Effects of physical exercise on glucose metabolism and related metabolic signatures in South Asian and Nordic women with previous gestational diabetes - DIAbetes and Exercise in South Asians (DIASA-Ex)

An 8-week exercise intervention trial

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Synopsis

Title of the study: Effects of physical exercise on glucose metabolism and related metabolic signatures in South Asian and Nordic women with previous gestational diabetes - DIAbetes and Exercise in South Asians (DIASA-Ex)
Background: South Asians have a high prevalence of type 2 diabetes (T2D), which develops ~10 years earlier than in Nordic subjects (NO) and remain poorly regulated with increased risk of complications. South Asians with previous gestational diabetes (GDM) have a high degree of insulin resistance and a high risk for developing T2D.
Research hypothesis Physical activity will improve insulin sensitivity in South Asians with previous GDM.
Primary objective: To assess the effect of physical exercise on insulin sensitivity in South Asian women with previous GDM, compare them to Nordic women, also with previous GDM, and perform extensive multi-tissue multi-omics analyses to unravel the molecular basis of insulin resistance in post-GDM women in general and in South Asians in particular.
Study design: Single-centre physical exercise intervention trial. Whole body, hepatic, muscle and adipose tissue insulin sensitivity will be assessed by oral glucose tolerance test (OGTT) with [6,6-D_2]glucose and [1,1,2,3,3-D_5]glycerol tracers. Insulin secretion and hepatic insulin clearance will be estimated using C-peptide deconvolution. Upper and lower maximum body strength, and VO2max, including indirect calorimetry will be tested. Subcutaneous adipose tissue and skeletal muscle biopsies will be harvested, in addition to blood and urine samples. CT or MRI/MRS will be performed to quantify fat depots, muscle mass, bone density and organ lipid deposition.
Recruitment: South Asian and Nordic women with previous GDM.
Duration of study: 8 weeks intervention, with study visits before and after for OGTT, strength/VO2max-tests, CT/MRI/MRS scans, questionnaires, blood samples and tissue biopsies. The project is expected to last a maximum of 4 years.
Power calculations: For the main outcome %-change in whole body insulin sensitivity from baseline to 8 weeks, based on previous data, we expect mean 44.4% increase with a SD of 58.8%. Using a one-sample *t*-test for the treatment effect, and assuming 20% drop-out, we aim for 20 SA, given >80% likelihood of a statistically significant result with α = 0.05. We will also include 20 Nordic controls to perform exploratory analyses.
Study population: Sedentary women ≥18 years of age of South Asian or Nordic ancestry with previous GDM.
Criteria for evaluation: 80% or higher attendance rate for exercise sessions. No change in diet. Safety and tolerability will be assessed by clinical adverse events and laboratory measurements from start to 8 weeks.
Primary outcome: Change in whole body insulin sensitivity from baseline to 8 weeks in South Asians.
Secondary outcomes: Muscle strength, VO2max, body composition, fat oxidation, and multi-omics (DNA methylation, transcriptomics, proteomics, metabolomics, lipidomics) multi-tissue (muscle, fat, urine and blood) analyses. In addition to pre-post-tests, we will also explore cross-sectional comparisons: South Asians vs Nordics at baseline and 8-week, and South Asians at 8-week vs Nordics at baseline, and mixed-effects: Difference of change in response to physical activity in South Asians vs. Nordics.
Statistical Methods: Frequentist statistics (e.g. repeated measures mixed-modelling and linear regression) and artificial intelligence algorithms (e.g. machine/deep learning).

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Abbreviations

ADA	American Diabetes Association
AE	Adverse Event
ANOVA	Analysis of Variance
CRF	Case Report Form
CT	computed tomography
CTU	Clinical Trials Unit
DIASA	DIAbetes in South Asians research program
DXA	Dual-energy X-ray absorptiometry
EASD	European Association for the Study of Diabetes
eGFR	estimated Glomerular Filtration Rate
EGP	Endogenous glucose production
EOT	End of treatment
FFA	Free fatty Acids
FFM	Fat-free mass
FFQ	Food frequency questionnaire
FSFV	First Subject First Visit
GCP	Good Clinical Practice
GDM	Gestational diabetes mellitus
GI	Gastro-intestinal
GMP	Good Manufacturing Practice
Hb	Hemoglobin
HbA1c	Hemoglobin A_{1c}
Hct	Hematocrit
HDL	High Density Lipoprotein
HOMA	Homeostasis model assessment
IC	Indirect Calorimetry
ICH GCP	International Conference on Harmonization Good Clinical Practice
IDF	International Diabetes Federation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IRB/IEC	Institutional Review Board/Independent Ethics Committee
LDL	Low Density Lipoprotein
Leuc	Leucocytes
LSFV	Last Subject First Visit
LSLV	Last Subject Last Visit
MCV	Mean corpuscular volume
MI	Myocardial Infarction
MRI	Magnetic Resonance Imagery
MRS	Magnetic Resonance Spectroscopy
NAFLD	Non-alcoholic fatty liver disease
NEFA	Non-esterified fatty acid
NO	Nordic
PBMC	Peripheral Blood Mononuclear Cells
pGDM	previous gestational diabetes mellitus
Prot	Protein
SA	South Asian
SAE	Serious Adverse Event
SD	standard deviation
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2D	Type 2 diabetes
TGD	Total glucose disposal
TG	Triglycerrides
TC	Total Cholesterol
TSH	Thyroid-stimulating hormone

