Predictive markers of pre-pregnancy insulin resistance in women with polycystic ovary syndrome

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Abstract:

Polycystic ovary syndrome (PCOS) is a disorder affecting a lot of women around the world. PCOS patients can have difficulties becoming pregnant and have an increased chance of adverse pregnancy outcomes like developing gestational diabetes (GDM). Women with PCOS who develop GDM seem to have an increased insulin resistance before pregnancy, which increases to levels higher than seen during a normal pregnancy. To give an overview of how to predict insulin resistance in these women, pre-pregnancy markers for insulin resistance will be discussed in this literature review by analysing what is known from the latest research. The insulin sensitivity chances of a normal pregnancy will be compared to a pregnancy with decreased insulin sensitivity.

The standard method to test for insulin resistance is the HOMA-IR test, but that requires the patient to fast for at least 12 hours. Serum markers that can be reliable to predict insulin resistance without the need of fasting would be more convenient, because fasting can be a burden and a nuisance for women who want to become pregnant or are pregnant. Recent studies have shown that an increased serum level of afamin, an increased free androgen index and a decreased adiponectin serum level have to highest reliability of predicting insulin resistance in a woman with PCOS. When a combination of these biomarkers is used, insulin resistance in PCOS patients can be predicted with a high certainty. When the presence of insulin resistance is known, adequate measures can be taken to prevent serious harm to mother and child during and after pregnancy.

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Introduction:

During a normal pregnancy a number of metabolic changes happen to accommodate the different necessities of the changing body and fetus. These metabolic adjustments are mainly driven by changes in circulating hormones and factors produced by the placenta. The metabolic changes secure adequate nutrient supply to the foetus, in particular during the third trimester when growth and hence nutrient needs are high(Barbour et al. 2007). The hormonal change primarily looked at in this paper is the change in insulin sensitivity during pregnancy. We were particularly interested to know if there are differences between normal pregnancies and pregnancies in women with polycystic ovary syndrome (PCOS). Insulin is a hormone that is produced by beta cells in the pancreas. It is secreted when glucose levels increase in the bloodstream. Blood sugar levels increase when glucose is absorbed in the gut or produced in the body. When insulin is secreted the blood insulin level increases and cells in the body, like the liver cells, are inhibited from producing and secreting more glucose. Insulin is also the regulator of the uptake of glucose from the blood into the cells. The liver, skeletal muscles and adipose tissue can store glucose under the influence of insulin. Insulin sensitivity refers to how strong tissues respond when presented with insulin. When there is insulin resistance it means that there is a weak response to insulin by cells (Sonksen and Sonksen 2000). In the first trimester of a healthy pregnancy insulin secretion increases while the insulin sensitivity stays unchanged or even increase a bit in some women. Small natural differences in the exact metabolism and homeostasis exist between humans. As long as these differences stay within an acceptable range, no problems occur. During the second and third trimesters of pregnancy the insulin sensitivity decreases (Barbour et al. 2007). The main driver for the normal physiological changes in insulin sensitivity is placental lactogen (Barbour et al. 2007). Hyperglycemia may occur when a metabolic disturbance leads to an inadequate control of these processes. Diabetes mellitus is the overarching name of metabolic disorders in which blood sugar levels are increased either due to a disturbed insulin production in the pancreas or a lower sensitivity of cells to insulin. In type II diabetes the disturbed insulin production is preceded by insulin resistance in the body, while in type I diabetes the patient has an impared insulin production as the start of the disorder (Sonksen and Sonksen 2000). When diabetes like symptoms occur during pregnancy it is called gestational diabetes mellitus (GDM).

