MEASURING INSULIN RESISTANCE IN CLINICAL PRACTICE

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ABSTRACT
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Insulin resistance is a fundamental factor in developing type 2 diabetes, which is a chronic metabolic disease with globally increasing number of diagnoses. Insulin resistance can still be reversed, therefore a sensor system capable of measuring insulin resistance in a clinical practice becomes increasingly desirable. This research defines the requirements of such a sensor system and evaluates the conventional methods available for measuring insulin resistance, furthermore possible innovative solutions are discussed. A clinical based sensor system requires a non-invasive measurement which can distinguish clearly between healthy, insulin resistant and diabetic test subjects. Furthermore no dietary preparations or extensive laboratory analysis should be required. All conventional methods for evaluating insulin sensitivity have limited value for clinical practice mainly due to their invasiveness, whereas new unconventional methods either lack significance in results or require more research. However both the $^{13}$C-glucose breath test and the urinary RBP4 measurement method have potential if further research can improve the significance of the results.
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1.1 Reason for this research

A worldwide diabetes epidemic is emerging, already responsible for close to 350 million people diagnosed with diabetes. The world health organization estimates 1.5 million deaths were directly caused by diabetes in 2012 and projects diabetes to be the 7th leading cause of death in 2030. From all cases of diabetes, around 90% comprises of type 2 diabetes which is caused by a reduced sensitivity of the body to insulin. This reduced sensitivity is called insulin resistance and occurred in most cases around the age between 50 and 60 years, however a steady trend of a significant increase in the number of obese and overweight individuals is responsible for lowering this age dramatically. Obesity and overweight are the primary causes of insulin resistance whereas physical activity and weight loss can increase the response to insulin.

Insulin binding to a target cell increases the ability of this cell to absorb glucose from the blood stream, in insulin resistance this cell’s ability to absorb glucose is impaired. A healthy pancreas will overcome the insulin resistance by increasing the insulin production maintaining healthy blood glucose levels. However over time the insulin secretion cells die trying to keep up with the insulin demand, which means healthy blood glucose levels cannot longer be maintained. At this point excess glucose will build up in the bloodstream and insulin resistance has evolved in diabetes type 2. Where insulin resistance is still reversible by primarily changes in lifestyle, is diabetes a chronic disease. Therefore the ability to detect insulin resistance becomes increasingly valuable.

Time intensive clinical methods such as, the euglycemic insulin clamp technique which measures insulin action, do already exist. However sensor system capable of measuring or detecting insulin resistance is not yet available and becomes increasingly desirable. This study evaluates the current possibilities to measure/detect insulin resistance and discusses the requirements a so called sensor system has to comply to.

1.2 Research questions

What are the current possibilities to measure insulin resistance?

What requirements would a sensor system, capable of measuring/detecting insulin resistance in a clinical setting, need to fulfill?
2.1 Insulin and metabolic effects

2.1.1 Chemistry

Insulin is a peptide hormone secreted by the β-cells (islets of Langerhans) in the pancreas, which is responsible for maintaining healthy blood glucose levels. Promoting cellular glucose uptake and regulating carbohydrate, fat and protein metabolism makes insulin an essential hormone within the human body. Insulin is secreted as a reaction to an abundance of energy, in form of carbohydrates, in the bloodstream. These carbohydrates are stored as glycogen in both muscles and the liver under the stimulus of insulin. Excess carbohydrates will be converted into fats and stored in adipose tissue, again with help of insulin. Insulin is composed of two amino acid chains which are connected by two disulfide links, these disulfide linkages are essential for the functional activity of insulin. In order for insulin to promote the cellular effects it has to bind to insulin receptors on target cells. Insulin binds and thereby activates a receptor protein, the activated receptor is responsible for the subsequent cellular effects(4,5).

The insulin receptor is a transmembrane receptor which consists of four subunits, two alpha and two beta glycoprotein subunits, all connected by disulfide linkages. The alpha subunits are located entirely external from the target cell, and are the receptor components which are in direct contact with insulin. The alpha subunits are connected to the beta subunits, which penetrate the target cell membrane. Insulin binding to the alpha subunit induces autophosphorylation of the beta subunits, which subsequently confers tyrosine kinase activity. Tyrosine kinase causes phosphorylation of intracellular enzymes including insulin receptor substrates (IRS), which now can bind signaling proteins to further mediate the insulin stimulated intracellular effects. Phosphatidylinositol 3-kinase (PI 3-kinase) is such a signaling enzyme which is responsible for promoting the translocation of glucose transporter proteins and glycogen, lipid and protein synthesis(2,6).
2.1.2 Translocation of glucose transporter proteins

