Longitudinal changes in circulating concentrations of inflammatory markers throughout pregnancy: are there associations with diet and weight status?

Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2021-0395.R1
Manuscript Type:	Article
Date Submitted by the Author:	25-Oct-2021
Complete List of Authors:	Savard, Claudia; Laval University, School of Nutrition Lemieux, Simone; Laval University, School of Nutrition Plante, Anne-Sophie; Laval University, School of Nutrition Gagnon, Marianne; Laval University, School of Nutrition; CHU de Québec-Université Laval, Endocrinology and Nephrology Unit; Laval University, Institute of Nutrition and Functional Foods Leblanc, Nadine; Laval University, Department of Food Science and Nutrition Veilleux, Alain; Laval University, School of Nutrition Tchernof, André; Laval University, Laval University Medical Center; Laval University, Institute of Nutrition and Functional Foods (INAF) Morisset, Anne-Sophie; Laval University, School of Nutrition
Novelty bullets: points that summarize the key findings in the work:	Circulating leptin and IL-6 concentrations increased across trimesters whereas CRP was stable, and adiponectin decreased., Variations in circulating leptin and adiponectin concentrations differed by ppBMI categories., Very few associations were observed between dietary scores and inflammatory markers.
Keyword:	leptin, interleukin-6, adiponectin, c-reactive protein, inflammation, diet, pregnancy
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

Longitudinal changes in circulating concentrations of inflammatory markers throughout pregnancy: are there associations with diet and weight status?

Claudia Savard^{1,2,3}, Simone Lemieux^{1,3}, Anne-Sophie Plante^{2,3}, Marianne Gagnon^{1,2,3}, Nadine Leblanc^{3,4,5}, Alain Veilleux^{1,3,4}, André Tchernof^{1,5} and Anne-Sophie Morisset^{1,2,3}

¹ School of Nutrition, Laval University, Quebec City, Canada.

² Endocrinology and Nephrology Unit, CHU of Québec-Laval University, Quebec City, Canada.

³ NUTRISS Centre - Nutrition, health and society, Institute on Nutrition and Functional Foods, Laval University, Quebec City, Canada.

⁴ Canada Excellence Research Chair in the Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND), Quebec City, Canada.

⁵ Quebec Heart and Lung Institute, Laval University, Quebec City, Canada.

Corresponding author: Anne-Sophie Morisset, R.D., Ph.D.
School of Nutrition, Laval University
2425, rue de l'Agriculture, local 2412
Québec (Québec) G1V 0A6
Tel: (418) 656-2131 ext. 13982
E-mail: anne-sophie.morisset@fsaa.ulaval.ca
ORCID handle: 0000-0002-9214-8912

ABSTRACT

The natural inflammation occurring during pregnancy can, under certain conditions, be associated with adverse pregnancy outcomes. This study aimed to: 1) quantify changes in circulating concentrations of leptin, adiponectin, interleukin-6 (IL-6) and C-reactive protein (CRP) across trimesters of pregnancy, according to pre-pregnancy body mass index (ppBMI); and 2) examine the trimester-specific associations between the inflammatory markers' concentrations, a Mediterranean diet score (MDS) and the dietary inflammatory index (DII). We measured leptin, adiponectin and IL-6 by ELISA, and CRP by high-sensitivity immunonephelometry, in blood samples from 79 pregnant women (age: 32.1 ± 3.7 years; ppBMI: 25.7 ± 5.8 kg/m²). Three web-based 24h recalls were completed at each trimester and used to compute the MDS and the DII. CRP concentrations remained stable across trimesters, whereas concentrations of leptin and IL-6 increased, and adiponectin concentrations decreased ($p < 0.001$). Changes in leptin and adiponectin concentrations also differed according to ppBMI categories ($p < 0.05$). As for the dietary scores, the only significant association was observed in the second trimester between leptin concentrations and the MDS ($r = -0.26$, $p < 0.05$). In conclusion, ppBMI and the progression of pregnancy itself probably supplant the potential associations between diet and the inflammation occurring during that period.

