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Outi Pellonperä, Ella Koivuniemi, Tero Vahlberg, Kati Mokkala, Kristiina Tertti, Tapani Rönnemaa, Kirsi Laitinen



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1 *Clinical Nutrition*

2 **Dietary quality influences body composition in overweight and obese**
3 **pregnant women**

4 Outi Pellonperä¹, Ella Koivuniemi², Tero Vahlberg³, Kati Mokkalä², Kristiina Tertti¹, Tapani
5 Rönnemaa⁴, Kirsi Laitinen²

6

7 ¹University of Turku and Turku University Hospital, Department of Obstetrics and Gynecology

8 ²University of Turku, Institute of Biomedicine

9 ³University of Turku, Department of Biostatistics

10 ⁴University of Turku and Turku University Hospital, Department of Medicine

11

12 Correspondence: Outi Pellonperä, Department of Obstetrics and Gynecology, Turku University
13 Hospital, Kiinamylynkatu 4-8, PL 52, 20521 Turku, Finland. E-mail outi.pellonpera@utu.fi. Tel.
14 +35823130384. Fax +358 2 3132340.

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18 Key words: body composition; gestational weight gain; nutrition; obesity; physical activity;
19 pregnancy

20 **Abbreviations:**

21 BF% body fat percentage

22 FFM fat free mass

23 FM fat mass

24 GWG gestational weight gain

25 IDQ-index index of diet quality

26 IOM The Institute of Medicine

27 MET-index metabolic equivalent index for leisure-time physical activity

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38 **ABSTRACT**

39 **Background & aims:** Excessive adiposity and gestational weight gain (GWG) have been linked
40 with maternal and offspring morbidity. We investigated the relation of maternal diet, physical
41 activity and GWG on body composition in overweight and obese pregnant women.

42 **Methods:** Fat mass (FM) and fat free mass (FFM) of 110 overweight and obese pregnant women
43 were measured by air displacement plethysmography in early and late pregnancy (mean 13 and 35
44 gestational weeks). At the same time points, the quality of overall diet was assessed by validated
45 index of diet quality (IDQ) questionnaire (score < 10/15 denotes poor dietary quality and score \geq
46 10/15 denotes good dietary quality), nutrient intakes by 3-day food diaries, and physical activity by
47 questionnaire. Weight gain between early and late pregnancy was compared to the gestational
48 weight gain guidelines issued by Institute of Medicine.

49 **Results:** Of the women, 77% gained more weight than recommended; this was related to greater
50 dietary fat consumption (80 ± 21 g/day vs. 67 ± 11 g/day, $p=0.010$) and greater increase in FM
51 (2.7 ± 3.0 kg vs. -1.0 ± 2.4 kg, $p<0.001$) compared to women with ideal GWG. Dietary protein intake
52 (g) correlated positively with FFM at both time points (early pregnancy: $r=0.31$, $p<0.002$, late
53 pregnancy: $r=0.39$, $p<0.001$). Women with higher dietary quality had more FFM, compared to
54 women with lower dietary quality (early pregnancy FFM: 48.8 ± 5.8 kg vs. 45.8 ± 4.7 kg, $p=0.004$, late
55 pregnancy FFM: 56.1 ± 6.4 kg vs. 53.4 ± 5.6 kg, $p=0.025$). No correlations were detected between total
56 energy intake or physical activity and FM or FFM at early or late pregnancy.

57 **Conclusions:** Body composition changes from early to late pregnancy were related to the amount of
58 weight gained and overall dietary quality during pregnancy. Higher dietary quality and protein
59 intake were associated with greater FFM, while dietary fat intake was related to excess weight
60 gain. Identification of these dietary determinants of body composition and weight offers new targets

61 for dietary counselling of pregnant women and thus potential for ensuing health benefits through
62 reduced adiposity.

63 **INTRODUCTION**

64 Over the last decades, the obesity epidemic has spread and grown worldwide. Approximately every
65 third woman of reproductive age in Europe and more than every other woman in the United States
66 is overweight or obese (BMI $>25\text{kg m}^{-2}$ and $\geq 30\text{kg m}^{-2}$ respectively)^{1,2}. Maternal obesity is
67 associated with a variety of health problems both to the mother and her child during pregnancy,
68 delivery and in later life^{3,4}. The mother is at risk of developing gestational diabetes, hypertension,
69 pre-eclampsia or peripartur complications, and in later life, type 2 diabetes and cardiovascular
70 disease⁵. The child is predisposed to suffering birth defects, prematurity, and macrosomia, and to
71 the programming of obesity and a range of metabolic conditions, which may influence the health
72 over the long term^{1,5,6}.

73 Body composition measurement by air displacement plethysmography (ADP) applies densitometric
74 principles of lean soft-tissues and fat to estimate fat mass (FM) and fat free mass (FFM), from
75 which body fat percentage (BF%) can be calculated. During the pregnancy the composition of the
76 weight gain includes gains in maternal FM and FFM, which involve expansion of the body water
77 compartments to support the pregnancy, and related tissues (placenta and amniotic fluid) and the
78 fetus. There is marked inter-individual variation in FM and FFM gain, addressing the importance
79 of measuring body composition⁷⁻⁹. Thus, body composition gives much more precise information
80 about the adiposity of the body than widely used BMI and reflects also nutritional status^{7,10}. It is
81 well known that age, diet, physical activity and chronic illness may affect body composition in the
82 non-pregnant state¹¹. Although less studied, these same features are also likely to be related to body
83 composition during pregnancy. The knowledge of the effects of lifestyle on gestational body

84 composition enables the development of counseling procedures for pregnant women by health care
85 professionals. This in turn would be expected to improve the health of both mother and child.