PCOS is an endocrine disorder in women of reproductive age. Women with this disorder have an increased androgen production in the ovary and have polycystic ovaries. Polycystic ovaries means that there are an increased, regularly more than 11, amount of immature follicles on a woman's ovaries that contribute to an altered hormonal output. PCOS is globally present in 6 to 15% of al women of reproductive age (Norman et al. 2007). No exact prevalence in the Netherlands is known. Common clinical features of PCOS are acne, hirsutism and androgenic alopecia. The latter two both involve hair growing or losing patterns normally only seen in men. PCOS can also cause infertility, with pregnancy requiring hormonal treatment and

sometimes in vitro fertilisation. (Diamanti-Kandarakis et al. 2012). The cause of PCOS is not known, although it seems to develop based on a combination of genetic and environmental factors. A Dutch twin study has reported a 79% heritability of PCOS by analysing the heritability of several of the previously mentioned clinical features as well as the prevalence of PCOS itself in monozygotic and dizygotic twins as well as in non twin sisters. The other 21% is due to environmental factors, like the lack or availability of food and the kind of food, exposure to harmful substances, excessive weight gain and even some psychological influences may play a role (Vink et al. 2006). It is, however, not yet known which genetic mutations are responsible for the heritability of PCOS. Another well-known risk factor for PCOS is ethnicity with women of african and middle-eastern descent having the highest prevalence of the disorder (Kakoly et al. 2018). The difference in prevalence based on ethnicity is likely to be mostly genetic, but environmental differences as well as gene environment interactions based on where most of these ethnic groups live does not rule out environmental factors.

GDM is a common disorder in women in which they develop a diabetes like phenotype during pregnancy. GDM has an average prevalence of 5.4% (3.8–7.8) in Europe according to a systematic review based on 40 studies (Eades et al. 2017). No exact prevalence in the Netherlands is known, 7.3% in western Europe. The exact causes of GDM are still unclear, but there is a higher prevalence in women with PCOS among GDM patients, odds ratio 2.94 (Boomsma et al. 2006). Women with PCOS who develop GDM are likely to already have reduced impared glucose tolerance before they become pregnant, odds ratio 3.26, independent of BMI (Kakoly et al. 2018). GDM is defined as high blood sugar levels during pregnancy and is caused by a pancreatic beta cell deficiency based on insulin resistance. Risk factors for GDM are a higher age at conception, the presence of obesity or overweight, ethnicity, excessive gestational weight gain, excessive central body fat deposition (which may be hormonally driven), short stature, excessive fetal growth and a family history of GDM or diabetes. The expression of GDM changes during the pregnancy, with more serious symptoms like increased fat deposits and more hormonal disturbances during the later stages of gestation and eventually adverse pregnancy outcomes. Blood glucose levels usually go back to normal after delivery. GDM is a risk factor for adverse pregnancy outcomes, like the baby being either too small or too large for its age and getting metabolic disturbances like hypoglycemia. Women with GDM have a 50% chance of developing type 2 diabetes later in life (Barbour et al. 2007).

The main aim of this paper is to describe different predictive markers in women with PCOS for insulin resistance before and during different stages of pregnancy. A secondary question answered in this paper is how insulin sensitivity changes during the course of pregnancy in women with PCOS.

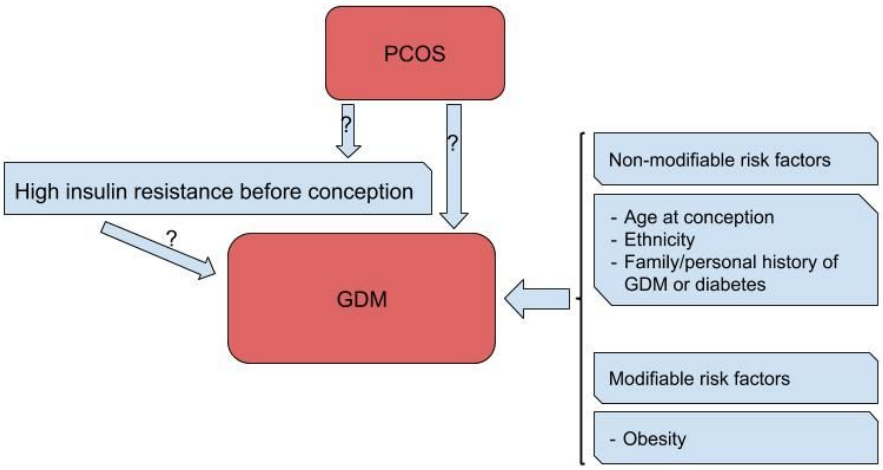

Figure 1: An overview of the known risk factors for developing gestational diabetes mellitus (GDM). On the right several risk factors independent of polycystic ovary syndrome (PCOS) are given, split into non-modifiable risk factors and modifiable risk factors. The influence of PCOS and insulin resistance on GDM will be discussed in this review paper.

