Interaction between rs10830962 polymorphism in *MTNR1B* and lifestyle intervention on maternal and neonatal outcomes: secondary analyses of the DALI lifestyle randomized controlled trial

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ABSTRACT

Background: Interactions between polymorphisms of the melatonin receptor 1B (*MTNR1B*) gene and lifestyle intervention for gestational diabetes have been described. Whether these are specific for physical activity or the healthy eating intervention is unknown.

Objectives: The aim was to assess the interaction between *MTNR1B* rs10830962 and rs10830963 polymorphisms and lifestyle interventions during pregnancy.

Methods: Women with a BMI (in kg/m^2) of ≥29 (n = 436) received counseling on healthy eating (HE), physical activity (PA), or both. The control group received usual care. This secondary analysis had a factorial design with comparison of HE compared with no HE and PA compared with no PA. Maternal outcomes at 24–28 wk were gestational weight gain (GWG), maternal fasting glucose, insulin, insulin resistance (HOMA-IR), disposition index, and development of GDM. Neonatal outcomes were cord blood leptin and C-peptide and estimated neonatal fat percentage. The interaction between receiving either the HE or PA intervention and genotypes of both rs10830962 and rs10830963 was assessed using multilevel regression analysis.

Results: GDM risk was increased in women homozygous for the G allele of rs10830962 (OR: 2.60; 95% CI: 1.34, 5.06) or rs10830963 (OR: 2.83; 95% CI: 1.24, 6.47). Significant interactions between rs10830962 and interventions were found: in women homozygous for the G allele but not in the other genotypes, the PA intervention reduced maternal fasting insulin (β: –0.16; 95% CI: –0.33, 0.02; $P = 0.08$) and HOMA-IR (β: –0.17; 95% CI: –0.35, 0.01; $P = 0.06$), and reduced cord blood leptin (β: –0.84; 95% CI: –1.42, –0.25; $P = 0.01$) and C-peptide (β: –0.62; 95% CI: –1.07, –0.17; $P = 0.01$). In heterozygous women, the HE intervention had no effect, whereas in women homozygous for the C allele, HE intervention reduced GWG (β: −1.6 kg; 95% CI: −2.4, −0.8 kg). No interactions were found.

Conclusions: In women homozygous for the risk allele of *MTNR1B* rs10830962, GDM risk was increased and PA intervention might be more beneficial than HE intervention for reducing maternal insulin resistance, cord blood C-peptide, and cord blood leptin. *Am J Clin Nutr* 2021;00:1–9.

Keywords: pregnancy, lifestyle intervention, insulin sensitivity, gestational diabetes, melatonin receptor 1B, polymorphism

Introduction

The prevention of gestational diabetes (GDM) and the associated fetal overgrowth is important for reducing future risk of obesity and type 2 diabetes in both mother and offspring (1). So far, lifestyle interventions have had modest effects on reducing both GDM risk (2, 3) and fetal adiposity (4). However, the risk

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Supplemental Figures 1–4 and Supplemental Table 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: DALI, Vitamin D and Lifestyle Intervention; GDM, gestational diabetes mellitus; GWG, gestational weight gain; HE, healthy eating; MTNR1B, melatonin receptor 1B; PA, physical activity; SNP, single-nucleotide polymorphism; UC, usual care.

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of GDM is not only related to lifestyle but also partly determined by genetic background (5).

Variants of the melatonin receptor gene, melatonin receptor 1B (*MTNR1B*), are strongly associated with increased risk of type 2 diabetes or GDM (6, 7). Common variants of *MTNR1B* include rs10830962 and rs10830963 with C to G base pair conversions. The rs10830963 single-nucleotide polymorphism (SNP) is intronic and therefore unlikely to cause a structural change in the receptor. However, the G variant results in a greater expression of MTNR1B mRNA in human islets of Langerhans and is associated with a reduced early response insulin secretion in response to a glucose challenge (8) and increased fasting glucose (9). Although similarly associated with a decreased insulin release (10), rs10830962 is located in the 5′-flanking region of the *MTNR1B* gene (11) and is likely to have a different pathway of upregulating *MTNR1B* expression than rs10830963.

Although there is strong evidence for the presence of rs10830963 and rs10830962 G variants increasing risk for GDM (12–14), there are also data to support rs10830963 having a moderating effect on the effectiveness of lifestyle (15) or lifestyle intervention (16) designed to reduce GDM risk during pregnancy. Similarly, the maternal rs10830962 genotype can moderate the association of gestational weight gain (GWG) on the risk of childhood obesity (17). These emerging data suggest that an interaction may exist between the effectiveness of lifestyle intervention in reducing the risk of GDM and maternal genotype, especially in relation to rs10830962 and rs10830963 genotypes. However, whether interactions exist for maternal parameters not related to glucose, such as insulin response or insulin sensitivity, is unknown. Furthermore, interactions for neonatal outcomes have not been described. In-depth knowledge of such interactions may improve targeting and cost-effectiveness of future intervention strategies (18).

In the pan-European Vitamin D and Lifestyle Intervention (DALI) lifestyle trial, counseling on healthy eating (HE), physical activity (PA), and HE + PA were compared with usual care (UC) (19, 20). We previously reported improvements of lifestyle behavior in all 3 intervention groups, in addition to a substantial reduction in GWG in the HE + PA group (20). The HE + PA intervention was also associated with a reduction in neonatal adiposity (4).

Here we wanted to study whether lifestyle intervention effects are associated with the presence of the SNP rs10830962 and rs10830963 polymorphisms of the *MTNR1B* gene. Given the unique design of the DALI lifestyle study, it is possible to determine whether interactions are specific for HE or PA interventions.

Methods

Design and participants

The DALI lifestyle trial is a multicenter parallel randomized trial conducted in 9 European countries [Austria, Belgium, Denmark (Odense, Copenhagen), Ireland, Italy (Padua, Pisa), Netherlands, Poland, Spain, and United Kingdom] during 2012–2015 (registered as ISRCTN 70,595,832). Local ethics committee approval and written informed consent of all women were obtained. Pregnant women with a prepregnancy BMI (in kg/m^2) of ≥29, <20 wk of gestation, a singleton pregnancy, and age ≥18 y were invited to participate. Exclusions included diagnosis with early gestational diabetes (21), preexisting diabetes, and chronic medical conditions.

Randomization, masking, and interventions

Women were randomly allocated to HE + PA, HE, PA, or UC using a computerized random number generator, prestratified for site. Staff involved with measurements, but not participants, were blinded to the intervention.

