**Association between Maternal Mid-gestation Vitamin D status and Neonatal Abdominal**

**Adiposity**

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1 **Abstract**

2 **Objectives:**

3 Lower vitamin D status has been associated with adiposity in children through adults.

4 However, the evidence of the impact of maternal vitamin-D status during pregnancy on

5 offspring’s adiposity is mixed. The objective of this study was to examine the associations

6 between maternal vitamin-D [25(OH)D] status at mid-gestation and neonatal abdominal

7 adipose tissue (AAT) compartments, particularly the deep subcutaneous adipose tissue linked

8 with metabolic risk.

9 **Methods:**

10 Participants (N=292) were Asian mother-neonate pairs from the mother-offspring cohort,

11 Growing Up in Singapore Towards healthy Outcomes. Neonates born at ≥34 weeks gestation

12 with birth weight ≥2000g had magnetic resonance imaging (MRI) within 2-weeks post-

13 delivery. Maternal plasma glucose using an oral glucose tolerance test and 25(OH)D

14 concentrations were measured. 25(OH)D status was categorized into inadequate

15 (≤75.0nmol/L) and sufficient (>75.0nmol/L) groups. Neonatal AAT was classified into

16 superficial (sSAT), deep subcutaneous (dSAT) and internal (IAT) adipose tissue

17 compartments.

18 **Results:**

19 Inverse linear correlations were observed between maternal 25(OH)D and both sSAT (r=-

20 0.190, *P*=0.001) and dSAT (r=-0.206, *P*<0.001). Each 1 nmol/L increase in 25(OH)D was

21 significantly associated with reductions in sSAT (β= -0.14 (95%CI: -0.24,-0.04) ml, P=0.006)

22 and dSAT (β= -0.04 (-0.06,-0.01) ml, P=0.006). Compared to neonates of mothers with

23 25(OH)D-sufficiency, neonates with maternal 25(OH)D-inadequacy had higher sSAT (7.3

24 (2.1, 12.4) ml, P=0.006), and dSAT (2.0 (0.6, 3.4) ml, P=0.005) volumes, despite similar

25 birth weight. In the subset of mothers without gestational diabetes, neonatal dSAT was also

26 greater (1.7 (0.3, 3.1) ml, P=0.019) in neonates with maternal 25(OH)-inadequacy. The

27 associations with sSAT and dSAT persisted even after accounting for maternal glycemia

28 (fasting and 2-hour plasma glucose).

29 **Conclusions:**

30 Neonates of Asian mothers with mid-gestation 25(OH)D inadequacy have a higher

31 abdominal subcutaneous adipose tissue volume, especially dSAT (which is metabolically

32 similar to visceral adipose tissue in adults), even after accounting for maternal glucose levels

33 in pregnancy.

34 **Introduction**

35 Obesity in childhood leads to a wide range of long-term health complications.

36 Without intervention, obese infants and young children are more likely to remain obese in

37 adolescent and adulthood, and at increased risk of metabolic diseases later in life (1). The

38 developmental origins of health and disease (DOHaD) concept posits an important role for

39 early life environmental factors in programming offspring’s metabolic susceptibility over the

40 lifecourse (2). The in-utero environment may partly influence offspring’s health by altering

41 materno-fetal transfer of glucose and fatty acids leading to fetal fat deposition (3). Therefore,

42 body composition at birth may be an indicator of the in-utero environment during

43 development, and may form the basis for future cardio-metabolic risk (4).

44 With the recognition of developmental influences on obesity risk and the increasing

45 prevalence of childhood obesity, research has focused on identifying potentially modifiable

46 early life risk factors as a basis for timely interventions. Vitamin D has been increasingly

47 studied for its potential influence on a range of chronic diseases including type 2 diabetes

48 mellitus (T2DM) and cardiovascular diseases beyond its known role in bone mineral

49 metabolism (5)*.* Vitamin D is required for insulin secretion by pancreatic β-cells and thus

50 may also have a role in insulin sensitivity (6). Subjects with hypovitaminosis D had higher

51 risk of insulin resistance and the metabolic syndrome (7). Similarly, low serum 25-hydroxy

52 vitamin D [25(OH)D] is associated with obesity, insulin resistance and β-cell dysfunction in

53 children and adolescents (8). These findings suggest a potential role for 25(OH)D in

54 maintaining glucose metabolism and pancreatic β-cell function during pregnancy.