Glucose enters a cell through a glucose transporter protein (GLUT), different types of GLUT differ in characteristics such as insulin dependency and its affinity for glucose molecules. The brain cells mainly contain GLUT 1 as transporter protein which is capable of transporting glucose into the cell at very low blood glucose levels and is completely insulin independent. This means the neurons, which depend on glucose for intracellular energy, can extract glucose at any moment irrelevant of the blood glucose and insulin levels. Both adipose and muscle cells, which are responsible for the majority of cellular glucose uptake, use GLUT 4 as glucose transporter protein. GLUT 4 is capable of transporting high concentrations of glucose and is entirely insulin dependent. The high affinity to glucose of GLUT 4 allows the cells to respond adequately to high blood glucose levels, for example after a meal. Unlike the GLUT 1, GLUT 4 is located in intracellular vesicles and needs to translocate to the plasma membrane to allow glucose uptake. As mentioned before, insulin binding to an insulin receptor results in phosphorylation of insulin receptor substrates which subsequently bind signaling enzymes such as PI 3-kinase. PI 3-kinase is responsible for the translocation of GLUT 4 to the cell membrane in both muscle and adipose cells. The insulin stimulated effect on intracellular translocation of GLUT 4 is reversible, a lack of insulin induces GLUT 4 to be removed from the membrane and restored in the vesicles within an hour. Afterwards GLUT 4 is ready to be activated by insulin again (2,6,7).

2.1.3 Effect of insulin on carbohydrate metabolism

Blood glucose is absorbed from carbohydrate intake and causes secretion of insulin, subsequently the insulin causes glucose uptake, storage and use by primarily muscles, adipose tissue and the liver. Muscle cells represent the majority of glucose uptake, however in resting state muscle cells do not rely on glucose but on fatty acids for its energy provision. Only during intensive exercise and during high blood glucose levels, after a meal, muscle cells will absorb glucose in high quantities. During exercise the muscle fibers become more permeable to glucose as a result of the muscle contraction process, however with high blood glucose levels an elevated blood insulin concentration is responsible for the increase in glucose uptake by the muscle cells. When blood glucose levels are high but the muscles are in rest state, glucose is still transported into the muscle cells. This abundance of intracellular glucose is stored as muscle glycogen, which can later be used energy source for the muscle cells. Apart from the muscles, insulin also promotes the uptake, storage and use of glucose in the liver. Insulin causes glucose to be absorbed by the liver cells and stored in form of glycogen almost immediately after a meal. When blood glucose levels are decreasing, insulin secretion is decreased correspondingly. The stored glycogen is transformed back into glucose and secreted into the bloodstream to prevent unhealthy low blood glucose concentrations (2,3,6). When an abundance of glucose is absorbed in liver cells it is mainly stored as glycogen, however excess glucose is converted into fatty acids under the stimulus of insulin. These fatty acids are packaged as triglycerides and transported to adipose tissue where they are deposited as fat.
2.1.4 Effect of insulin on fat metabolism

As well as carbohydrate metabolism, insulin also affects fat metabolism by promoting the synthesis and storage. The role of insulin in fat metabolism is significantly important, a structural lack of insulin can lead to vascular diseases. As mentioned before, insulin increases the transport of glucose into liver cells where it primarily is stored as glycogen and excess glucose becomes available for fat syntheses. This excess glucose is split into pyruvate through glycolysis, pyruvate is in turn converted to acetyl coenzyme A (acetyl-CoA) which serves as a substrate for fatty acid synthesis. When glucose is used for energy, citrate and isocitrate ions are formed. These ions activate the enzyme acetyl-CoA carboxylase which is responsible for forming malonyl-CoA, the first stage of fatty acid synthesis. The fatty acids are synthesized in the liver and form triglycerides which in turn need to be transported to adipose tissue for storage. The triglycerides are secreted from the liver in the bloodstream in form of lipoproteins and transported to adipose tissue. The capillary wall of adipose tissue is not permeable to triglycerides, therefore insulin activates lipoprotein lipase which splits the triglycerides back into fatty acids which can be absorbed by adipose cells. Once the fatty acids are absorbed, they are converted back to triglycerides to be stored. Furthermore insulin promotes the glucose uptake in fat cells, the absorbed glucose forms α-glycerol phosphate which together with the absorbed fatty acids are responsible for the intracellular conversion into triglycerides. Stored fat can be broken down and used for providing energy, this process is significantly enhanced in the absence of insulin. All insulin stimulated storage related processes are reversed in the absence of insulin. The intracellular hormone-sensitive lipase enzyme becomes activated and causes hydrolysis of the stored triglycerides, resulting in fatty acids and glycerol being released into the bloodstream. The now free fatty acids become the primary energy source for all tissues except for the brain cells, since neurons exclusively rely on glucose as energy provision. Generally the relation between glucose and fatty acids as energy source is regulated in a healthy manner, however in insulin deficiency an excess of fatty acids is located in the blood plasma. This promotes the liver conversion of fatty acids in phospholipids and cholesterol, together with excess triglycerides they are secreted from the liver into the blood in lipoproteins. A lack of insulin therefore results in an increased blood cholesterol concentration, which promotes the development vascular diseases.(2,3,6)