Novelty

- Circulating leptin and IL-6 concentrations increased across trimesters whereas CRP was stable, and adiponectin decreased.
- Variations in circulating leptin and adiponectin concentrations differed by ppBMI categories.
- Very few associations were observed between dietary scores and inflammatory markers.

Key words

leptin, interleukin-6, adiponectin, C-reactive protein, inflammation, diet, pregnancy.

24 INTRODUCTION

25 Pregnancy is associated with a naturally induced low-grade inflammatory state that ensures
26 metabolic adaptation and success of specific physiological processes like implantation (Romero et al.,
27 2007). That inflammation is important and inevitable, but it must remain under control. Indeed, an
28 amplified and soaring inflammatory state can alter the body's homeostatic control systems. In that case,
29 inflammation has been associated with the development of various pregnancy complications (Poston et
30 al., 2011, Villar et al., 2006, Walker, 2011, Lekva et al., 2016). Markers such as the C-reactive protein
31 (CRP), a pro-inflammatory protein, and adipokines like interleukin-6 (IL-6) and leptin, a satiety signaling
32 hormone secreted by white adipose tissue and the placenta, are all indicative of a low-grade pro-
33 inflammatory state. On the contrary, circulating concentrations of adiponectin, an adipokine associated
34 with glucose level regulation, are inversely associated with inflammation. The evolution of those markers
35 throughout pregnancy has been somewhat documented in the past (Highman et al., 1998, Lepsch et al.,
36 2016, Andersson-Hall et al., 2020, Stokkeland et al., 2019). Still, how this evolution varies according to
37 pre-pregnancy body mass index (ppBMI) has not been investigated yet. Considering that ppBMI and
38 adipose tissue are associated with inflammation, it is possible that the progression of inflammatory
39 markers during pregnancy differs according to ppBMI category (Kac et al., 2011, Ramsay et al., 2002).
40 Moreover, women with lower ppBMI are required to gain more weight during pregnancy (National
41 Research Council, 2009b). Is this greater weight gain associated with a larger increase in concentrations
42 of inflammatory markers? Inversely, are women of higher ppBMI predisposed to an altered metabolic
43 profile that could translate into a greater increment in inflammation? These questions remain
44 unanswered.

45 It is also of interest to investigate specific dietary patterns that may have the potential to attenuate
46 an elevated pro-inflammatory state in pregnancy. In fact, in non-pregnant adults, adherence to dietary
47 patterns such as the Mediterranean diet was found to be inversely associated with concentrations of

inflammatory markers (Esmailzadeh et al., 2006, Galland, 2010, Chrysohoou et al., 2004, Panagiotakos et al., 2009, Salas-Salvado et al., 2008). Similarly, a higher adherence to an anti-inflammatory diet, assessed with the dietary inflammatory index (DII), was correlated with lower circulating CRP concentrations and various cytokines (Shivappa et al., 2017, Shin et al., 2019). Similar associations were identified in pregnant women (Spadafranca et al., 2018, Sen et al., 2016, Shin et al., 2017). However, most studies among pregnant women did not analyze trimester-specific associations between diet and inflammatory markers. This is problematic, since diet and concentrations of inflammatory markers have been reported to change during pregnancy (Mor et al., 2011, McGowan and McAuliffe, 2013, Moran et al., 2013). It is also important to document trimester-specific associations between diet and inflammation because associations can differ from one trimester to another and thus influence the nature, timing and intensity of future nutritional interventions during pregnancy.

Therefore, this study aimed to: 1) quantify changes in circulating concentrations of leptin, adiponectin, IL-6 and CRP across trimesters of pregnancy, according to ppBMI; and 2) examine the trimester-specific associations between the inflammatory markers' concentrations, a Mediterranean diet score (MDS) and the DII. We tested the hypothesis that circulating leptin, IL-6 and CRP concentrations increase, whereas circulating adiponectin concentrations decrease across trimesters, and that those fluctuations vary according to ppBMI categories. We also hypothesized that, at each trimester, circulating leptin, IL-6 and CRP concentrations are positively associated with DII and inversely with MDS while associations in opposite directions are found with circulating adiponectin concentrations.

