UNIVERSITA' DEGLI STUDI DI VERONA

DEPARTMENT of MEDICINE

GRADUATE SCHOOL of

Life and Health Sciences

DOCTORAL PROGRAM in

Clinical and Experimental Biomedical Sciences

XXIX cycle/2014

DOCTORAL THESIS

Effects of a supervised exercise training in the management of women with gestational diabetes: preliminary results from a randomized controlled trial

S.S.D. MED13

Coordinator: Prof. Paolo Moghetti

Tutor: Prof. Paolo Moghetti

Doctoral Student: Dott.ssa Silvia Donà

Effects of a supervised exercise training in the management of women with gestational diabetes: preliminary results from a randomized controlled trial

Silvia Donà
PhD thesis
Verona, 12 June 2017
ISBN

UNIVERSITA' DEGLI STUDI DI VERONA

DIPARTIMENTO di MEDICINA

SCUOLA DI DOTTORATO in

Scienze della Vita e della Salute

DOTTORATO DI RICERCA in

Scienze Biomediche Cliniche e Sperimentali

Ciclo XXIX /2014

TESI DI DOTTORATO

Effetti di un programma di esercizio supervisionato nel trattamento delle donne con diabete gestazionale: risultati preliminari di uno studio randomizzato controllato

S.S.D. MED13

Coordinatore: Prof. Paolo Moghetti

Tutor: Prof. Paolo Moghetti

Dottoranda: Dott.ssa Silvia Donà

CONTENTS

LIST OF PUBLICATIONS

1) *Donà S*, Bacchi E, Moghetti P.
   Is cardiorespiratory fitness impaired in PCOS women? A review of the literature.
   J Endocrinol Invest. 2016 Dec 27. doi: 10.1007/s40618-016-0599-1. [Epub ahead of print] Review.

2) Bacchi E, Bonin C, Zanolin ME, Zambotti F, Livornese D, *Donà S*, Tosi F, Baldisser G, Ihnatava T, Di Sarra D, Bonora E, Moghetti P.
   Physical Activity Patterns in Normal-Weight and Overweight/Obese Pregnant Women.
   PLoS One. 2016 Nov 9;11(11):e0166254.

3) Dona' S, Bacchi E, Coratella G, Moretta R, Flamigni S, Livornese D, Tosi F, Negri C, Kaufman JM, Schena F, Moghetti P.
   PCOS and Muscle Strength: Preliminary Results in Normal Weight Women.
   Med Sci Sports Exerc. June 2, 2016 May;48(5 Suppl 1):397 (1471 Board #124).

4) Moghetti P, Bacchi E, Brangani C, *Donà S*, Negri C.
   Metabolic Effects of Exercise.
   Front Horm Res. 2016;47:44-57.

LIST OF ABBREVIATIONS

BMI	**Body Mass Index**
FPG	**Fasting Plasma Glucose**
GDM	**Gestational Diabetes Mellitus**
HbA1c	**Haemoglobin A1c**
HRR	**Heart Rate Reserve**
IPAQ	**International Physical Activity Questionnaire**
MET	**Metabolic Equivalent Unit**
OGTT	**Oral Glucose Tolerance Test**
PWV	**Pulse Wave Velocity**

ABSTRACT

English Version

Background. Gestational diabetes mellitus (GDM) is becoming increasingly common and it is associated with a number of metabolic and cardiovascular alterations, including endothelial dysfunction and increased arterial stiffness. To date, only a few, small randomized controlled trials (RCTs) have investigated the effects of aerobic exercise training on maternal glyco-metabolic control and perinatal outcomes in women with GDM. Therefore, given the relatively limited availability of data, it remains still unclear whether these vascular and metabolic alterations may be reversed by regular exercise. In this study we aimed at investigating the effects of low-intensity aerobic exercise on the glycometabolic and cardiovascular outcomes in women with GDM.

Subjects and Methods. Twenty women with GDM were recruited in the GDM-FIT Study, a RCT (Clinicaltrials.gov ID: NCT03067662) to be randomly assigned between 18^{th}-28^{th} gestational week to structured aerobic exercise intervention (n=10) or standard care (n=10). Women in the control group received diet and physical exercise recommendations according to standard care protocols. Women in the intervention group received standard diet counseling and performed low intensity aerobic exercise three times per week at 30% heart rate reserve, under continuous heart rate monitoring. Exercise duration increased 2 min/week up to 40 minutes per session. Both the intervention and control groups underwent structured medical interview and psychological assessment in two separate occasions, at study entry and between 35^{th}-37^{th} gestational week, together with clinical and anthropometric measures. In the same circumstances weekly energy expenditure (expressed as metabolic equivalent, MET) and carotid-to-femoral (CF) and radial (CR) pulse wave velocity (PWV) were also measured. Blood samples were stored for the determination of circulating pro-inflammatory metabolites and miRNA. In addition, a continuous glucose monitoring (CGM) system was applied to measure capillary blood glucose levels at 5-min timed intervals over 5 days at 32^{th}-34^{th} gestational week. Glucose readings were interpreted by separately analyzing the glucose time course during each 40-min exercise session (2 slots), the night thereafter (2 slots from 1 AM to 5 AM) and over the 24-hours period after exercise start, as well as during the corresponding periods of a

non-exercise day. Glucose variability measures and time spent in hypo- or hyperglycemia were also calculated.

Results. Age, body-mass index (BMI) and HbA1c were similar between the two groups at study entry (standard care *vs.* exercise intervention group, mean±SE; $P \geq 0.05$ for all comparisons): age (years) 34.5±1.4 *vs.* 34.3±0.9, BMI (kg/m^2) 29.8±1.9 *vs*. 30±0.8, HbA1c (mmol/mol) 35±1.42 *vs*. 33.8±0.8, as well as in terms of carotid-radial CR-PWV (m/sec; 7.49±0.66 *vs*. 9.5±1.1) and carotid-femoral CF-PWV (m/sec; 5.57±0.83 *vs*. 5.56±0.96). The metabolic parameters were similar between groups at 35th-37th gestational week. However, 4 women in the intervention group *vs.* 8 in the control group required insulin to reach the glycemic targets for pregnancy. Compared to the control group, women assigned to the exercise intervention showed a significant increase in weekly energy expenditure (135 MET at study entry *vs.* 759 MET at the end of protocol) and a reduction in delta CR-PWV (1.4±0.74 *vs.* -2.2±1.4 m/sec) - $P<0.05$ for both. As compared to the corresponding non-exercise day, the CGM readings in the exercise group showed a progressive and significant decline of glucose levels during physical activity sessions, particularly after 30 minutes of exercise ($P<0.05$) up to 180 min after completion of the exercise session ($P<0.005$).

With regard to the nocturnal and 24-h mean blood glucose levels, as well as time spent in hypo-/hyperglycemia and 24-h glucose variability, no significant differences were observed between the exercise *vs.* non-exercise days. Notably, as compared to the standard care arm, significantly lower 24-h mean blood glucose levels were observed during exercise days in the intervention group ($P<0.05$).

Conclusions. These preliminary data indicate that a structured program of low-intensity aerobic exercise may have favorable effects on blood glucose and arterial stiffness in women with gestational diabetes.

ABSTRACT

Italian Version

Background. Il diabete mellito gestazionale (GDM) sta aumentando in termini di prevalenza e incidenza e si associa a numerose alterazioni metaboliche e cardiovascolari a carico della madre e del nascituro. Ad oggi pochi studi randomizzati controllati hanno valutato gli effetti dell'esercizio aerobico sul controllo glico-metabolico materno e sugli outcome fetali in donne con diabete gestazionale. Pertanto, considerata la relativa limitatezza dei dati ad oggi disponibili, rimane ancora da chiarire se tali alterazioni possano o meno essere reversibili con l'esercizio fisico. Questo studio si è proposto di studiare se un programma di esercizio aerobico a bassa intensità possa avere effetti favorevoli sull'assetto glico-metabolico e sul profilo di rischio cardiovascolare in donne con GDM.

Soggetti e Metodi. Venti donne gravide con GDM sono state reclutate nello studio randomizzato controllato "GDM-FIT" (Clinicaltrials.gov ID: NCT03067662) tra la 18-28ma settimana gestazionale, e randomizzate ad uno dei seguenti gruppi: gruppo di intervento, che svolgeva un esercizio aerobico strutturato (n=10) o gruppo di controllo, seguito secondo la normale pratica clinica (n=10). Le donne del gruppo di intervento hanno svolto esercizio aerobico a bassa intensità tre volte a settimana al 30% della frequenza cardiaca di riserva, sotto continuo monitoraggio della frequenza cardiaca. La durata dell'esercizio aumentava di 2 min/sett fino a raggiungere 40 min per sessione. Le donne appartenenti al gruppo di controllo hanno ricevuto raccomandazioni dietetiche e di esercizio fisico standard. Al momento dell'ingresso nello studio e alla 35-37ma settimana gestazionale in tutte le donne sono stati misurati parametri clinici, metabolici, antropometrici, spesa energetica settimanale, e rigidità vascolare carotido-femorale e carotido-radiale. Sono stati inoltre raccolti campioni di sangue per la misurazione di metaboliti pro-infiammatori e miRNA. Tra la 32ma e 34ma settimana, sono stati registrati i profili glicemici (ogni 5 min e per i successivi 5 giorni) tramite applicazione di un monitor per la rilevazione in continuo della glicemia (CGM). Sono state pertanto misurate le concentrazioni di glucosio durante i 40 min di esercizio fisico, il periodo notturno successivo e le 24 ore successive all'esercizio, così come i corrispondenti periodi di non esercizio. Sono stati, infine, calcolati il tempo trascorso in ipo- o iper-glicemia e la variabilità glicemica.

Risultati. All'inizio dello studio, i due gruppi erano simili per età, indice di massa corporea (BMI) ed emoglobina glicosilata (gruppo di controllo *vs.* gruppo di intervento, media±ES; $P \geq 0.05$ per tutti le variabili): età (anni) 34.5±1.4 *vs.* 34.3±0.9, BMI (kg/m^2) 29.8±1.9 *vs*. 30±0.8, HbA1c (mmol/mol) 35±1.42 *vs*. 33.8±0.8, valori di PWV carotide-radiale (m/sec; 7.49±0.66 *vs.* 9.5±1.1) e di PWV carotide-femorale (m/sec; 5.57±0.83 *vs.* 5.56±0.96). Tra la 35-37ma settimana di gestazione i parametri metabolici erano ancora simili tra i due gruppi. Tuttavia, per 4 donne del gruppo di intervento *vs.* 8 del gruppo di controllo è stata necessario il trattamento insulinico per raggiungere i target glicemici raccomandati. Confrontate con il gruppo di controllo, le donne assegnate al gruppo di esercizio hanno mostrato un significativo incremento nella spesa energetica settimanale (135 MET all'inizio dello studio *vs.* 759 MET a fine protocollo) ed una riduzione nel delta PWV carotide-radiale (1.4±0.74 *vs.* -2.2±1.4 m/sec) - $P<0.05$ in entrambi i confronti. Nel confronto con le rilevazioni ottenute in un giorno di non-esercizio, i livelli di glucosio durante la sessione di esercizio nel gruppo di intervento hanno mostrato un progressivo e significativo declino, in particolare dopo 30 min ($P<0.05$) e sino a 180 min ($P<0.005$) dall'inizio dell'esercizio.

Per quanto riguarda la glicemia media nel periodo notturno e nelle 24h, non ci sono state differenze significative tra il giorno di esercizio e il giorno di non-esercizio, come per il tempo speso in ipo- o iper-glicemia e nella variabilità glicemica. Tuttavia, confrontando la media glicemica nelle 24h con il gruppo di controllo, i livelli medi di glicemia sono significativamente più bassi nei giorni di esercizio del gruppo di intervento ($P<0.05$).

Conclusioni. Questi dati preliminari indicano che un programma di esercizio aerobico a bassa intensità può avere effetti favorevoli sulla glicemia e sulla rigidità vascolare in donne con diabete gestazionale.

CHAPTER 1

Introduction

Preface

Pregnancy represents a relatively limited period during the individual lifetime of women. However, it represents a pivotal step not solely for the anatomical development of a healthy newborn, but also for the definition of its individual metabolic set-point. The impact of pregnancy on the metabolism of the pregnant woman is also relevant for its future metabolic trajectory, as represented, for instance, by glucose regulation.

Dramatic anatomic and physiologic adaptations are typical of normal pregnancy, as they are necessary to grow and develop a healthy fetus and placenta. The relevance of the individual metabolic adaptations for the regulation of glucose homeostasis is of particular interest nowadays, as pre-diabetes and overt diabetes are becoming more prevalent in the general population.

This work explored the potential effects of structured exercise in women with gestational diabetes, i.e. a particular form of altered glucose regulation which is widely recognized as a high-risk condition for adverse fetal and perinatal outcomes and for the development of overt diabetes (mostly type 2) and other metabolic syndrome traits in both the mother and the newborn.

1.1. Physiologic and metabolic effects of normal pregnancy

During early pregnancy, cardiac output increases about 30-50% as a result of both increased heart rate and stroke volume and remains elevated until term (Hytten and Chamberlain, 1991). As pregnancy progresses, blood flow increases to the uterus, kidney, skin, and also probably to the gastro-intestinal tract. The attainment of adequate perfusion in these organs passes through increased peripheral vasodilatation during mid-pregnancy. As a result, arterial blood pressure decreases at the increase of cardiac output. Venous blood pressure rises in the lower limbs due to mechanical and hydrostatic pressure in the pelvis, thus leading to subcutaneous edema. Importantly, plasma volume expansion is correlated with both maternal clinical performance and newborn birth weight.

Indeed, the remarkable physiological changes in peripheral circulation during pregnancy (Gabbe et al., 1991; Hytten and Chamberlain, 1991) lead to a progressive plasma volume increase of about 50 percent by 30-34 weeks of gestation. These adaptations may be responsible for the occurrence of maternal dyspnea as a symptom of reduced tolerance to physical exercise. On the contrary, suboptimal plasma volume expansion is associated with fetal distress and poor perinatal outcomes.