2.1.5 Effect of insulin on protein metabolism

Likewise carbohydrates and fats, proteins are also stored synthesized in tissues under stimulation of insulin. The procedure of insulin stimulation on protein storage is not yet understood completely, however a few insulin stimulated processes promoting protein synthesis are known. Insulin increases the translation of messenger RNA, thereby promoting the forming of proteins. The ribosomal activity, which is responsible for protein synthesis, seems to be insulin dependent. Insulin also prevents protein degradation. A lack of insulin will result in an increased protein depletion, and protein storage will stop entirely just like protein synthesis. This effect on protein metabolism can cause weakness and affect the functionally of organs.(2,3,6)
Insulin is secreted by the pancreatic beta cells as a response to blood glucose levels. Glucose enters the beta cells and promotes insulin secretion. The beta cells have many glucose transporter proteins (GLUT 2), enabling the beta cells to absorb glucose levels proportional to the blood glucose concentration. Intracellular glucose is phosphorylated by glucokinase into glucose-6-phosphate. This phosphorylation step is considered to be responsible for glucose sensing and thereby controlling the level of insulin secretion. The phosphorylation step results in ATP, which in turn closes the ATP-sensitive potassium channels and thereby depolarizes the cell membrane. The depolarization activates voltage-dependent calcium channels, resulting in an increased intracellular calcium concentration. The calcium stimulates the translocation and fusion of insulin containing vesicles with the cell membrane, thereby secreting the insulin through exocytosis. Some amino acids and hormones increase the effect of glucose on insulin secretion by promoting the calcium intake. For example glucagon and cortisol promote the effect of glucose on insulin secretion, however extended secretion in high quantity can cause exhaustion of the beta cells which can lead to diabetes mellitus. (2,3,6,7)

Figure 2 Schematic of insulin secretion mechanism(2)
Diabetes Mellitus

Diabetes mellitus is a chronic disease resulting in impaired metabolism of carbohydrate, fat and protein which is caused by either a lack of insulin secretion or a decreased sensitivity to insulin. Diabetes mellitus can be classified into two types; type 1 diabetes which is caused by a lack of insulin secretion, and type 2 diabetes which is caused by a decreased insulin sensitivity also called insulin resistance. In both types of diabetes mellitus the insulin-stimulated effects on glucose metabolism are impaired or completely inhibited.\(^{(5)}\)

Type 1 diabetes accounts for around 10 percent of all diabetes cases and is often a result of viral infections or autoimmune disorders which damage the pancreatic beta cells, inhibiting the production and secretion of insulin. Type 1 diabetes generally develops mainly at teenagers and young adults, and can develop within weeks.\(^{(5)}\) First blood glucose levels increase significantly since the lack of insulin decreases glucose uptake and metabolism, furthermore a lack of insulin promotes the glucose production by the liver. The elevated blood glucose levels cause loss of glucose through the urinary tract. The high glucose concentration cannot be completely reabsorbed in the renal tubules and will partly be lost in urine, which means losing potential energy source. Additionally excessive glucose concentrations in the renal tubules cause considerable decrease in tubular reabsorption of fluid. This results in a substantial loss of fluid through urine, this causes dehydration of the extracellular fluid. The body tries to compensate by dehydrating the intracellular fluid. Besides the effect of dehydration, excessive glucose concentrations in diabetes also affect the blood supply to tissues. Prolonged excessive glucose concentrations affect the functionality of blood vessels in many tissues, structural changes in these blood vessels leads to a decreased blood supply to tissues. Decreased blood supply can have severe consequences such as increased risk for heart attack, stroke or blindness. Prolonged excessive glucose concentrations in diabetes can have destructive consequences on many tissues, however the exact processes are not completely understood yet.\(^{(2,3)}\)

After increasing blood glucose levels, diabetes causes an increase in use of fats and metabolic acidosis. The lack of insulin in diabetes inhibits glucose metabolism, therefore a compensatory shift to fat metabolism occurs. Fat metabolism releases keto acids in the plasma which normally are absorbed and oxidized by tissue cells, however an increased fat metabolism in diabetes results in an abundance in released keto acids. The excess keto acids cannot be absorbed by tissue cells and result in an increased acidity of the blood. This increased blood acidity is called acidosis and combined with the dehydration can lead to diabetic coma or even death. Besides fats, also proteins are used to compensate for the failure of glucose to produce energy in diabetes.\(^{(3,7)}\)