MATERIALS AND METHODS

Participants

The data used in the present study is part of the ANGE (*Apports Nutritionnels durant la Grossesse*) project, a prospective cohort of 86 French-Canadian pregnant women followed from their first to their

third trimester of pregnancy. Participants were recruited through e-mail lists and social media ads, from April 2016 to May 2017. The primary aim of that study was the validation of a Web-based 24h dietary recall (Savard et al., 2018a). The ANGE study took place in 2016-2017 in Quebec City, Canada, and consisted of trimester-specific, on-site visits as well as self-administered Web questionnaires (described below). Exclusion criteria were multiple pregnancies, previous diagnosis of a severe medical condition (i.e., type 1 or type 2 diabetes, renal or liver disease, inflammatory and autoimmune disorders) and age <18 years. The ANGE study was conducted according to the Declaration of Helsinki guidelines and was approved by the CHU de Québec-Université Laval Research Centre's Ethics Committee (Reference number: 2016-2866). All participants gave their informed written consent.

Measurement of inflammatory markers

Fasting blood samples were drawn at each trimester (9.9–14.7, 20.7–25.3 and 31.6–34.6 gestational weeks) and centrifuged for serum. All serum samples were stored at -80° C for future analyses. Circulating concentrations of leptin (EMD Millipore Corporation, Billerica, MA, USA.), adiponectin (Bridge International Inc., Santa Clara, CA, USA) and IL-6 (R&D Systems Inc., Minneapolis, MN, USA) were measured by commercial ELISA kits, according to the manufacturers' instructions. Circulating concentrations of CRP were measured by high sensitivity immunonephelometry at Quebec's Heart and Lung Institute's clinical laboratory (Siemens Medical Solutions USA Inc., Malvern, PA, USA). Since no known specific value or threshold of inflammatory markers' concentration is associated with an acute inflammatory state in pregnancy, values of circulating leptin (n=1), adiponectin (n=3), IL-6 (n=6) and CRP (n=5) with more than two standard deviations compared to the mean were considered as extreme and were excluded from analysis.

95 *Assessment of dietary intakes*

96 Dietary intakes were assessed by self-administered Web-based 24-hour dietary recalls, through the
97 R24W (*Rappel de 24h Web*) platform. Three dietary recalls were completed in each trimester (8.4–14.0,
98 19.3–28.3 and 31.9–37.7 gestational weeks). The R24W has been described elsewhere (Jacques et al.,
99 2016, Lafreniere et al., 2018) and validated among pregnant women (Savard et al., 2018a). Briefly, the
100 participants receive an automatically generated e-mail on three random days, alerting them they have to
101 report all foods and drinks consumed the day before. The database includes food items that are linked to
102 the 2015 Canadian Nutrient File (Health Canada, 2015), therefore enabling automatic extraction of
103 nutritional information. The participants' trimester-specific dietary intakes were published elsewhere
104 (Savard et al., 2018b).

105

106 *Mediterranean diet score*

107 The MDS was calculated on the basis of the Mediterranean pyramid as previously described (Goulet et
108 al., 2003, Willett et al., 1995). Briefly, the Mediterranean diet is characterized by higher intakes of plant-
109 based foods, moderate intakes of fish, poultry and dairy products and lower intakes of red meat and
110 processed foods (Willett et al., 1995). Thus, the food groups included in the MDS are 1) whole grain
111 products, 2) vegetables, 3) fruits, 4) legumes, nuts and seeds, 5) olive oil, olives and margarine made
112 from olive oil, 6) milk and dairy products, 7) fish and seafood, 8) poultry, 9) eggs, 10) sweets and 11)
113 red and processed meats. Mean numbers of daily or weekly servings for each food group were directly
114 obtained from the dietary recalls and compared with the MDS criteria to compute the total MDS and its
115 subscores¹. The subscores for whole grains, vegetables, fruits, legumes, olive oil and fish are positively
116 correlated with the total score, i.e. a greater consumption of those foods leads to a higher MDS. The

¹ Information regarding the MDS's scoring criteria is presented in a supplementary file (Table S1).

117 subscores for poultry, eggs, sweets and red meats are inversely correlated with the total score. Finally
118 intakes of milk and dairy have more of an inverse U shape association with the subscore, i.e. the lowest
119 score (0) is attributed to both a lower (<1/day) and higher (>4/day) intake of dairy products.