The increases in maternal plasma volume account for a significant proportion of the increase in total body water during pregnancy. Red blood cell mass also increases about 18% by term without iron supplementation and 30 percent with iron supplementation. Pulmonary ventilation increases 30-40% by late pregnancy due to increased tidal volume. Oxygen consumption increases only 15-20%, resulting in an increased alveolar and arterial PaO_2 (partial pressure of oxygen) and a fall in $PaCO_2$ (partial pressure of carbon dioxide) levels (Gabbe et al., 1991).
The adaptations in maternal renal physiology during gestation are among the primary mechanisms accounting for the increase in plasma volume and hence total body water during gestation. In detail, renal plasma flow increases 70% over pregravidic levels by 16 weeks of gestation until late pregnancy (Gabbe et al.,

1991). Accordingly, the glomerular filtration rate (GFR) is characterized by a progressive increase (up to 50 percent by term) inversely paralleling the circulating levels of urea and creatinine. Plasma osmolarity declines due to a reduction in serum sodium and associated anions. There is a large increase in tubular sodium reabsorption during pregnancy, promoted by increased aldosterone, estrogen, and deoxycorticosterone levels. Plasma renin activity, renin substrate, and angiotensin levels increase five- to tenfold above the pregravidic values. The renin-angiotensin system changes dramatically during pregnancy. The adrenal gland remains responsive to the trophic action of angiotensin II, although the earliest gestational stages are characterized by a refractory effect of pressors to angiotensin II, thus providing a probable explanation for the expansion of plasma volume during pregnancy.

Other metabolic changes in the hormonal *milieu* occur during pregnancy, thus overall decreasing the whole-body insulin sensitivity. As a result, a temporary worsening of cardiovascular risk profile occurs, including increased oxidative stress and higher circulating levels of pro-inflammatory cytokines such as IL-6.

For instance, the estrogen-dependent plasma concentration of corticosteroid-binding globulin (CBG) increases significantly (Gabbe et al., 1991), thus resulting in a (up to) three-fold increase in plasma cortisol concentration by the end of the third trimester. The metabolically active free cortisol increases accordingly, due to increased synthesis and decreased clearance. In turn, adrenocorticotropic hormone levels are suppressed by circulating estrogen and progesterone. The plasma concentration of dehydroepiandrosterone sulfate falls due to increased metabolic clearance rate by the placenta and maternal liver.

With regard to the adipose tissue, several adipokines are known to exert profound effects on metabolism and fertility, but their role in reproductive performance is yet to be fully understood. In addition to adipose tissue as a main source of adipokines and mediators of inflammation, the placenta plays a major role in the determining the concentration of circulating leptin, TNF-α, and resistin (Mitchell et al., 2005).

Serum adiponectin is lower in the third trimester and this correlates with decreased insulin sensitivity (Catalano et al., 2006). Increased maternal fat mass is most likely related to decreased concentrations of circulating adiponectin.

Many of the metabolic adjustments of pregnancy are already established during early pregnancy, as fetal nutrient demands are still minor. Changes in carbohydrate and lipid metabolism occur to ensure a continuous supply of nutrients to the growing fetus (Butte, 2000). Minimal nutrient balances are usually positive, reflecting the anabolic state of the fetus and the mother. In the absence of nausea or "morning sickness," most women experience an increase in appetite in the beginning of pregnancy (Gabbe et al., 1991). Several gastrointestinal changes occur, including decreased tone and motility of the stomach, reduced gastric acid secretion, delayed gastric emptying, and increased gastric mucous secretion as a function of increased progesterone. Motility of small intestine is also reduced during gestation; however, except for enhanced iron absorption, nutrient absorption is unchanged. These physiologic changes may affect the pattern of gestational weight gain in early gestation.

In fact, pregnancy is also characterized by reduced peripheral insulin sensitivity and an increase in diabetogenic hormones, such as human placental lactogen, cortisol, estrogen, progesterone, and prolactin. The secretion of prolactin from the pituitary gland and uterine decidua increases steadily during pregnancy. In contrast, luteinizing hormone and follicle-stimulating hormone are suppressed to levels similar to the luteal phase of ovulation. Growth hormone secretion is inhibited presumably by placental growth hormone production. Notably, the early release of the gestational hormones starts already in the first trimester, in order to support the metabolic demands of the fetus and mother (Weissberger, 2006).

The cascade of subsequent hormonal events promotes an increase in maternal blood glucose, accompanied by a decreased maternal liver glycogen storage with an elevation in hepatic glucose release (Mottola, 1991) and increased stimulation of the beta cells of the maternal pancreas to increase insulin production (Catalano,

1991). By mid to late pregnancy, maternal fat deposition increases further, and the hyperinsulinemia found at this stage leads to the emergence of insulin resistance at the level of skeletal muscle (Buchanan, 1990), with a reduction by 45 to 70% in total body insulin sensitivity (Freemark, 2006). Insulin resistance may also be associated with leptin resistance and fat mass accumulation (Newbern, 2011). Ultimately, these events lead to a decrease in the utilization of maternal glucose by the peripheral tissues to ensure more maternal glucose left for fetal usage (Lesser, 1994).

These adaptations in the mother are part of normal metabolic physiology and are relevant to the feto-placental unit as it uses 30 to 50% of maternal glucose pool in late gestation for growth and development (Clapp, 2002). The return of normal physiologic function, including a dramatic improvement in insulin sensitivity, may occur rapidly over a matter of days after delivery (Ryan et al., 1985).

The most distinct change during pregnancy is gestational weight gain (GWG) directly related to the alterations in maternal physiology. In 2009, the Institute of Medicine (IOM) released specific recommendations for pre-pregnancy BMI and for appropriate GWG to assist pregnant women in attaining healthier pregnancy through healthy diet and physical activity. The new guidelines for GWG are formulated as a range of each category of prepregnancy BMI, based on the World Health Organization (WHO) and including specific, relatively narrow range of recommended gain for obese women (IOM, 2009).

1.2 Gestational diabetes mellitus

Most pregnant women are able to regulate blood glucose concentrations due to adequate β-cell compensation with increased insulin release (Abell, 2015).

As normal pregnancy-induced insulin resistance is accompanied by metabolic dysregulation carbohydrate intolerance of varying severity ensues, thus unmasking the onset of β-cell disfunction and ultimately leading to overt GDM (Catalano, 1993)

For many years GDM has been defined as any degree of glucose intolerance recognized during pregnancy (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997), regardless of whether the condition may have been antecedent to or persisted after the pregnancy. This definition facilitated a uniform strategy for detection and classification of GDM, but it was limited by inaccuracy.

The ongoing epidemic of obesity and diabetes has led to an increased prevalence of type 2 diabetes (T2DM) in women of childbearing age, with a remarkable increase of pregnant women with undiagnosed T2DM (Lawrence, 2008). In fact, obesity and insulin-resistance are important contributors to both GDM and T2DM.

Given the rising prevalence of pregnant women with undiagnosed T2DM, it is reasonable to test women for T2DM risk factors at the initial prenatal visit, using standardized diagnostic criteria. Women being diagnosed during the first trimester as affected by overt diabetes should be classified as having pre-existing pre-gestational diabetes (mostly T2DM or, more rarely, type 1 diabetes).

Diabetes is classified as GDM in case the first diagnosis of overt diabetes occurs during the second or third trimester of pregnancy, after exclusion of pre-existing overt diabetes before gestation (American Diabetes Association - Classification and Diagnosis of Diabetes, 2017).

Gestational diabetes affects an estimated 2-10% of pregnancies in the United States, with rates varying by population subgroup and diagnostic test utilized. Prevalence of GDM varies by race-ethnicity, ranging from a low of 6.8% among non-Hispanic whites to 16.3% among Asian and Pacific Islanders (DeSisto 2014).

Gestational diabetes mellitus is associated with increased risk of fetal macrosomia, large-for-gestational-age neonates, preeclampsia, perinatal mortality, and cesarean delivery as well as long-term hazard, including a higher risk of maternal T2DM and obesity, glucose intolerance, and metabolic syndrome in the offspring of women with GDM (Lindsey 2015).

Furthermore, although pregnancy is associated with positive cardiovascular adaptations, such as increased stroke volume, heart rate, cardiac output and decreased total peripheral resistance, the development of GDM has been found to be associated with decreased endothelial function and increased arterial stiffnes (Paradisi, 2002; Savvidou, 2010).

As in T2DM, GDM is associated with both insulin resistance and impaired insulin secretion. The two disorders share the same risk factors and have the same genetic susceptibility. Therefore, they are assumed to be etiologically indistinct, with GDM preceding T2DM. In accordance to this, women with GDM have a 17-63% increased risk of developing T2DM within 5-to-16 years following pregnancy (Ben-Haroush, Diabet Med, 2004).

Pregnancy complicated by GDM is at increased risk of hypertensive disorders (Joffe, 2000), which in turn are associated with increased maternal stiffnes (Kaihura, 2009).

Savvidou et al (2010) showed that both GDM and T2DM are characterized by higher maternal augmentation index compared to control, suggesting increased stiffness of the small muscular arteries/arteriols at the major sites of pressure wave reflection. Furthermore, they observed a significant trend of elevated PWV with progressively increasing arterial stiffness from controls to GDM to T2DM. The increase in arterial stiffness was 10% in GDM and 17% in T2DM compared to non-diabetic pregnant women.

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (Metzger, 2008), a large-scale multinational cohort study including over 23,000 pregnant women, demonstrated that risk of adverse maternal, fetal, and neonatal outcomes continuously increased as a function of maternal glycemia at 24–28 gestational weeks, even within ranges previously considered normal for pregnancy. For most complications, there was no threshold for risk. These results have led to careful reconsideration of the diagnostic criteria for GDM.

According to the most recent ADA guidelines, GDM diagnosis (Table 1) can be accomplished with either of two strategies:

1) "One-step" 75-g OGTT

   or

2) "Two-step" approach with a 50-g (nonfasting) screen followed by a 100-g OGTT for those who screen positive

Different, country-specific, diagnostic criteria identify different degrees of maternal hyperglycemia and maternal/fetal risk, leading some experts to debate, and disagree on, optimal strategies for the diagnosis of GDM.

Nevertheless, even with strict glycaemic control, women with GDM still have excess risks of adverse pregnancy outcomes. Although the pathophysiology is not completely clear, there are a range of potentially contributing factors, including chronic low-grade inflammation. Growing evidence suggests that inflammation is a central features of insulin-resistance, that is also linked to obesity. Plasma levels of several markers of inflammation, C-reactive protein, interleukin-6 (IL-6), TNFα and leptin are elevated in individuals with obesity and T2DM (Ramsey, 2002) Pregnancy, and furtermore GDM, are characterized by an increased inflammatory markers. Research into inflammation and biomarkers is advocated to provide important clues and further insights into pathophysiology and risk prediction for pregnancy outcomes in GDM.

1.3 Exercise during pregnancy

Regular physical activity in all phases of life, including pregnancy, provides health health/fitness benefits during pregnancy to the mother and child. Regular physical activity consisting of planned, structured, and repetitive body movement improves and/or maintains one or more components of physical fitness. Exercise may also reduce the risk of developing conditions associated with pregnancy such as pregnancy-induced hypertension and GDM (ACSM, 2014). Women beginning pregnancy with a healthy lifestyle (e.g. regular exercise, balanced nutrition, non-smoking) should be encouraged to maintain those healthy habits. Women who do not have healthy lifestyle should be encouraged to view the pre-conception period and pregnancy as opportunities to embrace healthier routines (ACOG 2015).

The 2015 American College of Obstetricians and Gynecologists (ACOG) and the 2008 Physical Activity Guidelines for Americans (PAG) recommended that healthy pregnant women achieve ≥30 min/day moderate-intensity exercise, most days of the week. Although there are insufficient data on the effects of vigorous-intensity exercise, ACOG and PAG advise that women who habitually participate in vigorous-intensity exercise can continue to do so during pregnancy, as long they remain healthy and receive medical care.

The American College of Sport Medicine (ACSM) recommends 150 min/wk of aerobic individual exercise as a general rule for the general population. Research suggests ≥15 min/day for 3-4 days/week as ideal frequency of exercise, gradually increasing to a maximum of 30 min/day with an aggregate 120 min/wk of moderate intensity exercise. A 10-15 min warm-up and a 10-15 min cool-down of light intensity, physical activity is suggested before and after the exercise session, respectively, resulting in approximately 150 min/wk of accumulated exercise.
Pregnant women should perform dynamic, rhythmic physical activity that use large muscle groups such as walking and cycling. Since maximal exercise testing is rarely performed by pregnant women, moderate intensity exercise is recommended for women with a pre-pregnancy BMI <25 kg/m^2. Light intensity exercise is recommended for women with pre-pregnancy BMI ≥25 kg/m^2, starting at 25

min/day, adding 2 min/wk, until 40 min 3-4 day/wk is achieved. Age-adjusted and pre-pregnancy fitness-adjusted nomograms for heart rate ranges have been developed and validated for low-risk pregnant women corresponding to light and moderate intensity exercise.

In particular, American College of Obstetricians and Gynecologists guidelines suggested the following recommendations (ACOG 2015):

- Physical activity in pregnancy has minimal risks and has been shown to benefit most women, although some modification to exercise routine may be necessary because of normal anatomic and physiologic changes and fetal requirements.
- A thorough clinical evaluation should be conducted before recommending an exercise program to ensure that patient does not have a medical reason to avoid exercise.
- Women with uncomplicated pregnancies should be encouraged to engage in aerobic and strength-conditioning exercises before, during, and after pregnancy.
- Obstetricians-gynecologists and other obstetric care providers should carefully evaluate women with medical or obstetric complications before making recommendations on physical activity participation during pregnancy. Although frequently prescribed, bed rest is only rarely indicated and, in most cases, allowing ambulation should be considered.
- Regular physical activity during pregnancy improves or maintains physical fitness, helps with weight management, reduces the risk of gestational diabetes on obese women, and enhances psychological well-being.