Type 2 diabetes accounts for around 90 percent of all cases of diabetes and unlike type 1 diabetes, type 2 diabetes is primarily caused by an unhealthy lifestyle and develops gradually. Obesity is the most significant risk promoting the development of type 2 diabetes. Generally the onset of type 2 diabetes occurs at ages around 50 years, however the increasing prevalence of obesity has lowered this age dramatically.\(^{(1)}\) Whereas type 1 diabetes is associated with a lack of insulin secretion, type 2 is associated with a decreased sensitivity to insulin by the tissue cells which is also called insulin resistance. In insulin resistance either the amount of insulin receptors on a target cell is reduced but generally the signaling phosphorylation steps are impaired, both resulting in a reduced uptake and use of glucose which leads to higher blood glucose levels. The insulin secretion is regulated by the pancreatic beta cells which synthesize and secrete insulin proportional to blood glucose levels. An increased blood glucose concentration due to insulin resistance is therefore compensated by the pancreatic beta cells by secreting more insulin. In some cases the pancreas is able to keep compensating even in prolonged and severe insulin resistance, however generally as time progresses the pancreatic beta cells become exhausted and possibly die. At this point full blown type 2 diabetes has developed with the similar consequences as type 1 diabetes.\(^{(5)}\)
Insulin resistance

Insulin binding to an insulin receptor leads to phosphorylation of the insulin receptor substrates (IRS) which activates the glucose transport proteins resulting in an increased glucose uptake. In insulin resistance these phosphorylation steps are impaired, the signal to the glucose transporter proteins is reduced(7). This inhibits the glucose uptake in both skeletal muscle and adipose tissue and is referred to as systemic insulin resistance. Furthermore in the liver stored glucose can be secreted, controlled by insulin levels. High insulin levels suppresses this glucose secretion, however in insulin resistance this inhibition is impaired. The liver does not respond adequately to the insulin and glucose can be secreted by the liver without restraints, even in high blood insulin concentrations. The insulin resistance in the liver is called hepatic insulin resistance. Both the hepatic and systemic insulin resistance are compensated for by the pancreatic beta cells by secreting more insulin(6,7). As mentioned before, the increased insulin secretion can have a detrimental effect on the pancreatic beta cells resulting in depletion of these cells. Furthermore, prolonged increased blood insulin levels also have a detrimental effect on the insulin sensitivity of target cells caused by the process of down regulation. When insulin binds to a receptor, together with the receptor it is internalized into the cell through endocytosis. Intracellular the insulin and receptor are dissociated, afterwards the insulin is degraded and the receptor is recycled back into the cell membrane. However at high prolonged insulin concentrations, the synthesis and recycling of the insulin receptors cannot follow the level of internalization thereby resulting in a decreased amount of insulin receptors(8). The amount of insulin receptors does influence the insulin sensitivity of the target cells significantly, therefore a lack of insulin receptors does increase the insulin resistance. However it is believed that inhibition of the intracellular signaling phosphorylation steps is the fundamental and dominant element of insulin resistance instead of decreased receptors due to down regulation(7). The impaired signaling between IRS-1 and PI-3 kinase seems to be the primary intracellular defect in insulin resistance. An increased externally induced phosphorylation of serine residues of the IRS is believed to cause the impaired signaling. Mutations in the GLUT 4 gene can have significant effects on the glucose uptake as well, however these mutations very rarely found in type 2 diabetics(7).

Looking at the pathogenesis of insulin resistance, obesity seems to be the most significant risk promoting insulin resistance. Obese subjects have an increased level of adipose tissue in which fats are stored. Besides storing fat, adipose tissue also operates as an endocrine organ secreting cytokines (cell signaling proteins) such as adipokines. In obese subjects excess in adipose tissue is generally a result of enlarged fat cells or adipocyte, adipocyte enlargement can result in insufficient blood supply to these adipocytes. Insufficient blood supply leads to adipose tissue dysfunction which results in alterations in cytokines secretion. The secretion of pro-inflammatory adipokines is increased, whereas the secretion of anti-inflammatory adipokines in decreased, resulting in a pro-inflammatory state of the adipose tissue. This pro-inflammatory state is believed to promote the development of insulin resistance(9)(10). The pro-inflammatory cytokines interfere with the intracellular pathways of insulin stimulated glucose uptake. The pro-inflammatory cytokines can activate the intracellular c-Jun NH2-terminal kinase (JNK) through receptor pathways. JNK seems to promote insulin resistance by phosphorylation of serine residues in insulin receptor substrate 1 (IRS-1). Normally the tyrosine kinase activity induces and enables the IRS to bind to signaling enzymes resulting in for example glucose uptake through GLUT 4. However the JNK stimulated increase in serine phosphorylation of IRS 1 inhibits the tyrosine kinase activity of the receptor(7,9,10). Besides pro-inflammatory cytokines, obese subjects also have increased free fatty acid concentrations which likewise inhibit the insulin induced IRS signaling pathway. Excess of circulating free fatty acids are stored in muscle cells as triglycerides. Intramyocellular accumulation of these triglycerides induces the presence of lipid intermediates such as fatty acyl-CoA. Fatty acyl-CoA in turn activates the protein kinase C, which inhibits the tyrosine kinase activity of the insulin receptor similarly like the JNK. Kinase C protein is likewise responsible for the phosphorylation of serine residues in the IRS 1.(11,12)
CHAPTER 3

