diabetesresearchandclinicalpractice 202 (2023) 110780

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.journals.elsevier.com/diabetes-research-and-clinical-practice

Metformin versus insulin for gestational diabetes: Adiposity variables and adipocytokines in offspring at age of 9 years

Elisa Paavilainen [a,*], Harri Niinikoski [a], Riitta Parkkola [b], Kalle Koskensalo [c], Hilkka Nikkinen [d], Riitta Veijola [e], Marja Vääräsmäki [d], Britt-Marie Loo [f], Päivi Tossavainen [e], Tapani Rönnemaa [g], Kristiina Tertti [h]

[a] Department of Pediatrics and Adolescent Medicine, University of Turku and Turku University Hospital, Turku, Finland
[b] Department of Radiology, University of Turku and Turku University Hospital, Turku, Finland
[c] Department of Medical Physics, Turku University Hospital, Turku, Finland
[d] Department of Obstetrics and Gynecology, Research Unit of Clinical Medicine, University of Oulu and Oulu University Hospital, Oulu, Finland
[e] Department of Pediatrics, Research Unit of Clinical Medicine, Medical Research Center, University of Oulu and Oulu University Hospital, Oulu, Finland
[f] Joint Clinical Biochemistry Laboratory of University of Turku and Turku University Hospital, Turku, Finland
[g] Department of Medicine, University of Turku and Division of Medicine, Turku University Hospital, Turku, Finland
[h] Department of Obstetrics and Gynecology, University of Turku and Turku University Hospital, Turku, Finland

ARTICLE INFO

Keywords:
Gestational diabetes mellitus
Offspring
Body composition
Visceral adipose tissue
Liver fat
Low-grade inflammation

ABSTRACT

Aims: To compare body composition, visceral adiposity, adipocytokines, and low-grade inflammation markers in prepubertal offspring of mothers who were treated with metformin or insulin for gestational diabetes mellitus (GDM).

Methods: 172 offspring of 311 mothers randomized to receive metformin (n = 82) or insulin (n = 90) for GDM were studied at 9 years of age (follow-up rate 55%). Measurements included anthropometrics, adipocytokines, markers of the low-grade inflammation, abdominal magnetic resonance imaging (MRI), magnetic liver spectrometry (MRS), and whole body dual-energy X-ray absorptiometry (DXA).

Results: Serum markers of low-grade inflammation, visceral adipose tissue volume, total fat percentage, and liver fat percentage were similar between the study groups. Serum adiponectin concentration was higher in children in the metformin group compared to insulin group (median 10.37 vs 9.50 μg/ml, $p = 0.016$). This difference between groups was observed in boys only (median 12.13 vs 7.50 μg/ml, $p < 0.001$). Leptin/adiponectin-ratio was lower in boys in the metformin group than in the insulin group (median 0.30 vs 0.75; $p = 0.016$).

Conclusions: Maternal metformin treatment for GDM had no effects on adiposity, body composition, liver fat, or inflammation markers in prepubertal offspring compared to maternal insulin treatment but was associated with higher adiponectin concentration and lower leptin/adiponectin-ratio in boys.

1. Introduction

Gestational diabetes mellitus (GDM), often combined with maternal obesity, increases the risk of adverse outcomes during pregnancy and delivery [1]. Prenatal exposure to GDM increases total and abdominal adiposity in 6-year old offspring [2] and induces long-term metabolic effects in the offspring such as obesity, impaired glucose tolerance, and higher blood pressure [3–4]. Adequate treatment for GDM reduces these adverse effects [5–6].

Metformin has been increasingly used in the treatment for GDM. Metformin seems to be a safe option for the mother, newborn, and toddler [5–12], although it crosses the placenta with foetal levels similar

Abbreviations: ALT, Alanine aminotransferase; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FFMI, fat-free mass index; FM, fat mass; FMI, fat mass index; GDM, gestational diabetes mellitus; GlycA, glycoprotein acetyls; HDL, high-density lipoprotein; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IQR, interquartile range; L/A ratio, leptin/adiponectin ratio; MRI, magnetic resonance imaging; MRS, magnetic spectrometry; NAFLD, non-alcoholic fatty liver disease; NMR, nuclear magnetic resonance; ROI, regions of interest; VAT, visceral adipose tissue; VOI, volume of interest; WHtR, waist-to-height ratio.

* Corresponding author at: Department of Pediatrics and Adolescent Medicine, Turku University Central Hospital, Savitehtaankatu 5, FIN-20521 Turku, Finland.
E-mail address: elisa.paavilainen@tyks.fi (E. Paavilainen).

https://doi.org/10.1016/j.diabres.2023.110780
Received 6 January 2023; Received in revised form 30 May 2023; Accepted 13 June 2023
Available online 16 June 2023
0168-8227/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

to maternal concentrations [13]. It is also important to understand long-term effects of prenatal exposure to metformin in children. However, prepubertal data of body composition, adipose tissue distribution, and serum low-grade inflammation markers in offspring of metformin treated mothers with GDM are very limited [14]. This perspective is clinically important, because obesity [15–16], abdominal obesity [17], adverse concentrations of adipocytokines [18–19], and low-grade inflammation [20] in childhood are recognized as strong risk factors for the development of metabolic syndrome, type 2 diabetes, and cardiovascular diseases later in life.

We have previously shown that metformin treatment of GDM has no adverse effects on anthropometry and glucose and lipid metabolism in 9-year-old offspring [21]. Our previous results suggest that metformin exposure in pregnancy compared to maternal insulin treatment for GDM may relate to a more favourable lipid metabolism in male offspring [21].

To investigate in detail the possible late effects of prenatal metformin exposure on body composition and adiposity, we studied adipocytokines, markers of the low-grade inflammation, volume of visceral adipose tissue (VAT), liver fat percentage, and regional fat distribution in 9-year-old offspring. The study subjects were children born to mothers with GDM who were randomized to metformin or insulin in two Finnish trials [8–9].

2. Subjects, materials and methods

2.1. Study subjects

This was a longitudinal follow-up study in the offspring of two previously published Finnish randomized controlled trials with similar study designs [8–9], comparing metformin and insulin treatment for GDM. Study design of this follow-up study has been previously described in detail and published elsewhere [21]. Briefly, a total of 172 children participated in this follow-up study, comprising 55% of all eligible children (n = 311) from the two original trials (Fig. 1). The population was almost entirely of white Caucasian ethnicity (99%). In total 82 (48%) of the participating children were born to mothers with GDM who had been treated with metformin, and 90 (52%) were born to mothers treated with insulin. Furthermore, in the metformin group, 27% of the mothers (22/82) received additional insulin (17 in Turku University Hospital and five in Oulu University Hospital). In all analyses, children born to mothers originally randomized to receive metformin as initial drug therapy were handled as one group, including also those whose mothers needed additional insulin. One child with type 1 diabetes was excluded from the analyses.