55 25(OH)D deficiency is common in pregnant women worldwide (9) including Asian

56 women (10). The fetus does not produce vitamin D and thus depends on maternal vitamin D

57 supply through the placenta. Maternal 25(OH)D status during pregnancy is strongly

58 correlated with the offspring’s 25(OH)D status (11) and has been linked with the offspring’s

59 growth and body composition (12-14). Although growing evidence suggests a role of

60 maternal vitamin D on offspring growth and adiposity, findings are mainly from a small

61 number of studies in Western populations and are inconsistent. In addition, “adiposity” in

62 most of those studies was measured by body mass index (BMI), which reflects both fat mass

63 and fat free mass and does not separate abdominal and non-abdominal adiposity. Abdominal

64 adiposity is especially relevant in Asians who are at higher metabolic risk than the Western

65 population, even at lower or similar BMI or waist circumference (15, 16). Therefore, we

66 hypothesized that maternal vitamin D inadequacy during pregnancy would be associated with

67 higher abdominal adiposity in the newborn Asian infants. This study aimed to focus on the

68 association of 25(OH)D status of Asian mothers at mid-gestation with neonatal abdominal

69 adiposity measured by abdominal adipose tissue (AAT) compartment volumes using

70 magnetic resonance imaging (MRI).

71

72 **Materials/Subjects and Methods**

73 **Study design**

74 Participants were from a prospective birth cohort study in Singapore, Growing Up in

75 Singapore Towards healthy Outcomes (GUSTO)(17). A total of 1247 pregnant women in

76 their first trimester were recruited between June 2009 and September 2010 at the two major

77 public maternity units; KK Women’s and Children’s Hospital (KKH) and National

78 University Hospital (NUH). Details of the inclusion and exclusion criteria were discussed

79 earlier (17). This study was approved by the Domain Specific Review Board of National

80 Health Care group and Centralized Institutional Review Board, SingHealth. Written informed

81 consent was given by all participants.

82 **Subjects**

83 1115 mothers who attended the 32-34 week antenatal ultrasound scan were

84 approached for MRI of their newborns. 478 (43%) mothers signed consent to neonatal

85 magnetic resonance imaging (MRI) of their neonates. Neonates born earlier than late preterm

86 i.e.<34 weeks gestational age with birth weight <2000g were excluded from this study as

87 they were more prone to complications such as hypothermia. Neonates included in this study

88 were not on special neonatal care or neonatal intensive care unit at the time of MRI. MRI was

89 performed preferably within 2 weeks after birth. 416 neonates were eligible; 379 neonates

90 successfully competed MRI and 37 neonates dropped out. 46 data sets did not pass initial

91 quality control for image analysis (18). Neonates of mothers who did not have 25(OH)D at

92 26-28 weeks gestation (N=41) were also excluded. A total of 292 mother-neonate pairs

93 remained for this analysis: 161 male (55.1%), 131 female (44.9%); 131 Chinese (44.9%), 115

94 Malay (39.4%) and 46 Indian (15.7%). We considered this study an exploratory comparison

95 and no a priori sample size calculations were performed. However, from the data we

96 observed in vitamin D sufficiency (N=156)and inadequacy (N=136) groups, we are able to

97 show a difference of 0.35 Cohen’s effect with 80% power and two sided 5% type 1 error.

98 In Supplemental Table 1, we compare the neonates included (N=292) and those not

99 included (N=801) in this study. A total of 823 (1115-292) mothers were approached in

100

pregnancy about taking part in the MRI sub-study but are not included in the final analysis, of

101

whom 22 were born <34 gestational weeks with birth weight <2000 g. Therefore, the final

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non-participant group comprises 803 neonates. Participating neonates had mothers who were

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younger, more likely to be of Malay ethnicity and less likely to have higher than a secondary

104

education than those non-participating infants. Mothers of participating neonates also had

105

marginally higher FPG and 2-h OGTT glucose and lower 25(OH)D levels. Compared with

106

non-participants, participating neonates had a similar mean birth weight and similar

107

proportions of male and female neonates, but a marginally lower gestational age at delivery

108

(38.7 vs 38.9 weeks) (**Supplemental Table 1**).

109

**Maternal and infant characteristics**

110

Demographic data, lifestyle, obstetric and medical history of mothers were collected

111

at 11-12 and 26-28 weeks during antenatal visits using interviewer administered

112

questionnaires. Birth weights measured by midwives at KKH and NUH were obtained from

113

hospital medical records.

114

**Maternal biosample collection for glucose and vitamin D assessment**

115

Blood samples of mothers were collected after at least 8-10 hours of fasting, at 26-28

116

weeks antenatal clinic visit, processed within 4 hours of collection and stored at -80̊ C for

117

subsequent analysis.