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Table 1. Key contacts

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Table 4. Study overview

	Visit 1 Health	Visit 2 OGTT	Visit 3 Biopsies	Visit 4 Fitness	Intervention	Visit 6 Fitness	Visit 7 Biopsies	Visit 8 OGTT
Time	Days -14-1	Days -14-1	Days -14-1	Day -14-1	Weeks 1-8	Week 9-11	Week 9-11	Weeks 9-11
Informed consent	x							
Incl./excl. criteria	x							
Group assignment	x							
Medical history	x							
Concomitant medication	x							
Urine	x							x
HCG, USTIX, biobank	x							x
Height, weight, hip, waist	x							x
Blood pressure, heart rate	x							x
BIA/DXA/CT/MR	(dxa)	x						x
Point-of-care	x							x
Glucose	x							x
Clinical chemistry	x							x
HbA1c, Hb, Hct, MCV, MMA, folate, B12, transaminases, albumin, creatinine, Cystatin C, eGFR, TSH, LH, FSH, oestrogen, progesterone	x							x
TC, HDL, LDL, TG,		x						x
OGTT w/tracers		x						x
IPAQ		x						
24h recall	x	x					x	x
Fat biopsy			x				x	
Indirect calorimetry		x						x

RER, and cycling test for VO2max				x		x		
Muscle biopsies			x				x	
Chest press, pull down, leg press testing				x		x		
Patient information				x		x		

Adverse events will be continuously monitored at all visits, including all exercise sessions. For biobanking we will collect fasting serum/plasma/whole blood samples. Whole blood will be used for e.g. HbA1c, DNA methylation and flow cytometry. Serum/plasma will be used for e.g. clinical chemistry, incretins, cytokines, lipases, free fatty acids, extracellular vesicles, and multi-omics (e.g. proteomics, metabolomics and lipidomics). Biopsies will be used for e.g. histology and multi-omics. Patient information: After relevant visits, the participants gets weight, height, waist, waist-hip ratio, BMI, BIA measures of fat% and muscle%, fasting glucose and HbA1c, and BMD. The patient is explained the results. After VO2max, the patient gets O2 per kg per millilitre and an explanation. After strength testing, gets the values and an explanation. 24h recall will be done two days at random both before and after intervention. We will try to do DXA on day 1 to get a more accurate measure of FFM than from BIA.

Trial Design
An 8-week supervised exercise trial in SA and NO women with previous GDM. Gold standard methods for measurement of insulin sensitivity, physical fitness, body composition and multi-tissue multi-omics. 20 women will be recruited in each of the two ethnic groups.

Milestones

Planned First Subject First Visit (FSFV):	01 Oct 2024
Planned duration of recruitment period FSFV-LSFV:	24 months
Planned Last Subject Last Visit (LSLV):	01 Jan 2027

End of trial is defined as LSLV.

Background
South Asians (SA) (i.e. individuals with ancestral origins from India, Pakistan, Bangladesh or Sri Lanka) constitute the second largest immigrant population in Norway. More than 67,000 first and second generation immigrants from SA live in Norway [1], the majority in the larger Oslo area. Diabetes is prevalent among SA in their countries of origin, and particularly after migrating to Western countries [2]. SA immigrants to Western countries develop type 2 diabetes at a younger age than Western Europeans, and generally have poorer glycaemic control than Westerners, despite more intense treatment [3,4]. In Norway the prevalence of type 2 diabetes was higher than 25% in 30 to 60-year-old SA women and 20% in SA men residing in Oslo, compared to 2.7% in Norwegian (NO) women and 6.4% in NO men [5].

The development of type 2 diabetes is a gradual process with several years of worsening glucose metabolism before reaching the type 2 diabetes diagnostic limit. Hence, it is generally recommended to study individuals before they have developed frank type 2 diabetes when the purpose is to elucidate pathophysiological mechanisms behind the disease. Women with previous gestational diabetes mellitus (GDM) constitute a population where such prediabetes is prevalent [6], in the form of impaired glucose tolerance or fasting glucose [7,8]. Furthermore, we now have data showing that SA women has substantially increased risk for type 2 diabetes 11 years after a GDM pregnancy compared to NO women (manuscript in preparation). In DIASA-Ex we therefore aim to study SA women with a history of GDM.

There is evidence of pathophysiological differences in risk factors of type 2 diabetes between SA and white Westerners [3], such as differences in body composition, and glucose and lipid metabolism [9,10]. For patients with type 2 diabetes SA may have higher endogenous (mostly hepatic) glucose production than NO [10,11]. We have demonstrated that, compared to NO, SA appear to have lower insulin secretion in relation to their insulin resistance. Additionally, they show decreased clearance of insulin by the liver across all stages, from normal blood glucose levels to type 2 diabetes [12]. We also have data indicating that reduced insulin clearance in SA might be related to adipose tissue insulin resistance (manuscript in preparation).

Although SA and NO populations differ in risk factors and outcomes for type 2 diabetes, there's a gap in our understanding of the specific disease processes in SA. Currently, we also lack targeted prevention and treatment strategies for type 2 diabetes in SA, although some studies suggest an improved effect of incretin based therapy for type 2 diabetes in SA compared to other ethnicities [13]. However, the effect of physical activity in SA subjects is so far largely unknown, and there is a clear gap in the literature on controlled exercise interventions in SA [14]. To address this, our goal is to enhance the prevention and management of type 2 diabetes among SA. In the past, we started a medication trial for

those at risk of diabetes in the SA group, but it was unsuccessful in recruiting participants and had to be stopped. The main reason was that the women considered themselves healthy and preferred not to take medication, expressing a stronger interest in lifestyle changes instead. In addition, studying how physical activity affect the early stages of type 2 diabetes, before the onset of hyperglycaemia, glucose toxicity, diabetic complications, and permanent changes in insulin secretion and action, will provide valuable insights. Therefore, we are now launching a physical activity program tailored for SA women with previous GDM, but who have not yet developed type 2 diabetes.

Our research group has extensive experience with physical activity trials and mechanisms for improved insulin sensitivity [15], and SA are a main focus in our research centre (https://www.oslodiabetes.no/). We have previously showed that a 12-week exercise trial in sedentary NO men improved insulin sensitivity with ~40%, which is more than any other known treatment [16]. The men also improved their physical fitness and substantially [16]. Associated changes with these improvements were related to improved mitochondrial function [17], reduced liver fat content [18], less inflammation in adipose tissue [19], extensive remodelling of muscle [20], and substantial changes in the serum proteome [21].