The follow-up study was conducted at two sites, Turku University Hospital in Southwest Finland, and Oulu University Hospital in Northern

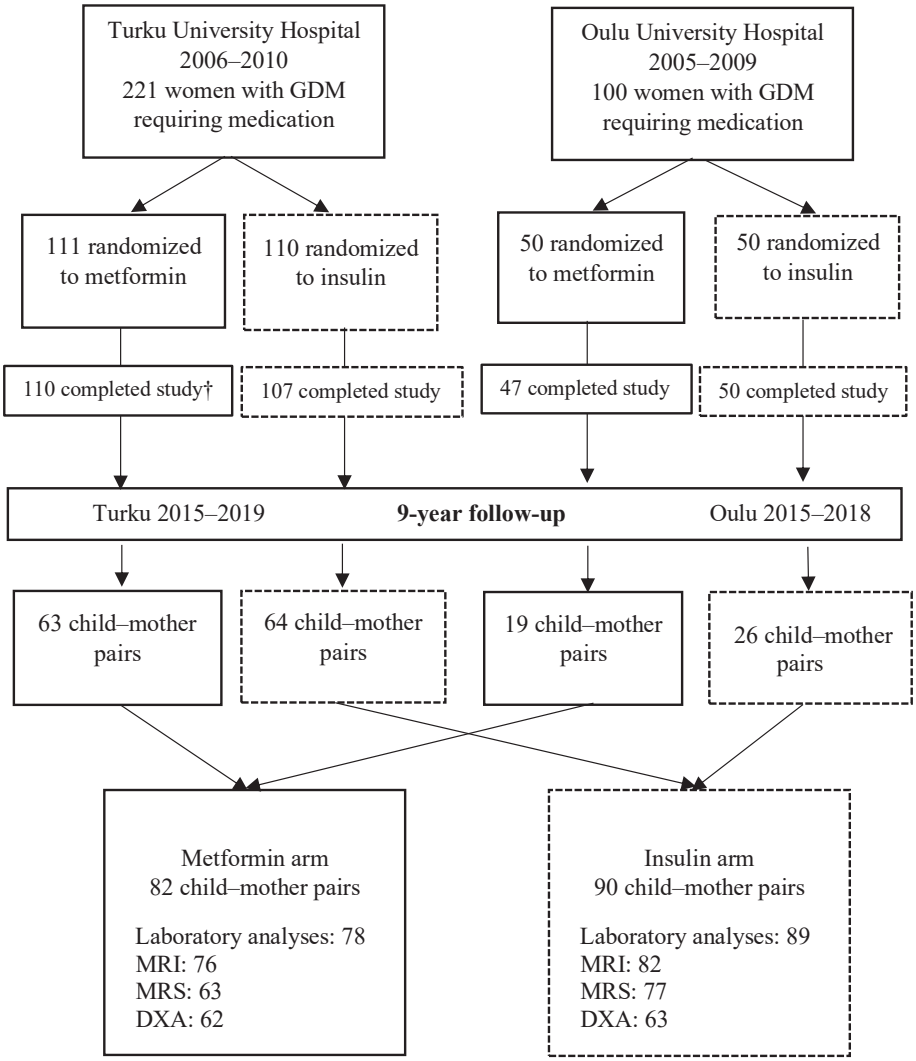

Fig. 1. Participants of the two original randomized controlled trials and those of the 9-year follow-up study. Abbreviations: DXA, dual energy X-ray absorptiometry; GDM, gestational diabetes mellitus; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy. † From 110 participants, who completed the original study in the metformin group in Turku, three offspring were excluded: one child had valproate syndrome, one child had Down syndrome and one child was stillborn.

Finland, between 2015 and 2019. The age of 9 years, just before the onset of puberty, was considered the most appropriate age to compare the adiposity variables between the offspring of the two treatment groups. Written informed consent was obtained from each mother, child, and father. The assessors were blinded to the treatment allocation of the mothers. This 9-year follow-up study was registered with the Clinical Trials Registry (NCT02417090) and approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK 31/2015, April 27, 2015).

2.2. Measurements

The follow-up visit included fasting blood samples, anthropometric measurements [21], whole body dual-energy X-ray absorptiometry (DXA), abdominal magnetic resonance imaging (MRI), and magnetic liver spectrometry (MRS). BMI and waist-to-height ratio (WHtR) were calculated in those children who had the data of the laboratory and imaging studies shown in Table 1 and 2.

2.3. Laboratory analysis

Venous blood was collected after an overnight fast. The processing of blood samples is described elsewhere [21]. Adiponectin, leptin, and interleukin-6 (IL-6) were assayed from serum samples using Quantikine ELISA Human Total Adiponectin/Acrp30, Quantikine ELISA Human Leptin and Quantikine HS Human IL-6 kits (all from R&D Systems, Minneapolis, USA). Serum IL-6 concentrations were analysed by ELISA (Quantikine HS Human IL-6, R&D Systems Inc, Minneapolis, USA). Alanine aminotransferase (ALT), ferritin, and high sensitivity C-reactive protein (hsCRP) were determined from serum samples using system reagents (ALT/GPT [IFCC]), Ferritin, and CRP High Sensitivity (all from Thermo Fisher Scientific,Vantaa, Finland) on an Indiko Plus analyzer (Thermo Fisher Scientific, Vantaa, Finland).The calculated coefficients of variation for these analyses were as follows: adiponectin 6.0%, leptin 4.3%, ALT 3,7%, ferritin 3,4%, IL-6 3.8%, and hsCRP 5,5%. The hsCRP concentrations were measured with detection limit of 0.25 mg/L. Serum concentrations below this detection limit (n = 21) were set to 0.24 mg/L for the calculations. Serum concentrations above 10 mg/L in the hsCRP (n = 1) and above 10 pg/ml in the IL-6 (n = 2) were interpreted to be due to infection and thus excluded from analysis. For IL-6 we used values < 1.5 pg/mL as normal for healthy 9-year-old children [22–23] in Table 1. Glycoprotein acetyls (GlycA) were measured as part of an NMR (nuclear magnetic resonance) metabolomics platform (Nightingale Health, Helsinki, Finland) as described elsewhere [24].

Table 1
Median (IQR) for BMI, waist-to-height ratio, concentrations of adipocytokines, markers of inflammation, ALT, VAT volumes (MRI) and liver fat percentages (MRS) in the 9-year-old offspring of metformin or insulin treated mothers with GDM.