In the intervention groups, participants were assigned to a single coach, with whom they discussed 5 PA and/or 7 HE messages, depending on group allocation, and were advised to keep gestational GWG <5 kg (19). Coaching, inspired by motivational interviewing, took place during 5 face-to-face sessions of 30–45 min each, alternated with up to 4 optional telephone calls. In the UC group, participants received no DALI interventions.

Assessments

At baseline (before 20 wk gestation) and at 24–28 and 35–37 wk of gestation, assessments took place. Information on demographics, prepregnancy weight, maternal/paternal smoking, alcohol consumption, past/current medical and obstetric history, and medication use was gathered by questionnaire. Women attended the 3 assessments fasted and undertook a standardized, 75-g oral glucose tolerance test, with blood samples taken at 0, 60, and 120 min after glucose ingestion. Not all recruitment sites were able to take additional samples at 30 and 90 min because of logistic or financial reasons. Therefore, these samples are available only for a subgroup of women (n = 188). Venous cord blood samples were taken immediately after delivery. Blood samples were stored at –20°C or colder until further analysis in the central trial laboratory in Graz, Austria.

Height was measured at baseline with a stadiometer (SECA 206; SECA; Leicester Height Measure), and the average value of 2 measurements was used. Women were weighed on calibrated electronic scales (SECA 888; SECA 877), wearing no shoes and light clothes, to the nearest 0.1 kg; the average value of 2 measurements was used. GWG was defined as the change in objectively measured weight and was calculated weight for 3 periods: baseline to 24–28 wk, baseline to 35–37 wk, and 24–28 to 35–37 wk.

Neonatal weight and length were measured at birth, and within 48 h, postpartum head, abdomen, upper and lower arm, and upper and lower leg circumferences were measured. Skinfold thickness was measured at 4 sites (i.e., triceps, subscapular, suprailiac, and quadriceps) with a Harpenden skinfold caliper (19). Each skinfold measurement was repeated once, and if a difference of more than 0.2 mm was registered, a third measurement was performed, and the average of the 3 was taken. Time between birth and measurements was registered in hours. Estimated fat mass in grams was calculated with a validated equation for neonates (22), with all neonates classified as "non-Hispanic." Estimated fat percentage was calculated by dividing estimated fat mass by total body mass × 100.

Biochemical analyses

Glucose was measured using the hexokinase method (DiaSys Diagnostic Systems) with a lower limit of sensitivity of 0.1 mmol/L. Central values are used for trial reporting.

Insulin was quantified by a sandwich immunoassay (ADVIA Centaur; Siemens Healthcare Diagnostics) with an analytical sensitivity of 0.5 mU/L, intra-assay CVs of 3.3–4.6%, and interassay CVs of 2.6–5.9%. All assays were carried out following the instructions of the manufacturer. HOMA-IR was calculated as [glucose $*$ insulin] / 22.5 mmol/L $*$ IU/mL. The oral disposition index was calculated as $\Delta Insulin_{0–30}$ / $\Delta Glucose_{0–30} \times 1$ / fasting insulin.

Cord blood leptin concentrations were quantified by solid-phase sandwich ELISA (E05-086-96; EIASON), according to the manufacturer's instructions. Analytical sensitivity was 1.0 ng/mL; intra- and interassay coefficients of variability (low/high concentrations) were 6.0/6.9% and 11.6/8.7%, respectively. Cord blood C-peptide was quantified by chemoluminometric solid-phase sandwich immune assay (ADVIA Centaur; Siemens Healthcare Diagnostics). Analytical sensitivity was 0.05 ng/mL, and intra- and interassay coefficients of variations (low/high concentrations) were 3.7/4.1% and 6.1/6.2%, respectively.

Genotyping

DNA was extracted from the buffy coat fraction of centrifuged blood with the QIAmp Blood Kit (Qiagen). Genotyping was performed by quantitative real-time PCR using the Agena platform (Agena Bioscience). IPLEX MassARRAY PCR and extension primers were designed from sequences containing each target SNP and 150 upstream and downstream bases with AssayDesign Suite (Agena Bioscience) using the default settings. Single-base extension reactions were performed on the PCR reactions with the iPLEX Gold Kit (Agena Bioscience) and 0.8 μL of the custom Unique Event Polymorphism pool. The kit contains mass-modified terminator nucleotides that increase the mass difference between extended UEPs, allowing for greater accuracy in genotyping. The mass difference with unmodified terminator nucleotides ranges from 9 to 40 kDa, depending on the 2 nucleotides compared. With the mass-modified terminator nucleotides, the mass difference increases to 16–80 kDa. The single-base extension reactions were cycled with a nested PCR protocol that used 5 cycles of annealing and extension nested with a denaturation step in a cycle that was repeated 40 times for a total of 200 annealing and extension steps. The goal was to extend nearly all of the UEPs. Following single-base extension, the reactions were diluted with 16 μL of water and deionized with 6 ng of resin. After deionizing for 20 min, the reactions were dispensed onto SpectroChipArrays with a Nanodispenser (Agena Bioscience). The speed of dispensation was optimized to deliver an average of 20 nL of each reaction to a matrix pad on the SpectroChip. An Agena Bioscience Compact MassArray Spectrometer was used to perform MALDI-TOF (Matrix–Assisted Laser–Desorption–Ionisiation (MALDI) time of flight (TOF))mass spectrometry according to the iPLEX Gold Application Guide (23). The Typer 4 software package (Agena Bioscience) was used to analyze the resulting spectra, and the composition of the target bases was determined from the mass of each extended oligo.

The two SNPs (rs10830962 and rs10830963) were selected based on their relation to the risk of GDM (6) and because of previous reported interactions with lifestyle (intervention) for the reduction of GDM incidence (15, 16).

Statistics

As primary outcomes of the lifestyle trial, GWG, fasting glucose, and insulin resistance (HOMA-IR) were used (20). For the purpose of this analysis, diagnosis of GDM, maternal fasting insulin, disposition index, 2 proxy measures for neonatal adiposity (estimated fat percentage and cord blood leptin), and cord blood C-peptide were added as primary outcomes.

The DALI lifestyle study had a 2 $\times$ 2 factorial design as previously described (24). The groups that received PA counseling were combined and compared with the combined groups that did not receive PA counseling, and similarly, HE groups were compared with no-HE groups.