86 The degree of adiposity and gestational weight gain (GWG) have been linked to the pregnancy-
87 associated maternal and offspring morbidity¹²⁻¹⁴. The Institute of Medicine (IOM) has issued
88 guidelines for GWG; these recommend less weight gain for women with higher pre-pregnancy BMI
89 to minimize the risks for pregnancy-related complications for both mother and child¹⁵. At the same
90 time, it is recognized that more information is needed regarding the association of lifestyle factors
91 with body composition in pregnant women, particularly in those in the upper BMI categories, as
92 this would help targeting ways of health promoting optimal GWG to those women most in need of
93 counselling.

94 The aim of this study was to investigate the changes in body composition over pregnancy and the
95 extent to which diet and physical activity influence body composition in overweight and obese
96 pregnant women. Secondly, we evaluated the differences in maternal body composition and
97 lifestyle in relation to the GWG recommendations issued by IOM¹⁵.

98 **MATERIALS AND METHODS**

99 This prospective study examined 110 pregnant women living in Southwest Finland. The data were
100 collected from overweight and obese women participating in a mother-infant dietary intervention
101 trial (ClinicalTrials.gov Identifier: NCT01922791). This study was conducted according to the
102 guidelines laid down in the Declaration of Helsinki and approved by the Ethics Committee of the
103 Hospital District of Southwest Finland. Written informed consent was obtained from all subjects.

104 Women at least 7 and maximum 17 gestational weeks and $\text{BMI} \geq 25 \text{ kg m}^{-2}$ were recruited in the
105 study. Exclusion criteria included: pre-pregnancy $\text{BMI} < 25 \text{ kg m}^{-2}$; more than 17 gestational weeks
106 at recruitment; chronic diseases impacting metabolic and gastrointestinal health such as diabetes or

107 inflammatory bowel diseases. We recruited generally healthy women. However, certain relatively
108 common conditions that were not thought to interfere with body composition results were allowed.
109 These were asthma and allergies, mild mental disorders, migraine and medically controlled
110 hypothyroidism. In this analysis, we included in chronological order the first 135 women who were
111 recruited. Study visits were conducted in early and in late pregnancy (mean 13.5 and 35.3
112 gestational weeks). Those women who were recruited but did not attend to the late pregnancy visit
113 were excluded (n=25). The baseline characteristics did not differ between the included and 25
114 excluded women (data not shown).

115 Height was measured at the first visit with a wall stadiometer to the nearest 0.1cm. Pre-pregnancy
116 weight was self-reported and was found to correlate highly with medical record values of measured
117 weight at the first antenatal visit in local maternity clinics (Pearson correlation 0.99, $p < 0.000001$).
118 On both study visits, women had their body composition and weight measured. Women were
119 classified into groups of excess, ideal and inadequate gestational weight gain according to the
120 recommendations issued by the IOM¹⁵. The actual measured weight gain between early and late
121 gestation visits was compared to recommended minimum and maximum weight gains taking into
122 account the specific weeks of gestation and the recommended weight gain over the follow-up
123 period. The IOM recommendation assumes a weight gain of 0.5-2kg in the first trimester for all
124 women and during the 2nd and the 3rd trimesters of pregnancy, overweight women (pre-pregnancy
125 BMI 25.0-29.9kg m⁻²) are advised to gain 0.23-0.33kg per week and obese women (pre-pregnancy
126 BMI ≥ 30.0 kg m⁻²) 0.17-0.27kg per week. Enrollment of some women in the first trimester (i.e. \leq
127 13+0 gestational weeks) was taken into account by multiplying the proportion of gestational weeks
128 that was left from the first trimester with either 0.5 kg (recommended minimum) or 2.0 kg
129 (recommended maximum) and by adding these first trimester weight gains respectively to
130 recommended minimum or maximum weight gains during 2nd and 3rd trimesters of pregnancy.

131 Questionnaires concerning physical activity and dietary quality were filled in by 107-110 of
132 women. Women were also asked to fill in questionnaires concerning their education, smoking
133 habits and obstetric medical history.

134 **Body composition measurements**

135 Air displacement plethysmography and an electronic scale (the Bod Pod system, software version
136 5.4.0, COSMED, Inc., Concord, CA, USA) were used to measure body volume and weight
137 according to the manufacturer's instructions. FM and FFM in kilograms were calculated from
138 density using the formulas devised by van Raaij et al.¹⁶, which take into account the length of
139 gestation and the presence of marked general swelling when necessary. Thoracic gas volume was
140 measured whenever possible (n=100/110 in early gestation and n=106/110 in late gestation) and
141 used in the calculations of FM and FFM, otherwise predicted thoracic gas volume was used in body
142 composition calculations. After overnight fasting and emptying the bladder, subjects entered the
143 measurement chamber wearing a tight cap and tight underwear. They were advised not to exercise
144 or to shower in the morning of measurements.