Results:

Difference about insulin sensitivity in women with a high insulin resistance during pregnancy

The normal decrease in insulin sensitivity during the second and mostly during the third trimester of pregnancy happens to spare glucose, ketones, fatty acids and amino acids for the fetus. This change in the glucose metabolism is made to ensure the body meets the energy needs of the fast growing fetus. Human placental lactogen (hPL) increases by 30 fold throughout pregnancy and mediates the insulin release from the pancreas from the second trimester of pregnancy. The link between hPL is insulin resistance in pregnant women with PCOS is however not clear yet (Barbour et al. 2007). Insulin resistance in pregnancy is made possible by an almost 50% decrease in insulin mediated glucose storage in adipose and muscle tissue and a 200% increase in insulin secretion (Diamanti-Kandarakis et al. 2012). The decrease in sensitivity is characterized by and decrease in insulin receptor substrate 1 (IRS1) expression and a lowered response of phosphoinositide 3-kinase (PI3 kinase) to insulin in adipose and muscle tissue (Vejrazkova et al. 2014). This change in the insulin signaling pathway is induced by alterations in cytokines in the blood. One of the major increases in blood cytokines is a sharp increase in tumor necrosis factor-α (TNFα) produced in the placenta, which is a proinflammatory cytokine. TNFα directly intervenes in the insulin signaling by phosphorylation of IRS1, mostly in skeletal muscle and adipose tissue (Vejrazkova et al. 2014). The glucose transporter type 4 (GLUT4) is an insulin regulated glucose transporter found in skeletal muscle and adipose tissue. During a pregnancy the production of GLUT4 is downregulated in adipose tissue, but not in skeletal muscle. However, both in skeletal and adipose tissue the translocation of the GLUT4 transporter is downregulated (Barbour et al. 2007). These changes in the insulin signaling pathway mean that during a pregnancy less glucose is absorbed into muscle and adipose cells to be used and stored in the mother. Less glucose going

into those tissues means there is more glucose available for the placenta.

In women with a higher insulin resistance than normal during pregnancy the IRS1 expression is lowered by 30% in muscle tissues compared to pregnant women with a normal insulin resistance. This results in a lower GLUT4 production and translocation in muscle cells of those pregnant women with a higher insulin resistance (Barbour et al. 2007). The consequence of this is that pregnant women with a higher insulin resistance have an even greater glucose availability in the body for other tissues. If those tissues cannot absorb all of the available glucose the blood glucose level will rise.

Influence of different stages of pregnancy on insulin sensitivity in women with PCOS

A study done by Falbo et al in 2010 measuring insulin and androgen levels during pregnancy in women with PCOS shows that women with PCOS already have a significant increased fasting blood insulin level in the first trimester, when glucose levels are normally appearing to be normal (1.93 ± 0.36 μU/mL in women with PCOS versus 1.02 ± 0.52 μU/mL in women without PCOS). Suggesting that these women have a decreased insulin sensitivity in the early stages of their pregnancy. This insulin level increases significantly during the second and third trimester (2.64 ± 1.13 μU/mL versus 1.41 ± 0.24 μU/mL in the second trimester and 2.71 ± 1.55 μU/mL versus 1.85 ± 0.73 μU/mL in the third trimester). 10% of women with PCOS have impaired glucose tolerance, which is 3,26 fold higher as healthy women with a normal weight (Kakoly et al. 2018). There is only a slight change in fasting blood insulin level in women without PCOS that served as control group (Falbo et al. 2010). This means that the extra insulin increase in PCOS patients is more than the normal rise in insulin driven by pregnancy hormones The same study shows that the HOMA-IR (homeostatic model assessment insulin resistance) index in the women with PCOS is increased in the first trimester and increases significantly more during the later stages of gestation, while the change in insulin resistance during the pregnancy of women without PCOS is lower. This group also measured the fasting glucose-to-insulin ratio (GIR) during the pregnancy. The GIR is another screening test for insulin resistance. A low GIR usually corresponds with a high insulin resistance. The women with PCOS had a small increase in the GIR in the first trimester, but it was reduced significantly during the second and third trimester. The women with PCOS already had a reduced GIR at the start of the measurements compared to the healthy control group. In the women without PCOS the GIR increased significantly more during the first trimester and only kept increasing during the second and third trimester.