It is important to be aware of contraindications for exercising during pregnancy.

Absolute contraindications to aerobic exercise during pregnancy are: hemodynamically significant heart disease, restrictive lung disease, incompetent cervix or cerclage, multiple gestation at risk of premature labor, persistent second- or third- trimester bleeding, placenta previa after 26 weeks of gestation, premature

labor during the current pregnancy, ruptured membranes, preeclampsia or pregnancy-induced hypertension, severe anemia.

Relative contraindications to aerobic exercise during pregnancy are: anemia, unevaluated maternal cardiac arrhythmia, chronic bronchitis, poorly controlled type 1 diabetes, extreme morbid obesity, extreme underweight, history of extremely sedentary lifestyle, intrauterine growth restriction in current pregnancy, poorly controlled hypertension, orthopedic limitations, poorly controlled seizure disorder, poorly controlled hyperthyroidism, heavy smoker.

ACSM gives other special considerations:

- Women who are pregnant and sedentary or have medical condition should gradually increase physical activity levels to meet the recommendation levels earlier as per pre-participation completion of the PARmed-X for pregnancy (PARmed-X for Pregnancy, 2002).
- Women who are pregnant should avoid contact sports and sports/activities that may cause loss of balance or trauma to the mother and fetus (e.g. soccer, basketball, roller blading, ice hockey, riding, skiing/snowboarding, scuba diving and vigorous intensity, racquet sports.
- Exercise should be terminated immediately with medical follow-up if any of these signs or symptoms occur: vaginal bleeding, dyspnea before exertion, dizziness, headache, chest pain, muscle weakness, calf pain or swelling, preterm labor, decrease fetal movement (once detected), and amniotic fluid leakage.
- Pregnant women should avoid exercising in a hot humid environment, be well hydrated, and dressed appropriately to avoid heat stress.
- During pregnancy, the metabolic demand increases by ~ 300 kcal/day. Women should increase caloric intake to meet the caloric costs of pregnancy and exercise.
- Women who are pregnant may participate in a strength training program that incorporates all major muscle groups with a resistance that permits multiple submaximal repetitions (i.e., 12-15 repetitions) to be performed to a point of moderate fatigue. Isometric muscle actions and the Valsalva

maneuver should be avoided as should the supine position after 16 wk of gestation. Kegel exercises and those that strengthen the pelvic floor are recommended to decrease the risk of incontinence.

- Generally, gradual exercise in the postpartum period may begin ~4-6 wk after normal vaginal delivery or about 8-10 wk (with medical clearance) after a cesarean section delivery. Deconditioning typically occurs during the initial postpartum period so women should gradually increase physical activity levels until pre-pregnancy physical fitness levels are achieved. Light-to-moderate intensity exercise does not interfere with breastfeeding.

Additional research is needed to study the effects of exercise on pregnancy-specific outcomes, and to clarify the most effective behavioral counseling methods and the optimal intensity and frequency of exercise. Similar work is needed to create an improved evidence base concerning the effects of occupational physical activity on maternal-fetal health (ACOG, 2015)

1.4 Exercise in pregnancy complicated with GDM

Physical activity and exercise during pregnancy have been shown to benefit most women by improving cardiovascular health and general fitness while reducing the risk of complications like preeclampsia and cesarean delivery (ACOG, 2015). Sufficient evidence supports the promotion of moderate-to-vigorous prenatal physical activity for maternal health benefits (Downs, 2012), and physical activity prior to and during pregnancy may lower the risk of developing GDM (Dempsey, 2004; Dempsey, 2004; Ruchat 2012, Tobias 2011, White 2013).

Not all studies have shown that physical activity can prevent the onset of GDM. In fact, a 2012 Cochrane review (Han, 2012) concluded that there was not enough evidence to support that physical activity decreases the risk of GDM or improves insulin sensitivity during pregnancy. As the authors of the review recognized, the lack of evidence could be attributed to weakness in the design and or sample size of the published studies.

On the contrary, Sanabria-Martinez (2015) et al. in a recent meta-analyses of 13 randomized controlled trials (RCT) provided evidence that physical exercise during pregnancy is associated with 31% reduction in risk of gestational diabetes, and also revealed that, as exercise program is conducted throughout pregnancy, the reduction in risk of gestational diabetes is even greater (36%). Similarly, Russo et al. (2015) in another meta-analysis suggested a 28% lower risk of GDM among those assigned to a physical intervention compared to those in a control group.

Notably, the meta-analyses by Sanabria-Martinez et al. (2015) and Russo et al. (2015) both provide support for the recommendation to advise mothers to engage in physical activity programs as an effective and safe strategy to have lower risk of GDM.

Effects of exercise on fasting blood glucose

However, few studies to date have evaluated the effects of exercise on fasting blood glucose in women with GDM and very few RCTs. For instance, a recent cross-sectional study by Leng et al (2016), recruited 11,450 pregnant women in China within the 12th gestational week. These women underwent a 50-g 1-h glucose challenge test (GCT) at 24-28 weeks of gestation and a 75-g 2-h OGTT if GCT glucose was ≥7.3%. Physical activity and sedentary behaviors data were collected at GCT time by a self-reported validated questionnaire. The study found that physical activity during pregnancy was associated with reduced risk of GDM, especially among women who were overweight or obese before pregnancy, and sedentary behaviors during pregnancy were also associated with the increased risk of GDM, both in normal weight and overweight/obese Chinese women.

Similarly, in a pilot-study by Davenport et al (2008), significantly lower fasting and post-prandial blood glucose were observed in 10 women assigned to the intervention group, who practiced 3 times/week 40 minutes of aerobic training at 30% HRR, as compared to 20 sedentary controls. This study also documented significantly lower insulin requirements in the intervention group compared to the control group.

Evidence from RCTs

Two recent meta-analyses by Harrison 2016 and Bgeginski 2016 have collected data of the few RCTs available on this topic. Both meta-analyses included the same 8 studies: five of them were RCTs of weekly supervised exercise (Avery, Bung, de Barros, Halse, Jovanovic-Peterson) and three were RCTs of physical activity counselling (Bo, Brankston, Youngwanichsetha). Four RCTs evaluated glycemic outcome with aerobic exercise programs and only one used resistance exercise.

Jovanovic-Peterson et al (1989) compared 19 women with gestational diabetes who followed an aerobic exercise program with women that followed the only dietary intervention. The training program included an arm-ergometer aerobic training 3 times/week. Duration of each session was 20 minutes at 70% predicted HRmax. Authors reported a reduction in HbA1c%, fasting and 1-h plasma glucose

concentration and significantly higher in the intervention group compared with the control group.

Halse et al (2014) randomized 40 women with GDM to exercise or control group. They found improvements in capillary glucose profiles after a home-based exercise program. Women in exercise group completed three supervised home-based exercise sessions each week plus two non-supervised ones. Duration of each session was 25-40 minutes at 55-85% of predicted HRmax (RPE 9-16).

Differently, Avery et al (1997) have not detected an improvement in glycemic control in women with gestational diabetes participating in an exercise program including 4 sessions/week (2 sessions were under supervision of an instructor) lasting 30 minutes at 70% HRmax.

De Barros et al (2010) evaluated the effects of a resistance exercise program on insulin requirements and glycemic control. Thirty-two women were randomized to the exercise group and thirty-two to a control group. Women in exercise group performed 3 times/week (two supervised sessions and one unsurpervised) a circuit resistance training with an elastic band for 30-40 minutes (RPE 5-6). Women in exercise group showed a significant decrease in the number of patients requiring insulin and a better glycemic control (in the percentage of weeks spent within the target glucose range).

Exercise was applied in addition to the normal clinical practice in all studies, with the trial of Bung (1991) as only exception. Bung and colleagues compared the metabolic effect of physical exercise performed by a group of women on insulin therapy against a control group not on insulin treatment. Eight weeks of moderate, supervised exercise at 50% VO2max, 3 times/wk by recumbent bicycle maintained blood glucose levels within normal limits without the need for exogenous insulin.

Among RCTs that evaluated the effect of a counseling physical activity program, Bo et al (2014) randomized 200 women to four different group: diet alone (D), diet plus behavioral recommendation (B), diet plus exercise (E), diet plus behavioral recommendation plus exercise (BE). Women in exercise group were instructed to perform briskly walk at least 20 minute/day every day (RPE 12-14). Exercise was

associated with a reduction of glucose, triglycerides and CPR (c-reactive protein) concentrations.

Brankston et al (2004) randomized 32 women to two groups: diet alone or diet plus resistance training. Women in exercise group performed three supervised sessions, then were instructed to perform, individually, a circuit training 3 times per week (at "somewhat hard" level). The amount of insulin prescribed was lower in the exercise group compared to the only diet group and showed a longer delay from diagnosis to the initiation of insulin therapy.

Youngwanichseta et al (2014) showed a significantly reduced fasting plasma glucose, 2-h post prandial glucose and HbA1c, in the exercise group which performed individually yoga breathing, postures and movements, 15-20 minutes five times per week.

In summary, the meta-analysis by Harrison et al. (2016) showed that aerobic or resistance exercise performed at moderate intensity, at least 3 times per week, helps to control post-prandial glucose levels and other glycemic outcomes, without maternal or fetal adverse effects. The meta-analysis by Bgeginski et al. (2016) reported also a significant effects of physical activity counselling compared to usual standard care group on fasting blood glucose.

Notably, none of these studies have evaluated blood glucose trend in the hours following the training, particularly during nocturnal period, in which increased the risk of hypoglycemia unnoticed.

CHAPTER 2

Aerobic exercise in women with gestational diabetes: a randomized controlled trial

2.1 Aim

To evaluate the efficacy of a supervised physical exercise program, as compared with standard care, in women with gestational diabetes.

Maternal glyco-metabolic control and overall maternal and perinatal outcomes were the specific study endpoints.

2.2 Materials and Methods

Subjects

Twenty women with GDM were recruited from the Outpatients Clinic of the Division of Endocrinology, Diabetes and Metabolism of Verona Hospital, Italy.

Inclusion criteria were singleton pregnancy, gestational diabetes diagnosis between 18^{th} and 28^{th} week of gestation, age 18-40 yrs, pre-pregnancy BMI $\geq$20 kg/m^2, Caucasian ethnicity.

Exclusion criteria were heart disease, restrictive lung disease, incompetent cervix/cerclage, persistent second or third trimester bleeding, placenta previa, threatened preterm labor, ruptured membranes, preeclampsia, hypertension, severe anemia, cardiac arrhythmias, history of epilepsy, chronic bronchitis, orthopedic limitations, type 1 diabetes mellitus, drugs that interfere with metabolic control (such as cortisone).

The study was approved by the Ethical Committee of the Verona hospital and written informed consent was obtained from all individuals before entering the protocol.

Protocol

Before inclusion in the study all subjects were screened for the exclusion criteria and the following information were collected: date of birth, parity, obstetric history, family history of diabetes, family history of cardiovascular disease, alcohol consumption (glasses/day), pre-pregnancy weight (kg) and use of medications. Oral Glucose Tolerance Test (OGTT) results were recorded.

After inclusion in the study and at 35-37th week of gestation, the following were assessed:

Physical and anthropometric measures

- Ambulatory blood pressure (mmHg) and resting heart rate (HR)
- Weight, height, BMI (kg)/m^2). Weight was taken at the nearest hg with an electronic scale (Tanita BWB-800, MA, USA); height was measured at the nearest mm with a Harpender stadiometer (Holtain Ltd., Crymych Pembs, UK)

Glycaemic control

- Fasting glucose (mg/dL), HbA1c (mmol/mol)

Circulating biomarkers and mediators of inflammation

- C-reactive protein (CRP, mg/dL), and adiponectin levels (μg/mL)
- Interleukin-1β (IL-1β), interleukin-6 (IL-6) and TNF-α levels, (pg/mL)

Ongoing therapies

- Insulin therapy, if any (insulin units and week of start)
- Other drugs/vitamins/supplements

Physical activity

- Total physical activity carried out was assessed by the International Physical Activity Questionnaire (IPAQ) (Hallal, 2004), and expressed as MET min*week

Arterial stiffness

- Pulse wave velocity (PWV, m/s). PWV is the gold standard non-invasive measure of arterial stiffness, at the carotid-radial and carotid-femoral level, by applanation tonometry (Laurent, 2006). Pulse wave acceleration is a preclinical predictor of atherosclerosis related to many disorders, including glucose intolerance (Henry, 2003), and a recognized cardiovascular risk marker (Crickshank, 2002). All vascular measurements were conducted in the morning, in a quiet room. Subjects refrained from drinking caffeine-containing beverage and fasting for at least 8 hours. On arrival at laboratory, subjects were to lie on a bed in left lateral position to avoid vena cava compression by the uterus. After at least 10 minutes of quiet rest, blood pressure (BP) was measured using an automated digital sphygmomanometer. Systolic and diastolic blood pressure were measured twice and averaged. For each woman the distance in millimeters between each artery location (carotid, femoral, and radial) and the suprasternal notch was measured.

Quality of life and depression

- The SF-36 questionnaire for the assessment quality of life (QoL) and the CES-D scale (Center of Epidemiological Studies Depression Scale) for the evaluation of depression state were employed. Depressive symptoms were assessed by the validated Italian version of the CES-D Scale and reported as CES-D score. The CES-D Scale is a self-reported measure of depression that is widely used in various settings and patient populations. It is composed of 20 items addressing symptoms of depression during the previous 4 weeks. Symptom frequency is rated from “none of the time” to “most or all of the time” on a four-point Likert scale. Values of the CES-D Scale range from 0 to 60; values ≥16 indicate the presence of depressive symptoms (Radloff, 1997).