145 **Dietary intake**

146 Three-day food diaries (2 weekdays and 1 weekend day) were recorded during the week preceding
147 the study visits. Subjects were given oral and written instructions on how to fill in the food diary
148 and diaries were checked for completeness and accuracy with the help of an illustrated portion
149 booklet. Mean daily intakes of energy (megajoules) and macronutrients (grams and E%) were
150 calculated by using computerized software (AivoDiet 2.0.2.3; Aivo, Turku, Finland) utilizing the
151 food composition database provided by the Finnish National Institute for Health and Welfare
152 (www.fineli.fi).

153 The quality of overall diet was assessed by validated index of diet quality (IDQ) questionnaire on
154 both study visits¹⁷. This questionnaire contains 18 questions regarding the frequency and amount of
155 consumption of food products during the preceding week. The health promoting criteria included:
156 use of wholegrain bread (≥ 4 slices/per day), saturated/unsaturated fatty acids (vegetable oil-based
157 margarine on bread, fish at least twice a week, use of low-fat $\leq 1\%$ dairy products, use of vegetable
158 oil-based salad dressing), dairy products (at least 4 dl/ day), consumption of vegetables, fruits and
159 berries (≥ 400 g/day), and use of sugar containing drinks and sweets (soft drinks max once/week,
160 sweets max once/week, fruit and berry juices max 1 glass/day), and less than 2 skipped meals/week.
161 The quality of the diet was defined as poor when index points were less than ten out of the
162 maximum 15 points and good when points were $\geq 10/15$ ¹⁷.

163 **Physical activity**

164 Physical activity was assessed by a questionnaire¹⁸. Women were asked to report the intensity,
165 frequency and duration of their habitual leisure-time physical activity during the preceding week. A
166 metabolic equivalent index for leisure-time physical activity (MET-index) was calculated from the
167 product of intensity x frequency x duration of activity (MET h/wk) on both study visits. The
168 coefficients for the intensity of physical activity were estimated from the existing tables¹⁹.

169 **Statistical analysis**

170 The normality of the data was checked visually from histograms. The data were summarized as
171 frequencies and percentages for categorical variables and as means and standard deviations for
172 normally distributed continuous variables. MET-indexes were generally non-normally distributed;
173 consequently, medians and interquartile ranges are reported. Ninety five percent confidence
174 intervals were calculated in cases where differences were reported.

175 In the comparisons, paired samples t-test was used to calculate change between early and late
176 pregnancy in body composition variables or daily energy/macronutrient intakes. Wilcoxon signed
177 ranks test was used to estimate if there were changes in physical activity (MET-index). To compare
178 the differences in body composition between women with inadequate, ideal or excess weight gain,
179 we used one-way ANOVA with Games-Howell post-hoc tests. When comparisons of energy intake,
180 IDQ- or MET-indices were made between subgroups of different GWG and BMI categories, one-
181 way ANOVA and independent samples t-test were used for normally distributed data and Mann
182 Whitney and Kruskal-Wallis tests for non-normally distributed data (Supplementary table 2). When
183 the subgroups were compared, adjustment for age and gestational weeks were not done, since these
184 variables did not differ significantly between the groups.

185 Possible associations between lifestyle variables and body composition measures (outcome
186 variables) were assessed using partial Pearson's correlation test adjusting for gestational weeks and
187 continuous pre-pregnancy BMI. In adjustments, gestational weeks at the point of measurement of
188 each variable were used and when change was evaluated, we also adjusted for weeks between early
189 and late gestation measurements. Associations between MET-index and body composition
190 (outcome variable) were assessed with Spearman's correlation test without adjustments, since no
191 correlation between body composition variables and gestational weeks or pre-pregnancy BMI
192 existed. Correlations of at least medium effect size ($r \geq 0.3$) were reported²⁰. We also conducted
193 linear models assessing the relationship between explanatory life style variables (physical activity,
194 dietary quality and macronutrient intake) and change in either FM, FFM or BF% (outcome
195 variables) (Supplementary table 2). We adjusted all models for intervention group, gestational
196 weeks at enrollment, age, height, and for each early gestation body composition variable.

197 A p-value < 0.05 was considered significant. Analyses were conducted with IBM SPSS statistics
198 version 22.0 for Windows (IBM SPSS Inc. USA, Chicago, IL, USA).

199 **RESULTS**

200 All participants were white Caucasians. The clinical characteristics of the women are presented in
 201 table 1; 55% were overweight and 45% were obese. Women participating in the study were
 202 generally in good health, although 29 reported having asthma or allergies, 5 mild mental disorders,
 203 5 migraine, 4 hypothyroidism controlled by medication and 2 psoriasis. No significant differences
 204 in body composition variables between women with or without asthma and allergies were found.