118

Plasma samples for 25(OH) D2 and D3 were extracted using vortex-mixing with

119

hexane (Chromonorm). The hexane layer was then evaporated to dryness and reconstituted

120

using a methanol-water (70:30 by volume) mixture (HyperSolv). Analysis of 25(OH)D

121

122

concentration was performed using Applied Biosystems (USA) liquid chromatography-

tandem mass spectrometry with its analystTM software version. 1.3. Chromatographic

123

separation was carried out with a BDS C8 reversed-phase column (ThermoHypersil) (19).

124

The intra- and inter-assay coefficient of variations for 25(OH)D2 and 25(OH)D3 were

125

≤10.3%, and the detection limit was <4 nmol/L for both metabolites. Maternal 25(OH)D

126

status i.e. combined 25 (OH)D2 and 25(OH)D3 was categorized into inadequacy ≤75.0

127

nmol/L vs. sufficiency: >75.0 nmol/L based on recommendations by the Endocrine Society

128

Clinical Practice Guidelines (20).

129

Mothers who attended 26-28week antenatal visit after overnight fasting underwent

130

75-g OGTT. Blood samples were collected at baseline for fasting plasma glucose (FPG) and

131

following a 75-g glucose challenge for 2 hour plasma glucose (2hPG). Glucose levels were

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measured by colorimetry [Advia 2400 Chemistry system (Siemens Medical Solutions

133

Diagnostics) and Beckman LX20 Pro analyzer (Beckman Coulter)]. Gestational diabetes

134

mellitus (GDM) was diagnosed using 1999 World Health Organization standard criteria: ≥7.0

135

or ≥7.8 mmol/l for FPG or 2hPG respectively (21). Pregnant women diagnosed with GDM

136

were managed according to the clinical protocols at KKH and NUH.

137

**Quantification of abdominal adipose tissue compartments**

138

MRI was performed using GE Signa HDxt 1.5 tesla magnetic resonance scanner (GE

139

Healthcare, Wauwatosa, Wisconsin, USA). The AAT from MRI images was categorized into

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superficial subcutaneous (sSAT), deep subcutaneous (dSAT) and internal (IAT)

141

compartments (**Figure 1**). sSAT had a clear anatomical outline following the contours of the

142

axial abdominal images. dSAT was located mainly posteriorly and was distinctly separated

143

from sSAT by a fascial plane. IAT was the internal fat including VAT i.e. amount of fat

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around the internal organs of abdominal cavity, inter-muscular, para-vertebral and intra-

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spinal fat within the abdominal region.

146

AAT compartments were segmented using semi-automated approach; with initial

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automated segmentation by in-house segmentation algorithm using MATLAB 7.13; The

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MathWorks Inc., Natick, Massachusetts, USA and followed by manual optimization by 2

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trained analysts who were blinded to all subject information. The mean inter-observer

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coefficients of variations (%) were 1.6% for sSAT, 3.2% for dSAT and 2.1% for IAT. The

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mean intra-observer coefficients of variations (%) were 0.9%, 2.1% and 4.0% for sSAT,

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dSAT and IAT, respectively. Total AAT volume for each compartment was derived from the

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sum of its volumes in each slice, from the level of dome of diaphragm to the superior aspect

154

of the sacrum (18).

155

**Statistical Analysis**

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As the neonatal AAT compartment volumes were normally distributed

157

**(Supplemental figure 1)**, multivariable regression models were used to assess the overall

158

associations between maternal 25(OH)D (either as continuous or dichotomous categorical

159

variables) and neonatal AAT compartment volumes. Covariates adjusted for included

160

ethnicity, sex, age on MRI day, gestational age, maternal age, maternal education, maternal

161

pre-pregnancy BMI and parity. We did not adjust for birth weight, as it may be on the causal

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pathway between maternal 25(OH)D status and neonatal AAT compartment volumes. In

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addition, we have shown that maternal 25(OH)D status was not associated with birth weight

164

in GUSTO cohort (22). Type of neonatal feeding (breastfeeding or formula feeding) was not

165

included in the multivariable models, as the types of neonatal feeding within 2 weeks of

166

delivery should not have a substantial influence on neonatal adiposity.