Health related quality of life. Health related quality of life were assessed by the validated version of the Short-Form Health Survey (SF-36). SF-36 is one of the most widely used measures of health-related QoL and consists of 36 items covering eight dimensions: physical functioning (PF), role limitations caused by physical health problems (RF), bodily pain (BP), general health perception

(GH), vitality (VT), social functioning (SF), role limitations caused by emotional health problems (RE), and mental health (MH). Scores on all the subscales are transformed linearly to a possible range of 0–100; higher scores indicate more favorable physical functioning/psychological well-being (Ware, 1992; Apolone, 1998). Score range from 0 to 60; values >=16 indicate the presence of depressive symptoms.

- RNA isolation, cDNA synthesis and qPCR from human plasma

Profiling of miRNA

- A total of 6 women with GDM were selected for miRNA profiling. For the selected patients the time of blood collections were at 19-29 weeks of pregnancy before the intervention and at 35-37 weeks of pregnancy after the physical training. Plasma was separated from peripheral blood samples by centrifugation at 3500 g for approximately 10 minutes and stored immediately at -80°C. The miRCURY RNA isolation kit (Exiqon, Vedbaek, Denmark) was used to extract miRNA from a constant volume of 250 µl of plasma samples with the addition, in the lysis reagent, of RNA carrier (1 µg/sample) and Spike-in UniSp-2,-4,-5 in order to evaluate the RNA isolation efficiency. The dissolved RNA was collected and stored at -80°C. The cDNA synthesis reaction was performed with the miRCURY LNA Universal RT microRNA PCR (Exiqon) starting from the same volume of purified microRNA with the addition of the quality control Spike-in Unisp6 and cel-miR-39-3p in a bulk reaction of 80 µl final volume. The cDNA was stocked at -20°C until use. To identify which miRNA were expressed in the circulation a global miRNA screening was performed using Exiqon miRNoma panel V.4 in 384 PCR well plates that includes primers for 752 miRNA and the primers targeting all the Spike-in controls in order to qualitatively validate the procedure at all the steps, ie RNA extraction, cDNA synthesis and real-time PCR efficiencies. cDNA samples were mixed with SYBR green, ROX, molecular grade water and the qPCR performed using the AB 7900HT, programmed to perform also a melting curve

analysis to check for primer specificity. All the procedures were conducted following the manufacturer's instructions.

At 32-34th week of gestation only:

- A continuous glucose monitoring system (CGM) (Dexcom G4 PLATINUM) was applied, and blood glucose was recorded every 5 min over the following five days. CGM was applied in the morning, by using the proper inserter, into the subcutaneous tissue of the arm, according to manufacturer's instructions. CGM is a thin, sterile, flexible, polyurethane device capable of measuring the concentration of glucose in the interstitial fluid and transmitting data, by radio-frequency, to the reader. The read-monitor, which is small and easy to wear, records blood glucose concentrations every 5 min over the following five days. The registration period included both a training day and a non-exercise day. During the five days, subjects were asked to maintain the same diet and to fill in a food recall. During the continuous blood glucose registration period, patients were required to conduct their usual life, do not exercise during the non-exercise day and to measured blood sugar levels by a finger sticks every 12 hours: before breakfast and before dinner, normally. Glucose concentrations during the 40-min exercise session, the subsequent night, and the 24-h period following exercise, as well as during the corresponding periods of a non-exercise day, were recorded, and glucose variability and time spent in hypoglycemia or hyperglycemia were calculated.

At 20, 30, 34-37 week of gestation the following data were also recorded:

- Fetal anthropometric parameters (bi-parietal diameter, head circumference, abdominal circumference, femur length, in cm)

Finally, three months after delivery the following information were recorded:

- Type of delivery (vaginal or caesarean section) and week of delivery
- Weight (kg) of child at birth
- Gender (M/F)
- Malformations and any neonatal diseases

Randomization and Intervention Program

Subjects were randomly assigned with 1:1 ratio – by using a computer-generated table of randomization – into two groups: a control group and an intervention group. Women assigned to the control group received verbal and written information about the benefits of physical activity during pregnancy and diet recommendations.

Women randomized to the intervention group received diet recommendations and performed low intensity aerobic exercise three times per week at 30% HRR (Heart Rate Reserve), under continuous heart rate monitoring. Duration of each session progressed from 26 minutes the first week to 40 minutes (increasing 2 min/week).

Each woman was submitted to regular diabetes care examinations every 1-3 weeks. On these occasions, insulin therapy was prescribed, if necessary. At baselines and during pregnancy all medications were recorded.

Training modalities

Pregnant women in the exercise group performed low-moderate intensity aerobic physical activities, at 30% HRR, as assessed by the Karvonen formula (1957). Duration of each session progressed from 26 minutes the first week to 40 minutes (increasing 2 min/week) under continuous heart rate monitoring. Initially and for the first 2 weeks, all the exercise sessions were carried out in a gym, under the supervision of an exercise specialist. Subsequently, patients were free of performing one of the three weekly sessions autonomously, recording distance covered and heart rate. To this end, pregnant women used pedometers (Digiwalker, Yamax 800) and heart-rate monitors (Polar, S810i, Polar Electro, Kempele, Finland). All subjects were instructed to maintain a correct hydration before, during and after exercise. At the beginning and at the end of each training session, blood glucose was measured in order to both motivate the patient and minimize the risk of hypoglycemia. Before each exercise session, blood pressure and heart rate were measured, after 10 minutes rest. Each single exercise session was stopped or delayed, and notified to a doctor, in the presence of the following symptoms: fatigue or marked dyspnea, vaginal bleeding, dizziness, sharp abdominal pain, persistent headache, leg pain, absence of fetal movement after 4th month, chest pain. The

exercise was postponed in case of hypoglycemia (≤ 60 mg/dL), increased blood pressure (> 140/90 mmHg) or heart rate above 120 bpm before exercise session. Women were required to alert the medical staff in presence of such symptoms.

For each exercise session, duration of the training, speed, slope, heart rate, time from meal and insulin units, if any, were reported in a diary.

Overall physical activity practiced outside the supervised sessions was assessed by monitoring time, distance and intensity perceived, and blood glucose was measured before and after exercise. All data were reported in a diary.

SUMMARY of STUDY OUTCOMES

Maternal outcomes

Primary outcome

Fasting Plasma Glucose (md/dL), measured at 36 ±1 week of gestation.

Secondary outcomes

- **BMI**
- **Pulse wave velocity**
- **Total physical activity**
- **Medications**
- **Blood glucose readings by CGM**
- **Depressive symptoms**
- **Health related quality of life**
- **Circulating miRNA expression**

Fetal and neonatal outcomes

At 20, 30, 34-37 week of gestation:

- **Fetal anthropometric parameters** (bi-parietal diameter, head circumference, abdominal circumference, femur length, in cm), measured by ultrasonography.

At delivery:

- **Birth weight** (kg) of the newborn
- **Gender** (M/F)
- **Any malformations and/or neonatal disease**

In addition, type of delivery (vaginal or caesarean section), week of delivery, and adverse events that occurred both during the exercise sessions and throughout pregnancy were recorded. In particular, attention was given to: hypoglycemia (symptomatic, asymptomatic or severe) and musculoskeletal pain.

Statistical analysis

Data are presented as mean and standard error (SE), or as a percentage, as appropriate. Non parametric tests were used to compared standard care vs structured exercise groups in terms of maternal variables (HbA1c, body weight, BMI, blood pressure, questionnaire scores (quality of life and depression), energy expenditure, pulse wave velocity. Simple correlations were analyzed by Spearman's coefficient. Subsequently, the multiple regression analysis was used to estimate the predictors of observed variations. ANOVA for repeated measures was used to compare glycemic values during the 40-min exercise session, the subsequent night, and the 24-h period following exercise, as well as during the corresponding periods of a non-exercise day.

Analysis of miRNAs

Data were exported from the 7900HT and imported in the multi PCR data analysis system GenEx Pro (Exiqon, Denmark). To avoid run-to-run variations, the interplate calibration was performed using IPC mean values. Subsequent quality control steps were carried out by visually inspection of Spike-in (performed using Grubbs outlier test), the hemolysis test (monitoring DCq between hsa-miR-23-3p and hsa-miR-451a) and a cut off Cq value assignment of 35. The normalization was calculated against the global mean of all expressed miRNAs and the data converted to relative quantities on a linear scale, according to the formula n=2(Cqavg-Cq). Quantification of PCR data was performed using DDCq method in GenEx-Pro PCR calculation tool. for After testing for Gaussian distribution and equal variance, the differences in miRNA expression levels were tested for statistical significance by paired *t*-test.

Target prediction of miRNAs and functional annotation

To evaluate functions of candidate miRNAs changed after physical intervention in GDM patients target prediction and functional annotation were performed using miRSystem database (version 20160513, available at http://mirsystem.cgm.ntu.edu.tw/index.php), which is an integrated system for characterizing enriched functions and pathways of miRNAs targets. In target

prediction, the miRSystem database integrates 2 experimentally validated databases, TarBase and miRecords, and 7 target gene prediction algorithms, including Diana-microT, miRanda, miRBridge, PicTar, PITA, RNA22 and Targetscan. In functional annotation, miRSystem integrates 5 databases, including KEGG (Kyoto Encyclopedia of Genes and Genomes), Biocarta, Pathway interaction databases (human only), Reactome (human only), and GO (gene ontology) molecular function. To identify significantly enriched pathways or biological function, several statistical approaches, including O/E (observed to expected) ratios of gene targets and hypergeometric test were provided. The parameter settings were: validated miRNA-target interactions identified by at least three prediction algorithms were considered for annotation in KEGG pathways and GO molecular function, 25-500 genes in biological functions/pathways and O/E ratios > 2.

A two-tailed Pearson's P <0.05 was considered as Type I error rate threshold for statistical significance. Analysis were carried out using SPSS 22.0 (SPSS Inc., Chicago, IL).

2.3 Results

Maternal outcomes

Anthropometric and metabolic features

Table 2 shows the main baseline characteristics of subjects assigned to standard care or supervised exercise: age, BMI, HbA1c, fasting plasma glucose, and blood pressure were similar between groups. The two groups were also similar for family history of diabetes, family history of cardiovascular diseases, and previous gestational diabetes.

At the end of treatment, between 35th and 37th week of gestation, women in the two groups did not show any significant changes in BMI, HbA1c, fasting plasma glucose and blood pressure (Table 3).

Overall physical activity

Overall physical activity, estimated by the IPAQ questionnaire was higher in the control group than in the structured exercise group (500±129 vs 135±51 MET*min*wk; P= 0.01). At the end of treatment, the increase of physical activity was significantly higher in the intervention group (P=0.04).

Pulse wave velocity

At baseline, women in the standard care and in the exercise group showed similar PWV values. Mean carotid-to-radial pulse wave was 7.49±0.66 vs 9.5±1.15 m/sec, and carotid-to-femoral pulse wave was 5.57±0.83 vs 5.56±0.96)

At the end of study, change in carotid-to-radial pulse wave velocity was significantly higher in the intervention group (P=0.034).

IL-1β, IL-6 and TNFα

At study entry, women in control and exercise group presented similar IL-1β levels (2.12±0.37 *vs.* 1.61±0.27), IL-6 levels (4.28±0.40 *vs.*4.34±0.94) and TNFα levels (7.80±0.93 *vs.* 7.57±0.52), respectively. At 35-37th week of gestation women in control group showed a significantly higher values in TNFα compared to baseline

values (9.28±1.11 vs 7.80±0.93, P=0.009). No significant changes in interleukins levels in both groups (Table 4).

Continuous glucose monitoring system

In the exercise group, CGMS showed that glucose levels significantly declined during a session of physical activity, as compared with the corresponding period of a non-exercise day. Differences were statistically significant after 30 minutes of exercise (p=0.03) (Figure 1). Moreover, blood glucose remained still lower 180 min after completion of the session (p=0.003). Mean blood glucose in the 24-h and nocturnal periods were similar during the exercise and non-exercise days (Figure 2-3). Similarly, no differences were found in time spent in hypoglycemia or hyperglycemia, as well as in glucose variability, during the 24-h period of the exercise or non-exercise day.

However, 24h mean blood glucose during a non-exercise group was significantly lower in the intervention group than in the control group (Figure. 4).

Insulin therapy

At recruitment, no woman in both groups was on insulin therapy. At the end of gestation, 8 women in standard care group vs 4 women in the exercise group required insulin therapy.

Psychological assessment

The CES-D and SF-36 Questionnaire scores were not significantly different between the two groups. However, four dimensions of SF-36 showed a trend to higher values in the exercise group than in the control group at the end of gestation. In particular, the following items showed the higher changes: Physical functioning, general health perception, vitality and mental health (Figure 5).

MicroRNAs Profiling

Blood samples from 6 women with GDM were assessed before and after the intervention of physical training and evaluated for miRNA profiling by quantitative PCR array. The percentage of positive call rates (i.e. miRNA having more than 60%

T° melting validated data) was 49,5% on 752 total miRNA targeted sequences. The statistical analysis conducted with paired t-test on the logged values of relative quantities gave 26 miRNA differentially expressed ($p<0,05$) including 12 up-regulated and 14 down-regulated (Table 5 and Figure 6). The measured log2(FC) values spanned from 2,1 to -1,63.