Given the clustered structure of the data, with participants nested within 9 recruitment sites in 7 countries, differences between intervention groups were assessed with multilevel, multivariate linear regression analyses, in which 2 levels (site and individual) were defined. In the multilevel models, log-transformed data for fasting insulin, HOMA-IR, cord blood leptin, and C-peptide were used as the distribution of these variables was skewed. Analyses of maternal fasting glucose, insulin, HOMA-IR, and disposition index were adjusted for the baseline values, and analyses of GWG were adjusted for baseline BMI. Given the randomized controlled trial design and similarity of intervention groups at baseline, no further confounders were added to the models for maternal outcomes. Because sex differences in neonatal outcomes have been reported (25), all analyses of neonatal outcomes were adjusted for fetal sex, and analyses of estimated fat percentage were also adjusted for the time after birth (hours) the measurements were performed. Interaction between polymorphisms and intervention group was assessed by adding the interaction term in the models. When interaction was found (P-interaction < 0.10), analyses were performed for each polymorphism separately.

Because the 2 SNPs have a high linkage disequilibrium (26) and thus some of the moderation observed for rs10830962 might be due to rs10830963, we also assessed the intervention effects in women who were homozygous for the G allele of rs10830962 but not for rs10830963 ($n = 32$). Two-sided $P < 0.05$ was taken as significant. All analyses were performed in SPSS version 27 (SPSS, Inc.).

Results

A total of 436 women were randomly allocated to the lifestyle trial. Information on rs10830962 was available for 406 (93%) women, and information on rs10830963 was available for 357 (82%) women (**Table 1**; **Supplemental Figure 1**). The risk allele frequency (GG) was 39% for rs10830962 and 24% for rs10830963, and both genotype distributions were in Hardy–Weinberg equilibrium ($P > 0.05$). Women who received HE intervention were more often multiparous compared with women

TABLE 1 Characteristics of the study population and the offspring by maternal genotype[1]

	rs10830962 genotype			rs10830963 genotype		
Characteristic	CC ($n = 153$)	CG ($n = 186$)	GG ($n = 67$)	CC ($n = 211$)	CG ($n = 119$)	GG ($n = 27$)
PA intervention, *n* (%)	77 (50)	88 (47)	39 (58)	100 (47)	59 (50)	15 (56)
HE intervention, *n* (%)	79 (52)	95 (51)	32 (48)	110 (52)	58 (49)	12 (44)
Age, mean ± SD, y	32.5 ± 5.4	31.3 ± 5.3	32.4 ± 4.9	32.4 ± 5.3	31.5 ± 5.1	33.3 ± 4.6
Multiparous, *n* (%)	75 (49)	101 (54)	31 (46)	109 (52)	57 (48)	11 (41)
European descent, *n* (%)	133 (87)	168 (90)	55 (82)	178 (84)	110 (92)	25 (93)
Lives with partner, *n* (%)	145 (95)	177 (95)	63 (94)	200 (95)	115 (97)	25 (93)
Higher education, *n* (%)	87 (57)	105 (57)	39 (58)	124 (59)	65 (55)	18 (67)
Maternal smoking, *n* (%)	21 (14)	35 (19)	9 (13)	30 (14)	17 (14)	5 (19)
History of GDM, *n* (%)	7 (7)	8 (7)	2 (5)	7 (5)	6 (9)	1 (7)
First-degree FH DM, *n* (%)	30 (20)	46 (25)	15 (22)	39 (19)	34 (29)[2]	3 (11)
Chronic hypertension, *n* (%)	16 (11)	24 (13)	9 (13)	28 (13)	15 (13)	4 (15)
Baseline (15 wk)						
Gestation on entry, mean ± SD, wk	15.4 ± 2.3	15.1 ± 2.3	15.5 ± 2.3	15.3 ± 2.3	15.1 ± 2.3	15.5 ± 2.0
BMI at entry, mean ± SD, kg/m^2	34.3 ± 3.6	34.5 ± 4.0	34.0 ± 3.8	34.6 ± 4.1	34.1 ± 3.2	33.7 ± 3.7
Fasting glucose, mean ± SD, mmol/L	4.60 ± 0.35	4.62 ± 0.38	4.68 ± 0.43	4.62 ± 0.35	4.67 ± 0.40	4.76 ± 0.43
Fasting insulin, median (IQR), mU/L	12.8 (9.8, 17.2)	13.1 (10.2, 17.6)	11.4 (9.2, 15.8)	13.2 (10.4, 17.8)	12.2 (9.5, 16.3)	10.7 (8.2, 14.2)
HOMA-IR, median (IQR)	2.7 (1.9, 3.5)	2.7 (2.0, 3.7)	2.2 (1.8, 3.4)	2.7 (2.0, 3.7)	3.6 (2.0, 3.3)	2.2 (1.7, 3.2)
Oral disposition index, median (IQR)[3]	2.49 (1.64, 3.94)	2.39 (1.68, 3.51)	2.21 (1.28, 3.85)	2.63 (1.87, 3.72)	2.12 (1.31, 2.77)[2]	1.80 (1.09, 3.87)
24–28 wk	$n = 145$	$n = 171$	$n = 61$	$n = 208$	$n = 119$	$n = 27$
Gestational weight gain, mean ± SD, kg	3.4 ± 2.7	4.0 ± 2.8	4.3 ± 3.0	3.6 ± 2.9	4.3 ± 2.5	3.9 ± 3.1
Fasting glucose, mean ± SD, mmol/L	4.60 ± 0.37	4.65 ± 0.43	4.73 ± 0.49	4.61 ± 0.42	4.66 ± 0.41	4.80 ± 0.51
Fasting insulin, median (IQR), mU/L	14.3 (11.1, 19.2)	14.8 (11.9, 19.5)	13.4 (10.4; 18.9)	14.7 (11.4, 19.5)	14.5 (11.8, 20.0)	12.8 (10.4, 17.0)
HOMA-IR, median (IQR)	2.9 (2.2, 4.0)	3.0 (2.4, 4.1)	2.9 (2.1, 4.2)	3.0 (2.3, 4.1)	3.0 (2.3, 4.3)	2.6 (2.1, 3.7)
GDM, *n* (%)	27/143 (19)	35/170 (21)	23/61 (38)[4]	45/204 (22)	28/119 (24)	12/27 (44)[4]
Oral disposition index, median (IQR)[3]	2.10 (1.33, 2.95)	1.64 (1.20, 2.58)	1.45 (1.03, 2.40)	2.02 (1.40, 2.91)	1.51 (1.06, 2.37)[2]	1.35 (0.98, 1.78)[2]
Neonatal outcomes	$n = 141$	$n = 169$	$n = 61$	$n = 203$	$n = 118$	$n = 27$
Female sex, *n* (%)	66 (47)	83 (49)	37 (61)	93 (46)	67 (57)	14 (52)
Gestational age at birth, mean ± SD, wk	39.7 ± 2.8	39.5 ± 1.7	39.5 ± 1.6	39.6 ± 2.5	39.5 ± 1.8	39.4 ± 1.9
Estimated fat percentage, mean ± SD	11.4 ± 3.5	11.6 ± 4.0	10.0 ± 4.6	11.3 ± 3.4	11.5 ± 4.6	11.0 ± 5.2
Cord blood leptin, median (IQR), μg/L	7.6 (4.3, 13.1)	8.0 (4.2, 13.7)	6.5 (4.5, 11.2)	7.1 (4.3, 13.3)	8.9 (4.2, 13.5)	6.1 (4.1, 12.1)
Cord blood C-peptide, median (IQR), μg/L	0.61 (0.42, 0.81)	0.70 (0.43, 0.98)	0.64 (0.47, 0.91)	0.61 (0.42, 0.84)	0.62 (0.41, 0.91)	0.66 (0.28, 0.78)

