agree in suggesting that clustering of cardiovascular risk factors, as favoured by insulin resistance in the metabolic syndrome and in other high-risk conditions, synergistically enhances cardiovascular morbidity and mortality beyond expected levels from the impact of single factors (100).

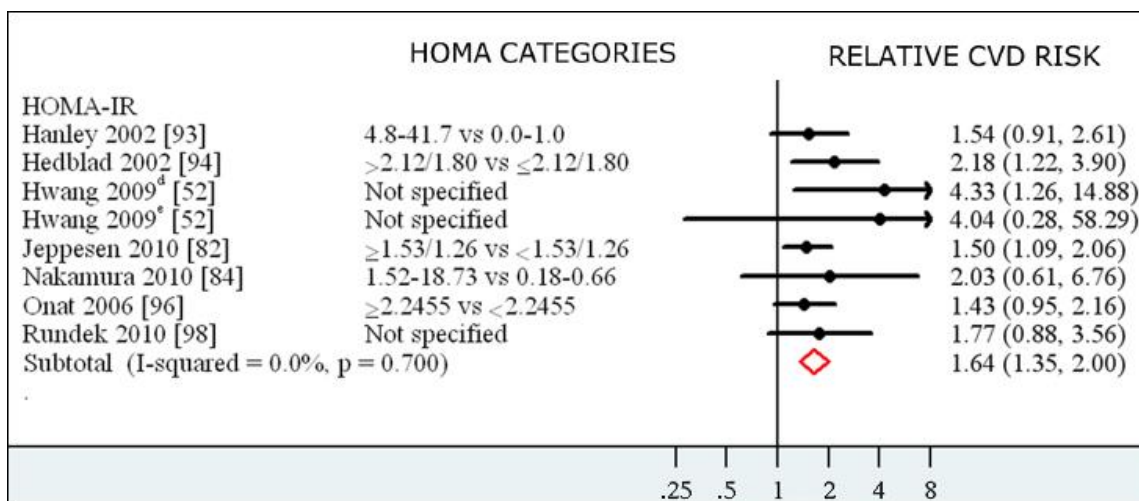


Fig. 7 Meta-analysis of risk for coronary heart disease in the highest- vs lowest HOMA category (from Ref 99)

5. Measurement of Insulin Resistance

Based on the above discussion, measurement of insulin resistance *in vivo* may provide crucial information on metabolic and cardiovascular risk in any given patient or population (101). Early detection of insulin resistance may be crucial in planning appropriate and effective prevention of progression to more advanced stages of cardiovascular risk. Repeated measurements may also provide useful clinical markers for evaluation of treatment effectiveness in modification of risk profile. While measurement of cellular and tissue events requires tissue collection through biopsy and is not commonly feasible in humans, the assessment of the effectiveness of insulin in promoting tissue glucose utilization may be performed *in vivo* through direct or indirect surrogate methods.

A number of established tests may indeed be used to measure insulin resistance in clinical research: the choice depends on sample size and type of study to be undertaken. The euglycaemic clamp is commonly considered the 'gold-standard' test, but it is highly labour-intensive and is most useful for physiological studies on small numbers of subjects. A simpler approach using HOMA is more appropriate for large epidemiological studies. In clinical practice however limitations usually apply, and simple tests that can be repeated over time are usually employed, based on measurements of fasting plasma glucose and insulin.

Euglycaemic Hyperinsulinaemic Clamp: this technique is usually considered the gold standard for measurement of insulin resistance (102). It is based on simultaneous infusions of insulin at a constant rate usually resulting in peak-physiological hyperinsulinaemia (comparable to post-prandial peak insulin levels) and of glucose at variable rates aimed at counteracting the physiological glucose-lowering effect of insulin and to maintain euglycaemia

(**Fig. 8**). Clamp duration may vary but it is commonly two to three hours and insulin action is inferred by the amount of glucose needed to maintain euglycaemia and prevent falls in glucose concentration: a higher glucose infusion rate per min·kg (GIR) reflects higher insulin sensitivity. Additional information may be inferred using constant infusions of tracers such as glucose stable isotopes, that allow calculation of glucose turnover rates and hepatic glucose production under fasting conditions.

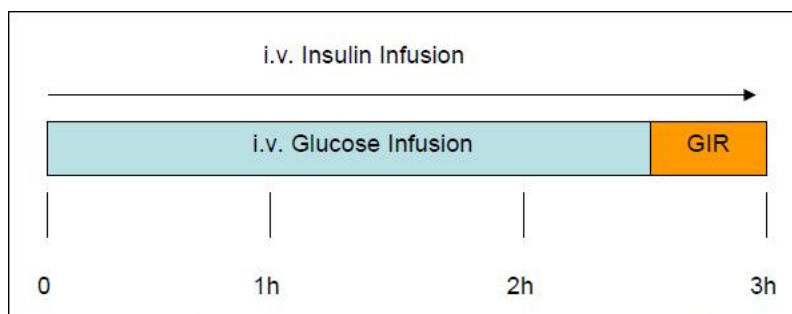


Fig. 8 The gold-standard hyperinsulinemic-euglycemic clamp technique to measure insulin sensitivity involves constant i.v. insulin infusion and variable glucose infusion to maintain basal glucose levels. The amount of glucose infused reflects insulin-mediated glucose disposal and is therefore an accurate measure of whole-body insulin sensitivity. Glucose infusion rate (GIR, mg/kg·min) may be commonly calculated in the last 30 minutes of a three-hour clamp.