205 Table 1. Characteristics of the pregnant women.

Characteristics	n=110
Primipara	49 (44.5)
Age (years)	30.2 ±4.8
Pre-pregnancy BMI (kg m ⁻²)	29.8 ±4.1
Overweight	61 (55.5)
Pre-pregnancy BMI (kg m ⁻²)	27.0 ±1.7
Obese	49 (44.5)
Pre-pregnancy BMI (kg m ⁻²)	33.3 ±3.3
Gestational age 1st visit (wk)	13.5 ±2.5
Gestational age 2nd visit (wk)	35.3 ±1.1
College or university education*	65 (60.7)
Smoking before pregnancy*	20 (18.7)
Smoking during pregnancy	7 (6.4)

206
 207 Data are expressed as numbers (percentages) or mean ±SD

208 Overweight BMI 25-29.9 kg m⁻², Obese BMI ≥ 30.0 kg m⁻²

209 *data available for n=107

210

211 **Weight and body composition**

212 The weight and body composition as well as their change over the follow-up period are presented in
 213 table 2. The mean weight gain was 9.1 kilograms (range 1.1-19.4kg) over a mean of 21.7 weeks. On
 214 average, 17% of the weight gain was FM and 83% FFM. Consequently, body fat percentage (BF%)
 215 decreased in the majority of the women (81%).

216 Table 2. Weight and body composition in 110 overweight and obese pregnant women measured with
 217 electronic scale and air displacement plethysmography.

	Early gestation	Late gestation		
	Mean \pm SD	Mean \pm SD	Mean change (95 % CI)	P-value ^b
All women n=110				
Weight (kg)	84.6 \pm 13.7	93.7 \pm 14.4	9.1 (8.4 ; 9.8)	<0.001
Fat mass (kg)	37.2 \pm 10.3	38.8 \pm 10.5	1.6 (0.9 ; 2.2)	<0.001
Fat free mass (kg)	47.4 \pm 5.4	54.9 \pm 6.2	7.5 (7.2 ; 7.9)	<0.001
Fat percentage	43.4 \pm 5.9	40.8 \pm 5.6	-2.5 (-3.0 ; -2.0)	<0.001
Overweight women n= 61				
Weight (kg)	76.6 \pm 8.3	86.6 \pm 10.1	9.9 (9.0 ; 10.9)	<0.001
Fat mass (kg)	30.7 \pm 5.7	33.1 \pm 6.8	2.5 (1.6 ; 3.3)	<0.001
Fat free mass (kg)	46.1 \pm 5.3	53.4 \pm 5.9	7.5 (7.0 ; 7.9)	<0.001
Fat percentage	39.9 \pm 4.9	38.0 \pm 4.8	-1.8 (-2.5 ; -1.2)	<0.001
Obese women n=49				
Weight (kg)	94.6 \pm 12.5	102.7 \pm 14.1	8.1 ^a (7.0 ; 9.2)	<0.001
Fat mass (kg)	45.4 \pm 8.9	45.8 \pm 10.0	0.4 ^a (-0.6 ; 1.5)	0.402
Fat free mass (kg)	49.2 \pm 5.1	56.8 \pm 6.1	7.6 ^a (7.0 ; 8.3)	<0.001
Fat percentage	47.7 \pm 3.8	44.3 \pm 4.4	-3.4 ^a (-4.1 ; -2.8)	<0.001

219 CI = confidence interval

220 Overweight BMI 25-29.9 kg m⁻², Obese BMI \geq 30.0 kg m⁻²

221 ^a Statistical significance of difference between overweight and obese women in the development of weight,
 222 fat mass, fat free mass and body fat percentage: p=0.011, p=0.002, p= 0.648 and p= 0.001, respectively.

223 ^b Paired Samples T-test

224 **Impact of dietary intake and dietary quality on body composition**

225 Although the total energy intake did not differ significantly between the early and late gestation, the
 226 intake of fat increased and that of carbohydrates as a proportion of energy intake (E%) decreased,
 227 (table 3). The dietary quality measured by IDQ did not change between the visits (9.8 \pm 2.2 vs.
 228 9.8 \pm 2.1, p=0.81) and was found to be poor in 50% and 42% of the women in early and late
 229 pregnancy, respectively.

230 When the effects of diet on body composition were evaluated, it was found that women with poor
 231 dietary quality had significantly less FFM compared to those women consuming a good quality diet
 232 (in early pregnancy 45.8 ± 4.7 kg vs. 48.8 ± 5.8 kg, $p=0.004$ and in late pregnancy 53.4 ± 5.6 kg vs.
 233 56.1 ± 6.4 kg, $p=0.025$). With respect to the macronutrients, protein intake (g) was found to correlate
 234 positively with FFM both in early and late gestation ($r=0.31$, $p=0.002$ and $r=0.39$, $p<0.001$
 235 respectively). No other correlations were detected between body composition measures and intakes
 236 of other macronutrients, total energy intake or dietary quality (Supplementary table 1). Linear
 237 models examining the association of dietary quality and macronutrient intake (in grams) with
 238 change in body composition variables did not change these results (Supplementary table 2).

239
 240 Table 3. Daily intakes of energy and macronutrients in early and late gestation.