What makes it that a person with PCOS develops GDM while others do not

Gestational diabetes is developed when the creasing need for insulin cannot be met during pregnancy in the absence of diabetes type I or II (de Wilde et al. 2015). de Wilde reported a 31% incidence of GDM in women with PCOS in a study done in the Netherlands, which means not all women with PCOS develop GDM. The GDM cutoff for the de Wilde study was set on fasting

glucose ≥5.3 mmol/l, 1 h glucose ≥10.0 mmol/l, 2 h glucose ≥8.6 mmol/l, 3 h glucose ≥7.8 mmol/l. 31% is however significantly and substantially higher than the reported average incidence of 7.3% of GDM among all pregnant women in western Europe (Eades et al. 2017). The exact mechanism why GDM does develop is not clear, most likely it is a combination of genetic and environmental factors. Women with PCOS who develop GDM do nearly always have significant increased levels of insulin and insulin resistance before they become pregnant (de Wilde et al. 2015). These levels of insulin and insulin resistance of the women with PCOS who got GDM were even more increased during the first trimester compared to pre-pregnancy values. The early insulin resistance is not related to hPL, as hPL is secreted from the placenta, which is not functional early on in pregnancy (Barbour et al. 2007).

Predictive markers of insulin resistance in women with PCOS

In order to try to predict which women will develop insulin resistance and in doing so have a significant risk of developing GDM, certain markers for insulin resistance have been proposed by researchers through the years to predict insulin resistance. Some of the predictive risk factors for GDM are a higher age at conception of the mother, the presence of obesitas, ethnicity and a family history of GDM or diabetes. These factors give however only an increased chance of developing GDM at a population level but do not give an individual risk analysis. Several measurable biomarkers before conception and in early pregnancy have been proposed which give an individual indication if someone is at increased risk of developing insulin resistance. HOMA-IR is a method used to quantify insulin resistance. Glucose and insulin are measured in plasma while the patient has fasted for at least 12 hours. The HOMA-IR index is increased in PCOS patients who develop GDM (sensitivity of 65.5% and a specificity of 88.2%) (Köninger et al. 2019). Suggesting that PCOS patients with insulin resistance have a high chance of developing GDM.

Sex hormone binding globulin (SHBG) levels rise thoughout the course of a normal pregnancy around by around 5 to 10 times. The main function of SHBG is binding testosterone and estrogen in the bloodstream to inhibit their biological activity. SHBG synthesis in the liver is inhibited when there is an excess of insulin circulating in the body. The exact mechanism of this inhibition is unknown (Deswal et al. 2017). It has been reported that women with PCOS who develop GDM have lowered SHBG levels before pregnancy compared to women who do not develop GDM (de Wilde et al. 2015). Although the SHBG level does rise during the first and second trimester it remains lower in GDM patients. The difference in the SHBG level is the most significant when measured pre-pregnancy, but still measurable in the first and second trimester (de Wilde et al. 2015). No data is available about SHBG levels during the third trimester. The odds ratio of the association between SHBG and HOMA-IR pre-pregnancy was determined in another study to be 0.845 (95% CI 0.741–0.993) in lean women and 0.942 (95% CI 0.895–0.992) in overweight/obese women (Huang et al. 2015). The predictive value of

a lower SHBG level for GDM in women without PCOS has been tested with a 90% sensitivity and a 96% specificity (Tawfeek et al. 2017).