Here, we propose a study on the effect of physical activity on glucose metabolism specifically in SA. As we have done previously [15], we aim to perform extensive phenotyping together with multi-tissues multi-omics [22] analyses to unravel the mechanisms behind increased type 2 diabetes risk in SA and the effect of physical activity.

Hypotheses, aims, outcomes

Primary hypothesis

Eight weeks of combined endurance and strength exercise will significantly improve insulin sensitivity in SA women with a history of GDM.

Secondary hypotheses

- Eight weeks of exercise will significantly improve maximum oxygen uptake and muscle strength in SA women with a history of GDM.
- Eight weeks of exercise will significantly improve body composition and reduce ectopic lipid deposition in SA women with a history of GDM.

Exploratory hypotheses

- The effect of exercise is associated with distinct multi-tissue multi-omics changes.
- The effects of exercise on clinical and molecular markers are different between SA and NO women.
- SA women are characterized by distinct metabolic features compared to NO women.
- Clinical characteristics and metabolic features predict of the response in insulin sensitivity and physical fitness after exercise.
- Exercise reduces future health risk as explored by changes in cardiovascular risk factors.

Primary aim

To evaluate the effects of a supervised exercise intervention on insulin sensitivity.

Secondary aims

- To evaluate the effects of the 8-week intervention in increasing physical fitness, reflected in improved maximum oxygen uptake and muscle strength, in SA women with a history of GDM.

- To evaluate the effects of the 8-week intervention in increasing muscle mass, reducing fat mass and reducing ectopic lipid deposition in SA women with a history of GDM.

Exploratory aims

- Identify and characterize the multi-tissue multi-omics changes occurring in response to exercise, such as related to epigenomics, transcriptomics, proteomics, lipidomics and metabolomics.
- Compare physiological and molecular responses to exercise between SA and NO women. This involves evaluating differences in changes to physical fitness, metabolic features, and other relevant biomarkers following the 8-week intervention.
- Explore metabolic profiles distinguishing SA women from NO women. This includes for example insulin resistance, physical fitness, energy expenditure, metabolic biomarkers, and substrate utilization rates.
- Develop predictive models based on clinical and metabolic features that can forecast improvements in insulin sensitivity and physical fitness following the 8-week exercise intervention. This requires collecting and analysing pre-exercise clinical data, metabolic features, and exercise outcomes to identify predictors of response.
- To analyse if exercise influence the likelihood of future cardiovascular disease risk.

Main outcome

Change in insulin sensitivity after 8 weeks of exercise in SA women with a history of GDM.

Secondary outcomes

- Quantifying and comparing VO2max, and chest press, pull down and leg press strength before and after the intervention in SA women with a history of GDM.
- Quantifying and comparing lean body mass, fat mass and fat stored in non-adipose tissues (e.g., liver, muscle, heart and pancreas) before and after the intervention in SA women with a history of GDM.

Exploratory outcomes

- Analyse responses in multi-omics (e.g., DNA methylation, mRNA, proteins, lipids and metabolites) related to multiple tissues (e.g., in blood, muscle, adipose tissue, and urine, and indirect liver-measures) in response to the 8-week intervention.
- Compare responses to the 8-week exercise trial in SA vs. NO women in terms of clinical and molecular features.
- Explore cross-sectional differences between SA and NO women in terms of e.g. insulin sensitivity, physical fitness, body composition, substrate utilization and multi-tissue multi-omics.
- Correlate pre-intervention clinical and metabolic features to change scores in response to exercise and construct predictive artificial intelligence/machine learning models.
- Calculate indices of future health risk, such as relevant cardiovascular disease scores, before and after the intervention.

Ethical reflection

In general, minority groups have an increased risk of disease. The reason our research group focuses on South Asians is that they have a significantly increased risk of diabetes, both in their home countries but especially when they migrate to Western countries like Norway, and also increased risk for diseases in general. This risk for type 2 diabetes is particularly high after gestational diabetes, which can be considered a "pre-diabetes"

condition with an accelerated pathogenesis towards type 2 diabetes. In Norway, South Asians develop type 2 diabetes at a younger age and with a lower BMI than Norwegians. Furthermore, treatment goals are more often not achieved in South Asians compared to Norwegians, and South Asians remain at a higher risk for diabetes complications and cardiovascular diseases. Therefore, there is a need to improve prevention and treatment for South Asians, especially after gestational diabetes. Lifestyle interventions, such as physical activity, are a cornerstone in the prevention and treatment of type 2 diabetes. However, there is generally a lack of data on lifestyle interventions, particularly physical activity, among South Asians, and it is still unknown whether exercise can reduce insulin resistance, the key pathogenic step towards type 2 diabetes, in South Asians. A major challenge when studying different ethnic groups is to avoid marginalization, which requires a sensitive and inclusive approach that respects cultural, social, and historical contexts. We strive not to reinforce stereotypes or contribute to discrimination by designing inclusive studies (all South Asian and Nordic countries), engaging with community leaders, and being culturally competent. We obtain informed consent and have systems in place to protect privacy. We focus on avoiding biases during data collection and interpretation, and we communicate findings in a way that does not stigmatize.

The relevance of muscle and fat tissue analysis to research objectives

The primary metabolic tissues related to insulin resistance (the key pathogenic step towards type 2 diabetes), and thus type 2 diabetes, are muscle, liver, and adipose tissue. Although blood can be a marker for systemic changes in terms of insulin resistance and exercise effects, we have previously observed clear differences in the amount and distribution of muscle and adipose tissue, as well as differences in peripheral (muscle/adipose) and central (liver) insulin resistance between South Asians and Norwegians. For example, South Asians have lower BMI than Norwegians but a higher risk of diabetes. Therefore, to truly understand the mechanisms behind the increased diabetes risk in South Asians and their potential response to exercise, we need tissue-specific data. Fat/muscle samples will provide unique data on, for example, mitochondrial function, intramuscular lipid distribution, cytokine secretion, and interactions between immune cells, all of which are linked to insulin resistance. Furthermore, tissue-specific data combined with blood samples can offer more detailed insights into which tissues contribute to systemic changes, thus enabling the identification of new biomarkers and mechanisms. Recent data indicate that insulin resistance and the effects of exercise result from intricate tissue-tissue cross-talk, for example, through cytokine signaling (“exerkines”), extracellular vesicles, miRNA, and certain fatty acids. To understand this interplay and how it contributes to diabetes risk and the effect of exercise in South Asians, we need more tissue samples. Therefore, we plan to collect blood (systemic changes), muscle and adipose tissue (specific changes), and urine (a marker for kidney function). Our study will also serve as a foundation for future studies, such as creating knockout mice for genetic targets discovered based on muscle data or following up with in vitro studies based on tissue-specific observed changes. The tissue samples are also crucial for identifying new drug targets and treatment options to enable personalized medicine for South Asians.