	Early gestation	Late gestation		
	Mean \pm SD	Mean \pm SD	Mean change (95% CI)	P-value ^a
Energy, MJ	8.1 ± 2.0	8.3 ± 2.2	0.2 (-0.2, 0.6)	0.395
Carbohydrates, E%	46.7 ± 6.2	44.2 ± 7.1	-2.5 (-4.2, 0.8)	0.005
Carbohydrates, g	225.2 ± 71.4	217.8 ± 73.0	-7.3 (-23.1, 8.5)	0.362
Fat, E%	33.6 ± 6.3	36.3 ± 7.0	2.7 (1.1, 4.3)	0.001
Fat, g	73.9 ± 22.0	81.6 ± 25.9	7.7 (2.6, 12.9)	0.004
Protein, E%	17.4 ± 4.4	17.1 ± 3.6	-0.3 (-1.2, 0.6)	0.496
Protein, g	81.5 ± 21.8	82.0 ± 21.7	0.5 (-4.1, 5.1)	0.834

242 Data obtained from 3-day food diaries filled in by 99 women in both early and late gestation

243 CI = confidence interval, E% = Proportion of energy intake

244 ^a Paired Samples T-Test

245

246 **The association of physical activity with body composition**

247 Physical activity reduced significantly over the pregnancy: the median MET-index was 5.0 h/wk
248 (2.0-12.0) in early gestation and 3.0 h/wk (0.2-11.0), $p < 0.001$ at late gestation. No correlations were
249 detected between physical activity or the change in physical activity and body composition
250 measurements in either early or late gestation (data not shown). Linear model examining the
251 relation of physical activity with change in body composition did not reveal significant associations
252 (Supplementary table 2).

253

254 **Differences in body composition and lifestyle between overweight and obese women**

255 As shown in table 2, the obese women gained less weight than overweight women ($p = 0.011$). This
256 was attributable to a lower increase in their FM ($p = 0.002$) whereas the gain of FFM did not differ
257 between the groups ($p = 0.648$). Consequently, obese women experienced a greater decrease in body
258 fat percentage ($p = 0.001$) over the follow-up period.

259 There were no significant differences in the total energy intake, dietary quality or physical activity
260 between the overweight and obese subjects ($p > 0.125$ in all comparisons, Supplementary table 3).

261 When intakes of macronutrients were compared, only the daily fat intake increased more between
262 early and late gestation in obese than in overweight women (mean 14.5 ± 27.3 g vs. 3.9 ± 25.3 g,
263 $p = 0.048$).

264 There was no significant difference between overweight and obese women in the distribution into
265 the inadequate, ideal or excess GWG class ($p = 0.207$). The overweight women with excess weight
266 gain acquired significantly more FM than obese women with excess weight gain ($p = 0.048$), but
267 there was no difference in the FFM gain ($p = 0.392$). Consequently, in the women with excess weight
268 gain, BF% decreased less in overweight women than in their obese counterparts ($p = 0.006$).

269 **Adherence to the weight gain recommendations**

270 Most of the women, 77%, exceeded the recommended weight gain whilst 11% had an inadequate
271 weight gain (table 4). Only women with excess GWG gained FM and the change in FM was
272 significantly different between women with excess and ideal weight gain. Although women with
273 excess GWG also gained more FFM, BF% decreased significantly less than in women with ideal
274 GWG. The average daily fat intake, calculated as a mean fat intake in early and late gestation, was
275 significantly higher in women with excess than ideal weight gain ($80\pm 21\text{g}$ vs. $67\pm 11\text{g}$, $p<0.010$),
276 while no significant differences in the intakes of other macronutrients were found (data not shown).

277 Compared with women with the ideal weight gain, the women with inadequate weight gain lost
278 significantly more FM, whilst no difference in the change of FFM was found (Table 4). When
279 intakes of macronutrients were compared, only a lower intake in the average gestational
280 carbohydrate intake as a percentage of energy intake ($41.9\pm 4.3\text{ E}\%$ vs. $47.3\pm 4.4\text{ E}\%$ respectively,
281 $p=0.018$) was detected in women with inadequate compared with ideal weight gain.

282 The energy intake, dietary quality or physical activity did not differ between the women with ideal,
283 inadequate and excess weight gain ($p\geq 0.092$ in all comparisons, Supplementary table 3).

284 Table 4. The change of weight and body composition in overweight and obese women with inadequate, ideal or excess GWG.

Adherence to GWG recommendations	N (%)	Δ weight	Δ FM		Δ FFM		Δ BF%	
		Mean (kg) \pm SD	Mean (kg) \pm SD	P-value*	Mean (kg) \pm SD	P-value*	Mean (%) \pm SD	P-value ^a
All women n=110								
Inadequate	12 (10.9)	2.79 \pm 0.89	-3.44 \pm 1.92	0.042	6.23 \pm 2.03	0.996	-5.45 \pm 1.86	0.100
Ideal	13 (11.8)	4.95 \pm 1.27	-1.21 \pm 2.38		6.16 \pm 1.86		-3.70 \pm 2.19	
Excess	85 (77.3)	10.64 \pm 2.90	2.70 \pm 2.97	0.0001	7.94 \pm 1.92	0.015	-1.94 \pm 2.39	0.041
Overweight n=61								
Inadequate	5/61 (8.2)	2.73 \pm 1.11	-2.97 \pm 1.25	0.061	5.70 \pm 1.11	0.987	-5.40 \pm 1.07	0.175
Ideal	5/61 (8.2)	6.27 \pm 0.74	0.42 \pm 2.30		5.85 \pm 1.89		-2.72 \pm 2.61	
Excess	51/61 (83.6)	11.01 \pm 3.04	3.22 \pm 2.87	0.106	7.79 \pm 1.75	0.169	-1.37 \pm 2.40	0.551
Obese n=49								
Inadequate	7/49 (14.3)	2.83 \pm 0.79	-3.78 \pm 2.32	0.372	6.61 \pm 2.51	0.974	-5.49 \pm 2.36	0.549
Ideal	8/49 (16.3)	4.12 \pm 0.67	-2.23 \pm 1.91		6.35 \pm 1.95		-4.31 \pm 1.78	
Excess	34/49 (69.4)	10.08 \pm 2.64	1.92 \pm 2.99	0.0004	8.16 \pm 2.16	0.094	-2.80 \pm 2.13	0.135