[1]Values are presented as number (%), mean ± SD, or median (IQR). CC = homozygous for the C allele; GC = heterozygous; GG = homozygous for the G allele. FH DM, family history of diabetes mellitus; GDM, gestational diabetes mellitus; HE, healthy eating; PA, physical activity.

[2]Significant difference compared with CC.

[3]Data on oral disposition index was only available for 188 women in total.

[4]Significant difference between GG and the other 2 genotypes. Differences were tested with *t* test (normally distributed variables) or Mann–Whitney *U* test (skewed data).

TABLE 2 Interactions between rs10830962 and lifestyle interventions and intervention effects on maternal and neonatal outcomes[1]

			Total population			Subgroup not homogeneous for G allele of rs10830963	
Characteristic	*n*	*P*-interaction	HE vs. no-HE, β (95% CI)	*P* value	*n*	HE vs. no-HE, β (95% CI)	*P* value
Gestational weight gain at 24–28 wk (kg)							
rs10830962 CC	145	0.03	–1.6 (–2.4, –0.8)	<0.001	135	–1.6 (–2.4, –0.8)	<0.001
rs10830962 CG	171	Reference	–0.3 (–1.2, 0.5)	0.40	159	–0.3 (–1.1, 0.6)	0.51
rs10830962 GG	61	0.20	–1.2 (–2.7, 0.3)	0.12	32	–1.8 (–3.9, 0.3)	0.09
			PA vs. no PA			PA vs. no PA	
Fasting insulin, mU/L (LN) at 24–28 wk							
rs10830962 CC	139	0.25	–0.01 (–0.12, 0.11)	0.91	129	–0.03 (–0.15, 0.10)	0.68
rs10830962 CG	163	0.04	0.09 (–0.02, 0.20)	0.12	152	0.09 (–0.03, 0.21)	0.12
rs10830962 GG	59	Reference	–0.16 (–0.33, 0.02)	0.08	30	–0.33 (–0.59, –0.06)	0.02
HOMA-IR (LN) at 24–28 wk							
rs10830962 CC	137	0.30	–0.03 (–0.16, 0.10)	0.64	127	–0.06 (–0.19, 0.08)	0.40
rs10830962 CG	163	0.04	0.08 (–0.04, 0.20)	0.17	152	0.09 (–0.04, 0.22)	0.17
rs10830962 GG	59	Reference	–0.17 (–0.35, 0.01)	0.06	30	–0.32 (–0.57, –0.07)	0.02
Cord blood leptin, μg/L (LN)[2]							
rs10830962 CC	85	0.05	–0.13 (–0.44, 0.18)	0.39	82	–0.18 (–0.49, 0.14)	0.27
rs10830962 CG	105	0.04	–0.12 (–0.44, 0.20)	0.45	97	–0.09 (–0.43, 0.25)	0.61
rs10830962 GG	36	Reference	–0.84 (–1.42, –0.25)	0.01	19	–0.89 (–1.85, 0.08)	0.07
Cord blood C-peptide, μg/L (LN)[2]							
rs10830962 CC	75	0.07	–0.04 (–0.34, 0.26)	0.79	72	–0.06 (–0.36, 0.25)	0.72
rs10830962 CG	97	0.12	–0.16 (–0.47, 0.15)	0.31	88	–0.16 (–0.49, 0.16)	0.32
rs10830962 GG	34	Reference	–0.62 (–1.07, –0.17)	0.01	19	–0.81 (–1.38, –0.24)	0.01

[1]HE, healthy eating; LN, natural logarithm; PA, physical activity.
[2]Adjusted for neonatal sex. Results from multilevel linear regression analysis.

who did not receive HE intervention (56% compared with 46%, $P = 0.04$) (**Supplemental Table 1**). No other significant differences in baseline characteristics or genotype frequencies were found between intervention groups.

Having a first-degree relative with diabetes was less common ($P = 0.03$) among the women homozygous for the rs10830963 C allele (19%) than among the rs10830963 heterozygous women (29%). Furthermore, for both SNPs, GDM risk was increased in women homozygous for the G allele, with ORs of 2.60 (95% CI: 1.34, 5.06 for rs10830962) and 2.83 (95% CI: 1.24, 6.47 for rs10830963), respectively, compared with women homozygous for the C allele. This increased risk was maintained after adjusting for first-degree relatives with diabetes. The oral disposition index at baseline was measured in 188 women and was significantly higher in women homozygous for the C allele of rs10830963 ($n = 93$) compared with heterozygous women ($n = 54$). Women homozygous for the G allele ($n = 13$) had the lowest oral disposition index, but this was not significantly different from the other genotypes. Otherwise, no differences were found between rs10830962 or rs10830963 genotypes at baseline.

Interactions between polymorphisms and lifestyle interventions for maternal outcomes

Positive interactions between rs10830962 and the PA intervention were found for fasting insulin and HOMA-IR (both CG compared with GG; *P*-interaction = 0.04; **Table 2**, **Figure 1**, **Supplemental Figure 2**). Although not statistically significant, the PA intervention reduced both fasting insulin and HOMA-IR in women homozygous for the G allele. A post hoc power calculation showed that the difference between the PA intervention and control group in maternal fasting insulin would have been significant with 94 participants homozygous for the G allele of rs10830962.

In the subgroup of women, who were homozygous for the G allele of rs10830963 but not homozygous for the G allele of rs10830963 ($n = 32$), the PA intervention had a significant effect on fasting insulin (β: –0.33; 95% CI: –0.59, –0.06; $P = 0.02$) and HOMA-IR (β: –0.32; 95% CI: –0.57, –0.07; $P = 0.02$).

A negative interaction was found with the HE intervention for GWG at 24–28 wk (CC compared with CG; *P*-interaction = 0.03). In heterozygous women, HE intervention had no effect, whereas in women homozygous for either the C or G allele, HE intervention reduced GWG, although not significantly in the GG genotype (Table 2, **Figure 2**, **Supplemental Figure 3**).