Recruitment

Participants will be recruited from the 216 SA and 108 NO women that participated in previous parts of the DIASA-program (DIASA1) and from external sources.

From DIASA1: We have the phone numbers of all the women who participated in DIASA1 and who consented to be contacted for future DIASA studies, along with their preferred language (the researcher most fluent in the woman's preferred language will make the call).

These numbers are written on paper and stored in a protected and locked research facility at the Diabetes Research Laboratory at Aker Hospital. We will contact each DIASA1 woman via phone. They will not be recruited during this call; they will only receive information about previous DIASA1 results and upcoming DIASA1 informational seminars. At the end of the call, they will receive brief information about DIASA-Ex and be asked if they would like written information. If they consent, we will send them the information via email (emphasizing that no sensitive information should be included in the email), a brochure (to better understand the concept of the study, see attachment "Flowchart.v3.1.05.07.24.PS"), and informed consent. We also offer a follow-up call/physical meeting/digital meeting for more information/to discuss further/answer questions, and potentially sign the informed consent.

From External sources: We will also recruit subjects who did not participate in DIASA 1. We will invite potential participants through posters at relevant locations, such as 'Helsestasjon' and participating hospitals, as well as through ads in media.

No study related procedures will be performed before a new signed informed consent form has been collected.

Participant compensation for time and expenses
The training is free of charge, and participants will be offered re-imbursement for travel expenses with public transportation. They will not receive compensation for lost wages unless there is a special agreement, but participants will be offered a small compensation for their use of time in test-days, in the form of giftcards and travel reimbursement for each of the test days before and after the training intervention. There are 6 test days (clinical tests x 2, oral glucose tolerance tests x 2, and tissue samples x 2), 3 before and 3 after the intervention. Two of these are full-day examinations, while the remaining four are half-day examinations. They will receive a total of 3100 NOK for participating in these test days as a combination of gift cards and reimbursements, in addition to travel reimbursements, as compensation for their time. They may also receive reimbursement/gift cards worth 500 NOK per day for each of the 6 test days if they need childcare for children not attending kindergarten.

Table 5. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Able and willing to give informed consent. Woman ≥18 years of age. Of SA or NO ancestry. Previous GDM.	Known type 1 or type 2 diabetes. Pregnant or fully lactating at inclusion or planned during study period. Concomitant use of any antidiabetic medication, fibrates or rifampicine. Known serious illness such as cancer (except in situ carcinoma) during past 5 years. Previous radiation therapy directed towards the pelvic area. Heart failure NYHA class I-IV. eGFR<60 ml/min/1,73m^2. Chronic liver disease with ASAT or ALAT >5xULN or known impaired liver function (INR >1.5, albumin <20 g/l, bilirubin >20 g/l). Active infectious disease at inclusion. Use of systemic corticosteroids >14 days within last three months before inclusion. Hypothyroidism where treatment has not been stable for the last 3 months or with TSH outside normal limits. Macroscopic haematuria not previously examined. History of major surgical procedures within 3 months prior to inclusion or planned during study period. Any condition

	which in the investigator's opinion would jeopardize the subject's safety or compliance with the protocol. Hard exercise >75 min per week.

Power calculations
For the main outcome %-change in whole body insulin sensitivity from baseline to 8 weeks, based on previous data, we expect mean 44.4% increase with a SD of 58.8%. Using a one-sample *t*-test for the treatment effect, and assuming 20% drop-out, we aim for 20 SA, given >80% likelihood of a statistically significant result with α=0.05.

For the secondary outcomes, %-change in VO2max and muscle strength (leg press, pull down and chest press), based on previous data, we expect mean 15% increase with a SD of 8% for all these outcomes after 8 weeks of exercise. Using a one-sample t-test for the treatment effect for 20 South Asians, we will obtain achieved power of >99% of a statistically significant result with α=0.05.

We will also include 20 Nordic controls to perform exploratory analyses. No power calculations are performed for exploratory outcomes because they will only serve hypothesis generation.

Study procedures and investigational methods
Group assignment
Women will be assigned to either the SA or NO groups based on their self-reported ethnicity.

External validity
After consent, basic clinical and sociodemographic data on non-included women, and potentially excluded women, will be used to analyse if the participant completing the study differ from the general population of women with previous GDM.

General info regarding intervention visits
The women will attend group-based exercise sessions with a trained instructor at NIH four times weekly for 8 weeks. Extra follow-up if needed is performed by phone/email. Alternatively, if deemed impossible to physically attend four sessions each week for 8 weeks, the participants may perform the endurance exercise sessions at home or at a local gym.

Diet
Participants will document their regular diet using validated 24h recall, twice before and twice after the intervention. They will be instructed to limit their alcohol consumption to no more than two units per day, and to not change their diet during the intervention.

Continuous glucose monitoring
We include continuous glucose monitoring (CGM) for the participants for 14 days at the start and end of the intervention. CGM data will provide high-resolution glucose profiles in relation to OGTT and physical activity. Participants will also receive feedback on their "time in range" and coefficient of variation both before and after the intervention. The participant information sheet will be updated to reflect this addition.