286 ^a Inadequate and excess weight gains are compared to ideal weight gain (one-way ANOVA with Games-Howell post-hoc tests)287 Women were divided into different GWG classes according to the recommendations issued by Institute of Medicine¹⁵

288 **DISCUSSION**

289 We demonstrated here that the good overall dietary quality and protein intake in grams were
290 positively associated with FFM in early and late pregnancy. Most overweight and obese women
291 exceeded gestational weekly weight gain recommendations, which led to an increase in body FM
292 and was also reflected in BF%. The most important macronutrient determinant of excess GWG was
293 fat intake. These results suggest that health counselling of overweight and obese pregnant women
294 should focus on optimizing their weight gain by improving the overall quality of diet and favoring a
295 proportional increment of protein intake instead of augmenting fat intake during gestation. The
296 subsequent benefits may be seen in maternal body composition and further in improved maternal
297 and child health.

298 Our results indicate, that among the overweight and obese pregnant women, the majority of weight
299 gain is FFM and only women exceeding the recommendations of GWG gain overall FM, which is
300 in line with a previous report with a similar setting²¹. Other studies also confirm the association of
301 excess GWG with the FM accrual^{22,23}. However, in our study, recommendations of GWG rate were
302 exceeded even more frequently than previously reported for total GWG among overweight and
303 obese women (77% vs. 41-45%)^{21,24}. Some observational studies^{25,26} have suggested that a higher
304 energy intake is associated with higher GWG, but studies are inconsistent²⁷. According to our
305 results, the increment in adiposity was not explained by the energy intake or physical activity per se.
306 Even after adjustments for possible confounding factors in the linear model, no significant
307 associations between gain of FM and several life style variables were found. In previous
308 reports^{15,24,28} as well as in the present study, it has been revealed that both GWG and increase in FM
309 are inversely proportional to pre-pregnancy BMI.

310 All things considered, energy intake and physical activity do not seem to account wholly for
311 gestational FM accrual or differences in FM gain between women with excess and ideal GWG or

312 overweight and obese women. Several mechanisms have been proposed to explain these
313 phenomena. Firstly, despite the lack of correlation with maternal body composition, physical
314 activity was strongly reduced during pregnancy, as reported also by others^{29,30}. In the third
315 trimester, the reduction in physical activity can account for nearly half of the estimated additional
316 energy needs of pregnancy, at least in normal weight women³¹. Secondly, differences in
317 macronutrient intakes or in the hormonal milieu could exert some influence³². In our study, women
318 increased their fat intake, and those with excess GWG were found to have significantly higher
319 average fat consumption than women experiencing an ideal GWG. Reports in the literature are
320 inconsistent regarding the relation of fat intake and GWG²⁷, but some studies suggest that there is
321 an association, at least among overweight women^{25,33}. Thirdly, presumably heavier women need
322 more energy to support their increased metabolic size. Additionally, it has been suggested that
323 cumulative increases in basal metabolic rate are significantly correlated with pre-pregnancy FM,
324 indicating that fatter women display the energy-profligate response. This indicates that the obese
325 women waste energy in pregnancy by increasing their basal metabolic rate, resulting in less FM
326 gain than non-obese women³⁴⁻³⁶. This could explain our finding with less FM accrual in obese
327 compared to overweight women despite their similar lifestyles. Finally, it has been postulated that
328 pregnancy alters homeostatic mechanisms, allowing for a more efficient storage of fat. The
329 proposed mechanism is pregnancy-induced alterations in gut microbiota allowing for highly
330 efficient energy extraction from the diet without major changes in energy intake or energy
331 expenditure³⁷⁻³⁹. All in all, it is apparent that there are several complex mechanisms behind GWG
332 and FM accrual and these mechanisms are probably dependent on pre-gestational fat stores or some
333 other unknown factors.