The clamp technique can be considered very precise and accurate. Its results represent a reliable evaluation of peripheral insulin resistance under stimulated conditions, with a prominent role for skeletal muscle tissue. It is however labour-intensive and time-consuming and it requires experienced personnel. Its application is therefore limited to relatively small sample sizes, and it may not be feasible in large epidemiological studies or in routine clinical practice. On the other hand, the clamp has been commonly used to test the reliability of alternative measurements that have been commonly compared to clamp results for validation.

Insulin Tolerance Test (ITT): this technique is similar in principle to the clamp, in measuring the slope of the decline in plasma glucose concentration over 40 minutes after the bolus i.v. administration of 0.1 U/kg regular insulin. The technique is accurate but risk of hypoglycaemia is a major drawback and ITT is not employed in clinical practice and clinical research.

Oral Glucose Tolerance Test (OGTT): this test is very commonly used in clinical practice since it is a cornerstone in the diagnosis of type 2 diabetes. 75 g glucose are administered orally under fasting conditions and plasma glucose and insulin concentrations are measured at baseline and 30, 60, 90, and 120 minutes after glucose ingestion. The results reflect both insulin sensitivity and beta cell function-insulin secretion, since changes in plasma glucose depend on both insulin sensitivity and the amount of insulin secreted in response to the glucose challenge. For clinical purposes, plasma glucose above 140 mg/dl (7.8 mmol/l) reflects impaired glucose tolerance, while plasma glucose of 200 mg/dl (11.2 mmol/l) or more indicates the presence of diabetes mellitus. For research purposes, several insulin resistance indices have been proposed based on OGTT results, generally using 120 minutes of glucose and insulin, and mean values of the areas under the curves for their concentrations (103).

FASTING Insulin-Glucose

Insulin resistance indices based on fasting insulin and glucose are easiest to obtain and therefore very commonly used in clinical practice. They should be used with the following limitations in mind: 1) they reflect insulin resistance under basal, non-stimulated conditions;

2) fasting plasma insulin not only reflects insulin resistance but is strongly dependent on insulin secretion, distribution and catabolism.

Fasting Insulin: elevated fasting plasma insulin strongly suggests insulin resistance. Its validity as surrogate measure should be however questioned in patients with altered, impaired beta-cell function such as insulin resistant type 2 diabetics. Plasma insulin is however useful in epidemiological studies and it may indicate elevated cardiovascular risk since it has been reported to be associated with high levels of common cardiovascular risk factors.

Homeostasis Model Assessment (HOMA): it is based on the product of fasting glucose (mmol/l) and insulin (mU/l) as follows (104):

$$\text{HOMA-R} = (\text{FG} * \text{FI}) / 22.5$$

Where R is the insulin resistance index, and this constant is derived from model calculations and assumptions that go beyond the scope of this module, taking into account variable distribution, production and disposal of glucose. HOMA has been validated against euglycaemic clamp in most available reports in adult populations, although weak or non-significant correlations have been reported (105,106). As stated above, it should be kept in mind that HOMA measurement reflects different aspects of insulin resistance compared to the clamp technique. Its cost-effectiveness has however made HOMA a very popular surrogate measure of insulin resistance that is widely employed in clinical research and can be used in clinical practice.

Other surrogate measures have been used to measure insulin resistance based on fasting plasma glucose and insulin, QUICKI and FIRI tests being among the most commonly utilized (107).

Mathematical modelling approach: more sophisticated modelling approaches have been sought by increasing the number of measurements following glucose i.v. bolus infusion, with or without insulin sampling. These models have yielded important insights towards our understanding of insulin resistance in human disease but their detailed description falls beyond the scope of this review.

6. Summary

Insulin resistance, defined as a lower than normal insulin effect on glucose metabolism or concentration at a given insulin concentration, is a very common alteration with profound implications for human disease. Causes of insulin resistance are complex, and recent views indicate a major involvement of inflammation and excess visceral adiposity with altered lipid deposition in non-adipose tissues. Insulin resistance is at the core of the metabolic syndrome, being associated with plausible causal roles in all of its components. Measurement of insulin resistance is relatively easy when surrogate measures are employed with full awareness of their limitations. Insulin resistance measurements may provide important tools for management of metabolic and cardiovascular risk in metabolic syndrome patients and in the insulin-resistant population at large.

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