	All children			Boys			Girls		
	Metformin	Insulin	P value	Metformin	Insulin	P value	Metformin	Insulin	P value
	n = 78	n = 89		n = 39	n = 43		n = 39	n = 46	
BMI, kg/m2	17.5 (16.3–19.3)	17.6 (16.1–20.1)	0.83	17.3 (16.2–18.6)	17.8 (16.2–20.8)	0.39	18.0 (17.90–19.6)	17.6 (16.0–20.0)	0.65
Waist-to-height ratio	0.431 (0.41–0.47)	0.444 (0.43–0.49)	0.19	0.429 (0.41–0.47)	0.444 (0.43–0.49)	0.032*	0.437 (0.41–0.47)	0.436 (0.42–0.47)	0.77
Adiponectin, μg/mL	10.37 (8.7–14.5)	9.50 (6.8–12.0)	0.016*	12.13 (9.7–14.7)	7.50 (5.8–11.3)	< 0.001*	9.37 (7.7–14.3)	9.98 (7.8–12.5)	0.85ǂ
Leptin, ng/mL	6.13 (2.7–11.7)	6.69 (3.1–11.8)	0.63	4.01 (2.1–6.8)	6.66 (2.3–10.8)	0.21	8.71 (4.7–14.5)	6.69 (3.7–13.8)	0.47
Leptin/adiponectin ratio	0.48 (0.2–1.1)	0.70 (0.3–1.4)	0.18	0.30 (0.2–0.7)	0.75 (0.3–1.3)	0.016*	0.75 (0.4–1.7)	0.70 (0.3–1.6)	0.44
hsCRP, mg/L	0.24 (0.2–0.7)	0.24 (0.2–1.1)	0.34	0.24 (0.2–0.4)	0.24 (0.2–0.8)	0.83	0.24 (0.2–0.8)	0.30 (0.2–1.3)	0.31
hsCRP < 0.25 mg/L, n (%)	46 (59)	47 (53)	0.47‡	24 (61.5)	26 (60.5)	0.92‡	22 (56)	21 (47)	0.37‡
hsCRP ≥ 0.25 mg/L, n (%)	32 (41)	41 (47)		15 (38.5)	17 (39.5)		17 (44)	24 (53)	
IL-6, pg/mL	1.18 (0.7–1.8)	1.31 (0.8–1.9)	0.23	0.87 (0.6–1.5)	1.30 (0.8–1.6)	0.23	1.44 (0.7–2.0)	1.31 (1.0–2.2)	0.63
IL-6 < 1.50 pg/mL, n (%)	51 (65)	51 (60)	0.48‡	29 (74)	26 (63)	0.29‡	22 (56)	25 (57)	0.97‡
IL-6 ≥ 1.50 pg/mL, n (%)	27 (35)	34 (40)		10 (26)	15 (37)		17 (44)	19 (43)	
Ferritin, ug/L	27.0 (20.9–40.1)	31.0 (24.0–41.4)	0.11	26.5 (21.3–36.3)	28.4 (23.5–40.5)	0.29	27.2 (20.8–43.9)	32.1 (24.5–44.9)	0.27
Glycoprotein acetyls, mmol/L	0.80 (0.8–0.9)	0.81 (0.7–0.9)	0.81	0.78 (0.7–0.9)	0.80 (0.7–0.8)	0.94ǂ	0.84 (0.8–0.9)	0.83 (0.7–0.9)	0.61
Alanine aminotransferase, U/L	16.0 (13.0–19.0)	17.0 (14.0–21.0)	0.22	16.0 (14.0–19.0)	17.0 (15.0–22.0)	0.07	16.0 (13.0–19.0)	16.0 (13.0–20.3)	0.99
MRI	n = 76	n = 82		n = 38	n = 42		n = 38	n = 40	
VAT volume, cm^3	246.3 (178.9–423.3)	254.5 (153.3–516.4)	0.82	204.7 (135.7–336.3)	286.0 (132.4–530.1)	0.46	284.3 (213.4–501.4)	216.5 (167.4–483.9)	0.16
VAT volume < 250 cm^3, n (%)	39 (51)	41 (50)	0.87‡	24 (63)	20 (48)	0.16‡	15 (39.5)	21 (52.5)	0.25‡
VAT volume ≥ 250 cm^3, n (%)	37 (49)	41 (50)		14 (37)	22 (52)		23 (60.5)	19 (47.5)	
MRS	n = 63	n = 77		n = 30	n = 39		n = 33	n = 38	
Liver fat, %	3.1 (2.5–4.1)	3.1 (1.7–4.0)	0.39	2.9 (2.5–4.1)	3.1 (1.5–3.9)	0.73	3.3 (2.5–4.1)	3.2 (1.9–4.0)	0.43
Liver fat < 5.0 %, n (%)	58 (92)	66 (86)	0.20‡	29 (97)	33 (85)	0.10‡	29 (88)	33 (87)	0.90‡
Liver fat ≥ 5.0%, n (%)	5 (8)	11 (14)		1 (3)	6 (15)		4 (12)	5 (13)	

Data are expressed as median (IQR) or n (%). Mann-Whitney *U* test was used unless stated otherwise [*T*-test (†), Chi-square or Fisher's exact test (‡)]. Abbreviations: ALT, alanine aminotransferase; GDM, gestational diabetes mellitus; hsCRP, high-sensitive CRP; IL-6, interleukin-6; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; VAT, visceral adipose tissue.

Table 2
Body composition by DXA in the 9-year-old offspring of metformin or insulin treated mothers with GDM.