334 Based on the gestational dietary reference intakes issued by the IOM⁴⁰, it is evident that fat intake
335 increased above the recommendations during the study. At the same time, protein intake remained

336 constant and at a sufficient, but not a particularly high, level. During pregnancy, the average protein
337 requirement increases to provide additional amino acids for protein synthesis in maternal, fetal, and
338 placental tissues. Protein intake correlated with FFM both in early and late gestation, which is
339 logical since FFM consists mainly of water and protein^{41,42}. Favoring protein intake over
340 augmenting fat consumption could be beneficial in reducing body adiposity also during pregnancy,
341 since FFM is the main determinant of basal metabolic rate, which in turn, is an essential component
342 of total energy expenditure⁴³⁻⁴⁵. Protein intake has been suggested to decrease GWG as a result of
343 higher energy expenditure because the thermogenesis of protein is larger than that of carbohydrate
344 or fat^{46,47}. Furthermore, a higher protein intake might also increase satiety and appears to be safe
345 during pregnancy^{48,49}. These adjustments to the diet are already possible within current nutritional
346 recommendations⁴⁰, and thus may be implemented by counseling the pregnant women.

347 The strength of this study was that this was a prospective study in a well-controlled setting with a
348 large sample size compared to most earlier studies involving air displacement plethysmography
349 measurements during pregnancy^{9,21,50}. As is the case with other techniques, body composition
350 measurements with air displacement plethysmography do not distinguish fetal from maternal
351 tissues. This can affect the results, since fetal growth is heterogenous particularly in late pregnancy
352 and, also the amount of amniotic fluid and size of the placenta varies. Nevertheless, ADP has been
353 found a valid method to measure adiposity in overweight and obese non-pregnant women⁵¹, and it
354 has also been suggested to be the preferred method for assessing maternal FM in late pregnancy⁹.

355 There was minimal previous knowledge regarding the effects of lifestyle on gestational body
356 composition of women in different BMI categories. Therefore, new information applicable for use
357 in interpreting gestational body composition was generated in this study. However, we acknowledge
358 that there are some limitations. The time of enrollment to the study varied somewhat (between 7
359 and 17 gestational weeks) and although we adjusted all correlations to gestational weeks, some

360 comparisons could have been affected by this. Some concern has also been raised about the
361 reliability of self-reported food diaries and questionnaires regarding lifestyle⁵². However, the
362 longitudinal design of the study with the same study personnel and the carefully collected data
363 should increase the reliability. In this study, we analyzed GWG rate instead of the whole gestational
364 weight gain and although this is an accurate method and in accordance to the IOM guidelines, this
365 can influence the comparisons with the other studies conducted in different methods. Also, diet and
366 physical activity were evaluated only twice during the pregnancy and this could be a study
367 limitation; more frequent exploration could improve the accuracy in evaluating the associations
368 with the body composition, although, the compliance in recordings could also be hampered.

369 Finally, although the participants in this study took part in an intervention study, the impact of the
370 intervention was not the focus in this sub-study. Therefore, to avoid bias caused by intervention, we
371 adjusted appropriate analyses for intervention group. Women in all intervention groups consumed
372 dietary supplements or placebo thus it is unlikely that this has induced any changes in behavior,
373 although it must be acknowledged as a potential study limitation.

374 In conclusion, dietary intake seems to influence body composition in pregnant women: we found
375 that both higher protein intake and dietary quality were positively associated with FFM. Excess
376 GWG and the related increase in FM were found in most overweight and obese women. Thus, we
377 would like to emphasize that overall good dietary quality and GWG according to the
378 recommendations could exert positive effects on body composition, and again may induce health
379 benefits in both mother and child.

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382 **Authors contributions**

383 All the authors have approved and made substantial contributions to the final version of the paper.
384 The study was designed and interpreted by K.L., O.P., T.R. and K.T. Acquisition of data and
385 analysis of food diaries were carried by E.K. and K.M. Statistical analysis and analysis of data were
386 performed by T.V. and O.P. The manuscript was drafted by O.P. and all the other authors revised it
387 critically.

388 **Conflict of interest**

389 The authors declare no conflict of interest.

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531

532 Supplementary table 1. The adjusted correlation coefficients* between energy intake, macronutrients and body composition variables.

	Early gestation						Late gestation						Change					
	FM (kg)		FFM (kg)		BF%		FM (kg)		FFM (kg)		BF%		FM (kg)		FFM (kg)		BF%	
	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p
Early gestation																		
Energy (kJ)	0.029	0.775	0.242	0.015	-0.103	0.304	0.004	0.971	0.243	0.015	-0.099	0.326	-0.036	0.724	0.095	0.349	-0.009	0.927
Fat (g)	0.106	0.289	0.157	0.118	0.030	0.766	0.109	0.280	0.139	0.168	0.075	0.459	0.049	0.627	0.018	0.857	0.070	0.487
Protein (g)	-0.082	0.414	0.308	0.002	-0.307	0.002	-0.085	0.398	0.293	0.003	-0.287	0.004	-0.044	0.667	0.056	0.582	0.001	0.994
Carbohydrates (g)	0.010	0.922	0.197	0.048	-0.089	0.378	-0.033	0.747	0.218	0.029	-0.118	0.241	-0.075	0.458	0.130	0.198	-0.061	0.544
Late gestation																		
Energy (kJ)							0.114	0.256	0.241	0.015	0.001	0.992	0.094	0.353	0.136	0.179	0.066	0.513
Fat (g)							0.112	0.223	0.207	0.038	0.032	0.753	0.068	0.501	0.072	0.478	0.064	0.528
Protein (g)							0.004	0.966	0.393	<0.0001	-0.186	0.062	-0.001	0.991	0.197	0.050	-0.031	0.756
Carbohydrates (g)							0.105	0.296	0.128	0.200	0.043	0.671	0.116	0.249	0.112	0.269	0.082	0.418
Change																		
Energy (kJ)							0.174	0.092	-0.037	0.721	0.164	0.113	0.157	0.126	0.043	0.678	0.095	0.359
Fat (g)							0.084	0.415	0.016	0.878	0.052	0.617	0.061	0.556	0.068	0.509	0.012	0.908
Protein (g)							0.151	0.142	0.053	0.606	0.139	0.176	0.072	0.486	0.141	0.170	-0.009	0.932
Carbohydrates (g)							0.195	0.057	-0.102	0.322	0.205	0.045	0.209	0.040	0.010	0.926	0.150	0.142