120

121 ***Dietary inflammatory index***

122 Nutritional data from the three dietary recalls were used to compute the DII at each trimester. The
123 DII is a literature-derived, population-based index developed to assess the inflammatory potential
124 properties of a population's diet (Shivappa et al., 2014). The revised version of the DII includes 45 food
125 parameters but as shown in various other studies, it is possible to calculate the DII with less than 45 food
126 parameters (Pieczynska et al., 2020, Sen et al., 2016, Shin et al., 2017, Yang et al., 2020). In the present
127 study, dietary data was available for the following 29 nutrients: energy, fat, alcohol, saturated fatty acids,
128 monounsaturated fatty acids, polyunsaturated fatty acids, n-3 fatty acids, n-6 fatty acids, trans fatty acids,
129 cholesterol, carbohydrate, protein, dietary fiber, caffeine, vitamins A, B₁, B₂, B₆, B₁₂, C and D, beta-
130 Carotene, alpha-tocopherol, folate, iron, magnesium, niacin, selenium and zinc. The development and
131 calculation of the DII is described elsewhere (Shivappa et al., 2014). In brief, each nutrient's intake has
132 to be converted to a z-score using its corresponding global daily mean and standard deviation provided
133 by the DII database. The resulting z-scores are converted into a percentile score (between 0 and 1) and
134 then into a centered proportion (between -1 and 1). Finally, all food parameters' centered proportions are
135 multiplied by their respective overall inflammatory effect score, which are then summed to obtain each
136 participant's total DII (Shivappa et al., 2014). Although the DII was not developed for pregnant
137 populations, it has been used in numerous cohorts of pregnant women (McCullough et al., 2017, Moore
138 et al., 2018, Pieczynska et al., 2020, Sen et al., 2016, Shin et al., 2017, Yang et al., 2020, Shivappa et al.,
139 2019).

Other Variables

Self-reported pre-pregnancy weight and measured height were used to calculate ppBMI. Because only two women were underweight, ppBMI was categorized as follows: <25 (normal weight), 25-29.9 (overweight) and ≥ 30 kg/m² (obesity). Total gestational weight gain (GWG) was calculated as the difference between the last weight measured before delivery (≥ 37 th week), retrieved from the participants' medical records (data available for 63 women), and the self-reported pre-pregnancy weight. Finally, information on education level, annual household income and ethnicity was collected through a self-administered Web-based questionnaire, and weeks of gestation were confirmed by ultrasound in the first trimester.

Statistical Analyses

Means, standard deviations and proportions were used to characterize the study sample. Mixed models for repeated measures were used to examine circulating concentrations of leptin, adiponectin, IL-6 and CRP across trimesters, with ppBMI included as a fixed effect. When the change in concentrations across trimesters was significant, the test slice analysis was used. This analysis performs the repeated measures mixed model for each ppBMI category to identify in which category the change in concentrations was significant. Pearson correlations were used to examine associations between ppBMI, GWG and circulating concentrations of inflammatory markers as well as associations between dietary scores and concentrations of inflammatory markers. Multivariate linear regression analyses were used to identify factors associated with inflammation, in models that included trimesters of pregnancy, ppBMI, weight gain and MDS as independent variables. Inflammatory markers were included in the models as dependent variables either as individual circulating concentration or globally, by using Z-scores. The global inflammatory Z-score corresponded to the averaged Z-scores $[(X - \text{mean}) / \text{standard deviation}]$

164 that were computed independently for each inflammatory marker. The residuals of all statistical analyses
165 were normally distributed; therefore, we did not normalize any of our data. All analyses were performed
166 with and without those two women and it did not impact the interpretation of our data. Statistical analyses
167 were performed with JMP Pro version 15 (SAS Institute Inc., Cary, NC, USA) and differences were
168 considered statistically significant at $p \leq 0.05$.