	All children			Boys			Girls		
	Metformin	Insulin	P value	Metformin	Insulin	P value	Metformin	Insulin	P value
	n = 62	n = 63		n = 32	n = 30		n = 30	n = 33	
BMI, kg/m2	17.6 (16.6–19.3)	17.9 (16.2–20.7)	0.51	17.8 (16.5–19.1)	18.0 (16.8–20.8)	0.57	17.6 (17.0–19.4)	17.9 (16.2–20.1)	0.53‡
Waist-to-height ratio	0.431 (0.41–0.47)	0.444 (0.42–0.49)	0.18	0.435 (0.41–0.47)	0.448 (0.43–0.49)	0.15	0.429 (0.41–0.47)	0.442 (0.41–0.47)	0.65‡
Total fat-free mass, kg	23.98 ± 4.08	23.94 ± 3.78	0.96‡	25.16 ± 3.71	25.41 ± 3.99	0.80‡	22.71 ± 4.14	22.60 ± 3.06	0.91‡
Total fat, kg	10.12 (8.2–11.9)	10.98 (8.7–14.6)	0.15	8.83 (8.0–11.6)	11.08 (7.8–14.0)	0.24	10.69 (9.0–12.6)	10.77 (8.9–15.2)	0.62
Total fat percentage, %	30.25 (25.6–34.7)	32.1 (28.3–36.7)	0.14‡	27.0 (24.6–31.2)	31.2 (24.1–34.7)	0.17	32.4 (29.3–35.5)	33.1 (29.4–38.0)	0.27‡
Arm fat, kg	1.33 (3.4–5.3)	1.45 (3.8–6.0)	0.21	1.21 (1.0–1.6)	1.43 (1.0–1.9)	0.39	1.39 (1.2–1.8)	1.49 (1.1–2.1)	0.46
Leg fat, kg	4.54 (3.4–5.3)	4.55 (3.8–6.0)	0.24‡	3.80 (3.3–5.0)	4.75 (3.6–5.6)	0.32	4.74 (3.4–5.4)	4.40 (3.9–6.2)	0.48
Trunk fat, kg	3.36 (2.6–4.4)	3.79 (2.9–5.5)	0.12	2.86 (2.5–4.2)	3.78 (2.3–5.6)	0.23	3.54 (3.1–4.9)	3.79 (2.9–5.8)	0.50
Trunk fat/leg fat, %	0.77 (0.7–0.9)	0.85 (0.7–1.0)	0.11	0.76 (0.7–0.9)	0.87 (0.7–1.0)	0.11	0.78 (0.7–1.0)	0.85 (0.7–1.0)	0.54
Android fat, kg	0.50 (0.4–0.7)	0.60 (0.4–0.9)	0.14	0.44 (0.4–0.64)	0.57 (0.4–1.0)	0.17	0.56 (0.4–0.8)	0.62 (0.4–1.0)	0.65
Android fat, %	25.7 (20.8–32.5)	28.0 (24.1–34.5)	0.07	22.5 (20.3–26.7)	26.7 (19.2–34.4)	0.15	30.7 (24.9–34.8)	30.5 (26.0–374)	0.83‡
Gynoid fat, kg	1.66 (1.3–2.0)	1.75 (1.5–2.4)	0.18	1.58 (1.3–1.9)	1.73 (1.4–2.3)	0.28	1.91 (1.4–2.1)	1.79 (1.5–2.5)	0.56
Gynoid fat, %	35.24 ± 5.68	36.76 ± 5.52	0.13‡	33.27 ± 5.72	34.28 ± 6.09	0.50‡	37.3 ± 4.9	39.0 ± 3.8	0.83‡
Android/gynoid ratio	0.293 (0.27–0.35)	0.340 (0.28–0.40)	0.073	0.293 (0.26–0.35)	0.342 (0.29–0.40)	0.063	0.308 (0.27–0.37)	0.332 (0.28–0.40)	0.55
FMI, kg/m^2	5.41 (4.4–6.4)	5.88 (4.6–7.7)	0.15	4.63 (4.1–5.9)	5.71 (4.2–6.8)	0.23	5.78 (5.0–6.88)	5.88 (4.8–7.9)	0.55
FFMI, kg/m^2	12.71 ± 1.47	12.66 ± 1.20	0.84‡	13.12 ± 1.37	13.10 ± 0.19	0.96‡	12.28 ± 1.46	12.27 ± 1.21	0.34‡

Data are expressed as median (IQR), mean ± SD or n (%). *T*-test was used unless stated otherwise [Mann-Whitney *U* test (†), Chi-square or Fisher's exact test (‡)]. DXA values are from Turku. Abbreviations: DXA, dual energy X-ray absorptiometry; FMI, fat mass index; FFMI, fat-free mass index; GDM, gestational diabetes mellitus.

2.4. Body composition

2.4.1. MRI and MRS acquisition

MRI and MRS scans were performed on a Siemens MAGNETOM Sola fit 1.5 T MRI system (Siemens Healthcare, Erlangen, Germany) in Turku University Hospital and a Siemens MAGNETOM Aera 1.5 T MRI system (Siemens Healthcare, Erlangen, Germany) in Oulu University Hospital using a similar scan protocol in both sites. First, a 2-point Dixon scan (see Supplementary Table 1 for parameters) was performed on the thighs and another one on the pelvic area. Then the abdominal and thorax areas were scanned with respiration compensated 2-point Dixon sequences. Finally, single-voxel proton MRS was performed to determine the liver fat content. Parameters of the MRS sequence are listed on Supplementary Table 2. To positioning the spectroscopic voxels in the liver tissue, three orthogonal views of the liver were produced with T2 HASTE sequences. Altogether, the MRI and MRS scanning session took 30 min.

2.4.2. VAT segmentation

For every child, the separate water and fat images produced by the Dixon sequences were combined into single water and fat images covering the total abdominal cavity. This was done using Osirix (version 6.0.2, Pixmeo SARL, Bernex, Switzerland) software.

Fat fraction maps [25] of the visceral area were constructed from water and fat images by performing the following calculation for each voxel in Vinci (version 4.9) software [26]: F/(W + F), where F is the signal intensity of the fat image and W is the signal intensity of the water image. Thus, each voxel in the fat fraction map represents the fraction of fat signal intensity in relation to the signal intensity of both water and fat.

Carimas (version 2.9, PET Centre, Turku University Hospital, Finland) software was used for defining VAT volume from fat fraction maps. The VAT was segmented as follows: 2-dimensional regions of interest (ROI) covering the VAT area were drawn on every 6 sagittal slices, typically a total of 22 ROIs were drawn for each subject. Then, a 3-dimensional volume of interest (VOI) covering the VAT region was constructed from the ROIs using the interpolation feature of the Carimas software. Artifacts or bright areas inside the gastrointestinal tract (stool and air) were segmented correspondingly and excluded from the volume of interest. Finally, the voxels with an intensity value above 0.6 (i.e., fat fraction over 60 %) within the volume of interest were considered to represent VAT (Fig. 2).

The segmentation of the VAT area was defined with anatomical landmarks for consistency: the most superior slice was chosen so that the diaphragm forms a unified pattern and epicardial fat remains above the area. The most inferior slice was chosen on the top of the S1 vertebra. Fat behind vertebral bodies were left outside of the ROI. Segmentations were generated by the first author and 15% of the masks were validated by RP with 32 years of experience with MRI. Segmentation time per patient was 1.5 h.

To further illustrate possible differences in the total VAT volumes between the two medication groups, the median VAT of all study offspring (250 cm3) was used as cut off (Table 1). To our knowledge, no reliable VAT reference values for 9-year-old children are reported.

2.4.3. MRS data analysis

Liver magnetic resonance spectroscopy (MRS) data was exported from the MR system in Siemens.rda-format and quantified using LCModel software (Version 6.3–1 N) [27]). To determine a composite lipid signal amplitude the lipid signal amplitudes from 0.9 to 2.8 ppm were summed up and the contribution of the lipid signals residing under the water peak was corrected by multiplying the sum by 1.086 [28]. Water and the composite lipid signal amplitudes were corrected for T2 relaxation using the T2 times determined by Hamilton et al. [29]. T1 correction was not applied since the TR time of 3000 ms was considered long enough to make the correction unnecessary [30–31]. Fat fraction was calculated by dividing the corrected composite lipid signal amplitude by the sum of the corrected water and composite lipid signal amplitudes. We considered liver fat level <5% as normal in MRS (Table1). Di Martino et al. have shown that 5% is a threshold value between healthy children and those with non-alcoholic fatty liver disease (NAFLD) [32]. MRS analysis was successful in 140/171 children; nine children refused to participate in MRI imaging and for 21 children MRS was unsuccessful due technical reasons or for poor co-operation of the child.