REQUIREMENTS FOR A CLINICAL SENSOR SYSTEM

The aim of this research is to define requirements for a sensor system capable of measuring or detecting insulin resistance in a clinical practice. The global prevalence of obesity and diabetes causes the ability to measure and detect insulin resistance on a large number of potential patients to become increasingly desirable. A sensor system would be able to scan a potential patient as a precautionary measure in clinical practice. Insulin resistance shows little to no symptoms, therefore many subjects do not realize their condition. The lack of symptoms does not promote potential patients to visit the hospital for a precautionary check, especially when the diagnostic tests are time intensive and invasive proceedings. Therefore the measurement through the sensor system should be a noninvasive procedure. To maintain an approachable testing procedure, little to no preparation from the test subject should be required. Generally insulin resistance tests measure the effect of insulin on blood glucose concentration, therefore the test subjects have to retain a strict diet and cannot ingest anything often up to eight hours before the test. Eliminating these dietary preparations for the test subject promotes the accessibility of the test. Therefore the measurement through the sensor system should be independent from the ingestion of glucose before the measurement.

Furthermore the measurement system should have an accuracy which at least can distinguish between a healthy, an insulin resistant and possibly a type 2 diabetic subject. An accuracy capable of quantifying the level of insulin resistance would be ideal, however a significant distinction between healthy and insulin resistant is sufficient in early stages of future research. The system should be able to distinguish between healthy and insulin resistant subjects, in order to supply a method capable of population based screening. Further examination can be performed with use of conventional measuring methods. Besides eliminating intensive preparation for the test subjects, the system should also preferably result in real-time data. Extensive laboratory analysis slows down diagnostic tests, therefore a measurement system which can generate diagnostic data without help of laboratory analysis becomes desirable for screening tests.
Measuring insulin resistance is a complicated procedure since the performance of intracellular signaling mechanisms are evaluated. However several processes or substances can be used as indicator reflecting on insulin resistance. A number of testing procedures are available which look into the blood glucose levels when insulin levels are being controlled, thereby reflecting the body’s response to insulin. Most of these tests are generally used in research projects instead of clinical practice because of the invasiveness and time intensiveness of the procedures.

4.1 Euglycemic Clamp

The euglycemic clamp is a technique which provides whole body in vivo insulin response of the test subject. This test is based on controlling both blood insulin and glucose concentration and measuring the tissue response, controlling the concentrations requires injecting both glucose and insulin directly in the bloodstream. Up to 12 hours before the test the subject cannot eat or drink anything to both minimize and stabilize the glucose and insulin concentrations. During the measurement which typically takes around three hours, first the blood insulin concentration is raised and accurately maintained around 100 μU/ml by continuous infusion of insulin. To compensate for the abundance of insulin and to prevent hypoglycemia, glucose is infused. Blood glucose concentrations are kept constant through the infusion to prevent unhealthy low glucose levels, hypoglycemia. The amount of glucose used to regulate the glucose concentration reflects the uptake by all tissues in the body is thereby a measure of tissue insulin sensitivity. High levels of used glucose mean that the subject is sensitive to glucose, whereas low levels of used glucose relate to insulin resistance.

Although the euglycemic insulin clamp technique is often seen as the golden standard in measuring insulin resistance, it is an intensive procedure which is not often used in clinical practice due to its invasiveness and complexity. (13,14) The test takes around three hours in which both patient and performer of the test have to be present, this makes it expensive. Furthermore the test is invasive and requires glucose and insulin infusion in peripheral veins which can be unpleasant for the test subject. During the insulin infusion symptoms of hypoglycemia can occur which include; dizziness, hunger and headache (15). Besides the possible side effects, the test subject also needs to retain strict dietary preparations. Nevertheless, the test does result directly in diagnostic data which represents the insulin sensitivity. The euglycemic insulin clamp technique might be an accurate and reliable method for measuring insulin resistance, it is not desirable for clinical practice because it is invasive, time intensive and complex.