167

25(OH)D and its deficiency were related to glucose homeostasis (23, 24), which may

168

in turn influence on their offspring’s adiposity. In our study, maternal 25(OH)D and glucose

169

levels were measured using blood samples collected at the same time, and we are therefore

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unable to analyze their temporal relationship (24). Nonetheless, to examine whether the 25

171

(OH)D-neonates adiposity association was mediated by concurrently measured glycemia, we

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performed a secondary analysis by further adjusting for maternal glycemia, both FPG and

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2HrPG, in multivariable regression analyses. We performed these analyses both in the overall

174

group of 292 neonates and as a sensitivity analysis in the subset (N=237) of mothers without

175

GDM (non-GDM). Statistical analyses were performed using SPSS Statistics for Windows,

176

177

Version 21.0. (IBM Corp., Armonk, NY).

178

**Results**

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**Table 1** summarizes the baseline characteristics of study participants (N=292). 136 (46.6%)

180

of 292 mothers were classified as having 25(OH)D inadequacy although 183 (62.7%)

181

reported taking supplements containing vitamin D at the time of blood collection. 25(OH)D

182

inadequacy was higher in Malay (55.9%) than Chinese (25.7%) and Indian mothers (18.4%)

183

184

(*P*<0.001). Mothers with 25(OH)D inadequacy were younger, mean ±SD: 29 ±5 vs 30 ±5

years (*P*=0.010) and had a higher pre-pregnancy BMI 23.8 ± 5.1 vs 22.4 ± 4.6 kg/m2

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(*P*=0.019) than those who had sufficient 25(OH)D. Mean FPG and 2hPG levels at 26-28

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weeks of gestation were similar in the 25(OH)D sufficient and inadequate groups. Neonates

187

between 2 groups had similar mean birth weight and weight on MRI day, and were born at

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similar gestational weeks. However, neonates with maternal 25(OH)D inadequacy had

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greater sSAT and dSAT compared to those with maternal 25(OH) sufficiency; 81.3 ±22.5 vs

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74.7 ±21.0 ml, *P*=0.010 for sSAT and 14.3 ±5.8 vs 12.2 ± 5.2 ml, *P*=0.002 for dSAT.

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Inverse linear correlations were observed between maternal 25(OH)D and

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subcutaneous AAT volumes: r= -0.190, *P*=0.001 for sSAT and r= -0.206, *P*<0.001 for dSAT.

193

**Table 2** shows AAT volumes in different quartiles (Q) of 25(OH)D (Q1: 20.0 to 58.2, Q2:

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58.3 to 78.0, Q3: 78.1 to 95.8, Q4: 95.9 to 155 nmol/L). sSAT and dSAT volumes decreased

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with increasing quartiles of 25(OH)D. Adjusting for relevant covariates (ethnicity, sex, age

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on MRI day, gestational age, maternal age, maternal education, maternal pre-pregnancy BMI

197

and parity), multivariable regression analysis showed that each 1 nmol/L increase in

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25(OH)D was associated with a reduction in sSAT (-0.14 ml; 95% CI: -0.24, -0.04 ml,

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*P*=0.006) and dSAT (-0.04 ml; 95%CI: -0.06, -0.01 ml, *P*=0.006), but was not associated

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with IAT.

201

Neonates of mothers with 25(OH)D inadequacy had greater abdominal sSAT and

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dSAT volumes than those of mothers with 25(OH)D sufficiency, but had similar IAT

203

volumes. The associations remained significant after adjusting for above-mentioned

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covariates; differences (95%CI) were: 7.3 (2.1, 12.4) ml, *P*=0.006 and 2.0 (0.6, 3.4) ml,

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*P*=0.005 for sSAT and dSAT respectively (**Table 3**). Sensitivity analyses were performed to

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examine the robustness of the results by restricting our analyses to subgroups of neonates

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born >37 weeks gestational age (N=272) and non-small for gestational age neonates born >37

208

weeks gestational age (N=240) (**Supplemental table 2**). The associations between 25(OH)D

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status and the neonatal abdominal adiposity persisted in both subgroups.

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Table 3 also demonstrates the associations of maternal 25(OH)D groups and

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neonatal adiposity in a subset of non-GDM mothers. The effect size for these associations

212

declined by 34% for sSAT, 15% for dSAT and 46% for IAT. Maternal 25(OH)D

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insufficiency remained significantly associated with neonatal dSAT: difference (95%CI); 1.7

214

(0.3, 3.1) ml, *P*=0.019 in this subset.