Target prediction of miRNAs and functional annotation

Only the most significant miRNA ($p< 0,01$), i.e. hsa-miR-512-3p, hsa-miR-107 and hsa-miR-103a-3p, were considered for the targets prediction and functional annotation by miRSystem database. Results indicated that 1.117 putative targets were regulated by these miRNAs. Among these targets, O/E ratios of 520 targets were more than 2. Functional annotation showed that these 520 targets were significantly enriched in 25 KEGG pathways and 1 GO molecular functions (Table 6 and Figure 7). Among the KEGG pathways involved is worthy to notice the presence of T2DM ($p= 0,02$), insulin ($p=0,01$) and adipocytokine ($p=8,9 \times 10^{-4}$) signalling pathways. The only GO item was related to protein binding and transcription factor activity ($p=0,004$). The three microRNAs interact with the transcripts of some key genes related with the above mentioned metabolic pathways, in particular we highlight the gene insulin receptor substrate 2 (IRS2, targeted by hsa-miR-103a-3p and hsa-miR-107), the gene solute carrier family 2 (SLC2A4, facilitate glucose transporter targeted by hsa-miR-512-3p) and the gene mitogen-activated protein kinase 10 (MAPK10 targted by hsa-miR-512-3p) that are in common between the three pathways and illustrated in Figure 8.

Fetal and neonatal outcomes

No differences were observed in terms of newborn outcomes between groups, including body weight and incidence of SGA (small for gestational age) or macrosomia. Newborns in the control group were more likely to be delivered earlier as compared to the structured exercise group (38.2 ±0.2 vs 39.1 ± 0.4 weeks, respectively, P=0.05).

Adverse events

No patient experienced musculoskeletal pain, maternal-fetal diseases or clinically significant hypoglycemia during or after exercise.

Several additional investigations were also carried out as part of this study. For instance, caloric intake was assessed by a 3-day food recall questionnaire and C-reactive protein and adiponectin levels were measured. However, these results are not available yet. Therefore, these features have not been included in this thesis.

2.4 Discussion

In the present study we evaluated the efficacy of a supervised physical exercise program, as compared with standard care, in women with gestational diabetes. The specific endpoints of the study were maternal glyco-metabolic control and overall maternal and perinatal outcomes.

As a main finding, our study showed a significant and sustained decline in blood glucose during physical activity, as compared with the corresponding period of a non-exercise day, measured by a CGM system. Differences were statistically significant after 30 minutes of exercise ($p=0.03$). Blood glucose remained still lower 180 min after completion of the session ($p=0.003$). Moreover, mean blood glucose toward 24-h between the two groups presented a lower glycemic trend in the exercise compared to standard care group.

Notably, at the end of pregnancy only 4 women in the intervention group required insulin to reach the recommended glycemic targets, a proportion 2-fold lower than what observed in the control group, where insulin was needed in 8 women.

Physical activity and exercise during pregnancy are currently known to benefit women by improving individual cardiovascular health and general fitness, by reducing the risk of complications like preeclampsia, cesarean delivery and gestational diabetes mellitus. Management of GDM consists of dietary modification, self-monitoring postprandial capillary glycaemia and, when necessary, insulin therapy. The most recent guidelines published by the most important scientific societies in this field, such as the American College of Obstetricians and Gynecologists and the American Diabetes Association, recommend exercise programs including at least 20-30 min of moderate-intensity exercise on most or all days of the week. There is evidence that either aerobic or resistance training can improve insulin action and glycemic control (Colberg, 2013). However, the evidence has been equivocal due to small sample size and heterogeneity of exercise type and outcome measures. Recent meta-analysis supports the importance of exercise in post-prandial glycemic control and lowered

fasting blood glucose (Harrison, 2016), although only eight randomized controlled trials have investigated on this topic yet.

With regard to the preceding literature, our study adds several novel insights on the vascular and metabolic effects of a moderate intensity exercise program in pregnant women. Our study is also one of the first RCTs examining with considerable detail the effects of structured exercise on metabolic parameters and gene expression levels during gestational diabetes.

Indeed, Jovanovic-Peterson et al. (1989) have previously shown that women, who exercise 3 times per week (on an arm ergometer) had significantly lower fasting and postprandial blood glucose than those on diet alone. Further investigations by Bung et al. (1991) demonstrated that a moderate, supervised, exercise at 50% VO2max, 3 times/wk by recumbent bicycle, is just as effective as insulin in maintaining blood glucose levels within normal limits.
On the contrary, Avery et al (1997) did not find significant improvements in blood glucose level after a partially home-based exercise program. However, in a subsequent study (2001), the same group evaluated the effects of a single session of exercise on blood glucose (sampled every 15 min). They found significant declines in blood glucose level during low and moderate exercise (cycling) compared to rest. These differences were gone by 45 minutes after exercise.
Moreover, gestational diabetes seems to be associated with endothelial dysfunction and increased arterial stiffness, but data are limited and it remains unclear whether this alteration may be reversed by regular exercise training.

In our study, delta CR-PWV measured from baseline to the end of gestation was significantly higher in the exercise intervention than in the standard care group (-2.27±1.42 vs 1.40±0.74; p=0.034), thus highlighting that a structured program of low-intensity aerobic exercise may have favorable effects on arterial stiffness in women with GDM.

With regard to the gene expression profiling, as provided by the analysis of circulating miRNAs, the analysis of the RT-qPCR low density arrays we employed identified 26 differentially expressed molecules in GDM patients after physical training among almost 370 plasma miRNAs.
Functional annotation analysis of these 26 miRNAs revealed that three metabolic pathways related to lipids and glucose were modified by exercise training.
These promising results add novel insights on the epigenetic modulation due to physical exercise. However, further research is needed to better understand the regulatory activity of miRNAs and their implications on metabolic parameters.

It should be however acknowledged as a major limitation that the sample size of our study is somewhat limited, an issue which is amenable to solution in the near future as the RCT is still ongoing and open for recruitment. Additionally, although the presented data pertain to 20 subjects only, these preliminary results suggest a remarkable and beneficial effect of exercise on lowering plasma glucose levels.

2.5 Conclusions

In conclusion, our preliminary data indicate that a structured program of low-intensity aerobic exercise may have favorable effects on glycemic control during 24-h and on arterial stiffness in women with gestational diabetes.
Further studies and a larger sample are needed to definitely support our conclusions, which would be crucial for designing optimal exercise programs in women with gestational diabetes.

CHAPTER 3

References

Abell SK, De Courten B, Boyle J, Teede H. Inflammatory and other biomarker: role in pathophysiology and prediction of gestational diabetes mellitus. Int J Mol Scie 2015; 16: 13442-73

ACOG, Committee Opinion. Physical Activity and Exercise during pregnancy and the postpartum period. Vol. 126, no. 6, december 2015

American College of Sports Medicine (2014). Guidelines for exercise testing and prescription, 9th edn. Lippincott Williams & Wilkins, Baltimore.

Apolone G., Mosconi P. (1998). The Italian SF-36 Health Survey: Translation, Validation and Norming. Journal of Clinical Epidemiology, 51(11):1025-1036.

Avery MD, Leon AS, Kopher RA. Effects of a partially home-based exercise program for women with gestational diabetes. Obstet Gynecol. 1997 Jan;89(1):10-5.

Avery MD, Walker AJ. Acute effect of exercise on blood glucose and insulin levels in women with gestational diabetes. J Matern Fetal Med. 2001 Feb;10(1):52-8.

Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with T2DM. Diabet Med. 2004 Feb;21(2):103-13.

Bgeginski R, Ribeiro PA, Mottola MF, Ramos JG. Effects of weekly-supervised exercise or physical activity counseling on fasting blood glucose in women diagnosed with gestational diabetes mellitus: a systematic review and meta-analysis of randomized trials. J Diabetes. 2016 Dec 29.

Bo S, Rosato R, Ciccone G, Canil S, Gambino R, Poala CB, Leone F, Valla A, Grassi G, Ghigo E, Cassader M, Menato G Simple lifestyle recommendations and the outcomes of

gestational diabetes. A 2 × 2 factorial randomized trial. Diabetes Obes Metab. 2014 Oct;16(10):1032-5. doi: 10.1111/dom.12289. Epub 2014 Apr 7.

Brankston GN, Mitchell BF, Ryan EA, Okun NB. Resistance exercise decreases the need for insulin in overweight women with gestational diabetes mellitus. Am J Obstet Gynecol. 2004 Jan;190(1):188-93.

Buchanan TA, Metzer B, Frienkel N. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or gestational diabetes. Am J Obstet Gynecol 1990;162: 1008-14

Buchanan TA, Metzger BE, Freinkel N. Accelerated starvation in late pregnancy: a comparison between obese women with and without gestational diabetes mellitus. Am J Obstet Gynecol. 1990 Apr;162(4):1015-20.

Bung P, Artal R, Khodiguian N, Kjos S. Exercise in gestational diabetes. An optional therapeutic approach? Diabetes. 1991 Dec;40 Suppl 2:182-5.

Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr. 2000 May;71(5 Suppl):1256S-61S.

Catalano PM, Hoegh M, Minium J, Huston-Presley L, Bernard S, Kalhan S, Hauguel-De Mouzon S. Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. Diabetologia. 2006 Jul;49(7):1677-85. Epub 2006 May 11.

Catalano PM, Hollenbeck C. Energy requirements in pregnancy: a review. Obstet Gynecol Surv. 1992 Jun;47(6):368-72.

Catalano PM, Nizielski SE, Shao J, Preston L, Qiao L, Friedman JE. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. Am J Physiol Endocrinol Metab. 2002 Mar;282(3):E522-33.

Catalano PM, Tizbir E, Roman N. Longitudinal changes in insulin release and insulin resistance in non-obese pregnant women. Am J Obstet Gynecol 1991; 165: 1667-72.

Catalano PM, Tyzbir ED, Wolfe RR, Calles J, Roman NM, Amini SB, et al. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. Am J Physiol 1993; 264: E60-7

Clapp J. Maternal carbohydrate intake and pregnancy outcome. Proc Nutr Soc 2002; 61:45-50

Classification and Diagnosis of Diabetes. American Diabetes Association. Diabetes Care 2017 Jan; 40 (Supplement 1): S11-S24

Colberg SR, Castorino K, Jovanovic L. Prescribing physical activity to prevent and menage gestational diabetes. World J Diabetes 2013;4(6):256-262.

Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. 2002 Oct 15;106(16):2085-90.

Davenport MH, Mottola MF, McManus R, Gratton R. A walking intervention improves capillary glucose control in women with gestational diabetes mellitus: a pilot study. Appl Physiol Nutr Metab. 2008 Jun;33(3):511-7.

de Barros MC, Lopes MA, Francisco RP, Sapienza AD, Zugaib M. Resistance exercise and glycemic control in women with gestational diabetes mellitus. Am J Obstet Gynecol. 2010 Dec;203(6):556.

Dempsey JC, Butler CL, Sorensen TK, Lee IM, Thomson ML, Miller RS, Frederick IO, Williams MA. A case-control study of maternal recreational physical activity and risk of gestational diabetes mellitus. Diabetes Res Clin Pract 2004; 66:203-215.

Dempsey JC, Sorensen TK, Williams MA, Lee IM, Miller RS, Dashow EE, Luthy DA. Prospective study of gestational diabetes mellitus risk in relation to maternal recreational physical activity before and during pregnancy. Am J Epidemiol 2004; 159: 663-670.

DeSisto CL, Kim SY, Sharma AJ. Prevalence estimates of gestational diabetes mellitus in the United States, pregnancy risk assessment monitoring system (PRAMS), 2007-2010. Prev Chronic Dis 2014; 11:E104

Downs DS, Chasan-Taber L, Evenson KR, Leiferman J, Yeo S. Physical activity and pregnancy: past and present evidence and future recommendations. Res Q Exerc Sport. 2012 Dec;83(4):485-502.

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197

Freemark M. Regulation of maternal metabolism by pituitary and placental hormones: roles in fetal development and metabolic programming. Horm Res Paediatr 2006; 65 (Suppl. 3): 41-9

Friedman JE, Ishizuka T, Shao J, Huston L, Highman T, Catalano P. Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. Diabetes. 1999 Sep;48(9):1807-14.

Friedman JE, Kirwan JP, Jing M, Presley L, Catalano PM. Increased skeletal muscle tumor necrosis factor-alpha and impaired insulin signaling persist in obese women with gestational diabetes mellitus 1 year postpartum. Diabetes. 2008 Mar;57(3):606-13.

Gabbe S, Niebyl JR, Simpson JL (1991). Obstetrics: Normal and problem pregnancies. Edinburgh: Churchill Livingstone.

Glinoer D. The regulation of thyroid function during normal pregnancy: importance of the iodine nutrition status. Best Pract Res Clin Endocrinol Metab. 2004 Jun;18(2):133-52.

Hallal PC, Victora CG. Reliability and validity of the International Physical Activity Questionnaire (IPAQ). Med Sci Sports Exerc. 2004 Mar;36(3):556.

Halse RE, Wallman KE, Newnham JP, Guelfi KJ. Home-based exercise training improves capillary glucose profile in women with gestational diabetes. Med Sci Sports Exerc. 2014 Sep;46(9):1702-9

Han S, Middleton P, Crowther CA. Exercise for pregnant women for preventing gestational diabetes mellitus. Cochrane Database Syst Rev. 2012 Jul 11;(7):CD009021

Harrison AL, Shields N, Taylor NF, Frawley HC. Exercise improves glycaemic control in women diagnosed with gestational diabetes mellitus: a systematic review. J Physiother. 2016 Oct;62(4):188-96.

Henry RM, Kostense PJ, Spijkerman AM, Dekker JM, Nijpels G, Heine RJ, Kamp O, Westerhof N, Bouter LM, Stehouwer CD; Hoorn Study. Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study Circulation. 2003 Apr 29;107(16):2089-95. Epub 2003 Apr 14.

Hytten F, Chamberlain G (1991). Clinical Physiology in Obstetrics. Oxford: Blackwell Scientific Publications.

Joffe GM, Esterlitz JR, Levine RJ, Clemens JD, Ewell MG, Sibai BM, Catalano PM. The relationship between abnormal glucose tolerance and hypertensive disorders of pregnancy in healthy nulliparous women. Calcium for Preeclampsia Prevention (CPEP) Study Group. Am J Obstet Gynecol. 1998 Oct;179(4):1032-7.