4.2 Fasting Plasma Insulin Concentration

A much simpler and less invasive testing method is measuring fasting plasma insulin concentrations. After a period of time of around 8-12 hours where the test subject cannot eat or drink anything, plasma insulin concentrations are measured by taking blood samples. High plasma insulin levels indicate the presence of insulin resistance. The relation between high plasma insulin levels and insulin resistance is derived from comparing the results with euglycemic clamp studies, resulting in a relatively good correlation between the methods (14). However fasting insulin concentrations can only represent up to 50% of the variation in insulin action since plasma insulin concentrations are also a result of insulin secretion, distribution and degradation instead of just insulin sensitivity. For example, when insulin resistance evolves into type 2 diabetes, the pancreatic beta cells get exhausted and insulin secretion decreases. The fasting insulin concentration method would not result in diagnosis of insulin resistance whereas this is present in the test subject. Furthermore insulin is often secreted in pulses, measuring right after an insulin pulse might lead to measurement values which are not representable for the actual insulin concentration (6,14).

Although this test is much simpler and less invasive compared to the euglycemic clamp technique, the accuracy of the method is questionable. Furthermore, interpretation of the results can be difficult because the source of increased plasma insulin cannot be determined. Dietary preparation by the test subject is still required in order to get reliable and significant results. Besides the practicality of this method, the remaining value for clinical practice is limited.
4.3 The Homeostasis Model Assessment (HOMA)

Looking solely at fasting insulin does not always lead to reliable results, therefore the homeostasis model assessment includes fasting plasma glucose in the method. The insulin sensitivity is calculated by a formula which incorporates both the fasting insulin (FI in µU/mL) and fasting glucose (FG in mmol/L) concentrations resulting in the HOMA insulin resistance (HOMA\textsubscript{IR}).

\[
\text{HOMA}_{\text{IR}} = \frac{\text{FI} \times \text{FG}}{22.5}
\]

A low HOMA\textsubscript{IR} corresponds to a high insulin sensitivity whereas a low HOMA\textsubscript{IR} indicates low insulin sensitivity. The relation between insulin resistance and the HOMA\textsubscript{IR} value correlates well with the euglycemic insulin clamp method at normal glucose levels, however at really high blood glucose levels (hyperglycemia) this correlation decreases. This limits the value of this method since high blood glucose level is one of the symptoms of insulin resistance. Although glucose levels are incorporated as well in this formula, the fasting insulin concentrations still play a major role in the calculation resulting in similar limitations as the previous method. However taking multiple fasting insulin samples within a small time frame can prevent the error caused by the pulsation property of insulin secretion. This method is less invasive than the euglycemic insulin clamp method and gives more significant results than the fasting plasma insulin resistance, however the method does not produce reliable results for test subjects with high blood glucose levels. Dietary preparation by the test subject is still required and although being less invasive, the method still requires several blood samples making this method not ideal for clinical practice on all subjects.

4.4 Quantitative Insulin Sensitivity Check Index (QUICKI)

Like HOMA, QUICKI is also a mathematical model relating insulin sensitivity to both fasting plasma insulin and glucose concentrations and is defined by the following formula

\[
\text{QUICKI} = \frac{1}{\log(I_0) + \log(G_0)}
\]

Where \(I_0\) is the fasting plasma insulin in µU/mL and \(G_0\) is the fasting plasma glucose in mg/dL. Besides a different way of calculation, the QUICKI practically is not any different from the HOMA method. However in obese and diabetic subjects, this method seems to have a slightly higher correlation with the results of the euglycemic insulin clamp method. Nevertheless, this method does not seem to be highly valuable in clinical practice either.

4.5 Continuous Infusion of Glucose with Model Assessment (CIGMA)

The CIGMA model assesses insulin sensitivity through measuring insulin and glucose concentrations after continuous infusion of glucose. The model compares the measured insulin and glucose concentrations with known physiologic data derived from healthy test subjects, which describes the insulin and glucose response to glucose infusion. The continuous infusion of glucose increases the accuracy of the test compared to the previous mentioned mathematical models, low basal insulin concentrations can result in inaccurate insulin measurements. CIGMA results correlate well with euglycemic insulin clamp results, however in subjects with severe insulin resistance this correlation decreases. CIGMA is more invasive than HOMA and QUICKI due to the continuous infusion of glucose, however it does give a better reflection of insulin sensitivity. CIGMA is an invasive and complex procedure, thereby limiting the clinical feasibility.
4.6 Oral Glucose Tolerance Test (OGTT)