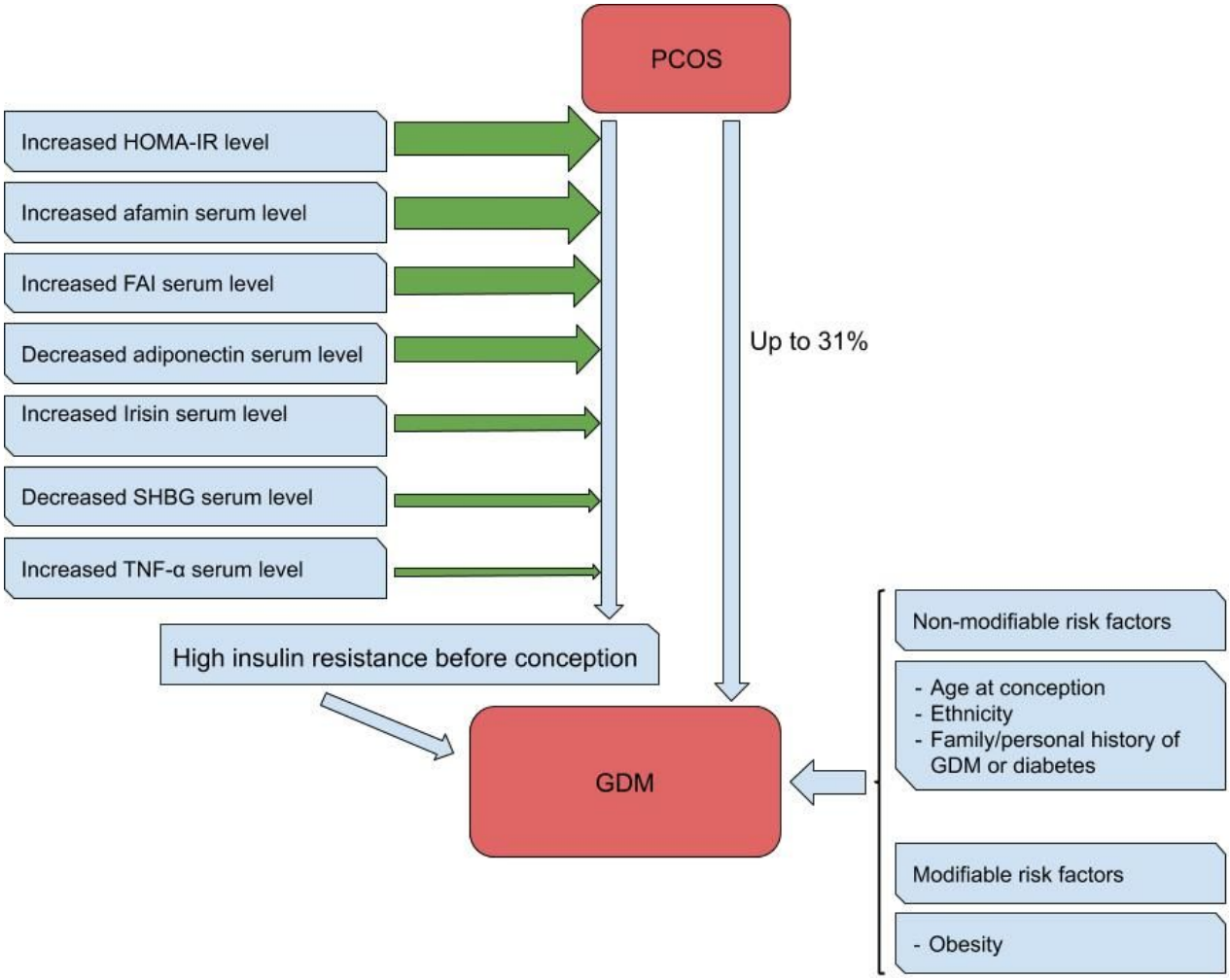

Figure 2: An overview of risk factors for developing GDM. PCOS is seen as an independent risk factor for developing GDM with reported up to 31% prevalence of GDM in PCOS patients (15% average for PCOS patients, <5% for non PCOS patients). On the right several risk factors independent of PCOS are given, split into non-modifiable risk factors and modifiable risk factors. PCOS patients with a high insulin resistance before pregnancy have a nearly certain risk of developing GDM. The thickness of the green arrows represent the certainty of the marker of a PCOS patient having a high insulin resistance and developing GDM. HOMA-IR gives the best indication of insulin resistance, followed by afamin, FAI and adiponectin serum levels which also give a good indication of the insulin resistance before conception. Of the latter 3 afamin gives the best indication of insulin resistance. Irisin is not investigated enough to give a certain prediction. SHBG serum levels give a lower certainty compared to markers like afamin. There is conflicting data of the predictive value of TNF-α and other inflammatory factors, this might depend on ethnicity.