Jovanovic-Peterson L, Durak EP, Peterson CM. Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes. Am J Obstet Gynecol. 1989 Aug;161(2):415-9.

Kaihura C, Savvidou MD, Anderson JM, McEniery CM, Nicolaides KH. Maternal arterial stiffness in pregnancies affected by preeclampsia. Am J Physiol Heart Circ Physiol. 2009 Aug;297(2):H759-64. Epub 2009 May 22.

Kalhan SC. Protein metabolism in pregnancy. Am J Clin Nutr. 2000 May;71(5 Suppl):1249S-55S.

Kalkhoff R et al (1979). Carbohydrate and lipid metabolism during normal pregnancy: relationship to gestational hormone action. In the Diabetic Pregnancy: A Perinatal Perspective. I. Merkatz and P.Adam. New York, Grune& Straton: pp.3-21.

Karvonen JJ, Kentala E, Mustala O. The effects of training on heart rate: a "longitudinal" study. Ann Med Exp Biol Fenn 1957; 35:307-315.

Kirwan JP, Hauguel-De Mouzon S, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, Kalhan SC, Catalano PM. TNF-alpha is a predictor of insulin resistance in human pregnancy. Diabetes. 2002 Jul;51(7):2207-13.

Kühl C. Insulin secretion and insulin resistance in pregnancy and GDM. Implications for diagnosis and management. Diabetes. 1991 Dec;40 Suppl 2:18-24.

Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H; European Network for Non-invasive Investigation of Large Arteries.Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006 Nov;27(21):2588-605.

Lawrence JM, Contreras R, Chen W, Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999–2005. Diabetes Care 2008;31:899–904

Leng J, Liu G, Zhang C, Xin S, Chen F, Li B, Tian H, Yu Z, Tuomilehto J, Hu G, Yang X. Physical activity, sedentary behaviors and risk of gestational diabetes mellitus: a population-based cross-sectional study in Tianjin, China. Eur J Endocrinol. 2016 Jun;174(6):763-73

Lesser KB, Carpenter MW. Metabolic changes associated with normal pregnancy and pregnancy complicated by diabetes mellitus. Semin Perinatol. 1994 Oct;18(5):399-406.

McIntyre HD, Sacks DA, Barbour LA, et al. Issues with the diagnosis and classification of hyperglycemia in early pregnancy. Diabetes Care 2016;39:53–5448

Metzger BE, Lowe LP, Dyer AR, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002.

Mitchell M, Armstrong DT, Robker RL, Norman RJ. Adipokines: implications for female fertility and obesity. Reproduction. 2005 Nov;130(5):583-97.

Mottola MF, Christopher PD. Effects of maternal exercise on liver and skeletal muscle glycogen storage in pregnant rats. J Appl Physiol 1991; 71: 1015-9

Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. Curr Opin Endocrinol Diabetes Obes. 2011 Dec;18(6):409-16.

Paradisi G, Biaggi A, Ferrazzani S, De Carolis S, Caruso A. Abnormal carbohydrate metabolism during pregnancy : association with endothelial dysfunction. Diabetes Care. 2002 Mar;25(3):560-4.

PARmed-X for Pregnancy. Ottawa (Ontario): Canadian Society for Exercise Physiology; 2002 (cited 2011 feb 4). 4p. Available from http://www.csep.ca/english/view.asp?x=698

Physical Activity Guidelines Committee. (2008). Physical activity guidelines advisory committee report. Washington, DC: Department of Health and Human Services.

Radloff, L.S. (1977). The CES-D Scale: A self-report depression scale for research in the general population. Applied Psychological Measurement , 1, 385-401.

Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. J Clin Endocrinol Metab. 2002 Sep;87(9):4231-7.

Ruchat SM, Davenport MH, Giroux I, Hillier M, Batada A, Sopper MM, McManus R, Hammond JA, Mottola MF. Effect of exercise intensity and duration on capillary glucose responses in pregnant women at low and high risk for gestational diabetes. Diabetes Metab Res Rev. 2012 Nov;28(8):669-78

Russo LM, Nobles C, Ertel KA, Chasan-Taber L, Whitcomb BW. Physical activity interventions in pregnancy and risk of gestational diabetes mellitus: a systematic review and meta-analysis. Obstet Gynecol. 2015 Mar;125(3):576-82

Ryan EA, Enns L. Role of gestational hormones in the induction of insulin resistance. J Clin Endocrinol Metab. 1988 Aug;67(2):341-7.

Ryan EA, O'Sullivan MJ, Skyler JS. Insulin action during pregnancy. Studies with the euglycemic clamp technique. Diabetes. 1985 Apr;34(4):380-9.

Sanabria-Martinez G, Garcia-Hermoso A, Poyatos-Leon R, Alvarez-Bueno C, Sanchez-Lopez M, Martinez-Vizcaino V. Effectiveness of physical activity interventions on preventing gestational diabetes mellitus and excessive maternal weight gain: a meta-analysis. BJOG 2015; 122:1167-1174.

Savvidou MD, Anderson JM, Kaihura C, Nicolaides KH. Maternal arterial stiffness in pregnancies complicated by gestational and T2DM mellitus. Am J Obstet Gynecol. 2010 Sep;203(3):274.e1-7.

Sibai BM, Caritis SN, Hauth JC, MacPherson C, VanDorsten JP, Klebanoff M, Landon M, Paul RH, Meis PJ, Miodovnik M, Dombrowski MP, Thurnau GR, Moawad AH, Roberts J. Preterm delivery in women with pregestational diabetes mellitus or chronic hypertension relative to women with uncomplicated pregnancies. The National institute of Child health and Human Development Maternal- Fetal Medicine Units Network. Am J Obstet Gynecol. 2000 Dec;183(6):1520-4.

Sims EA, Krantz KE. Serial studies of renal function during pregnancy and the puerperium in normal women. J Clin Invest. 1958 Dec;37(12):1764-74.

Tobias DK, Zhang C, van Dam RM, Bowers K, Hu FB. Physical activity before and during pregnancy and risk of gestational diabetes mellitus: a meta-analysis. Diabetes Care. 2011 Jan;34(1):223-9

Ware JE, Sherbourne CD (1992). The MOS 36-item Short-Form Health Survey (SF-36): Conceptual Framework and Item Selection. Medical Care, 30(6), 473-483.

Weissberger TL, Physiological adaptations in early pregnancy: adaptations to balance maternal-fetal demands. Appl Physiol Nutr Metab 2006; 31:1-11

White E, Pivarnik J, Pfeiffer K. Resistance training during pregnancy and perinatal outcomes. J Phys Act Health. 2014 Aug;11(6):1141-8.

Youngwanichsetha S, Phumdoung S, Ingkathawornwong T. The effects of mindfulness eating and yoga exercise on blood sugar levels of pregnant women with gestational diabetes mellitus. Appl Nurs Res. 2014 Nov;27(4):227-30.

CHAPTER 4

Tables and Figure

Table 1. Screening for and diagnosis of GDM (Diabetes Care 2017)

One-step strategy

Perform a 75-g OGTT, with plasma glucose measurement when patient is fasting and at 1 and 2 h, at 24−28 weeks of gestation in women not previously diagnosed with overt diabetes.

The OGTT should be performed in the morning after an overnight fast of at least 8 h.

The diagnosis of GDM is made when any of the following plasma glucose values are met or exceeded:

- Fasting: 92 mg/dL (5.1 mmol/L)
- 1 h: 180 mg/dL (10.0 mmol/L)
- 2 h: 153 mg/dL (8.5 mmol/L)

Two-step strategy

Step 1: Perform a 50-g GLT (nonfasting), with plasma glucose measurement at 1 h, at 24–28 weeks of gestation in women not previously diagnosed with overt diabetes.

If the plasma glucose level measured 1 h after the load is ≥130 mg/dL, 135 mg/dL, or 140 mg/dL* (7.2 mmol/L, 7.5 mmol/L, or 7.8 mmol/L), proceed to a 100-g OGTT.

Step 2: The 100-g OGTT should be performed when the patient is fasting.

The diagnosis of GDM is made if at least two of the following four plasma glucose levels (measured fasting and 1 h, 2 h, 3 h after the OGTT) are met or exceeded:

	Carpenter/Coustan (59)	or	NDDG (60)
• Fasting	95 mg/dL (5.3 mmol/L)		105 mg/dL (5.8 mmol/L)
• 1 h	180 mg/dL (10.0 mmol/L)		190 mg/dL (10.6 mmol/L)
• 2 h	155 mg/dL (8.6 mmol/L)		165 mg/dL (9.2 mmol/L)
• 3 h	140 mg/dL (7.8 mmol/L)		145 mg/dL (8.0 mmol/L)

Table 2. Main baseline characteristics of subjects. Values are mean ± SE unless otherwise specified (N=20)

Variables	Standard care (n=10)	Structured exercise intervention (n=10)
Age (yr)	34.5 ±1.41	34.3 ± 0.96
Family history of diabetes (%)	80%	70%
Family history for CVD (%)	40%	50%
Pre-pregancy BMI (kg/m^2)	27.52 ±2.45	28.5 ± 2.68
BMI (kg/m2)	29.88 ± 1.95	30.01 ±2.29
Systolic BP (mmHg)	116 ± 6.5	108 ± 3.64
Diastolic BP (mmHg)	71.8 ±3.91	67 ± 2.88
Fasting plasma glucose (mg/dL)	91.88 ±3.81	82.2 ± 2.67
HbA1c (mmol/mol)	35 ±1.42	33.8 ± 0.87
PWV CR (m/s)	7.49 ± 0.66	9.5 ± 1.1
PWV CF (m/s)	5.57 ±0.83	5.56 ± 0.96

Table 3. Changes observed during the study in standard care and structured exercise intervention group. Values are mean±SE

Variable	Standard care (n=10)		Structured exercise intervention (n=10)		P-value
	Baseline	35th -37th wk	Baseline	35th -37th wk	
BMI (kg/m2)	29.88 ± 1.95	30.92 ± 1.96	30.01 ±2.29	30.77 ±2.34	n.s.
Systolic BP (mmHg)	116 ± 6.5	110 ± 3.6	108 ± 3.64	108 ±4.25	n.s.
Diastolic BP (mmHg)	71.8 ±3.91	73.5 ± 3.9	67 ± 2.88	74 ±2.46	n.s.
Fasting plasma glucose (mg/dL)	91.88 ±3.81	92.57 ± 11.08	82.2 ± 2.67	78.4 ± 2.72	n.s.
HbA1c (mmol/mol)	35 ±1.42	38 ± 3.17	33.8 ± 0.87	35.7 ± 0.8	n.s.
PWV CR (m/s)	7.49 ± 0.66	8.52 ± 0.59	9.5 ± 1.1	6.92 ± 1.01	n.s
PWV CF (m/s)	5.57 ±0.83	6.87 ± 1.7	5.56 ± 0.96	5.6 ± 0.66	n.s.
Week of delivery (wk)	-	38.24 ±0.2		39.15 ± 0.4	**0.05**
Baby weight (gr)	-	3317.5 ±186		3167. 77 ± 161	n.s.

Table 4. Changes observed during the study in standard care and structured exercise intervention group. Values are mean±SE

Variables	Standard care (n=10)		Structured exercise intervention (n=10)	
	Baseline	**35th -37th wk**	**Baseline**	**35th -37th wk**
IL-1β (pg/mL)	2.12±0.37	2.04±0.30	1.61±0.27	1.44±0.33
IL-6 (pg/mL)	4.28±0.40	4.95±0.61	4.34±0.94	4.34±0.89
TNF-α (pg/mL)	7.80±0.93	9.28±1.11 *	7.57±0.52	7.72±0.91

***P<0.01 vs baseline**

Table 5. Summary of the RT-qPCR results for the significant microRNAs

	(POST) vs (PRE)	Fold change	Difference (A-B log scale) Log2FC	P-value (paired *t*-test)	P-value (Benjamini-Hochberg)	P-value (Westfall & Young)	P-value (Benjamini-Yekutieli)	Frequency missing data
1	hsa-miR-518a-3p	4,26	2,09	*0,015*	0,178	0,08354	> 0.99	0,00%
2	hsa-miR-95-3p	3,83	1,94	*0,036*	0,218	0,5675	> 0.99	0,00%
3	hsa-miR-525-5p	3,37	1,75	*0,028*	0,202	0,23161	> 0.99	0,00%
4	hsa-miR-518c-3p	2,99	1,58	*0,044*	0,237	0,80817	> 0.99	0,00%
5	hsa-miR-518f-3p	2,89	1,53	*0,011*	0,178	0,0667	> 0.99	0,00%
6	**hsa-miR-512-3p**	**2,77**	**1,47**	***0,005***	0,129	0	> 0.99	0,00%
7	hsa-miR-100-5p	2,72	1,44	*0,011*	0,178	0,04947	> 0.99	0,00%
8	hsa-miR-141-3p	2,61	1,39	*0,036*	0,218	0,60744	> 0.99	0,00%
9	hsa-miR-483-5p	2,55	1,35	*0,030*	0,202	0,26293	> 0.99	0,00%
10	hsa-miR-518e-3p	2,52	1,33	*0,019*	0,202	0,13301	> 0.99	0,00%
11	hsa-miR-193b-3p	2,21	1,14	*0,048*	0,252	0,84057	> 0.99	0,00%
12	hsa-miR-210-3p	1,61	0,69	*0,010*	0,178	0,04947	> 0.99	0,00%
13	hsa-miR-140-5p	-1,65	-0,72	*0,042*	0,232	0,80817	> 0.99	0,00%
14	hsa-miR-26b-5p	-1,74	-0,80	*0,035*	0,218	0,53477	> 0.99	0,00%
15	hsa-miR-545-3p	-1,88	-0,91	*0,046*	0,244	0,84057	> 0.99	0,00%
16	hsa-miR-26a-5p	-2,15	-1,10	*0,035*	0,218	0,53477	> 0.99	0,00%
17	hsa-miR-191-5p	-2,19	-1,13	*0,038*	0,223	0,72818	> 0.99	0,00%
18	hsa-let-7e-5p	-2,26	-1,17	*0,021*	0,202	0,13301	> 0.99	0,00%
19	hsa-miR-338-3p	-2,30	-1,20	*0,015*	0,178	0,08354	> 0.99	0,00%
20	hsa-miR-361-3p	-2,36	-1,24	*0,024*	0,202	0,13301	> 0.99	0,00%
21	hsa-miR-329-3p	-2,40	-1,27	*0,029*	0,202	0,26293	> 0.99	0,00%
22	**hsa-miR-107**	**-2,41**	**-1,27**	***0,001***	*0,006*	0	> 0.99	0,00%
23	hsa-miR-221-3p	-2,76	-1,46	*0,026*	0,202	0,16555	> 0.99	0,00%
24	hsa-miR-28-3p	-2,80	-1,49	*0,041*	0,232	0,80817	> 0.99	0,00%
25	**hsa-miR-103a-3p**	**-2,84**	**-1,51**	***0,009***	0,178	0,01573	> 0.99	0,00%
26	hsa-miR-454-3p	-3,10	-1,63	*0,027*	0,202	0,23161	> 0.99	0,00%