215

**Supplemental table 3** shows the association of maternal 25(OH)D status with

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neonatal adiposity after adjusting for both maternal FPG and 2HrPG for both the entire MRI

217

cohort (N=292) and in the non-GDM subset (N=237). For maternal FPG in the entire MRI

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cohort, the associations between maternal 25(OH)D status and neonatal abdominal adiposity

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declined by only 19% for sSAT to 5.9 (1.0, 10.8) ml and 15% for dSAT to 1.7 (0.4, 3.0) ml

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compared to the unadjusted associations. However, these associations increased by 12% for

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sSAT, 8.3 (3.0, 13.5) ml and by 13% greater for dSAT, 2.3 (0.9, 3.7) ml after adjusting for

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2HrPG.

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In the non-GDM subset, the associations between maternal 25(OH)D status and

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neonatal adiposity persisted for dSAT; 1.6 (0.2, 2.9) ml and 1.8 (0.4, 3.2) ml after adjusting

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for maternal FPG and 2HrPG, respectively. For sSAT, the association persisted after

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227

adjusting for FPG: 5.4 (0.6, 10.6) ml (Supplemental table 3).

228

**Discussion**

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In this cohort study of mother-neonate pairs, inverse associations were observed between

230

maternal mid-gestation 25(OH)D levels and neonatal abdominal subcutaneous adipose tissue

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compartment volumes; both sSAT and dSAT. These observed associations were present even

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after adjusting for maternal glycemia, both FPG and 2HrPG levels. These findings are

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consistent with those of previous studies in adolescents and adults, which observed inverse

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associations between vitamin D levels and visceral adiposity measured by computed

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tomography or MRI (25-28). Maternal 25(OH)D inadequacy was also associated with greater

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neonatal abdominal subcutaneous adipose tissue (sSAT and dSAT) despite similar birth

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weight and weight on MRI day. For non-GDM mothers, the association between maternal

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25(OH)D inadequacy and neonatal SAT was less pronounced but persisted for dSAT, which

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is more metabolically active and similar to VAT in adults. In the GUSTO cohort, lower

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maternal 25(OH)D status was associated with higher FPG levels (24) with a continuous

241

gradient between maternal glycemia and excessive neonatal adiposity that extended across

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the range of maternal glycemia (29). Therefore, it is expected that association between

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maternal 25(OH)D and neonatal adiposity would be less pronounced in non-GDM group

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compared to that of whole MRI cohort.

245

Abdominal adiposity is known to be associated with higher risks of insulin resistance,

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T2DM and coronary heart disease in adult life and has been widely studied in relation to

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metabolic diseases (30, 31). Abdominal adiposity is relevant especially in Asians who are at

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higher metabolic risk than the Western population even at lower or similar BMI or waist

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circumference (15, 16), a conventional measure for abdominal adiposity. AAT is classically

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grouped into subcutaneous adipose tissue (SAT) and visceral (VAT) or internal adipose

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tissue. Further subdivision of SAT into sSAT and dSAT has been studied only recently.

252

dSAT could be more relevant to metabolic parameters than sSAT and is increasingly

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suggested to be strongly related to increase in insulin resistance and cardio-metabolic

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parameters in a similar manner to VAT (32-34). IAT in neonates includes VAT which is the

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fat around the organs, intermuscular fat, paravertebral and intra-spinal fat (Figure 1).

256

However, amount of VAT is minimal compared to other types of fat within abdominal region

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in neonates (18). Therefore, the presence of IAT in neonates might not reflect metabolically

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active visceral adiposity, as it does in adults. Our findings of increased dSAT in the neonates

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of mothers with 25(OH)D inadequacy suggest a potential role for maternal 25(OH)D in the

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offspring’s susceptibility to metabolic diseases later in life.

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Reported associations between maternal or umbilical cord plasma 25(OH)D status and

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adiposity in neonates have been mixed. Godang et al studied total fat mass (FM) measured by

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dual-energy X-ray absorptiometry in 202 Norwegian mother-neonate pairs. They found no

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association with maternal 25(OH)D at 30-32 weeks gestation but a positive association with

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umbilical cord plasma (UCP) 25(OH)D (13). Josefson et al observed a cross-sectional

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association between UCP 25(OH)D at 36-38 weeks gestation and FM of newborns measured

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by air displacement plethysmography in 61 mother-newborn pairs in Chicago (14). The same

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authors found no relationship between maternal or cord blood 25(OH)D levels measured at

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28 weeks and neonatal adiposity measured by skinfold thickness in a subset of subjects

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(N=360) of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study (35). In the

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Southampton Women’s Survey, Crozier et al found maternal 25(OH)D insufficiency at 34

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weeks of gestation to be associated with lower FM in 977 neonates but greater adiposity at

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age 6 years (12). Other studies observed no association between maternal 25(OH)D and

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neonatal FM measured by skinfolds or bio-electrical impedance analysis (36, 37). All these

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studies measured total adiposity of the offspring, not abdominal adiposity, which is known to

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be more strongly associated with metabolic risk. Therefore, our findings add to the limited

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published information on the association between maternal 25(OH)D and abdominal

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adiposity in Asian neonates, and our study is the first to report on these associations.