In a study done among a Chinese Han population of women with PCOS free androgen in the bloodstream was linked to insulin resistance. The androgen is quantified by the free androgen index (FAI) which is the total testosterone level divided by the SHBG level. The FAI decreases during a normal pregnancy because of the rising SHBG blood level. However, because SHBG is lowered in PCOS patients even before pregnancy the FAI is increased in this group. The women examined in this study were not pregnant and the results are independent of the body mass index (BMI). The odds ratio of the association between FAI and HOMA-IR was 1.302 (95% CI 1.061–1.765) in lean women and 1.132 (95% CI 1.008–1.270) in overweight/obese women (Huang et al. 2015). A significant link between blood testosterone levels and insulin resistance in PCOS patients has not been found yet.

Irisin is a recently discovered protein secreted by muscle cells in response to exercise. It functions probably involve increasing energy expenditure and regulating the glucose tolerance. Not many studies have been done on irisin, but Li et al. suggest that increased irisin levels can predict insulin resistance in PCOS patients (Li et al. 2015).

Tumor necrosis factor-alpha (TNF-α) is a cytokine usually associated with inflammation. One of the functions of TNF-α in the body is inducing insulin resistance by promoting the phosphorylation of IRS1, making it a good predictor of insulin resistance independent of fat mass. During a normal pregnancy serum TNF-α is decreased in the first trimester and rises during the third trimester, corresponding to the insulin sensitivity changes of a normal pregnancy (Kirwan et al. 2002). TNF-α is produced in the placenta during pregnancy. The placenta becomes active later in the pregnancy, correlating to the rise in the third trimester (Kirwan et al. 2002). PCOS patients have been reported to have an increased TNF-α serum level independent of pregnancy and independent of BMI (Xiong et al. 2011). The same study by Xiong et al also a link between developing GDM and C-Reactive protein (CRP) and between GDM and Interleukin 6 (IL6) was made. This link between GDM development and inflammatory factors is however questioned as the link is only made in Caucasian studies (Kim et al. 2014).

Adipose tissue secretes a whole range of hormones. Several of these have been linked to insulin resistance in PCOS patients. For adipocytokines like leptin the correlation is disputed. The best correlation of these adipocytokines is between decreased serum adiponectin levels and increased insulin resistance. This connection is independent of BMI in PCOS patients (Polak et al. 2016).

Afamin is a protein that is a member of the albumin family that binds vitamin E in extravascular fluids. Vitamin E has antioxidant functions in cell membranes and Afamin seems to play an important role in oxidative stress-related anti-apoptotic cellular processes (Köninger et al. 2019). Plasma concentrations of afamin are independent of sex, age or fasting status. Afamin concentrations are however strongly correlated with increases of BMI, plasma glucose concentrations, hypertension and the metabolic syndrome. During a normal pregnancy afamin doubles in a linear trend (Köninger et al. 2019). An analysis study including more than 20000 individuals, both men and women with type II diabetes, shows that afamin is strongly correlated with

the prevalence of insulin resistance (odds ratio 1.47) and the incidence of type 2 diabetes (odds ratio 1.19) (Kollerits et al. 2017). A more recent study confirmed that afamin is a strong predictor of GDM in PCOS patients. Afamin concentrations were increased significantly before pregnancy in women who had PCOS and developed GDM independent of BMI and (99.4 mg/L; 95% CI 88.6–110.1 in GDM patients against 80.1 mg/L; 95% CI 70.3–89.9 in the controls) (a sensitivity of 79.3% and a specificity of 79.4%) (Köninger et al. 2019). The controls were women with PCOS who did not develop GDM. The predictive value of afamin for GDM in women without PCOS is shown to have potential, but is not yet shown to contribute significantly (Ravnsborg et al. 2019).

Discussion:

The aim of this study was to describe different predictive markers in women with PCOS for insulin resistance before and its relation to changes that occur during different stages of pregnancy. Studies have shown that directly testing for insulin resistance by performing a HOMA-IR test, which is the golden standard for determining insulin resistance at the moment, is still the most reliable. The HOMA-IR test can however be a burden for the patient as it requires fasting for at least 12 hours before the blood test to be completely reliable. Especially when the patient is already pregnant this can be a big constraining factor. Therefore, only markers which can be examined without the need of fasting will be discussed below.