The "OGTT"
Table 4 provides an overview of all examinations at all visits. Three days prior to these visits the subjects will abstain from alcohol, unusual diet and strenuous physical exercise. The first

of these visits will be performed within two weeks before the intervention starts. The end-of-study visit is three days after the last bout of exercise. The subjects arrive at the study site in the morning after an overnight fast of at least 8 h (including no smoking or use of snuff). The participant is informed about the study in writing and orally, and signs an informed consent before any study related activities takes place. Height, weight, waist, hip and BIA etc. are measured (see Table 4). She will empty her bladder, urine samples are collected, and a pregnancy test is performed. All patients will remain fasted throughout the OGTT.

OGTT. After collection of basal blood samples, an infusion of stable isotope-labelled glucose ([6,6-2 H2]glucose; priming dose of 17.6 μmol kg−1 × Y/5, where Y stands for fasting plasma glucose in mmol/l, and continuous infusion of 0.6 μmol kg−1 min−1) and glycerol ([1,1,2,3,3-2H5]glycerol; priming dose of 2.0 μmol/kg and continuous infusion of 0.1 μmol kg−1 min−1) was initiated at time −120 min. Isotope enrichment at different time points was calculated by subtracting basal enrichment. After 2 h of tracer infusions (to obtain tracer steady state), at time 0 min, the participants drank 75 grams of anhydrous glucose added 2.5 grams of [U-13C6]glucose. Fasting blood biobanking will be done at times -120 (before tracer infusion starts). Samplings of c-peptide and insulin (2.5 ml) will be done at times 0, 15, 30, 60, 90, and 120. Tracer samples (2 ml) are drawn at times -120, -30, -15, 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 240. A glucometer will be used at times -120 and 120 for patient information.

Resting indirect calorimetry is done by using a computerized flow-through canopy gas analyser system. After a 10-minute adaptation period, a 10-minute registration will be performed. Based on findings from DIASA2, which show that women with previous GDM exhibit imparied switch from fat to carbohydrate metabolism in response to insulin and glucose, we also add indirect calorimetry after the OGTT for selected participants. These measurements will be performed both before and after the intervention. It will allow us to assess metabolic flexibility and the impact of the exercise intervention.

The biopsy visits
Abdominal adipose tissue biopsies will be obtained in the fasted state. After assuring that the participant has not previously had an allergic reaction to lidocaine, subcutaneous adipose tissue will be collected from the periumbilical region using a suction-based biopsy procedure. The skin will be sterilized, and a semi-circular area of the underlying tissue will be anesthetized by injecting 5 mL of a local anaesthetic with lidocaine without adrenaline. A needle attached to a vacuum syringe will be used for the insertion. The adipose tissue will then be placed into a 50 mL tube filled with cold NaCl. Subsequently, the NaCl will be removed by decanting the contents through a Gauze cloth. This cloth will be used for the dissection of the tissue. Adipose tissue samples will be quickly frozen in 1.5 mL cryotubes and stored at -80°C.

Muscle biopsies will be obtained from the M. vastus lateralis using a modified Bergström procedure. The area will be sterilized using chlorhexidine-ethanol, followed by the careful injection of a local lidocaine with adrenaline anaesthetic into the subcutaneous and superficial tissues. Initially, biopsies will be taken from the right leg, and after 8 weeks, from the left leg. A 6 mm diameter muscle biopsy needle attached to a 50 mL syringe will be used to manually create a vacuum for the biopsy. The muscle biopsies will then be promptly washed in cold PBS and dissected on a chilled aluminium plate using a magnifier. Tissue designated for RNA isolation will be immediately placed in RNA-later (Qiagen, Hilden, Germany) overnight, after which the solution will be removed before the samples are stored at -80°C.

The "Physical fitness visits"
The participants arrive at NIH fasted were they will be served a standardized meal before performing strength tests followed by a VO2max test. They can drink water freely during the testing day.

Muscle strength will be tested as one repetition maximum (1 RM) in the leg press and chest press. Familiarization to the tests will be performed within one week before testing. After 10 min of brisk walking on a treadmill, a range of warm-up sets is performed before both exercises with 10, 6, 3, and 1 repetitions at 50%, 70%, 80%, and 90% of the expected 1 RM, respectively. 1 RM should be achieved within 2 to 5 attempts, with 2 to 3 min of rest separating attempts. Knee flexion during leg press is set to 90 degrees and grip width in the chest press is standardized. Load increments during tests can be made by a minimum of 5 kg and 1 kg for leg press and chest press, respectively.

Peak oxygen uptake (VO2peak) will be assessed by a cardiopulmonary exercise test performed by walking and running on a treadmill, or using a cycle ergometer, using a modified Balke protocol [23-25]. Gas exchange and ventilatory variables will be measured continuously breath-by-breath. Peak heart rate will be measured through a 12-lead ECG, and blood pressure and transcutaneous oxygen saturation will be measured after five-minutes rest in sitting position, during the test, and shortly after termination. Borg rating of perceived exertion will be assessed at the end of the test. VO2peak is defined as the highest oxygen consumption value sampled over a 30-sec interval. The ventilatory threshold will be calculated using the ventilatory equivalent method expressed as VO2. The minute ventilation (VE) and carbon dioxide (CO2) responses during exercise will be used to calculate the VE/VCO2 slope. The breathing reserve will be calculated using measured maximal voluntary ventilation (MVV) and the maximal minute ventilation (VE) using the following equation: ((MVV-VE)/MVV) x 100 and oxygen pulse will be calculated by dividing VO2peak (in mL) by the peak heart rate [23-25]. Blood samples will be taken before and after the test.

At the end of the testing day, the participants will get an information hand out with their leg press, chest press and VO2max results together with a brief explanation and e.g. a comparison to a reference population.

Body composition
Examinations will be performed fasted without and no strenuous exercise is permitted the 24h before. The participants arrives at the radiology department and must fill out a safety check-list (if not already filled out), including metal objects in the body, as is the standard before any MR procedures. An ancle-to-neck MRI and an MRS protocol will be performed to quantify adipose tissue depots, muscle mass and ectopic fat. In a worst case scenario, if we cannot coordinate MR for the women, a simpler 4 slice CT abdomen/thigh (excluding the pelvic area) protocol will be used. DXA and BIA will also be performed and the results will be readily available for the study participates and they will receive written and oral explanations of the results at the visit.