Tab. 2 Functional annotation summary reports of the most significant (p< 0.02) KEGG pathways and Gene Ontology (GO) molecular functions

Category	Term	Targets in the term	miRNAs in the term	P value
KEGG	Cell cycle	12	3	0,00003
KEGG	Oocyte meiosis	11	3	0,00006
KEGG	Ubiquitin mediated proteolysis	12	3	0,00007
KEGG	Hedgehog signalling pathway	7	3	0,0003
KEGG	Regulation of actin cytoskeloton	14	3	0,0004
KEGG	Pathways in cancer	18	3	0,0005
KEGG	Adipocytokine signalling pathway	7	3	0,0009
KEGG	p53 signalling pathway	7	2	0,0009
KEGG	MAPK signalling pathway	15	3	0,001
KEGG	Hypertrofic cardiomyopathy (HCM)	7	3	0,003
GO	Protein binding transcription factor	17	3	0,003
KEGG	Thyroid cancer	4	3	0,004
KEGG	RNA transport	9	3	0,005
KEGG	Focal adhesion	11	3	0,005
KEGG	Alanine aspartate glutamate metabolism	4	2	0,006
KEGG	WNT signalling pathway	9	3	0,006
KEGG	Basal transcription factors	4	2	0,008
KEGG	Insulin signalling pathway	8	3	0,010
KEGG	Progesterone mediated oocyte maturation	6	3	0,012
KEGG	Endocytosis	10	3	0,013
KEGG	Prostate cancer	6	3	0,013
KEGG	Dilataed cardiomyopathy	6	3	0,014
KEGG	Type 1 diabetes mellitus	4	3	0,014
KEGG	Melanoma	5	3	0,02
KEGG	Notch signalling pathway	4	3	0,02
KEGG	T2DM mellitus	4	3	0,02

Figure 1. Glucose levels during a 40 min exercise session and up to 3 hours after, as compared with the same day-time of a non-exercise day. (Interaction time*period P=0.003)

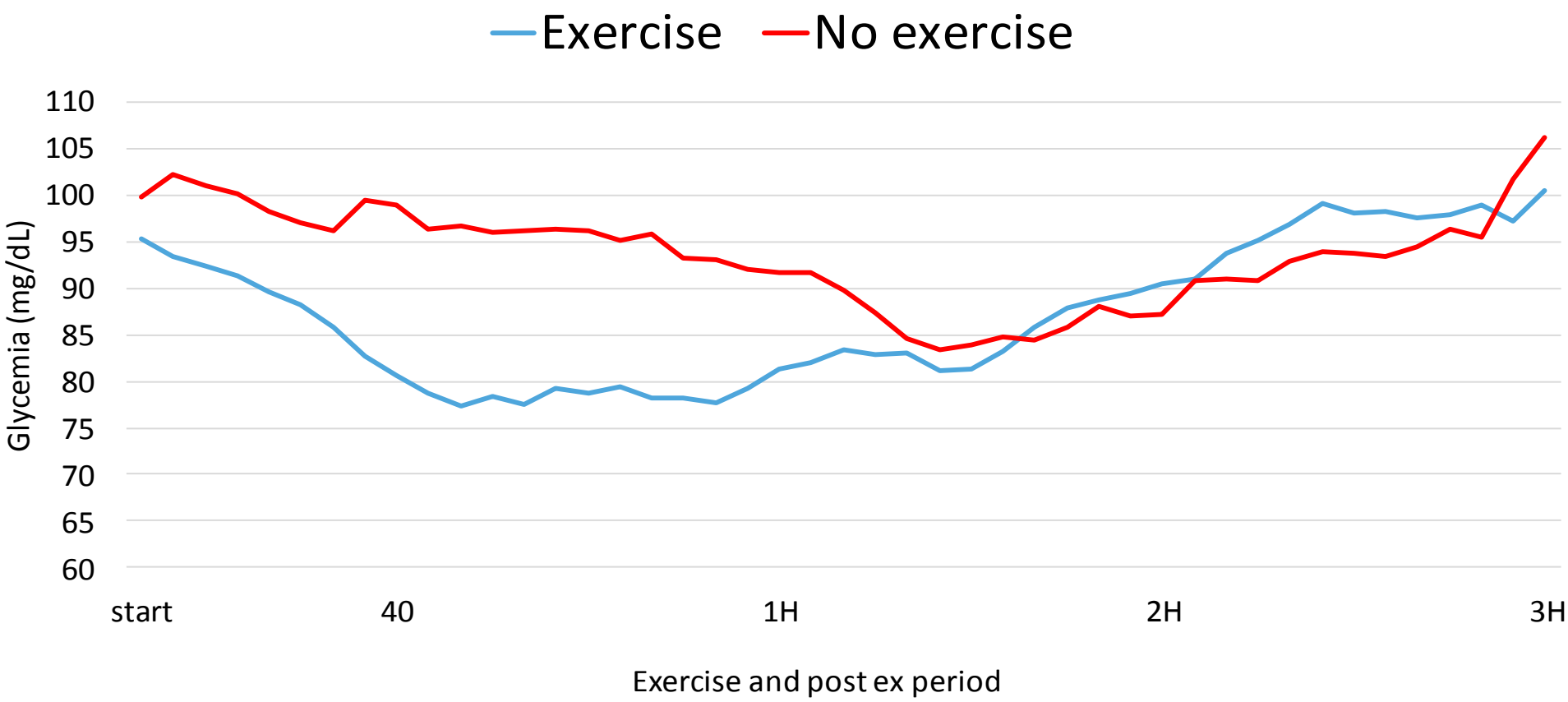

Figure 2. Glucose levels during 24 hours in an exercise day compared with a non-exercise day (P=N.S.)

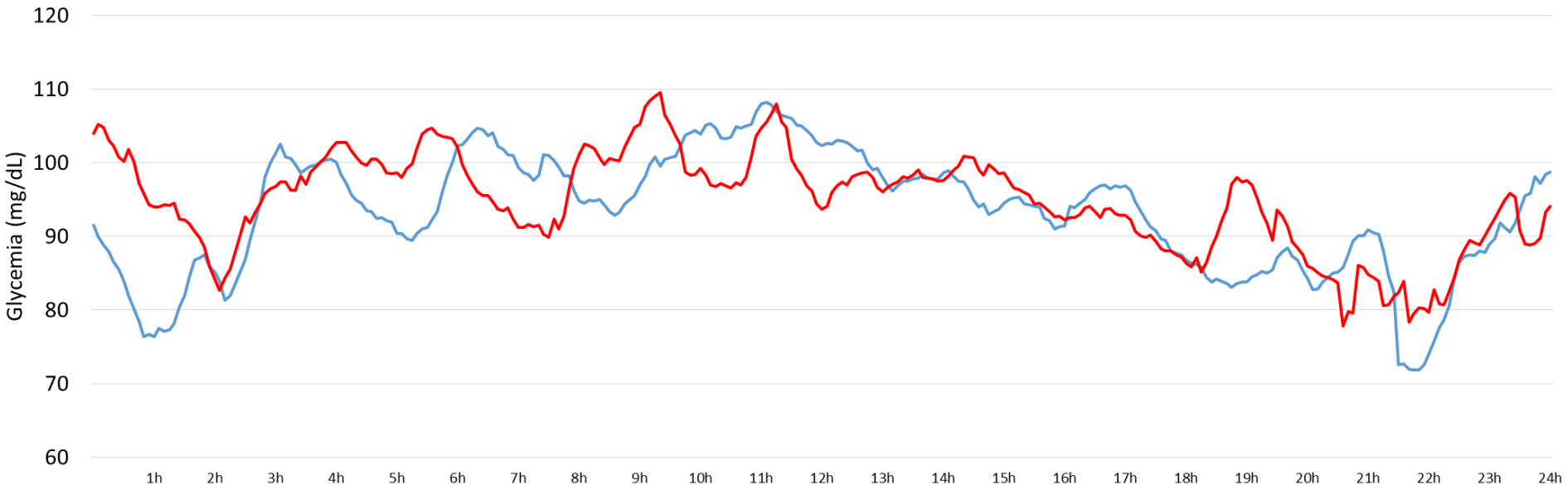

Figure 3. Glucose levels during the nocturnal period of an exercise day compared with a non-exercise day (P=N.S.)

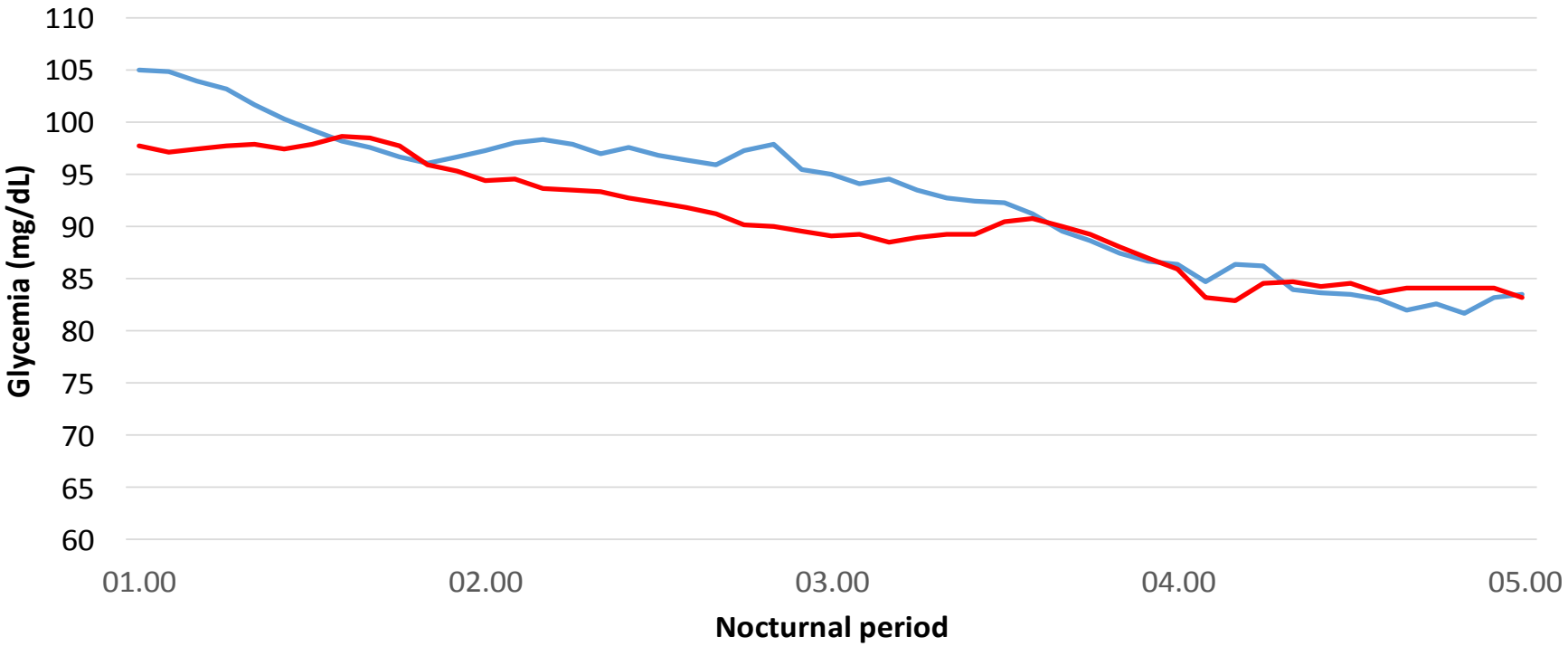

Figure 4. Glucose levels during 24 hours in exercise group compared with control group (P<,001) .