The OGTT method evaluates insulin sensitivity by measuring plasma glucose and insulin concentrations after oral ingestion of a known amount of glucose. Instead of intravenous infusion, in OGTT glucose is orally ingested. This reduces the required preparation for the measurements and makes the method less invasive, making it more valuable in clinical practice. Several mathematical models result in indices describing insulin sensitivity. The mathematical models use insulin and glucose concentrations measured after 0, 30, 60 and 120 minutes after ingestion of glucose. OGTT results correlate well with euglycemic insulin clamp results; however in cases of advanced type 2 diabetes the correlation is decreased. (14) This is caused by the impaired ability of insulin secretion in these diabetic subjects. Insulin concentration response to oral glucose ingestion involves gut hormones, neural stimulation and pancreatic response. (6,7) Therefore a set amount of glucose intake can result in non-insulin resistance induced differences in response; this causes the reproducibility of the test fairly low around 60%. Nevertheless, the high practicality of the test increases the clinical value.

4.7 Insulin Tolerance Test (ITT)

The ITT method assesses insulin sensitivity by measuring plasma glucose concentrations over a period of time after an intravenous infusion of insulin. Over a period of 60 minutes the plasma glucose concentrations are recorded, larger decrease in plasma glucose indicates higher insulin sensitivity. Test subject cannot eat or drink anything up to 10 hours before the test. Ingesting insulin in subjects which already have a low glucose concentration can lead to extreme low blood glucose levels (hypoglycemia) which can interfere with the insulin sensitivity measurement through hormonal responses. (14) Therefore the ITT should always be used with great caution to prevent hypoglycemia and generate reliable results. ITT gives an indirect estimate of the overall insulin sensitivity which correlates well with the euglycemic insulin clamp results. ITT is an invasive method which in some cases can give questionable results due to hypoglycemia and dietary preparation is required for the test subject. ITT has a limited value for clinical practice.
CHAPTER 5
POSSIBLE SOLUTIONS

All described methods do not entirely fulfill the set requirements, mainly the methods are invasive and require too much preparation. Although great results on insulin sensitivity can be produced with for example the euglycemic insulin clamp method, the clinical value is very limited. All described methods try to assess insulin sensitivity by evaluating the plasma glucose concentration, insulin concentration or a combination of both. However insulin resistance has more effects besides direct insulin and glucose action, indirect measurement of insulin resistance by measuring biomarkers corresponding to insulin resistance might result in less invasive and clinical valuable methods.

5.1 Detecting insulin receptors

The previously methods all measure the effect of insulin sensitivity by measuring insulin and glucose concentrations, measuring the actual sensitivity might lead to more reliable results. Direct measurement of insulin sensitivity involves measuring either the insulin receptors or evaluating the intracellular phosphorylation steps. Measuring intracellular processes is very complex and possible results can differ each cell, measuring insulin receptors is a more feasible possibility. This would be an invasive method which probably requires a biopsy of the desired tissue. The insulin receptors are well defined and can possibly be detected through spectroscopy measurements. The insulin receptors contain disulfide bonds which have specific absorption properties around 260 and 550nm. (16–18) An advantage of quantifying the insulin receptors is that it allows for tissue specific measurements, results only reflect insulin sensitivity of the used tissue. Quantifying the insulin receptors practically has no value for clinical practice due to the invasiveness. Furthermore it is believed that in insulin resistance the intracellular signaling steps are the dominant factor affecting insulin sensitivity instead of the amount of receptors.

5.2 Condition of the pancreatic beta cells

Insulin resistance generally leads to exhaustion and depletion of the pancreatic beta cells which normally synthesize and secrete insulin. The ability to quantify the condition of the pancreatic beta cells can give a reflection on the insulin sensitivity of the subject. Recent research by the department of radiology and nuclear medicine at the RadboudUMC reveals a method to visualize the pancreatic beta cells(19,20). The pancreatic beta cells have DLP-1 receptors present, this method uses radionuclide labeled exendin-4 which specifically binds to the DLP-1 receptors. The exendin is labeled by 111-indium or 68-gallium isotopes which can be visualized by single positron emission computed tomography (SPECT). The radinuclide labeled exendin is intravenously injected in the test subject, after four hours the subject is scanned by SPECT. The method results in a known pancreatic uptake of exendin, which in turn correlates to beta cell mass. The beta cell mass is not a direct indication for beta cell condition, however an increase or decrease in beta cell mass does correlate with the insulin secretion. Furthermore the beta cell mass can be different for every patient without indicating a difference in insulin secretion conditions. Nevertheless, measuring the beta cell mass at different moments in time can give an indication on beta cell depletion. This method is less invasive than the euglycemic insulin clamp method, but it requires multiple expensive scans in order to obtain any significant results. Furthermore the possible results only reflect insulin resistance in an advanced stage where the beta cells start to die. The required scans and lack of significant results make this method not suitable for clinical practice.
5.3 $^{13}$C-Glucose breath test