Strength and endurance exercise intervention
Participants will engage in a weekly exercise regimen that includes two sessions of strength training and two sessions of spinning bike intervals, totalling four hours of vigorous activity. The strength training will include with a 10-minute aerobic warm-up, followed by three sets of various exercises: leg presses, leg curls, chest presses, cable pulldowns, shoulder presses, seated rows, abdominal crunches, and back extensions. Initially, exercises will be performed

using a weight that can be lifted 12 times (12-RM), which will be adjusted to 10 repetitions (10-RM) starting from the fourth week. However, abdominal crunches and back extensions will be done for 12–20 repetitions. Weights will be continuously modified to ensure maximum effort for the designated repetitions.

The endurance component will consist of spinning bike intervals: one session with 7-minute intervals at 85% of estimated maximum heart rate (HRmax), and another with 2-minute intervals exceeding 90% of HRmax. Recovery between intervals will involve rest or light cycling, with rest periods set at 3 minutes for the 7-minute intervals and 2 minutes for the 2-minute intervals. The frequency of 7-minute intervals will increase from 3 to 4 after two weeks, and then to 5 after six weeks, while the 2-minute intervals will increase from 6 to 7 after two weeks and further to 10 after six weeks.

End of treatment visits
After 8 weeks of exercise all women will again be subjected to the OGTT, body composition estimations, fat and muscle biopsies, and VO2max and strength testing etc. Please see Table 4 for details. Adverse events will be assessed. Participants will get a final report to take home, including pre and post values of e.g. glucose, HbA1c, weight, BMI, BIA, VO2max and strength measures etc. They will also get information on what the results may indicate regarding their health and effect of the intervention.

Post-intervention interview
The interviews aim to gather insights from participants of the DIASA-EX study, focusing on their experiences with the intervention and investigations. For participants who completed the study, the goal is to understand their motivations, encountered challenges, and suggestions for improving clinical trials, exercise sessions, and other interventions. For those who dropped out, the focus shifts to identifying barriers and reasons for discontinuation.

This feedback is essential for tailoring future interventions, ensuring they are participant-friendly and addressing potential challenges. The interviews are guided by structured questions and are conducted with strict confidentiality, using data solely for research purposes. The study's findings will support the development of more effective interventions in similar contexts.

1-year follow-up
We will invite participants who completed the 8-week exercise intervention to a simplified 1-year follow-up visit. This single follow-up visit will include a shortened oral glucose tolerance test (OGTT) without tracers with a total duration of 2 hours, assessment of body composition by bioelectrical impedance analysis, measurement of body weight, waist and hip circumference, and blood pressure. Blood sampling will be limited to five time points. Continuous glucose monitoring (CGM) will be repeated for a new monitoring period. Participants will also complete questionnaires including a 24-hour dietary recall, physical activity during the last 7 days (IPAQ), and a short questionnaire assessing physical activity over the past year (PYTPAQ). No imaging, indirect calorimetry, fitness testing, or tissue biopsies will be performed at the 1-year follow-up. The aim is to evaluate the longer-term maintenance of metabolic and clinical changes induced by the intervention using a participant-friendly follow-up protocol. Participants will be re-consented prior to follow-up assessments.

Premature discontinuation

If a participant needs to stop the trial prematurely she will be asked to inform the study personnel as soon as possible. The study investigator will decide whether full end of study visits should be performed. Attendance to exercise sessions should be at least 80%, be lower than this may be accepted if close to 80% and/or the participant has obtained expected improvements in physical fitness. Despite attending end of study visits or not, the participant will be contacted e.g. by phone to document reasons for discontinuation.

Some methodological considerations
Aliquots of whole-blood is stored for flowcytometry. Aliquots of platelet-free plasma will be stored for analyses of EVs by centrifuge plasma at 2500 g for 15 minutes to remove most of the platelets before freezing. These samples will serve to e.g., isolate EVs with size exclusion chromatography, and then characterize nanoparticle tracking, size distribution, and various exosomal markers, such as miRNA.

Adverse events
The investigator or site staff will be responsible for detecting and documenting events that meet the definition of an Adverse Event (AE) and for detecting, documenting and reporting Serious Adverse Event (SAE).

Definition of an adverse event:
An AE is defined as any untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject participating in a clinical trial, which does not necessarily have to have a causal relationship with this clinical trial. An AE can therefore be any unfavorable and unintended sign (an abnormal laboratory finding), symptom or disease temporally associated with the participation in a trial, whether or not associated with the clinical trial.

Definition of a Serious Adverse Event (SAE)
- An SAE is any untoward medical occurrence that:
- Results in death,
- Is life-threatening,
- Requires hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

All identified AEs must be recorded and described on the appropriate Non-serious or Serious AE page of the CRFs and in AE and SAE tables. If known, the diagnosis of the underlying illness or disorder should be recorded rather than its individual symptoms. The following information should be captured for all AEs: date of onset and resolution, severity of the event (see definition), investigator's opinion of the relationship to the medicinal product (see definition), treatment required for AE, cause of event (if known) and information regarding resolution/outcome (see definition) The investigator shall supply the Sponsor with any additional requested information.

The following categories and definitions of severity should be used:
- Mild (grade 1) – Awareness of event but easily tolerated: Asymptomatic or mild symptoms. Clinical or diagnostic observations only. Intervention not indicated.
- Moderate (grade 2) – Discomfort enough to cause some interference with usual activity: Minimal, local or noninvasive intervention indicated. Limiting age-appropriate instrumental ADL.

- Severe (grade 3) – Inability to carry out usual activity: Severe or medically significant but not immediately life-threatening. Hospitalization or prolongation of hospitalization indicated. Disabling, limiting self-care ADL.
- Life-threatening (grade 4) – Urgent intervention needed. Debilitating, significantly incapacitates subjects despite symptomatic therapy.
- Death (grade 5) – Death related to AE.