No interactions between rs10830962 genotypes with GDM, fasting glucose, or oral disposition index were found. Furthermore, no interactions between rs10830963 genotypes and the PA or HE intervention were found for any of the maternal outcomes.

Interactions between polymorphisms and lifestyle interventions for neonatal outcomes

A positive interaction between maternal rs10830962 and PA intervention was found for cord blood leptin (CC and CG compared with GG; *P*-interaction = 0.05 and 0.04, respectively) and nonsignificantly for cord blood C-peptide (CC compared with GG; *P*-interaction = 0.07) (Table 2, **Figure 3**, **Supplemental Figure 4**). The PA intervention reduced both cord blood leptin and C-peptide in neonates born of women homozygous for the G allele (both $P = 0.01$). No interactions between rs10830962 genotypes and estimated fat percentage were found, and no interactions between rs10830963 genotypes and PA or HE intervention were found for any of the neonatal outcomes.

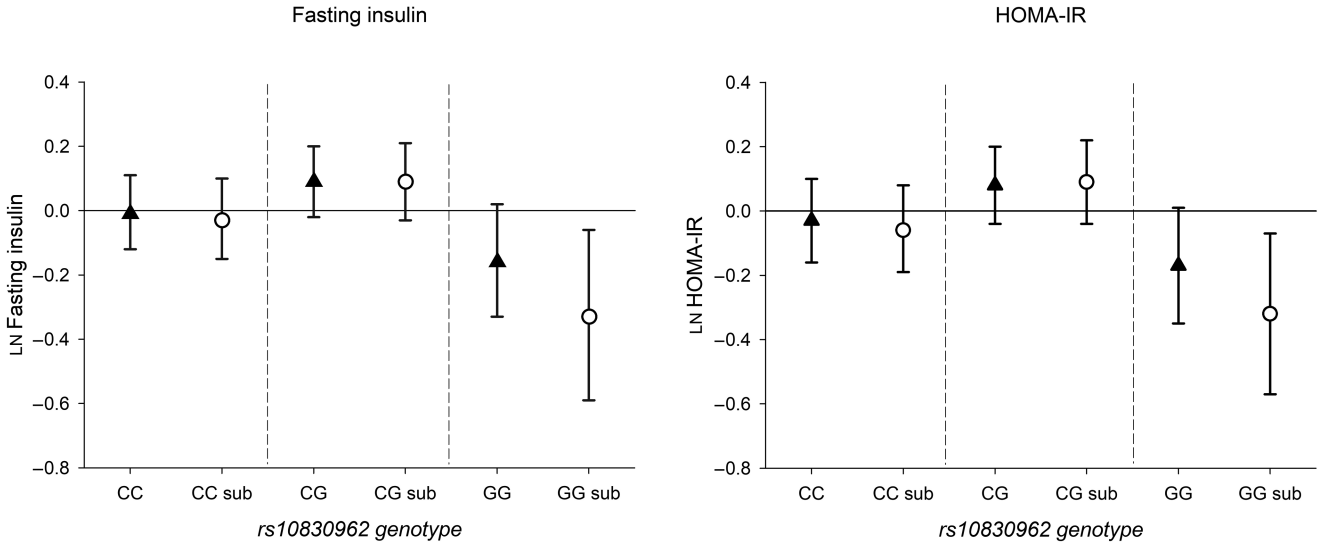

FIGURE 1 The effects of physical activity (PA) compared with no PA on maternal fasting insulin and HOMA-IR at 24–28 wk by rs10830962 genotype. CC = homozygous for the C allele of rs10830962; GC = heterozygous; GG = homozygous for the G allele of rs10830962. Black triangles: total group with a specific rs10830962 genotype (n = 361); white circles: subgroup (sub) of women with a specific rs10830962 genotype who are not homozygous for the rs10830963 G allele (n = 311). Intervention effects were larger in women homozygous for the G allele of rs10830962, especially in the subgroup that was homozygous for the G allele of rs10830962 but not for rs10830963. Differences between intervention groups were derived from multilevel linear regression models. LN, natural logarithm.

Discussion

In this population of European overweight/obese women, those homozygous for the G allele of both rs10830962 and rs10830963 were at higher GDM risk than those with 1 or more C alleles. Similar findings were reported previously (12, 27). However, in the present study, the risk of developing GDM was not affected by lifestyle intervention, and no interactions between rs10830962 and rs10830963 genotypes and intervention for GDM risk were found. However, positive (synergistic) genotype–lifestyle interactions were found for some other outcomes related

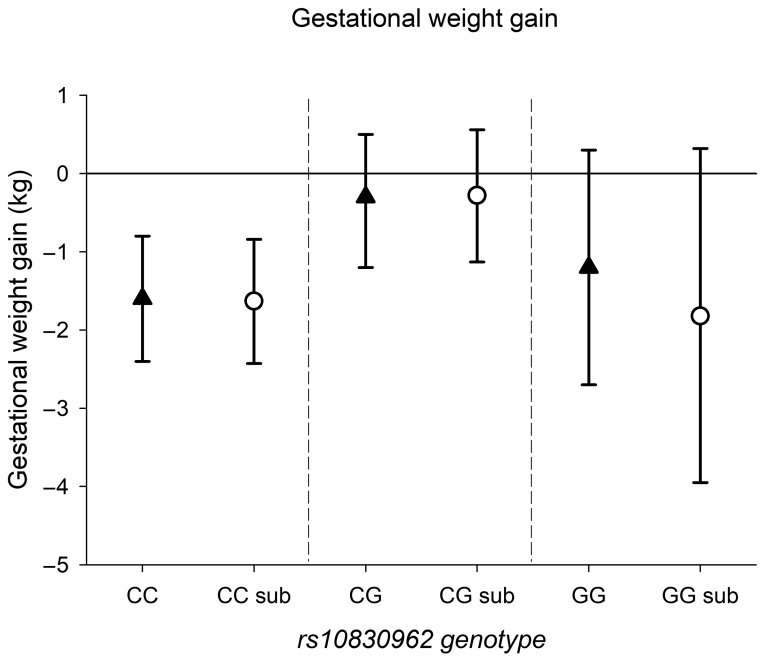

FIGURE 2 The effects of healthy eating (HE) compared with no HE on gestational weight gain at 24–28 wk by rs10830962 genotype. CC = homozygous for the C allele of rs10830962; GC = heterozygous; GG = homozygous for the G allele of rs10830962. Black triangles: total group with a specific rs10830962 genotype (n = 377); white circles: subgroup (sub) of women with a specific rs10830962 genotype who are not homozygous for the rs10830963 G allele (n = 326). Intervention effects were only significant in women homozygous for the C allele of rs10830962. Intervention effects in the subgroup were similar to the total group with that specific rs10830962 genotype. Differences between intervention groups were derived from multilevel linear regression models.

