Göteborg, 2018

Polycystic Ovary Syndrome
Androgen Excess and Insulin Resistance in Women: Identification of Molecular Targets to Improve Glucose Homeostasis

AKADEMISK AVHANDLING

som för avläggande medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Arvid Carlsson, Medicinaregatan 3, Göteborg, måndagen den 29 januari 2018 kl. 13:00

av

Milana Kokosar

Fakultetsopponent: Romain Barrés, Associate Professor
Novo Nordisk Foundation center for Basis Metabolic Research, Danmark

Avhandlingen baseras på följande delarbeten:

I. **Epigenetic and transcriptional alterations in human adipose tissue of polycystic ovary syndrome**
Milana Kokosar, Anna Benrick, Alexander Perfilyev, Romina Fornes, Emma Nilsson, Manuel Maliqueo, Carl Johan Behre, Antonina Sazonova, Claes Ohlsson, Charlotte Ling, Elisabet Stener-Victorin. *Sci Rep. 2016*; 6: 22883 | DOI: PMCID: PMC4791632

II. **Transcriptional and epigenetic changes influencing skeletal muscle metabolism in women with polycystic ovary syndrome**
Emma Nilsson, Anna Benrick, Milana Kokosar, Anna Krook, Eva Lindgren, Thomas Källman, Michaela Martis, Kurt Højlund, Charlotte Ling, Elisabet Stener- Victorin. Manuscript 2017

III. **Autonomic nervous system activation mediates increase in whole-body glucose uptake by electroacupuncture**
Anna Benrick, Milana Kokosar, Min Hu, M.D, Martin Larsson, Manuel Maliqueo, Rodrigo Marcondes, Marzia Soligo, Virginia Protto, Elisabet Jerlhag, Antonina Sazonova, Carl Johan Behre, Kurt Højlund, Peter Thorén, Elisabet Stener-Victorin. *FASEB J*. 2017 Apr 12. pii: fj.201601381R. doi: 10.1096/fj.201601381R.

IV. **Single bout of electroacupuncture remodels epigenetic and transcriptional changes in adipose tissue in polycystic ovary syndrome**
Milana Kokosar, Anna Benrick, Alexander Perfilyev, Emma A Nilsson, Thomas Källman, Claes Ohlsson, Charlotte Ling, Elisabet Stener-Victorin. Accepted, *Sci Rep. 2017*

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR NEUROVETENSKAP OCH FYSIOLOGI

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Polycystic Ovary Syndrome
Androgen Excess and Insulin Resistance in Women: Identification of Molecular Targets to Improve Glucose Homeostasis

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ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in women. Women with PCOS demonstrate metabolic morbidities such as obesity, insulin resistance with compensatory hyperinsulinemia and type 2 diabetes. Physical inactivity in women with PCOS aggravates the metabolic conditions. Pharmacological treatment is efficient but associated with side effects. The pathogenesis of PCOS is largely unknown and current research suggests that genetic and epigenetic factors are implicated. Epigenetics e.g. methylation is an important mechanism that regulates gene transcription and transcriptional alterations could explain metabolic aberrations associated with PCOS.

The aim of this thesis was to profile genome-wide gene expression and methylation pattern in adipose tissue and skeletal muscle in women with PCOS and controls, and to investigate if electroacupuncture could be used to restore altered CpG sites and transcriptional alterations. Furthermore, we aimed to investigate the effect and mechanisms of a single bout of low-frequency electroacupuncture (EA) on whole-body glucose uptake.

The results of the thesis demonstrate that women with PCOS have multiple differentially methylated sites that are associated to gene expression changes in adipose tissue. In subcutaneous adipose tissue, we found 30 differentially methylated genes that are associated with mRNA expression. We have shown that transcriptional alteration in adipose tissue are associated to circulating testosterone, glucose infusion rate and adipocyte size. Furthermore, in adipose tissue electroacupuncture reversed expression of 80 genes to a healthier phenotype. In skeletal muscle, we found 85 genome-wide transcriptional differences and 21 differentially methylated genes. We also showed that mRNA expression levels of *KLF10* and *COL1A1* are under hormonal regulation of insulin and testosterone respectively and both of these genes are involved in controlling glycogen accumulation in human skeletal muscle cells.

Women with PCOS have increased sympathetic nerve activity, which is related to aberrant androgen levels and this can lead to increased insulin resistance and obesity. We have shown that EA lowers protein expression of markers of sympathetic nerve activity: proNGF, serotonin and homovanillic acid in adipose tissue. We measured whole-body glucose uptake by euglycemic-hyperinslinemic clamp and we demonstrated that EA increases glucose infusion rate in both rats and in women with PCOS and controls. In the rat experiment, we show that administration of α and β blockers during clamp blocks glucose uptake suggesting that adrenergic receptors partly mediates the effect of EA by activating the autonomic nervous system. Overall, EA-induced glucose uptake was controlled by activation of both sympathetic and parasympathetic nervous system in rats. In women with PCOS we identified increased expression of several genes involved in regulation of glucose uptake, including *NR4A2* and *JUNB,* in adipose tissue after EA. We investigated transcriptional changes of those genes in rats receiving α and β adrenergic blockers, and the involvement of the sympathetic nervous system is supported by lowered expression of *Nr4a2* and *Junb* genes, suggesting that EA mediates it effect by affecting mRNA levels of those genes.

Keywords: PCOS, Epigenetics, Hyperinsulinemia, Hyperandrogenemia, Electroacupuncture

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