The following categories and definitions of causal relationship to clinical trial should be used:

- Probable: There is a reasonable causal relationship between the clinical trial and the AE. The event responds to dechallenge with or without rechallenge.
- Possible: There is a reasonable causal relationship between the clinical trial and the AE. Dechallenge information is lacking or unclear.
- Not likely: There might be a temporal relationship to clinical trial, but there is not a reasonable causal relationship between the clinical trial and the AE, or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE.

The following categories and definitions of outcome categories should be used:

- Recovered: Fully recovered, or by medical or surgical treatment the condition has returned to the level observed at the first trial related activity after the subject signed the informed consent.
- Recovering: The condition is improving and the subject is expected to recover from the event. This term should only be used when the subject has completed the trial.
- Recovered with sequelae: As a result of the AE the subject suffered persistent and significant disability/incapacity (e.g. became blind, deaf, paralyzed). Any AE recovered with sequelae should be rated as an SAE.
- Not recovered
- Fatal
- Unknown: This term should only be used in cases where the subject is lost to follow-up.

Collection, recording and reporting of adverse events

All events meeting the definition of an AE must be collected. At each contact with the trial site (visit or telephone), the subject must be asked about AEs. All AEs, either observed by the Investigator or reported by the subject, must be recorded by the Investigator on the standard adverse event form in the CRF and evaluated. If more than one sign or symptom is to be reported, use a separate adverse event form for each sign and symptom. AEs that are not serious will not be further reported. For SAEs, the serious adverse event safety information forms must also be completed. The Sponsor Detailed records of all serious adverse events relating to a clinical trial which are reported will be kept by the principal investigator.

The staff at the trial site must report initial information on all SAEs to the principal investigator within 24 hours of obtaining knowledge about the event. The investigator must inform the regulatory authorities and IECs/IRBs in accordance with the local requirements in force and ICH GCP.

Emergency preparedness plan

Acute cases (such as allergic reactions, cardiac arrest, bleeding, sports injuries, or fainting) will be handled through established routines at all visit locations. Necessary equipment (such as a heart defibrillator and adrenaline) will be available along with the phone number

for the ambulance service. During OGTT and blood/tissue sampling, a doctor will be present to manage any serious events. Emergency equipment, including a defibrillator and adrenaline for allergic reactions, will be available. Contact phone numbers for emergency services and ambulances will be readily accessible. Equipment to stop minor bleeding will be prepared. These cases will be treated, informed, and followed up by a doctor. Water and food will be available if needed. During exercise sessions/testing, a defibrillator will be available, and staff will be trained to handle cardiac arrest. Contact phone numbers for emergency services and ambulances will be easily accessible. Water and food will be available if necessary. Sports injuries will be assessed by a study nurse or doctor and referred to the appropriate facility (emergency clinic or general practitioner). All serious events will be documented, and participants will be followed up by a DIASA researcher who is also a doctor. The researcher will ensure that the participant's general practitioner is informed and that there is a follow-up plan. If a participant experiences anxiety or other mental health issues, a DIASA researcher who is also a doctor will provide support. If necessary, the participant will be referred to psychiatric care. For motivation (e.g., helping them understand that it will take time to achieve the benefits of exercise) and empathy during the exercise intervention, participants will be contacted, if they wish, every other week. Many of the women may lack family support during the time they are away and may feel guilty about leaving small children. They will also receive the number of the Diabetes Laboratory to contact us if needed.

Premature termination of the study
The Sponsor or the DIASA Steering Committee has the right to terminate the study prematurely. Reasons may include efficacy, safety or futility, among others. Should the Sponsor or Steering Committee decide to terminate the study, the investigator(s) will be notified in writing.

Statistics
Data will be analysed as per protocol. However, additional analyses will be performed to assess external validity of the results and to identify key factors for discontinuation.

For the main outcome, change in insulin sensitivity in SA, a random intercept linear mixed model with participants as a random effect and visits as a fixed effect, will be performed to study the trajectory during the trial. The secondary outcomes will be analysed similarly as the main outcome. We will perform hierarchical testing for reduce the problem with multiple testing. Explorative outcomes will not be hierarchically tested.

For exploratory outcomes, where appropriate, we will apply a correction for multiple testing. Our preferred method of choice is the Benjamini-Hochberg approach set at an false discovery rate of 25% (maximum of 1 out of 4 false positive results).

For large-scale data we will apply a range of methods such as artificial intelligence/machine learning algorithms, clustering, feature selection methods, variable reduction techniques, partial least square regressions and multi-omics integration mm..

Data management and monitoring
The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual examined in the trial. Data will be reported in each individual’s CRF and will be derived from and consistent with the source documents. The CRF will be completed electronically or legibly in ink. Subjects are to be identified by initials, date of birth and

subject identification number. All relevant questions will be answered, leaving no blank spaces.

The confidentiality of records that could identify subject must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirements. The investigator will maintain a Signature Sheet to document signatures and initials of all persons authorized to make entries and/or corrections to the CRFs. A correction must be made by striking through the incorrect entry with a single line and entering the correct information adjacent to the incorrect entry. The correction must be dated, signed and explained if necessary and must not obscure the original entry. The completed CRF must be promptly reviewed, signed and dated by the investigator or a sub-investigator. Data will be stored according to GCP and national authority regulations.

Source data verification
The Clinical Trials Unit at Oslo University Hospital Research Support will monitor the study. The investigator agrees to allow any local monitoring, audits, IRB/ERC reviews and regulatory authority inspection direct access to source documents (original documents, data and records). Direct access includes permission to examine, analyze, verify and reproduce any record(s) and report(s) that are important to the evaluation of the clinical trial. An Investigator Source Document Verification Agreement will be completed in order to describe where source data for each data item can be found. As a minimum requirement the following data must be source data verifiable in source documentation other than the CRF:

- Existence of subject (subject identifier, date of birth)
- Confirmation of participation in the trial (subject ID, trial ID and signed and dated informed consent form(s))
- Diagnosis/indication under investigation
- Visit dates
- Anthropometrics
- Results from fasting and procedure related blood tests
- OGTT glucose levels, including at the end of the OGTT before leaving the hospital
- Data from adverse event form(s)
- Relevant medical history/concomitant illness
- External questionnaires
- Reason for exclusion or withdrawal when given

Host institution
Oslo University Hospital, Department of Endocrinology, Morbid Obesity and Preventive Medicine.