533
534 *Pearson's partial correlation coefficients adjusted for prepregnancy BMI and gestational weeks and/or change in gestational weeks between early and late
535 gestation.

536 Early and late gestation visits were conducted at mean 13.5 and 35.3 gestational weeks respectively.

537 FM= fat mass, FFM= fat free mass, BF%= body fat percentage

538

539 Supplementary table 2. Linear models presenting the effects of physical activity, diet quality and macronutrient intake on change of body composition
 540 variables.

	Δ FM			Δ FFM			Δ BF%		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Physical activity (MET-index)	-0.013	-0.082;0.056	0.70	0.014	-0.027;0.055	0.49	-0.019	-0.066;0.029	0.44
Diet quality (IDQ-index)	-0.309	-0.662;0.043	0.13	-0.014	-0.224;0.196	0.89	-0.184	-0.428;0.060	0.14
Protein intake (g)	0.002	-0.035;0.039	0.92	-0.005	-0.027;0.017	0.64	-0.002	-0.028;0.024	0.89
Carbohydrate intake (g)	-0.008	-0.020;0.004	0.19	0.006	-0.001;0.014	0.08	-0.006	-0.014;0.002	0.15
Fat intake (g)	0.023	-0.014;0.061	0.22	-0.011	-0.033;0.011	0.31	0.019	-0.006;0.045	0.14

541
 542

543 A separate linear model analysis has been performed for each body composition variable.

544 The models are adjusted for intervention group, age, height, gestational weeks at enrollment and each body composition variable in early gestation.

545 Δ FM= change in fat mass

546 Δ FFM= change in fat free mass

547 Δ BF%= change in body fat percentage

548 CI= confidence interval

549

550

551

552 Supplementary table 3. Total energy intake, diet quality and physical activity of women with inadequate, ideal or excess gestational weight gain and women
 553 with BMI classified as overweight or obese.

	Gestational weight gain				BMI classification		
	Inadequate	Ideal	Excess		overweight	obese	
Energy intake (kJ)	n=11	n=12	n=76	p ^a	n=57	n=42	p ^b
-early gestation	7780 ± 1530	7460 ± 2160	8280 ± 2030	0.347	8070 ± 1840	8210 ± 2220	0.727
-late gestation	7340 ± 1760	7560 ± 1600	8570 ± 2250	0.092	8010 ± 1800	8730 ± 2560	0.125
-change	-440 ± 1430	100 ± 2750	290 ± 2140	0.575	-60 ± 2000	510 ± 2330	0.194
Diet quality (IDQ)	n=11	n=13	n=83	p ^a	n=59	n=48	p ^b
-early gestation	10.2 ± 1.6	9.2 ± 1.6	9.8 ± 2.2	0.486	9.9 ± 2.0	9.6 ± 2.1	0.508
-late gestation	10.2 ± 1.4	9.4 ± 2.3	9.8 ± 2.3	0.649	10.0 ± 2.2	9.7 ± 2.2	0.514
-change	-0.0 ± 1.5	0.2 ± 1.9	0.0 ± 1.3	0.933	0.0 ± 1.4	0.0 ± 1.5	0.966
Physical activity (MET-index)	n=10	n=13	n=84	p ^c	n=60	n=47	p ^d
-early gestation	7.8 (4.4-16.8)	4.8 (2.1-12.0)	4.9 (1.9-12.0)	0.615	6.3 (3.0-12.0)	4.8 (1.2-12.0)	0.583
-late gestation	4.5 (0.0-12.0)	4.8 (0.1-12.0)	3.0 (0.2-7.5)	0.930	3.0 (0.1-7.5)	1.3 (0.2-12.0)	0.771
-change	4.7 (-14.8-3.0)	0.0 (-3.7-0.0)	-1.5 (-5.0-0.0)	0.622	-1.8 (-4.7-0.0)	-0.3 (-7.5-0.0)	0.700

554

555 Numbers are expressed as mean ± sd or median (interquartile range)

556 ^aOne-Way ANOVA, ^bIndependent Samples T-test, ^cKruskal-Wallis, ^dMann Whitney

557 Early and late gestation visits were conducted at mean 13.5 and 35.3 gestational weeks respectively.

558 Overweight BMI 25-29.9 kg m⁻², Obese BMI ≥ 30.0 kg m⁻²

559 IDQ= index of diet quality

560 Women were divided into different GWG classes according to the recommendations issued by Institute of Medicine¹⁵