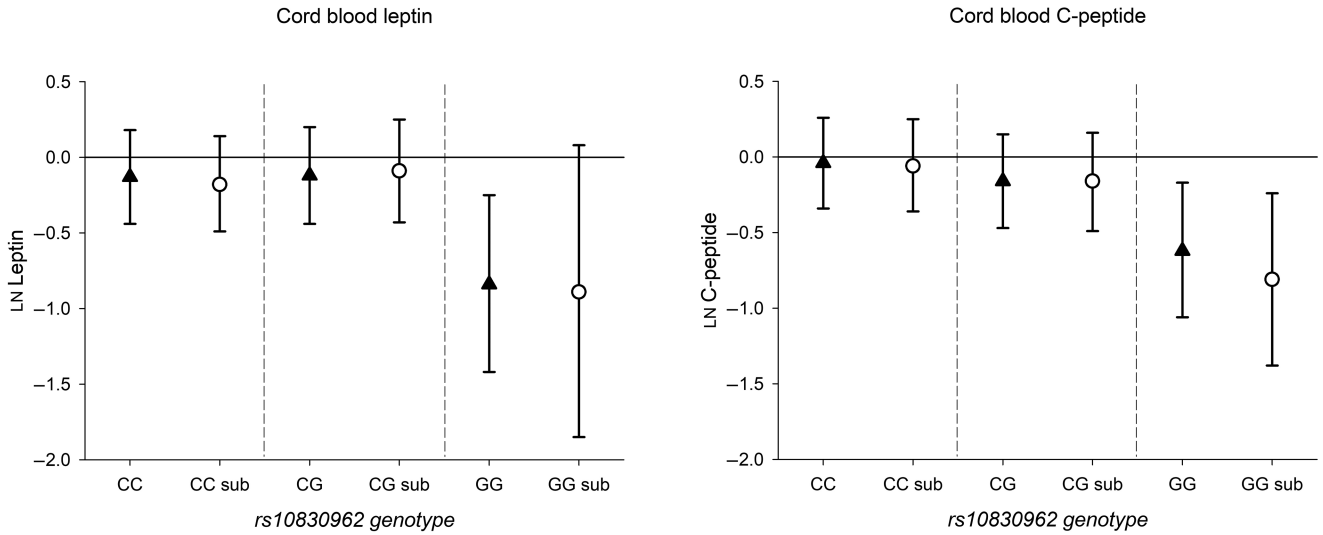

FIGURE 3 The effects of physical activity (PA) compared with no PA on cord blood leptin and C-peptide by maternal rs10830962 genotype. CC = homozygous for the C allele of rs10830962; GC = heterozygous; GG = homozygous for the G allele of rs10830962. Black triangles: total group with a specific rs10830962 genotype ($n = 226$); white circles: subgroup (sub) of women with a specific rs10830962 genotype who are not homozygous for the rs10830963 G allele ($n = 198$). Intervention effects were larger in women homozygous for the G allele of rs10830962. Intervention effects in the subgroup were similar to the total group with that specific rs10830962 genotype. Differences between intervention groups were derived from multilevel linear regression models. LN, natural logarithm.

to maternal metabolism. In women homozygous for the G allele but not in women with 1 or more C alleles of rs10830962, the PA intervention reduced maternal fasting insulin, insulin resistance, and cord blood leptin and C-peptide. An additional interaction was found between rs10830962 genotypes and the HE intervention for GWG. This interaction was negative, meaning that in women homozygous for the C allele, the HE intervention significantly reduced GWG but not in heterozygous women. Because both rs10830962 and rs10830963 mutations in *MTNR1B* have been linked to a deficiency of insulin release (10, 28), it is likely that the genomic effects on lifestyle intervention were mediated indirectly through improved insulin sensitivity and a possible reduction in cellular stress on the maternal β-cells. It is unknown whether these SNPs might also lead to an alteration on β-cell mass, which normally increases substantially during pregnancy.

Our findings that women homozygous for the G allele of both SNPs have a higher GDM risk and (nominally) higher fasting glucose, lower fasting insulin, and insulin resistance at baseline than women with other genotypes are consistent with earlier studies (6, 29). A novel finding is the interaction between the PA intervention and the rs10830962 genotype. The PA intervention resulted in a smaller increase in fasting insulin and insulin resistance from baseline to 24–28 wk compared with the control group in women having 2 G alleles of s10830962. Interestingly, no interactions were found with the HE intervention for these outcomes, which might indicate a specific responsiveness to higher PA levels in women with 2 G alleles of rs10830962.

The genotype has been linked to impaired β-cell function and reduced insulin secretion (6, 30). PA affects multiple processes related to glucose metabolism and insulin sensitivity, including increased non-insulin-dependent glucose uptake in skeletal muscle and improved muscle tissue insulin sensitivity (31). Although this might reduce the stress on β-cells and prevent further deterioration, one has to keep in mind that PA also improves aspects directly related to β-cell function, such as glucose sensing, and insulin secretion (32). It is possible that these effects of PA might benefit women with impaired β-cell function more than others. However, we did not find an interaction of the PA intervention with the disposition index, a measure of insulin secretion and β-cell function, possibly due to lack of power. The interaction of PA intervention with rs10830962 might be specific for the pregnancy period, since in Chinese women with previous GDM, no interaction between rs10830962 and combined HE + PA intervention was found for metabolic parameters (33). Currently, no other studies have reported interactions between rs10830962 and PA (interventions) in any population, pregnant or otherwise, and this deserves further investigation.

Previously, moderation by rs10830962 of the effect of GWG on offspring obesity was reported (17). Such interaction of maternal genotype with GWG can be at least partly explained by differential changes in maternal metabolism. In line with this notion, we found that the variant in the rs10830962 genotype, related to reduced maternal fasting insulin and insulin resistance after PA intervention, was also associated with a PA intervention effect on cord blood leptin, a proxy for neonatal adiposity. The reduction in cord blood leptin might be related to the reduction in C-peptide after PA intervention in this group. Cord blood C-peptide is a strong (statistical) mediator of the association between maternal and neonatal adiposity (34) and correlates with cord blood leptin in babies large for gestational age (35).