Collaborators
For recruitment, Akershus University Hospital, Department of Endocrinology, Vestre Viken Drammen Hospital, Department of Endocrinology and the Norwegian University of Sports Sciences.

For analyses, University of Glasgow, Scotland, University of Lyon, France. Other possible collaborators within or outside the EU can be added at a later stage.

Project period
The project period is until January 2033. There will be extensive analysis and publication work after the last participant has completed the intervention, and the tentative timeline is as follows:

Year	Activities
2024-2025	Recruitment, implementation of the intervention, and data collection.
2026-2027	Data analyses and presentations
2027-2028	Multi-omics, analysis, publication, presentations. First PhD.
2028-2029	Multi-omics, analysis, publication, presentations. Follow-up experiments.
2029-2030	Multi-omics, analysis, publication, presentations. Follow-up experiments.
2030-2031	Multi-omics, analysis, publication, presentations. Secund PhD.
2031-2032	Multi-omics, analysis, publication, presentations. Follow-up experiments.
2032-2033	Multi-omics, analysis, publication, presentations. Follow-up experiments.

This study is a multi-tissue multi-omic study, meaning that extensive data will be generated. Hence the need for a project period until January 2033.

Data collection
Quantitative data collected will be entered into approved electronic case report forms (CRF). All data will be stored deidentified with an individual code for each participant on an approved system, and later in the University of Oslo secure research server, accessible only to specified personnel.

The code key will only be stored digitally using an approved program at Oslo University Hospital, which is located in a protected data area on the hospital's network.

Collected information will be retained for 5 years after the end of study for documentation purposes, and will then deleted.

Human biosamples
Blood, adipose tissue, muscle and urine will be collected deidentified for direct analysis or for storage in a study specific biobank for later batch analysis. All biobank material will be kept de-identified until the end of the study and destroyed at the latest by the end of the study.

Retention of clinical trial documentation
Each participating subject’s medical records must be kept for 5 years after the end of the study (electronic and paper-based records pertaining to the trial). Medical records will be kept in the hospital’s electronic records and other source documents will be scanned and stored in Oslo University Hospital’s secure research server or sent to a secure storage facility after end of study. Collected information will be retained for 5 years after the project is completed and then deleted.

Responsibilities
The principal investigator is accountable for the conduct of the trial and must ensure adequate supervision of the conduct of the trial at the trial site. The investigator must maintain a log of appropriately qualified persons to whom he or she has delegated specified

trial related duties. The investigator must ensure that there is adequate and documented training for all staff participating in the conduct of the trial. It is the investigator's responsibility to supervise the conduct of the trial and to protect the rights, safety and well-being of the subjects.

Ethics

The study will be sought approved by the Regional Ethics Committee and will conform to the Helsinki declaration and the ICH-Good Clinical Practice guidelines. All participants will give informed consent before any study related procedure takes place. The study-related procedures planned are judged by the principal investigator and the steering committee to not pose an unacceptable risk to participants' health or well-being.

Subjects will be exposed to study procedures including peripheral venous catheters and a stable isotope tracers. In the event of incidental radiology findings unrelated to the study, our collaborating radiologist will make sure the scans are re-examined by dedicated radiologists at Oslo University Hospital, and the participant will be informed about the finding(s). Such incidental findings may cause distress in the participant. She will receive support and advice about any need of further investigations, and will be asked to involve her GP.

The participants will also be subjected to biopsies. Some of these procedures may cause moderate pain or discomfort, and in rare cases more serious adverse effects like allergic reactions and haematomas. The risk of these occurrences is deemed low, and measures to prevent them happening and to treat them and prevent permanent damage is of high priority.

Reports and publications

Results will be sought published in international high impact peer-reviewed journals, and abstracts presented at international scientific meetings such as the European Association for the Study of Diabetes (EASD) annual meeting, International Diabetes Federation (IDF) world conference, and the American Diabetes Association (ADA) annual meeting, and at national and regional scientific meetings. The results and publications will also become part of a PhD and one post doctorate.

Main planned publications in scientific, peer-reviewed journals:

- Enhancing Insulin Sensitivity and Physical Fitness in South Asian Sedentary Women with History of Gestational Diabetes: The Impact of a 8-Week Combined Endurance and Strength Training Program
- Body Composition and Ectopic Fat Reduction via Exercise: Outcomes of a 8-Week Exercise Program in South Asian Women with Previous Gestational Diabetes
- Multi-Tissue Multi-Omics Alterations Triggered by Exercise: Unveiling the Systemic Effects of Physical Activity
- Differential Responses to Exercise between South Asian and Nordic women with previous Gestational Diabetes: A Comparative Study
- Metabolic Signature of South Asian women with previous Gestational Diabetes: Distinct Features and Implications for Exercise-Induced Benefits
- Predicting Exercise Outcomes: The Role of Clinical and Metabolic Features in Enhancing Insulin Sensitivity and Physical Fitness

The steering committee oversees the approval process for all publications, both planned and future projects, ensuring they align with ethical standards, and contribute to academic excellence. This process is designed to maintain research integrity and quality. Proposed projects are encouraged and should not be dismissed prematurely, and no single member of

the steering group can deny a project proposal, it must be a majority vote and the PI has a double vote if needed. We encourage open dialogue, constructive critique, and detailed feedback for improvement.

User involvement
A user representative advisory group (URAG) has been established for the DIASA Research Programme. The SA group members have experience from participation in previous research projects. The DIASA URAG gives advice in the planning of the projects, in particular concerning the setting of the examinations, type of intervention and general recruitment strategies to reach SA participants.

Our research group has extensive experience in performing clinical research in immigrant groups and we pay particular care in all our communications not to label or stigmatize participants or the minority groups. The DIASA URAG also helps to guide us in this communication.

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