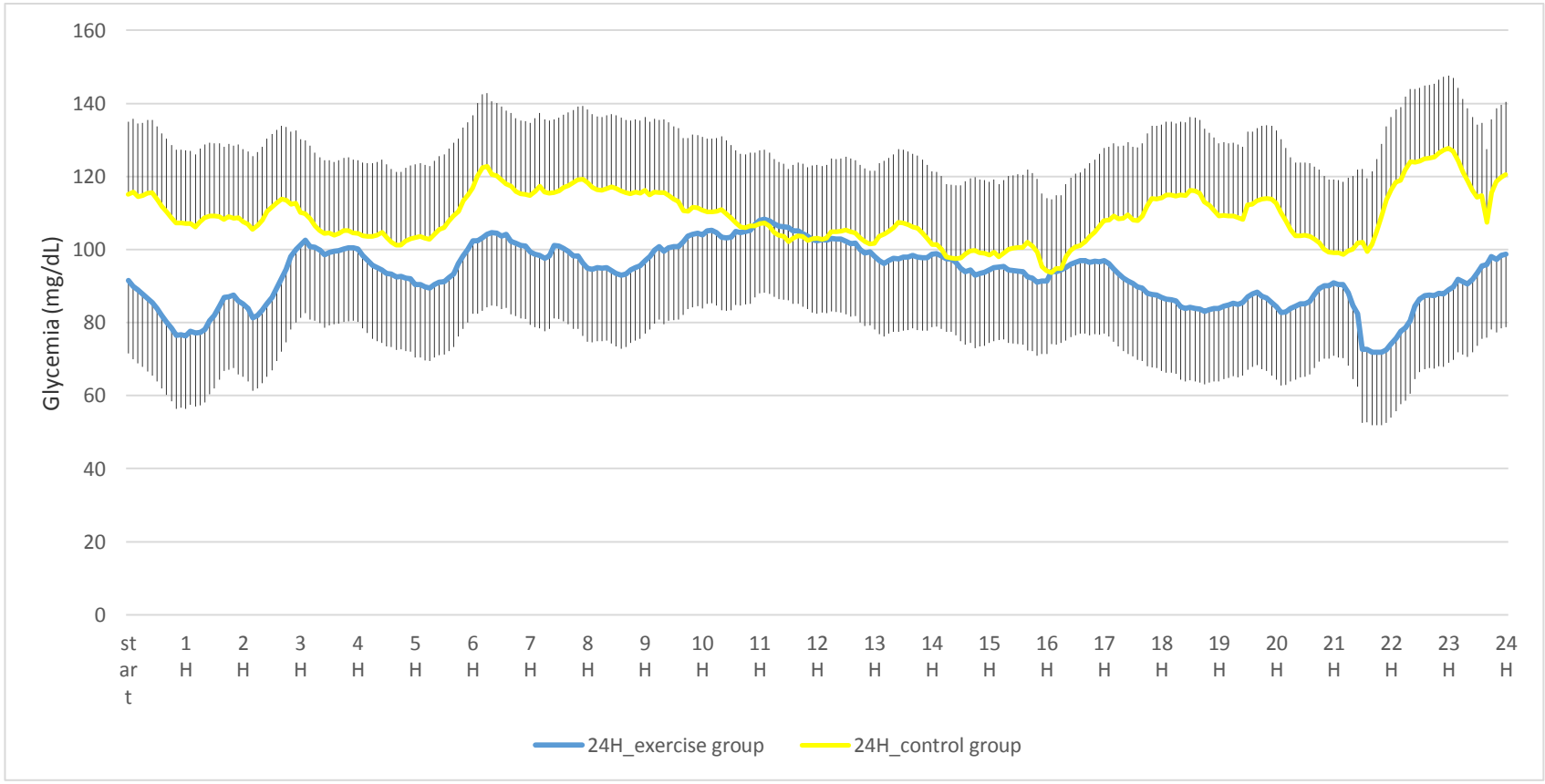

Figure 5. Changes in SF-36 questionnaire of the following items: Physical functioning (A), General health (B), Vitality (C) and Mental health (D).

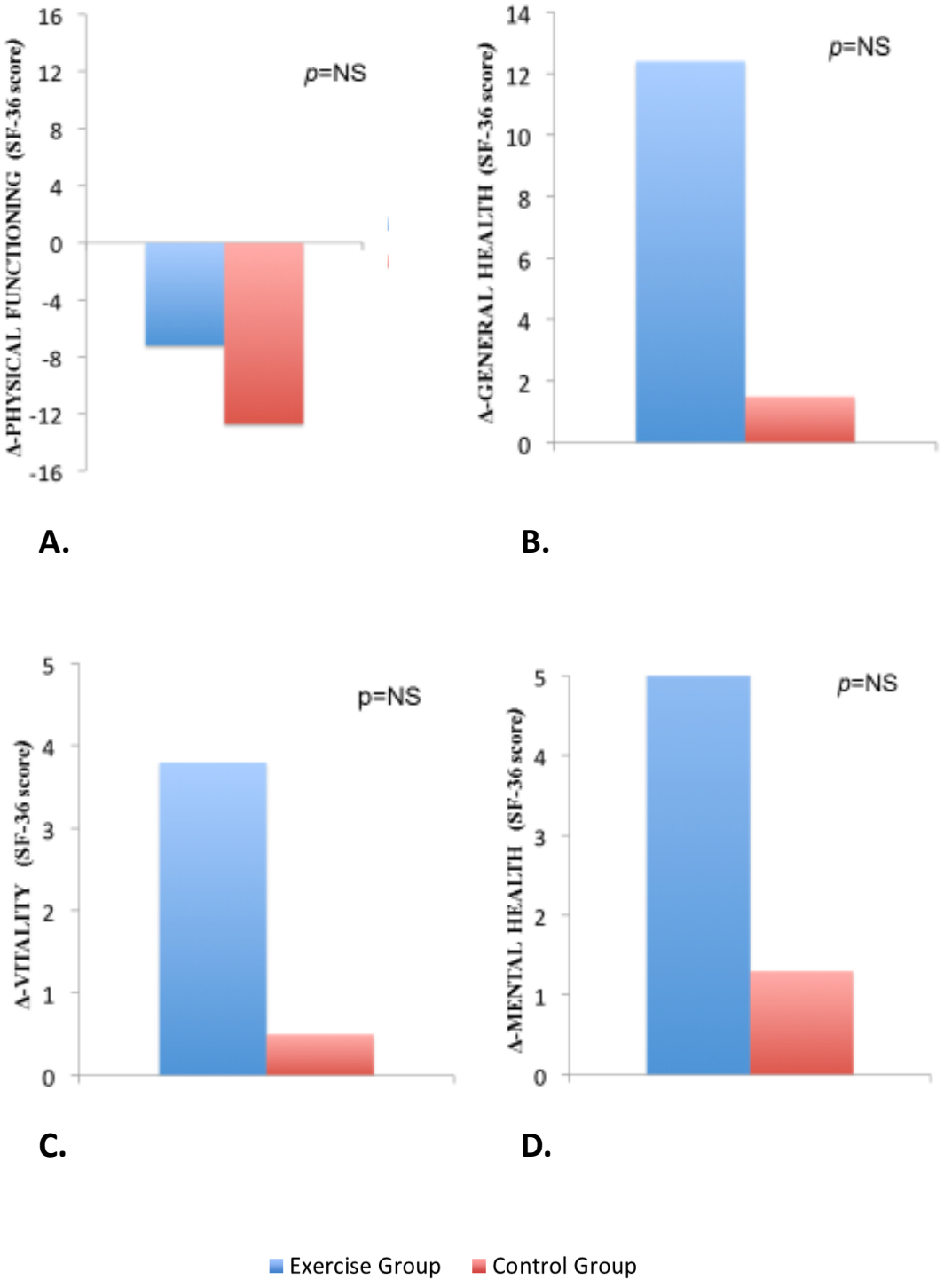

Figure 6. Log2FC (post versus pre) of the 26 miRNA differentially expressed (p<0.05 by paired t-test) in GDM patients after physical intervention

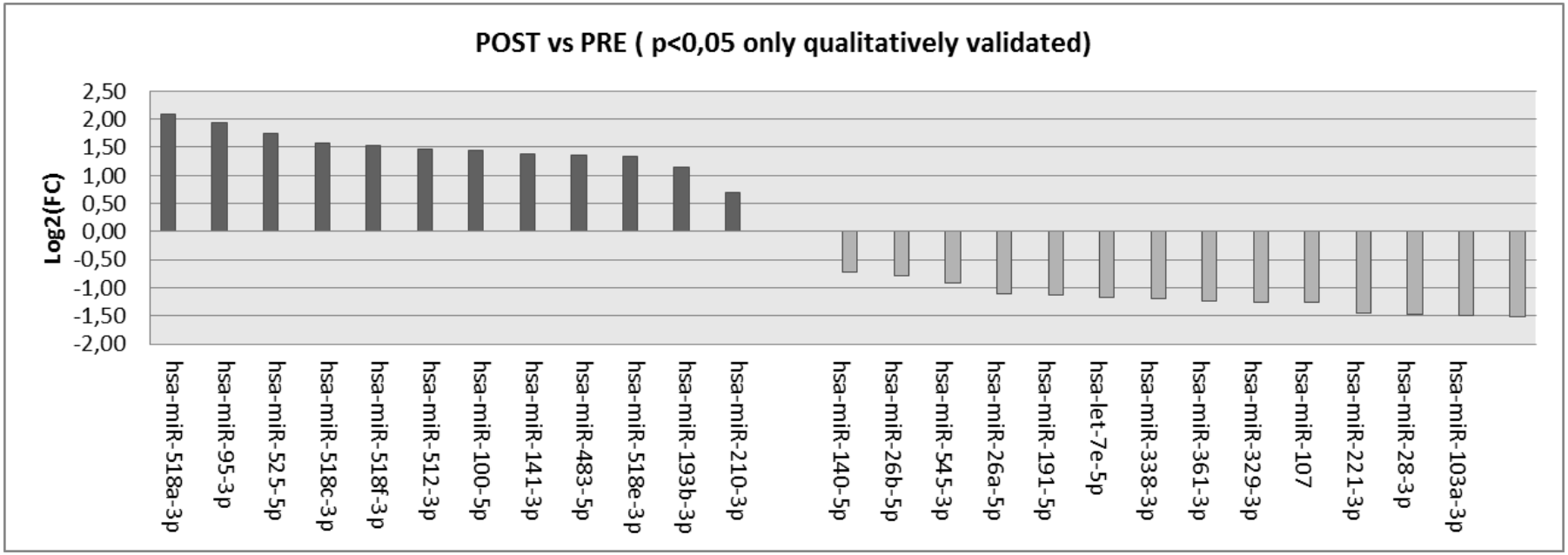

Figure 7. Graphical results of enriched pathways for miRNA target genes

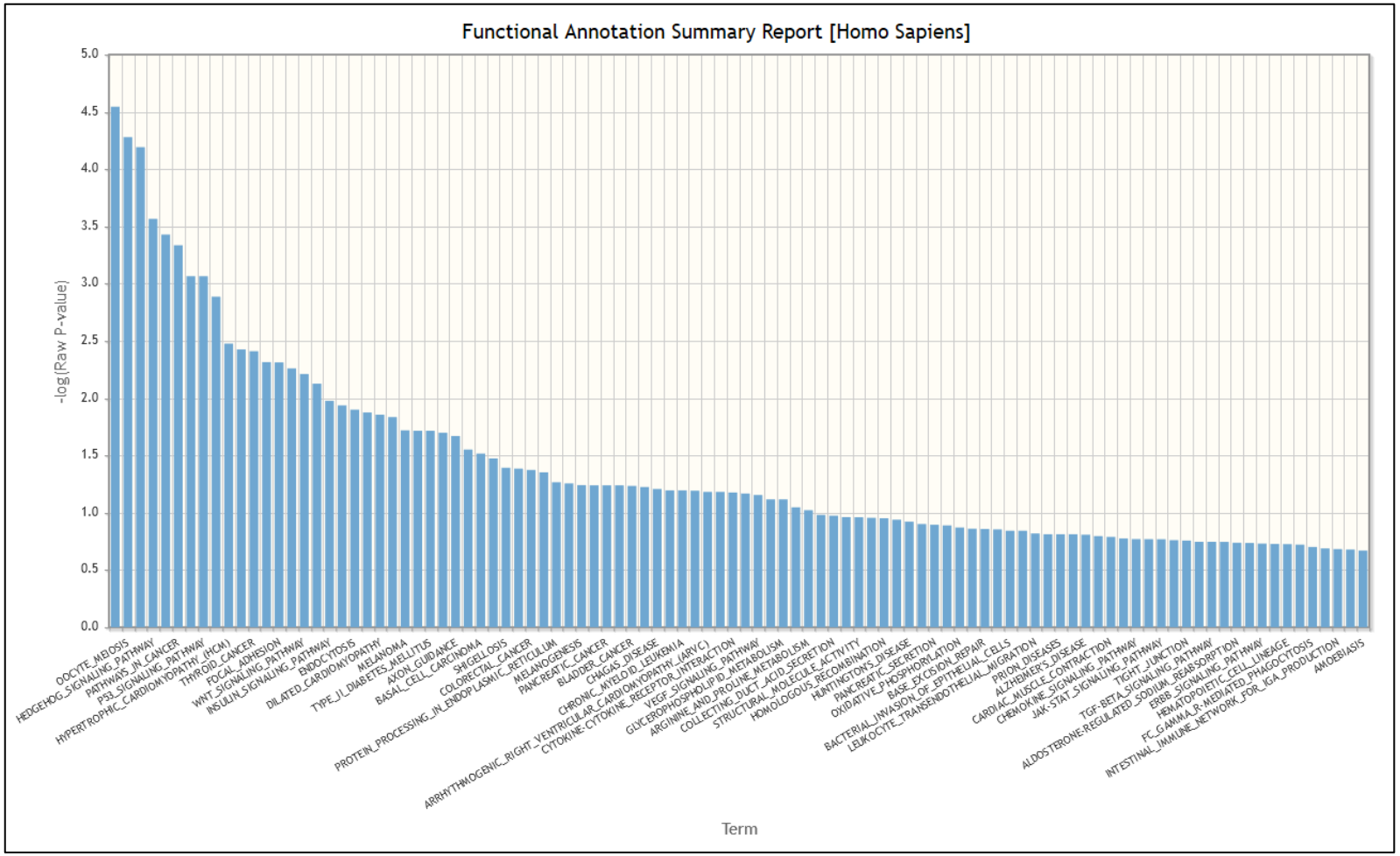

Figure 8. Venn diagram of KEGG metabolic related pathways based on common miRNA targets showing the sheared genes IRS2, MAPK10 and SLC2A4 for Type 2 diabetes, insulin and adipocytokine signaling pathways.