None of the previously reported interactions between rs10830963 and lifestyle interventions (15, 16) for the risk of developing GDM could be confirmed. This might be due to the relatively small sample size in our study, especially

of the group of women homozygous for the G allele of rs10830963.

Our results imply that for women homozygous for the G allele of rs10830962, a PA intervention, preferably combined with HE, might be most beneficial, given the effects on maternal insulin resistance and cord blood C-peptide. Higher insulin resistance is strongly related to increased risk of type 2 diabetes and cardiovascular disease (36). Furthermore, only the PA intervention had beneficial effects on neonatal outcomes in women with this genotype. Both maternal insulin resistance (37) and cord blood C-peptide (34) mediate the association of maternal BMI with neonatal adiposity. Furthermore, cord blood C-peptide is positively associated with childhood BMI and fat mass (38). However, the implications of a reduction in cord blood leptin and C-peptide after PA intervention for long-term health effects in the offspring remain to be seen.

The strengths of the DALI trial are the pan-European design and the possibility to determine interactions with HE and PA intervention separately. However, this design also led to smaller groups and more comparisons. Especially for the risk alleles that have the lowest frequency, intervention effects might be present, but we lack the power to demonstrate those, as shown in the post hoc power analysis. Small numbers also precluded testing for 3-way interaction between genotypes, interventions, and neonatal sex. We reported sex interactions previously for intervention effects on cord blood leptin and PA intervention (4), and such a 3-way interaction would be likely. Pooling of various studies or conducting larger studies will be needed to achieve this. Furthermore, our study sample was restricted to women with a BMI of 29 or more, and we do not have information on the fetal genotype and how this might contribute to intervention effects on neonatal outcomes. Last, we did not have other biochemical parameters [e.g., insulin-like growth factor I and placental growth hormone (39)] that might shed light on mechanistic pathways underlying the observed interactions.

In conclusion, for overweight and obese pregnant women homozygous for the risk allele of *MTNR1B* rs10830962, GDM risk was increased, and a PA intervention, either combined with HE or alone, might be more beneficial than HE intervention alone for reducing maternal insulin resistance and cord blood leptin and C-peptide.

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The authors' contributions were as follows—MvP: undertook the statistical analyses, wrote the first draft of the paper, and is the guarantor of this work; and all authors: contributed to the conception and/or design of the trial, read and corrected draft versions of the manuscript, and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript will not be made available because there is no informed consent from participants to do so.

References

1. Lowe WL, Scholtens DM, Lowe LP, Kuang A, Nodzenski M, Talbot O, Catalano PM, Linder B, Brickman WJ, Clayton P, et al. Association of gestational diabetes with maternal disorders of glucose metabolism and childhood adiposity. JAMA 2018;320(10):1005–16.
2. Mitanchez D, Ciangura C, Jacqueminet S. How can maternal lifestyle interventions modify the effects of gestational diabetes in the neonate and the offspring? A systematic review of meta-analyses. Nutrients 2020;12(2):353.
3. Song C, Li J, Leng J, Ma RC, Yang X. Lifestyle intervention can reduce the risk of gestational diabetes: a meta-analysis of randomized controlled trials. Obes Rev 2016;17(10):960–9.
4. van Poppel MNM, Simmons D, Devlieger R, van Assche FA, Jans G, Galjaard S, Corcoy R, Adelantado JM, Dunne F, Harreiter J, et al. A reduction in sedentary behaviour in obese women during pregnancy reduces neonatal adiposity: the DALI randomised controlled trial. Diabetologia 2019;62:915–25.
5. Powe CE, Nodzenski M, Talbot O, Allard C, Briggs C, Leya MV, Perron P, Bouchard L, Florez JC, Scholtens DM, et al. Genetic determinants of glycemic traits and the risk of gestational diabetes mellitus. Diabetes 2018;67(12):2703–9.
6. Powe CE, Kwak SH. Genetic studies of gestational diabetes and glucose metabolism in pregnancy. Curr Diab Rep 2020;20(12):69.
7. Tuomi T, Nagorny CLF, Singh P, Bennet H, Yu Q, Alenkvist I, Isomaa B, Östman B, Söderström J, Pesonen A-K, et al. Increased melatonin signaling is a risk factor for type 2 diabetes. Cell Metab 2016;23(6):1067–77.
8. Marchetti P, Syed F, Suleiman M, Bugliani M, Marselli L. From genotype to human β cell phenotype and beyond. Islets 2012;4(5):323–32.
9. Song J-Y, Wang H-J, Ma J, Xu Z-Y, Hinney A, Hebebrand J, Wang Y. Association of the rs10830963 polymorphism in MTNR1B with fasting glucose levels in Chinese children and adolescents. Obesity Facts 2011;4(3):197–203.
10. Staiger H, Machicao F, Schäfer SA, Kirchhoff K, Kantartzis K, Guthoff M, Silbernagel G, Stefan N, Häring H-U, Fritsche A. Polymorphisms within the novel type 2 diabetes risk locus MTNR1B determine beta-cell function. PLoS One 2008;3(12):e3962.
11. Xie K, Chen T, Zhang Y, Wen J, Cui X, You L, Zhu L, Xu B, Ji C, Guo X. Association of rs10830962 polymorphism with gestational diabetes mellitus risk in a Chinese population. Sci Rep 2019;9(1):5357.
12. Huopio H, Cederberg H, Vangipurapu J, Hakkarainen H, Pääkkönen M, Kuulasmaa T, Heinonen S, Laakso M. Association of risk variants for type 2 diabetes and hyperglycemia with gestational diabetes. Eur J Endocrinol 2013;169(3):291–7.
13. Ren J, Xiang AH, Trigo E, Takayanagi M, Beale E, Lawrence JM, Hartiala J, Richey JM, Allayee H, Buchanan TA, et al. Genetic variation in MTNR1B is associated with gestational diabetes mellitus and contributes only to the absolute level of beta cell compensation in Mexican Americans. Diabetologia 2014;57(7):1391–9.
14. Kim JY, Cheong HS, Park B-L, Baik SH, Park S, Lee SW, Kim M-H, Chung JH, Choi JS, Kim M-Y, et al. Melatonin receptor 1 b polymorphisms associated with the risk of gestational diabetes mellitus. BMC Med Genet 2011;12(1):82.
15. Popova PV, Klyushina AA, Vasilyeva LB, Tkachuk AS, Bolotko YA, Gerasimov AS, Pustozerov EA, Kravchuk EN, Predeus A, Kostareva AA, et al. Effect of gene-lifestyle interaction on gestational diabetes risk. Oncotarget 2017;8(67):112024–35.
16. Grotenfelt NE, Wasenius NS, Rönö K, Laivuori H, Stach-Lempinen B, Orho-Melander M, Schulz CA, Kautiainen H, Koivusalo SB, Eriksson JG. Interaction between rs10830963 polymorphism in MTNR1B and lifestyle intervention on occurrence of gestational diabetes. Diabetologia 2016;59(8):1655–8.
17. Liang Z, Liu H, Wang L, Chen Y, Zhou T, Heianza Y, Li W, Leng J, Wang J, Gao R, et al. Maternal MTNR1B genotype, maternal gestational weight gain, and childhood obesity. Am J Clin Nutr 2020;111(2):360–8.