2.4.4. DXA

Assessments were performed only for the participants at the Turku

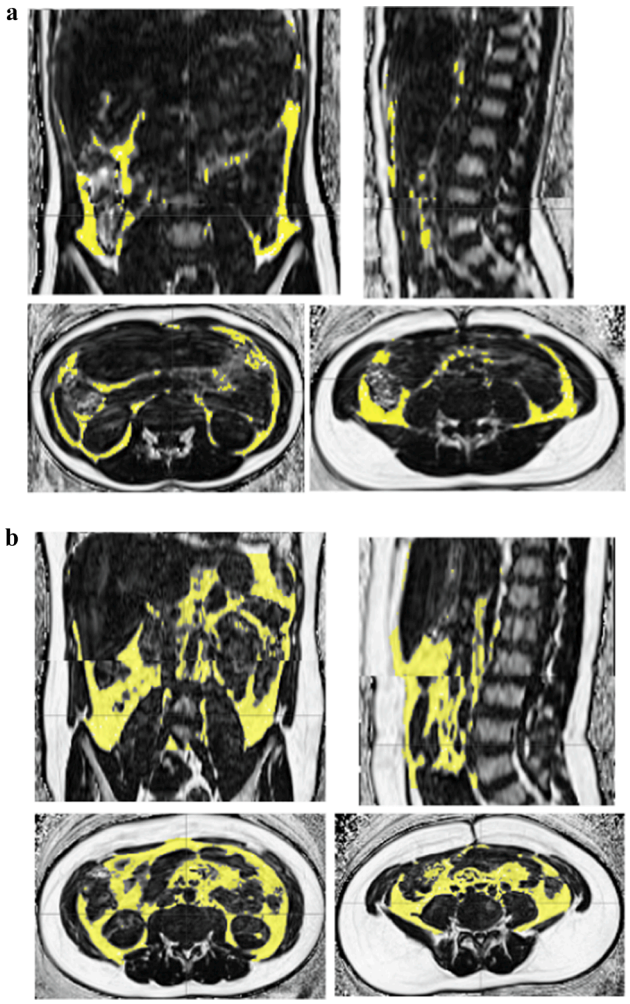

Fig. 2. A 9-year-old child with low (a) or high (b) visceral adipose volume. 2.a. Yellow colour represents visceral adipose tissue (VAT; fat fraction over 60 %) in abdominal cavity. Axial slice on the left side is from the L2-L3 level and axial slice on the right side is from the L4-L5 level. Fig. 2a. VAT volume is 195 cm^3 (BMI 17.6 kg/m^2, WHtR 0.43). Fig. 2b. VAT volume is 1048 cm^3 (BMI 22.6 kg/m^2, WHtR 0.50). Abbreviations: BMI, body mass index; VAT, visceral adipose tissue; WHtR, waist-to-height ratio.

University Hospital. Whole-body DXA for regional and total body fat, fat-free mass and percent body fat was performed using Discovery A System (Hologic, 123 Waltham, MA, USA) with standard imaging and positioning protocols. All metal items were removed before densitometry, and children were examined wearing only underwear and a cloth gown. Android fat, gynoid fat, and android/gynoid ratio by DXA are reported because they are found to highly correlate to risk factors for both metabolic and cardiovascular diseases in normal weight and overweight boys [17,33]. To report more clinically relevant height-normalized indexes, fat mass index (FMI) and fat-free mass index (FFMI) were calculated as fat-free mass (FFM) or fat mass (FM) divided by height squared, respectively [34].

2.5. Statistical methods

The Kolmogorov-Smirnov test was used to analyse whether the variables were normally distributed, and the Shapiro-Wilk test was used to test the normality of the subgroups of boys and girls ($n < 50$). Between-group comparisons were performed using Student's *t*-test for normally distributed data and the Mann-Whitney *U* test for skewed data and the results are expressed as median (interquartile range [IQR]) or mean ± SD unless otherwise stated. Most variables were not normally distributed in the subgroups. Chi-square or Fisher's exact test was used for categorical variables. Potential differences in boys and girls between the treatment groups were explored using subgroup analysis. The IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) software package was used and a P value of <0.05 was taken to indicate statistical significance.

3. Results

As published previously [21], there were no significant differences in maternal baseline characteristics (including socio-economic status measured by parental education level), pregnancy outcomes, and neonatal measures (including the number of children born small for gestational age, metformin group n = 4, insulin group n = 4) between the offspring who participated or did not participate in the follow-up study. The baseline characteristics were similar also between those offspring who participated in the 9-year follow-up study and were exposed to metformin and those whose mothers GDM were treated with insulin during the pregnancy [21]. Parental characteristics and the offspring's anthropometric measures were similar in both treatment groups at the 9-year follow-up [21].

3.1. BMI, WHtR

BMI did not differ between the study groups or sexes. In boys WHtR values were lower in metformin group than in insulin group (median 0.429 [0.41–0.47] vs 0.444 [0.43–0.49], p = 0.032; Table 1).

3.2. Adipocytokines

The offspring of the metformin group had higher median adiponectin concentration than those of the insulin group (10.37 vs 9.50 μg/ml, p = 0.016: Table 1). In an exploratory analysis the correlation between high-density lipoprotein (HDL) cholesterol concentration [21] and adiponectin concentration was 0.20 (p = 0.011) in all children and 0.32 in the metformin group (p = 0.005) and 0.08 (p = 0.87) in the insulin group. The median serum leptin concentration (6.13 vs 6.69 ng/mL, p = 0.63) and leptin/adiponectin (L/A) ratio (0.48 vs 0.70, p = 0.18) did not differ between the two treatment groups (Table 1). In a detailed analysis, a more favourable adipocytokine profile was evident only in the boys. Median adiponectin concentration was higher (12.13 vs 7.50 μg/ml, p < 0.001; Table 1; Fig. 3.a.) and L/A ratio was lower (0.30 vs 0.75; p = 0.016; Table 1; Fig. 3.c.) in the boys of the metformin group than in the boys of the insulin group.

3.3. Low-grade inflammation

For the assessment of low-grade inflammation, we used hsCRP, IL-6, ferritin, and GlycA as biomarkers. The median serum hsCRP was 0.24 mg/ml and no difference between the groups were seen. Concentrations of the other inflammation markers; serum IL-6, ferritin and GlycA, were similar in the two treatment groups. No sex-related differences were found in the inflammation markers.