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The mechanisms underlying the associations between maternal 25(OH)D levels and

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offspring abdominal adiposity are unclear. Current available data suggests a possible link

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between vitamin D level and glucose homeostasis, but the exact mechanism for role of either

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on the other is not well clear. Several mechanisms have been proposed including the possible

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effects of 25(OH)D on insulin sensitivity and glucose homeostasis. In animal studies,

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25(OH)D affects circulating glucose levels by altering insulin secretion from pancreatic β-

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cells through vitamin D receptors in the pancreas (38, 39). An association of 25(OH)D

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deficiency with β-cell dysfunction and insulin resistance has also been observed in adults. In

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NHANES III, a lower quartile of 25(OH)D (N=6228) was associated with higher fasting

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glucose levels. The prevalence of 25(OH)-deficiency is greater in T2DM patients, and serum

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25(OH)D levels are lower in newly-diagnosed adult diabetics and those with impaired

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glucose tolerance compared to controls (40). A cross-sectional study of healthy glucose-

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tolerant subjects found a significant positive correlation of 25(OH)D levels with insulin

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sensitivity and a negative correlation with first- and second-phase insulin response measured

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by hyperinsulinemic clamp (7). These findings suggest an important role of 25(OH)D on

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glycemia, perhaps via endocrine functioning of the pancreatic β-cells.

295

In our multi-ethnic Asian cohort GUSTO, maternal vitamin D maternal 25(OH)D

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inadequacy was associated with higher fasting plasma glucose levels (24). However, the

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associations between maternal 25(OH)D status and neonatal abdominal subcutaneous tissue

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persisted (though only for dSAT in neonates of the non-GDM mothers) after the adjustment

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for maternal FPG and 2HrPG.

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Our findings may help in developing strategies to prevent vitamin D deficiency in

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offspring of women with (or having a high risk of) 25(OH)D insufficiency. Our observation

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that neonatal adiposity in very early in life is influenced by maternal 25(OH)D insufficiency

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independently of its effect on maternal glycemia suggests that correcting mothers’ 25(OH)

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deficiency may be as important as optimizing their glucose levels.

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Evidence from randomized controlled trials (RCTs) is mixed on the effectiveness of

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vitamin D supplementation during pregnancy on offspring metabolic outcomes. Some RCTs

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have reported a benefit [newborns of pregnant women of the intervention groups had higher

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birth weight than the controls (41-44)], while others have not (45, 46). Systematic review of

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the observational data concluded there was modest evidence to support a relationship

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between maternal 25(OH)D status and offspring birth weight (47). The offspring’s vitamin D

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concentration is largely determined by maternal vitamin D status, and maintaining maternal

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vitamin D status through supplementation might therefore benefit neonatal metabolic

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outcomes such as adiposity. Further observational and maternal vitamin D supplementation

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studies are needed; for example, childhood adiposity assessment is currently underway in the

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MAVIDOS randomized control trial (48).

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Our study has several strengths. To our knowledge, it is the first study to examine the

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association between maternal 25(OH)D status and abdominal adipose tissue distribution in

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neonates using MRI. MRI is the only possible method to quantify AAT compartment

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volumes without radiation. We used a more robust semi-automated approach for

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segmentation of AAT; initial auto-segmentation by in-house segmentation algorithm

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followed by manual optimization performed by trained image analysts. Using MRI to

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measure AAT has enabled us to demonstrate the impact of maternal 25(OH)D levels on

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neonatal abdominal adiposity. This might not have been possible using conventional

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measures, such as birth weight or even total body fat. A recent report from a randomized

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controlled trial by Wagner and Hollis did not observe a difference in birth weight among

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neonates whose mothers received different doses of vitamin D supplementation during

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pregnancy, even though a sufficient vitamin D level (>80nmol/L) was achieved in the group

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receiving 4,000 IU/day (vs 400 and 2000 IU/day) (49).

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In addition, our sample size (N=292) is larger than those of the few previous studies using

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MRI to measure abdominal adiposity (50, 51). Another strength is the use of MS/MS, the

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gold standard for measuring 25(OH)D. The competitive binding assays commonly used for

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measuring 25(OH)D systematically underestimate vitamin D levels, owing to differences in

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antibody affinity.