The $^{13}$C-glucose breath test is a non-invasive test which correlates insulin resistance to CO$_2$ produced by glucose metabolism. The metabolism of glucose produces CO$_2$ which is eliminated through expiration by the lungs. Monitoring the amount of exhaled CO$_2$ which is produced by glucose metabolism can give an estimation on glucose uptake by the cells which in turn reflects the insulin sensitivity. In this method glucose is labeled with an isotope tracer ($^{13}$C). After ingestion of the $^{13}$C labeled glucose, glucose metabolism results in $^{13}$CO$_2$ to be exhaled. The difference in expired $^{13}$CO$_2$ before and after the ingestion of $^{13}$C labeled glucose gives an estimation of insulin resistance, where higher differences reflect a higher insulin sensitivity.(21).

All the conventional methods for assessing insulin resistance are invasive and require blood samples or complex infusion processes. The $^{13}$C-glucose breath test only requires oral ingestion of $^{13}$C-glucose and measurement of exhaled breath, therefore it seems to be ideal for clinical practice. However the results of this method only correlate moderate with the conventional methods(21). Furthermore results are most reliable when the test subjects undergo a fasting period of 12 hours before the test. The $^{13}$CO$_2$ is generally measured with help of a mass spectrometer but lately possibilities with infrared spectroscopy are being explored(22). The poor accuracy and required dietary preparations, limit the clinical value of $^{13}$C-glucose breath test.

5.4 Urinary RBP4 measurements

Instead of looking to the measurable effects of insulin resistance, this possible method evaluates the source causing insulin resistance. Adipose tissue secretes adipokines which can interfere with the intracellular signaling pathways which enable glucose uptake, thereby inducing insulin resistance(9,10,23). Retinol Binding Protein 4 (RBP4) is such an adipokine associated with insulin resistance. Recent extensive research shows that elevated RBP4 concentrations result in increased urinary RBP4 concentrations and are strongly related to insulin resistance. This enables a non-invasive measurement through urine samples, also glucose intake before the test would not interfere with the test results.(24) Urinary RBP4 concentrations are higher in cases of insulin resistance. The correlation between this method and the conventional methods is not yet extensively defined, therefore more research is required to evaluate the significance of the urinary RBP4 results as an evaluation on insulin resistance. The possibilities to measure urinary RBP4 concentrations are limited since the structure and properties of RBP4 are not well defined yet. This limits the measurement options significantly, currently only the use of immunoassays which use specific RBP4 antibodies results in reliable data(23,25,26). These immunoassays are relatively complex, sensitive and expensive laboratory tools which limit the clinical value for urinary RBP4 measurements moderately.

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*Table 1: Evaluation of measurement systems*
CHAPTER 6
POSSIBLE SOLUTIONS

All conventional methods for evaluating insulin sensitivity have limited value for clinical practice mainly due to their invasiveness, whereas new unconventional methods either lack significance in results or require more research. The conventional methods rely on regulating insulin and glucose concentration or measuring a response after infusion of a stimulation and require taking blood samples. The conventional invasive methods such as the euglycemic insulin clamp method are valuable in research projects and can achieve great results on measuring insulin sensitivity, however their value in a clinical setting is limited. Therefore a sensor system capable of measuring insulin resistance in a clinical practice is desirable. The sensor system should be non-invasive, independent of dietary preparation by the test subject, have a significant accuracy and does not require extensive laboratory analysis. None of the conventional methods fulfill all the requirements, therefore possibilities for new measurement methods have been researched. Detecting insulin receptors will not reflect insulin resistance significantly, whereas visualizing the pancreatic beta cells only has potential in advanced stages of insulin resistance. This leaves the only two methods which are truly non-invasive, the $^{13}$C-glucose breath test and the urinary RBP4 measurement. Both have great potential for performing in a clinical setting but both come with limitations. The $^{13}$C-glucose breath test requires dietary preparations by the test subject and results can be questionable when comparing to the conventional methods. The urinary RBP4 measurement is independent of dietary preparations, however more research is required to establish the significance of the results. Furthermore measuring RBP4 concentrations is still a complex procedure which limits the clinical value. An insulin resistance measurement method which fulfills all requirements for use in clinical practice is not yet available, however both the $^{13}$C-glucose breath test and the urinary RBP4 measurement method have potential if further research can improve the significance of the results.
REFERENCES CITED