3.4. Adiposity, liver fat, fat distribution

VAT volume measured by MRI, liver fat percentage measured by MRS, total and regional body fat percentages measured by DXA were similar in the offspring in both treatment groups (Table 1, 2). Only few offspring, five (7.9%) in metformin group and 11 (14.3%) in insulin group, had liver fat percentage higher than 5% which is considered to express significant liver fat content in MRS [33]. However, the proportion of boys with high liver fat content was numerically lower in the metformin group (3.3% vs 15.4%). The fat distribution measured by DXA and the total fat free mass (FFM) of the offspring were similar

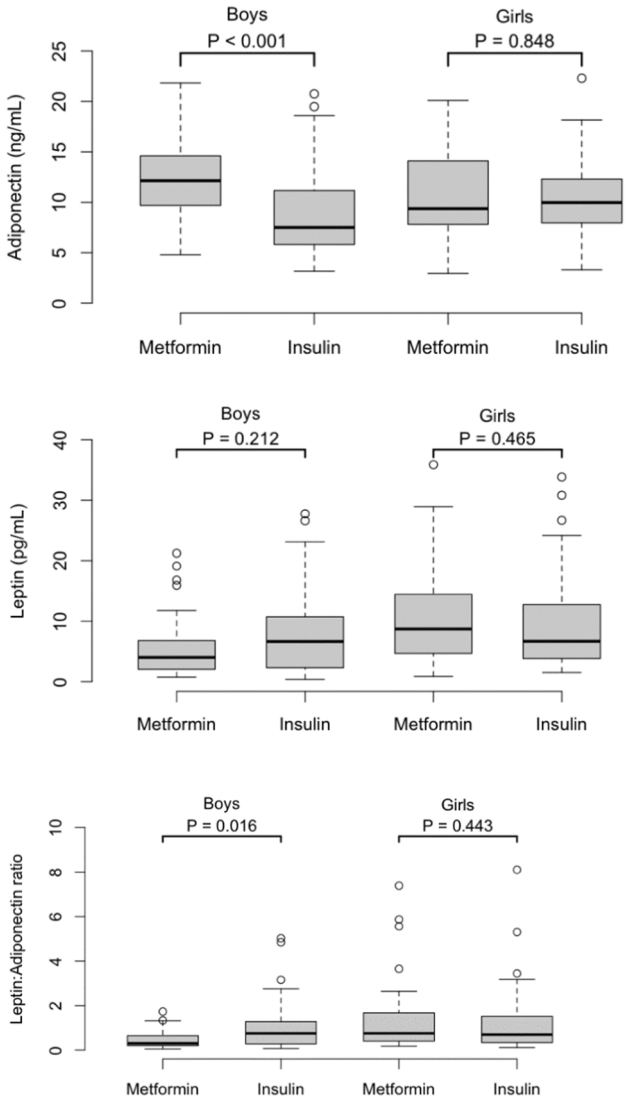

Fig. 3. Offspring (a) adiponectin and (b) leptin concentrations, and (c) leptin/adiponectin ratios in boys and girls. Box plot analysis.

between the groups. Similarly, body size adjusted fat mass and fat-free mass indexes (FMI and FFMI) did not differ between the two treatment groups (Table 2). There were no significant differences between sexes in MRI, MRS, or DXA studies, but a borderline difference was found in android/gynoid ratio by DXA between the metformin and insulin group boys (median 0.293 vs 0.342, p = 0.063). Serum ALT concentrations did not differ between the groups or sexes (Table 1).

4. Discussion

In the present follow-up study of offspring of mothers with GDM randomized to metformin or insulin treatment during pregnancy, we found that metformin does not adversely affect offspring adiposity at 9 years of age. Noteworthy, some adiposity related variables were more favourable in the boys of the metformin group compared to the boys of the insulin group.

Higher serum adiponectin concentration in the metformin group offspring is in-line with our previous result of higher serum high-density lipoprotein (HDL) cholesterol concentration in the offspring who were exposed to metformin during pregnancy [21]. These two favourable results were found only in the boys of the metformin group, and no differences were found between the two treatment groups in the girls. Similarly, significantly lower L/A ratio was found only in the metformin group boys compared to insulin group boys. WHtR was slightly lower in the boys of the metformin group than those of the insulin group, which may possibly be related to higher serum adiponectin concentration and lower L/A ratio in metformin group boys.

Adipose tissue is a highly active endocrine organ, and it has been shown that the characteristics of adipose tissue determine adiponectin secretion more than the amount of it [35]. Optimal adiponectin concentration enhances insulin sensitivity and also the adiponectin/leptin ratio is a reliable and predictive biomarker for several metabolic disorders, such as type 2 diabetes, and cardiovascular disorders [35]. Frithioff-Bøjsøe et al. [18] have indeed suggested that L/A ratio is more important risk marker of cardiometabolic comorbidities in children than adiponectin or leptin alone. The findings in present study raise questions about the mechanism by which metformin treatment for GDM during pregnancy could increase adiponectin values in prepubertal boys and why this effect was not observed in girls. Lu et al. [36] have indeed showed sex-discordant differences in adult mice offspring after being exposed to maternal GDM. They found that male mice offspring are more vulnerable to maternal hyperglycemia in pregnancy than female mice offspring. Autonomous correction of overgrowth, dyslipidemia and hyperglycemia was seen in female but not in male adult mice offspring. However, maternal metformin, insulin or glibenclamide treatment corrected metabolic alterations in male offspring [36]. This finding that the sexes can develop different metabolic phenotypes after similar exposure during pregnancy is interesting, but the setting of this experimental animal study was not aimed to examine the influence of the maternal medication for GDM to offspring.

To date, only one other randomized controlled trial of maternal metformin or insulin treatment for GDM [7] has reported offspring data of adipocytokines, markers of the low-grade inflammation, adiposity measures, regional fat distribution and liver fat percentage [14]. In the study of Rowan et al. [14] the offspring in metformin group had higher weight, mid-upper arm circumference, waist circumference and WHtR at the age of 9 years, but their study groups were smaller and participation rate was lower than in the present study. To the contrary to Rowan et al. [14], we found higher serum adiponectin values in boys of the metformin group. Furthermore, we found no differences in ferritin or other inflammation markers between the two treatment groups. Differences between our results and those of Rowan et al. [14], who found higher weight, WHtR and ferritin (median 52 μg/L vs 40 μg/L, p = 0.009) in the metformin group, might be due to differences in the ethnicity [37]: 99% of mothers of our children were Caucasian whereas more than 50% of the mothers in the study of Rowan were of other ethnicities.

The estimation of liver fat percentage might be dependent of the selected region for analysis in MRS. In present study median liver fat percentage was slightly higher (3.1% both in the metformin and insulin groups) compared to the study of Rowan et al. [14] (2.5% and 1.8% respectively, p = 0.10). Compatibly, Rowan et al. [14] found slightly higher VAT volume in the metformin group than in the insulin group offspring (mean 941 cm^3 vs 722 cm^3, p = 0.051). We found no differences in VAT volume between the two treatment groups. Neither we nor Rowan et al. [14] found differences between treatment groups in total or abdominal (or android) body fat measured by DXA. However, in the present study the android/gynoid ratio was slightly lower in the metformin group boys. High serum ALT concentration is characteristic for fatty liver but median serum concentrations of ALT in our study were low, and similarly in the study by Rowan et al., did not differ between the treatment groups.