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One limitation of our study is our single measurement of maternal 25(OH)D. 25(OH)D is

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known to increase throughout pregnancy to compensate the fetus’s increasing demand for

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growth and development (52). If the association between 25(OH)D and neonatal abdominal

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adiposity is more pronounced during the third trimester, we may have underestimated the

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magnitude of this association. Another limitation of our study is the absence of neonatal or

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cord blood 25(OH)D measurements, which would allow us to verify the levels transferred to

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the neonate. However, previous studies have found maternal 25(OH)D status during

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pregnancy to be strongly correlated with both cord blood (56, 57) and later offspring

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25(OH)D status (11, 58). Nor did we measure vitamin D binding protein (DBP) and therefore

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cannot determine whether 25(OH) levels in sufficient and inadequate groups were due to true

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differences in free 25(OH)D levels or variations in DBP levels. Another potential source of

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bias is that mothers of non-participating neonates had higher mean maternal 25(OH)D level

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than mothers of participants, despite a similar mean birth weight between neonates of these 2

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groups. It is possible that inclusion of non-participating neonates might have changed the

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observed associations between maternal 25(OH)D and neonatal abdominal adiposity. In

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addition, as in any other observational study, residual confounding may have affected our

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results, despite our adjustment for measured potential confounders.

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Our study supports an extended role of 25(OH)D in fetal development and the

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offspring’s body composition. Our findings provide novel insight into the associations

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between maternal vitamin D and neonatal adiposity, suggesting that these associations are

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largely independent of maternal glucose levels. Observed greater abdominal adiposity in

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neonates born to in mothers with 25(OH)D inadequacy may place the neonates at higher risk

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of cardio-metabolic diseases later in life. Since increased central fat deposition is a potentially

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modifiable risk factor for T2DM and CVD, our findings, if replicated in both Asian and

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Western population, may have important public health implications. Future randomized

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interventional trials with long-term follow-up will help establish if the observed associations

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are causal and to assess the value of vitamin D supplementation during pregnancy.

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**Authors’ contribution**

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MTT performed statistical analysis and wrote the manuscript. MTT and MVF

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contributed to MR image acquisition, analysis and interpretation of images. IMA contributed

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to statistical analysis by SAS. JK contributed to MR image acquisition and VSJ was

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responsible for neonatal data acquisition. MFC and PLQ were responsible for 25(OH)D data.

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MFC, KMG, MSK, CJH and YSL contributed to critical revision of the manuscript for

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important intellectual content*.* SSM, FY, LYS, KMG, PDG and YSC designed and led

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GUSTO study. MVF and YSL have primary responsibility for the final content of the

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manuscript. MVF and YSL are joint corresponding authors. All authors have read and

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approved the final manuscript.

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**Figure 1.** MRI image of the segmented abdominal adipose tissue compartments. Each

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compartment is filled with different colors: green denotes superficial

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subcutaneous adipose tissue, orange denotes the right and left deep

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subcutaneous adipose tissue; magenta denotes the internal adipose tissue.

**Table 1** Maternal and neonatal baseline characteristics by maternal plasma 25(OH)D levels among

292 participants in the GUSTO Study.

|  |  |  |  |
| --- | --- | --- | --- |
| N (%) | 25(OH)D sufficiency  > 75.0 nmol/L N=156 (53.4) | 25(OH)D inadequacy  ≤ 75.0 nmol/L N=136 (46.6) | *P* |
| **Maternal characteristics** |  |  |  |
| Ethnicity, N (%) |  |  | <0.001 |
| Chinese (N=131) | 96 (61.5) | 35 (25.7) |  |
| Malay (N=115) | 39 (25.0) | 76 (55.9) |  |
| Indian (N=46) | 21 (13.5) | 25 (18.4) |  |
| Parity, N (%) |  |  | 0.879 |
| Primiparous | 61 (39.1) | 52 (38.2) |  |
| Multiparous | 95 (60.9) | 84 (61.8) |  |
| Maternal Education, N (%) |  |  | 0.623 |
| Primary and below | 8 (5.3) | 6 (4.5) |  |
| Secondary/Technical education | 74 (49.0) | 72 (54.1) |  |
| Diploma/ University | 69 (45.7) | 55 (41.4) |  |
| Age (year) | 30 ± 5 | 29 ± 5 | 0.010 |
| Pre-pregnancy BMI (kg/m**2**) | 22.4 ± 4.6 | 23.8 ± 5.1 | 0.019 |
| Fasting plasma glucose (mmol/L) | 4.3 ± 0.4 | 4.5 ± 0.7 | 0.055 |
| 2-hour plasma glucose (mmol/L) | 6.3 ± 1.5 | 6.1 ± 1.4 | 0.250 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Neonatal characteristics**  Sex, N (%) |  |  |  | 0.240 |
| Male (N=161) |  | 91 (58.3) | 70 (51.5) |  |
| Female (N=131) |  | 65 (41.7) | 66 (48.5) |  |
| Birth weight (kg) |  | 3.1 ± 0.5 | 3.2 ± 0.4 | 0.081 |
| Gestational age (wk.) |  | 38.7 ± 1.2 | 38.7 ± 1.2 | 0. 526 |
| Age on MRI day (d) |  | 10 ± 3 | 10 ± 2 | 0.166 |
| Weight on the day of MRI | (kg) | 3.1 ± 0.7 | 3.2 ± 0.6 | 0.256 |
| sSAT (ml) |  | 74.7 ± 21.0 | 81.3 ± 22.5 | 0.010 |
| dSAT (ml) |  | 12.2 ± 5.2 | 14.3 ± 5.8 | 0.002 |
| IAT (ml) |  | 22.9 ± 8.1 | 22.9 ± 7.3 | 0.976 |