18. Firneisz G, Rosta K, Rigó J, Nádasdi Á, Harreiter J, Kautzky-Willer A, Somogyi A. Identification and potential clinical utility of the MTNR1B rs10830963 core gene variant associated to endophenotypes in gestational diabetes mellitus. Front Genet 2020;11:332.
19. Jelsma JGM, van Poppel MNM, Galjaard S, Desoye G, Corcoy R, Devlieger R, van Assche A, Timmerman D, Jans G, Harreiter J, et al. DALI: vitamin D and lifestyle intervention for gestational diabetes mellitus (GDM) prevention: an European multicentre, randomised trial—study protocol. BMC Pregnancy Childbirth 2013;13(1): 142.
20. Simmons D, Devlieger R, van Assche A, Jans G, Galjaard S, Corcoy R, Adelantado JM, Dunne F, Desoye G, Harreiter J, et al. Effect of physical activity and/or healthy eating on GDM risk: the DALI lifestyle study. J Clin Endocrinol Metab 2017;102:903–13.
21. WHO. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy. Geneva (Switzerland): WHO; 2013.
22. Deierlein AL, Thornton J, Hull H, Paley C, Gallagher D. An anthropometric model to estimate neonatal fat mass using air displacement plethysmography. Nutr Metab 2012;9(1):21.
23. Gabriel S. Ziaugra L Tabbaa D SNP genotyping using the Sequenom Massarray iPLEX platform. Curr Protoc Hum Genet 2009;2.12.1–2.12.18.
24. Harreiter J, Simmons D, Desoye G, Corcoy R, Adelantado JM, Devlieger R, Galjaard S, Damm P, Mathiesen ER, Jensen DM, et al. Nutritional lifestyle intervention in obese pregnant women, including lower carbohydrate intake, is associated with increased maternal free fatty acids, 3-β-hydroxybutyrate, and fasting glucose concentrations: a secondary factorial analysis of the European multicenter, randomized controlled DALI lifestyle intervention trial. Diabetes Care 2019;42:1380–9.
25. Taine M, Khalfallah O, Forhan A, Glaichenhaus N, Charles M-A, Heude B. Does cord blood leptin level mediate the association between neonatal body size and postnatal growth? Results from the EDEN mother-child cohort study. Ann Hum Biol 2020;47(2):159–65.
26. Kwak SH, Kim S-H, Cho YM, Go MJ, Cho YS, Choi SH, Moon MK, Jung HS, Shin HD, Kang HM, et al. A genome-wide association study of gestational diabetes mellitus in Korean women. Diabetes 2012;61(2):531–41.
27. Popova PV, Klyushina AA, Vasilyeva LB, Tkachuk AS, Vasukova EA, Anopova AD, Pustozerov EA, Gorelova IV, Kravchuk EN, Li O, et al. Association of common genetic risk variants with gestational diabetes mellitus and their role in GDM prediction. Front Endocrinol 2021;12:628582.
28. Li Y, Wu H, Liu N, Cao X, Yang Z, Lu B, Hu R, Wang X, Wen J. Melatonin exerts an inhibitory effect on insulin gene transcription via MTNR1B and the downstream Raf–1/ERK signaling pathway. Int J Mol Med 2018;41:955–61.
29. Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJF, Manning AK, Jackson AU, Aulchenko Y, et al. Variants in MTNR1B influence fasting glucose levels. Nat Genet 2009;41(1):77–81.
30. Müssig K, Staiger H, Machicao F, Häring H-U, Fritsche A. Genetic variants in MTNR1B affecting insulin secretion. Ann Med 2010;42(6):387–93.
31. Sylow L, Kleinert M, Richter EA, Jensen TE. Exercise-stimulated glucose uptake—regulation and implications for glycaemic control. Nat Rev Endocrinol 2017;13(3):133–48.
32. Curran M, Drayson MT, Andrews RC, Zoppi C, Barlow JP, Solomon TPJ, Narendran P. The benefits of physical exercise for the health of the pancreatic β-cell: a review of the evidence. Exp Physiol 2020;105(4):579–89.
33. Liang Z, Wang L, Liu H, Chen Y, Zhou T, Heianza Y, Leng J, Li W, Yang X, Shen Y, et al. Genetic susceptibility, lifestyle intervention and glycemic changes among women with prior gestational diabetes. Clin Nutr 2020;39(7):2144–50.
34. Lee I-L, Barr ELM, Longmore D, Barzi F, Brown ADH, Connors C, Boyle JA, Kirkwood M, Hampton V, Lynch M, et al. Cord blood metabolic markers are strong mediators of the effect of maternal adiposity on fetal growth in pregnancies across the glucose tolerance spectrum: the PANDORA study. Diabetologia 2020;63(3): 497–507.
35. Wolf HJ, Ebenbichler CF, Huter O, Bodner J, Lechleitner M, Föger B, Patsch JR, Desoye G. Fetal leptin and insulin levels only correlate inlarge-for-gestational age infants. Eur J Endocrinol 2000;142: 623–9.
36. Mechanick JI, Farkouh ME, Newman JD, Garvey WT. Cardiometabolic-based chronic disease, adiposity and dysglycemia drivers. J Am Coll Cardiol 2020;75(5):525–38.
37. Shapiro ALB, Schmiege SJ, Brinton JT, Glueck D, Crume TL, Friedman JE, Dabelea D. Testing the fuel-mediated hypothesis: maternal insulin resistance and glucose mediate the association between maternal and neonatal adiposity, the Healthy Start Study. Diabetologia 2015;58(5):937–41.
38. Josefson JL, Scholtens DM, Kuang A, Catalano PM, Lowe LP, Dyer AR, Petito LC, Lowe WL, Metzger BE. Newborn adiposity and cord blood C-peptide as mediators of the maternal metabolic environment and childhood adiposity. Diabetes Care 2021;44(5):1194–202.
39. McIntyre HD, Zeck W, Russell A. Placental growth hormone, fetal growth and the IGF axis in normal and diabetic pregnancy. Current Diabetes Rev 2009;5(3):185–9.