4.1. Strengths and limitations of the study

The major strength of the present follow-up study is that the 9-year-old offspring represents well the original cohort, allowing valid comparisons between the treatment groups. Moreover, the baseline data were similar between the 9-year study participants and the group of

nonparticipants and between the two study sites. In addition, the study protocol was similar at the two study sites as well as at the baseline (pregnancy, birth) and at the 9-year follow-up. Among the participating children, both sexes and treatment groups were evenly distributed, and all the children were prepubertal. All measurements were performed using strict procedures and all blood samples were stored under similar conditions and analysed at the same time in one laboratory [21]. Currently, this follow-up cohort of 172 9-year-old offspring whose mothers received either metformin or insulin for GDM is the largest published cohort comparing long-term effects of prenatal metformin exposure and maternal insulin treatment. The follow-up rate of 55% obtained for the total cohort was satisfactory, considering the period of 9 years between birth and follow-up. There were some limitations in the study. Firstly, the suboptimal follow-up rate may have led to some potential differences not being detected between the treatment groups. Secondly, the majority of the study subjects had hsCRP concentration below the detection limit. Thirdly, we were not able to get some results due to participant́s refusal or technical reasons. Furthermore, DXA assessments were performed only in children followed in Turku. These children, however, represent 74 % of all participants. Lastly, the participants were almost entirely of white Caucasian ethnicity, which may affect the applicability of the results to other ethnic groups.

4.2. Conclusions

In conclusion, treating GDM with metformin was not inferior to insulin in regard of prepubertal offspring body composition or inflammation markers. These main results support the hypothesis that using metformin for the treatment of GDM has no negative effects on the adiposity variables of the offspring during the prepubertal period. In addition, adipocytokine profiles were similar in the two treatment groups in the girls but more favourable in the boys of the metformin group than in the insulin group. Our results in the present and our previous study [21] leave open an interesting question as to whether exposure to metformin during pregnancy influences the offspring sexes differently regarding lipid, adipocytokine and glucose metabolism also in long term, e.g., after puberty and young adulthood.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We acknowledge Tommi Kauko for the graphics (Fig. 3), statistical advice, and support. We would also like to especially thank study nurse Ulla Torkko and study nurse Tiina Latva-aho for organizing the study, and Marja Perhomaa, Ekaterina Saukko, Esa Liukkonen, Jani Saunavaara, and Eveliina Lammentausta for practical and technical help. We would also like to acknowledge the expert work of the laboratory technicians Minna Romo and Elina Åkerblom. Finally, thanks are due to all the children and parents who agreed to take part in this study.

Funding.

This study was financially supported by the Diabetes Research Foundation, Finland; the Finnish Foundation for Paediatric Research; Special Governmental Grants for Health Sciences (Turku University Hospital and Oulu University Hospital, Finland); Yrjö Jahnsson Foundation, Finland and the Turku University Hospital Research Foundation, Finland.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2023.110780.

References

[1] HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358(19):1991–2002. https://doi.org/10.1056/NEJMoa0707943.

[2] Kearney M, Perron J, Marc I, Weisnagel SJ, Tchernof A, Robitaille J. Association of prenatal exposure to gestational diabetes with offspring body composition and regional body fat distribution. Clin Obes 2018;8(2):81–7. https://doi.org/10.1111/cob.12237.

[3] Lowe Jr WL, Scholtens DM, Kuang A, Linder B, Lawrence JM, Lebenthal Y, et al. HAPO Follow-up Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study (HAPO FUS): Maternal Gestational Diabetes Mellitus and Childhood Glucose Metabolism. Diabetes Care 2019;42(3):372–80. https://doi.org/10.2337/dc18-1646.

[4] Dabelea D. The predisposition to obesity and diabetes in offspring of diabetic mothers. Diabetes Care 2007;30(Suppl 2):S169–74. https://doi.org/10.2337/dc07-s211. Erratum. In: Diabetes Care. 2007 Dec; 30(12):3154.

[5] Guo L, Ma J, Tang J, Hu D, Zhang W, Zhao X. Comparative efficacy and safety of metformin, glyburide and insulin in treating gestational diabetes mellitus: a meta-analysis. J Diabetes Res 2019;2019:29. https://doi.org/10.1155/2019/9804708.

[6] Tarry-Adkins JL, Aiken CE, Ozanne SE. Neonatal, infant and childhood growth following metformin versus insulin treatment for gestational diabetes: a systematic review and meta-analysis. PLoS Med 2019;16:e1002848. https://doi.org/10.1371/journal.pmed.1002848.

[7] Rowan JA, Hague WM, Gao W, Battin MR, Moore MP, MiG Trial Investigators. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008;358(19):2003–15. https://doi.org/10.1056/NEJMoa0707193. Erratum in: N Engl J Med. 2008 Jul 3;359(1):106.

[8] Ijäs H, Vääräsmäki M, Morin-Papunen L, Keravuo R, Ebeling T, Saarela T, et al. Metformin should be considered in the treatment of gestational diabetes: a prospective randomised study. BJOG 2011;118(7):880–5. https://doi.org/10.1111/j.1471-0528.2010.02763.x.

[9] Tertti K, Ekblad U, Koskinen P, Vahlberg T, Rönnemaa T. Metformin vs insulin in gestational diabetes. A randomized study characterizing metformin patients needing additional insulin. Diabetes Obes Metab 2013 Mar;15(3):246–51. https://doi.org/10.1111/dom.12017.

[10] Rowan JA, Rush EC, Obolonkin V, Battin M, Wouldes T, Hague WM. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition at 2 years of age. Diabetes Care 2011;34:2279–84. https://doi.org/10.2337/dc11-0660.

[11] Ijäs H, Vääräsmäki M, Saarela T, Keravuo R, Raudaskoski T. A follow-up of a randomised study of metformin and insulin in gestational diabetes mellitus: growth and development of the children at the age of 18 months. BJOG 2015;122(7):994–1000. https://doi.org/10.1111/1471-0528.12964.

[12] Tertti K, Toppari J, Virtanen HE, Sadov S, Rönnemaa T. Metformin treatment does not affect testicular size in offspring born to mothers with gestational diabetes. Rev Diabet Stud 2016;13(1):59–65. https://doi.org/10.1900/RDS.2016.13.59.

[13] Tertti K, Ekblad U, Heikkinen T, Rahi M, Rönnemaa T, Laine K. The role of organic cation transporters (OCTs) in the transfer of metformin in the dually perfused human placenta. Eur J Pharm Sci 2010;39(1–3):76–81. https://doi.org/10.1016/j.ejps.2009.10.014.