Data shown are N (%) for categorical variables or mean ± SD for continuous variables.

P values are based on between group comparisons of 25(OH)D groups using ANOVA for continuous variables and Chi square test for categorical variables among 25(OH)D groups.

Abbreviations: sSAT: abdominal superficial subcutaneous adipose tissue, dSAT: abdominal deep subcutaneous adipose tissue, IAT: abdominal internal adipose tissue

**Table 2** Abdominal adipose tissue compartment volumes by quartiles of maternal plasma25(OH)D levels among 292 participants in the GUSTO Study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 25(OH)D quartiles | N | Mean *±* SD | P for trend |
| Superficial subcutaneous | 1 | 73 | 81.2 ± 20.9 | 0.013 |
| adipose tissue | 2 | 75 | 80.0 ± 23.9 |  |
|  | 3 | 71 | 76.9 ± 21.1 |  |
|  | 4 | 73 | 72.7 ± 20.9 |  |
| Deep subcutaneous | 1 | 73 | 13.8 ± 4.9 | 0.006 |
| adipose tissue | 2 | 75 | 14.4 ± 6.7 |  |
|  | 3 | 71 | 13.1 ± 5.1 |  |
|  | 4 | 73 | 11.5 ± 5.2 |  |
| Internal adipose tissue | 1 | 73 | 22.4 ± 6.3 | 0.900 |
|  | 2 | 75 | 23.1 ± 8.0 |  |
|  | 3 | 71 | 23.7 ± 8.3 |  |
|  | 4 | 73 | 22.4 ± 8.1 |  |

Data shown are mean ± SD.

25(OH)D quartile 1: 20.0 to 58.2, quartile 2: 58.3 to 78.0, quartile 3: 78.1 to 95.8, quartile 4: 95.9 to 155 nmol/L.

**Table 3** Mean difference in neonatal abdominal adiposity according to maternal plasma

25 (OH)D status (i.e. sufficiency vs. inadequacy) in GUSTO Study

**Overall MRI group (N=292)**

sSAT (ml) dSAT (ml) IAT (ml)

25(OH)D sufficiency (>75.0 nmol/L) Reference Reference Reference

25(OH)D inadequacy (≤ 75.0 nmol/L) 7.3 (2.1, 12.4) 2.0 (0.6, 3.4) 1.1 (-0.8, 2.9)

*P* =0.006 *P* =0.005 *P* =0.268

**Non-GDM group (N=237)**

25(OH)D sufficiency (>75.0 nmol/L) Reference Reference Reference

25(OH)D inadequacy (≤ 75.0 nmol/L) 4.8 (-0.6, 10.1) 1.7 (0.3, 3.1) 0.6 (-1.3, 2.4)

*P* =0.075 *P* =0.019 *P* =0.549

Abbreviations: sSAT: abdominal superficial subcutaneous adipose tissue, dSAT: abdominal deep subcutaneous adipose tissue, IAT: abdominal internal adipose tissue.

Associations shown are differences in mean (95% CI) of 25(OH)D inadequacy group vs the reference

25(OH)D sufficient group.

P values were determined with the use of multivariable regression models. Total sample size (N) is not always 292 or 237 due to the missing values.

Models controlled for ethnicity, sex, age on MRI day, gestational week, maternal age, maternal education,

maternal pre-pregnancy BMI and parity.