The secondary aim of this paper is to describe how insulin sensitivity changes during the course of pregnancy in women with PCOS. PCOS is a heterogeneous disease, meaning the diagnosis of PCOS itself is not a prediction of GDM or any other problems during pregnancy. This means that some PCOS patients have a normal and healthy pregnancy. Others might have a lot of issues ranging from difficulties conceiving to altered hormonal levels and adverse pregnancy outcomes. Adverse pregnancy outcomes can be damaging for the baby, like a higher birth weight, hypoglycemia or a defect in its on metabolism, but also for the mother, who has the risk of developing GDM. Normal alterations have to be made to keep the body's metabolism in check, which need flexibility to handle outside situations like starvation or overeating or more internal changes like pregnancy. When these normal alterations fail to get the body back to normal standards a disorder can occur. When women have a high insulin resistance during pregnancy several transporters and part of the signaling chain is altered. The changes in these proteins are not universal throughout the whole body. For the GLUT4 the biggest change is in de adipose tissue, where it is not only downregulated, but also the translocation is hindered. This all is probably due to the altered available amount of IRS1, which is the receptor substrate. It is not yet discovered where in the whole signaling or transport chain of insulin to glucose the fault is that creates a lower insulin resistance. The process to an eventual insulin resistance could have a genetic contributor. Environmental factors can play a role as well, because there is a

trigger which courses a healthy individual to form symptoms. The symptoms could also form over time when the damage of a genetic disturbance slowly accumulates over time until a tipping point is reached. More research could be done on which genetic factors increase the risk of insulin resistance and on which environmental factors influence the insulin sensitivity.

For nearly all biomarkers of insulin resistance is the timing of the measurement is extremely important since serum levels change naturally during the several stages of pregnancy. Pre-pregnancy serum levels of these markers can be easier to compare to the normal levels of a woman and help prediction insulin resistance.

SHBG, irisin and TNF-α serum levels are less reliable biomarkers for insulin resistance in PCOS patients as shown in figure 2. SHBG has a weaker connection to insulin resistance then afamin and HOMA-IR (Köninger et al. 2019). Irisin is a relatively new discovered protein, because of that not enough research has been done to be sure that irisin serum levels have a high predictability of insulin resistance (Li et al. 2015). TNF-α and other inflammatory markers have a potential to be good biomarkers, but are questioned mainly on grounds of difference between ethnical groups. TNF-α is involved in the insulin signaling pathway regulation, but changes in TNF-α might be independent of insulin resistance (Kim et al. 2014). Others reported that TNF-α serum levels are increased independent of pregnancy in PCOS patients, but made no direct link with insulin resistance (Xiong et al. 2011).

An increase in afamin, FAI and adiponectin serum levels seem to be more reliable biomarkers for insulin resistance in PCOS patients as shown in figure 2. All can be measured in blood plasma without the need of the patient to fast, meaning it can be measured reliably at any time. Both adiponectin and FAI have been the subject of several studies evaluating their potential as a biomarker for insulin resistance in PCOS patients. Both have been proven to do this reliable. One study showed that afamin has an even higher predictability of insulin resistance then HOMA-IR (Köninger et al. 2019). To draw definitive conclusions from one study is a bit premature, but afamin shows great potential as a marker for insulin resistance.

Conclusion:

In conclusion, this literature review showed that an increased serum level of afamin, an increased free androgen index and a decreased adiponectin serum level have to highest reliability of predicting insulin resistance in a woman with PCOS. These serum markers can be useful as they can be measured without the need of fasting, which can be a burden and a nuisance for women who want to become pregnant or are pregnant. When insulin resistance is detected before pregnancy adequate measures can be taken to prevent serious harm to mother and child during and after pregnancy. However, not all mechanisms are known of how these serum levels are affected during pregnancy. Only of increased FAI serum levels is known that it gives a good indication of insulin resistance during pregnancy. More studies with large cohorts should be done to standardise the use of these markers, as the combination of these biomarkers has not been tested and as there is no standardardised way in how measurements should be analysed.

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