[14] Rowan JA, Rush EC, Plank LD, Lu J, Obolonkin V, Coat S, et al. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition and metabolic outcomes at 7–9 years of age. BMJ Open Diab Res Care 2018;6:e000456. https://doi.org/10.1136/bmjdrc-2017-000456.

[15] Jin B, Lin H, Yuan J, Dong G, Huang K, Wu W, et al. Abdominal Adiposity and Total Body Fat as Predictors of Cardiometabolic Health in Children and Adolescents with Obesity. Front Endocrinol (Lausanne) 2020;11:579. https://doi.org/10.3389/fendo.2020.00579.

[16] Tompuri TT, Jääskeläinen J, Lindi V, Laaksonen DE, Eloranta AM, Viitasalo A, et al. Adiposity Criteria in Assessing Increased Cardiometabolic Risk in Prepubertal Children. Front Endocrinol (Lausanne) 2019;26(10):410. https://doi.org/10.3389/fendo.2019.00410.

[17] Samsell L, Regier M, Walton C, Cottrell L. Importance of android/gynoid fat ratio in predicting metabolic and cardiovascular disease risk in normal weight as well as overweight and obese children. J Obes 2014;2014:846578. https://doi.org/10.1155/2014/846578.

[18] Frithioff-Bøjsøe C, Lund MAV, Lausten-Thomsen U, Hedley PL, Pedersen O, Christiansen M, et al. Leptin, adiponectin, and their ratio as markers of insulin resistance and cardiometabolic risk in childhood obesity. Pediatr Diabetes 2020;21(2):194–202. https://doi.org/10.1111/pedi.12964.

[19] Ghadge AA, Khaire AA. Leptin as a predictive marker for metabolic syndrome. Cytokine 2019;121:154735. https://doi.org/10.1016/j.cyto.2019.154735.

[20] Christaki EV, Pervanidou P, Papassotiriou I, Bastaki D, Valavani E, Mantzou A, et al. Stress, Inflammation and Metabolic Biomarkers Are Associated with Body Composition Measures in Lean, Overweight, and Obese Children and Adolescents. Children 2022;9:291. https://doi.org/10.3390/children9020291.

[21] Paavilainen E, Tertti K, Nikkinen H, Veijola R, Vääräsmäki M, Loo B-M, et al. Metformin versus insulin therapy for gestational diabetes: Effects on offspring anthropometrics and metabolism at the age of 9 years: A follow-up study of two open-label, randomized controlled trials. Diabetes Obes Metab 2022;24(3):402–10. https://doi.org/10.1111/dom.14589.

[22] De Filippo G, Rendina D, Moccia F, Rocco V, Campanozzi A. Interleukin-6, soluble interleukin-6 receptor/interleukin-6 complex and insulin resistance in obese

children and adolescents. J Endocrinol Invest 2015;38(3):339–43. https://doi.org/10.1007/s40618-014-0176-4.
[23] Tam CS, Garnett SP, Cowell CT, Heilbronn LK, Lee JW, Wong M, et al. IL-6, IL-8 and IL-10 levels in healthy weight and overweight children. Horm Res Paediatr 2010;73(2):128–34. https://doi.org/10.1159/000277632.
[24] Soininen P, Kangas AJ, Würtz P, Tukiainen T, Tynkkynen T, Laatikainen R, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. Analyst 2009;134(9):1781–5. https://doi.org/10.1039/b910205a.
[25] Bray TJ, Chouhan MD, Punwani S, Bainbridge A, Hall-Craggs MA. Fat fraction mapping using magnetic resonance imaging: insight into pathophysiology. Br J Radiol 2018;91(1089):20170344. https://doi.org/10.1259/bjr.20170344.
[26] Vollmar S, Hampl JA, Kracht L, Herholz K. Integration of Functional Data (PET) into Brain Surgery Planning and Neuronavigation. Advances in Medical Engineering 2007;114:98–103. https://doi.org/10.1007/978-3-540-68764-1_16.
[27] Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Reson Med 1993;30(6):672–9. https://doi.org/10.1002/mrm.1910300604.
[28] Hamilton G, Yokoo T, Bydder M, Cruite I, Schroeder ME, Sirlin CB, et al. In vivo characterization of the liver fat ^{1}H MR spectrum. NMR Biomed 2011;24(7):784–90. https://doi.org/10.1002/nbm.1622.
[29] Hamilton G, Middleton MS, Bydder M, Yokoo T, Schwimmer JB, Kono Y, et al. Effect of PRESS and STEAM sequences on magnetic resonance spectroscopic liver fat quantification. J Magn Reson Imaging 2009;30(1):145–52. https://doi.org/10.1002/jmri.21809.
[30] Qayyum A. MR spectroscopy of the liver: principles and clinical applications. Radiographics. 2009;29(6):1653–64. https://doi.org/10.1148/rg.296095520.
[31] Leporq B, Ratiney H, Pilleul F, Beuf O. Liver fat volume fraction quantification with fat and water T1 and T 2* estimation and accounting for NMR multiple components in patients with chronic liver disease at 1.5 and 3.0 T. Eur Radiol 2013;23(8):2175–86. https://doi.org/10.1007/s00330-013-2826-x.
[32] Di Martino M, Pacifico L, Bezzi M, Di Miscio R, Sacconi B, Chiesa C, et al. Comparison of magnetic resonance spectroscopy, proton density fat fraction and histological analysis in the quantification of liver steatosis in children and adolescents. World J Gastroenterol 2016;22(39):8812–9. https://doi.org/10.3748/wjg.v22.i39.8812.
[33] Lätt E, Mäestu J, Jürimäe J. Longitudinal associations of android and gynoid fat mass on cardiovascular disease risk factors in normal weight and overweight boys during puberty. Am J Hum Biol 2018;30(5):e23171. https://doi.org/10.1002/ajhb.23171.
[34] Shypailo RJ, Wong WW. Fat and fat-free mass index references in children and young adults: assessments along racial and ethnic lines. Am J Clin Nutr 2020;112(3):566–75. https://doi.org/10.1093/ajcn/nqaa128.
[35] Zhao S, Kusminski CM, Scherer PE. Adiponectin, Leptin and Cardiovascular Disorders. Circ Res 2021;128(1):136–49. https://doi.org/10.1161/CIRCRESAHA.120.314458.
[36] Lu Y, Jia Y, Lu J, Liu J, Xu Y, Liu Y, et al. Progenies of gestational diabetes mellitus exhibit sex disparity in metabolism after respective therapies of insulin, glibenclamide, and metformin in dams during pregnancy. Arch Physiol Biochem 2021;23:1–13. https://doi.org/10.1080/13813455.2021.1991957.
[37] Tahmasebi H, Asgari S, Hall A, Higgins V, Chowdhury A, Thompson R, et al. Influence of ethnicity on biochemical markers of health and disease in the CALIPER cohort of healthy children and adolescents. Clin Chem Lab Med 2020;58(4):605–17. https://doi.org/10.1515/cclm-2019-0876.