169

170 **RESULTS**

171 Of the 86 pregnant women initially recruited, seven were lost to follow-up due to miscarriage (n=3)
172 or a lack of time to devote to the project (n=4). The remaining 79 pregnant women were predominantly
173 French-Canadian, multiparous, aged (mean $\pm$ SD) 32.1 ± 3.7 years old, had an average $\pm$ SD ppBMI of
174 25.7 ± 5.8 kg/m², a university degree and a household income $\geq 80,000$ CAD (**Table 1**). Mean total MDS,
175 and DII did not vary across trimesters, but the subscore for milk and dairy products, included in the MDS,
176 significantly decreased with time ($p = 0.02$)². That decrease was associated with an increase in milk and
177 dairy servings reported across trimesters (2.5 ± 1.1 , 2.7 ± 1.4 and 3.0 ± 1.4 servings per day in the first,
178 second and third trimesters; $p = 0.002$). The DII and MDS were inversely correlated in the second and
179 third trimesters ($r = -0.27$ and -0.29 ; $p < 0.05$).

180 Circulating leptin and IL-6 concentrations increased across trimesters whereas circulating
181 adiponectin and CRP concentrations decreased and remained stable, respectively (**Figure 1**). It can also
182 be noted in **Figure 1** that absolute changes in circulating leptin and adiponectin concentrations across
183 trimesters varied according to ppBMI categories (p trimester*ppBMI <0.05). Circulating leptin increased
184 significantly in women with a ppBMI ≥ 30 kg/m² (test slice p -value < 0.0001), but not in women of lower
185 ppBMI. Regarding adiponectin, circulating concentrations decreased significantly in all ppBMI

² Data on the MDS and DII are presented in Tables S2 and S3

categories (test slice p -values <0.01). The increase in circulating concentrations of IL-6 was only significant among women with a ppBMI $<25 \text{ kg/m}^2$ (test slice p -value <0.001).

In all trimesters, circulating leptin, IL-6 and CRP concentrations were positively associated with ppBMI (**Table 2**). Circulating adiponectin concentrations were inversely correlated with ppBMI in the first and second trimesters only. Total GWG was positively correlated with circulating concentrations of leptin in the second and third trimesters, and inversely correlated with circulating concentrations of IL-6 and CRP in the second trimester (**Table 2**). No associations were observed between total GWG and circulating concentrations of adiponectin.

In the first trimester, the MDS was inversely associated with circulating leptin concentrations, but that association was non-significant after adjustment for ppBMI (**Table 3**). In the second trimester, the MDS was inversely correlated with circulating leptin concentrations, but only after adjustment for ppBMI. The DII was not correlated with any of the inflammatory markers; therefore, it was not included in the regression models (see below).

Finally, in five distinct regression models, ppBMI was significantly associated with all markers' circulating concentrations and overall inflammation status (**Figure 2**). Pregnancy itself, as represented by advancing trimesters, was also significantly associated with overall inflammation status and circulating concentrations of inflammatory markers, with the exception of CRP. The MDS and the weight gain were only associated with circulating leptin concentrations.

DISCUSSION

This study assessed the changes in circulating leptin, adiponectin, IL-6 and CRP concentrations across trimesters of pregnancy, according to ppBMI. We observed a significant increase in circulating leptin and IL-6 and a significant decrease in circulating adiponectin concentrations across trimesters, which is in line with the progression of an inflammatory state during pregnancy. However, circulating

210 CRP concentrations remained stable. Changes in circulating leptin and adiponectin concentrations across
211 trimesters varied according to ppBMI, which is in accordance with our initial hypotheses. Furthermore,
212 we believe this study is the first to examine trimester-specific associations between circulating
213 concentrations of leptin, adiponectin, IL-6 and CRP and inflammation-related dietary scores. Overall,
214 there were close to no association between the dietary scores and the concentrations of inflammatory
215 markers. The regression analyses performed suggest that inflammation is strongly associated with ppBMI
216 and maybe the pregnancy itself, which may supplant potential associations between inflammation and
217 dietary variables.

218 Firstly, the observed changes in circulating leptin concentrations are in accordance with the current
219 literature (Masuzaki et al., 1997, Perez-Perez et al., 2018). Further analyses in our sample showed that
220 leptin concentrations only increased significantly among women with obesity. Misra and Trudeau
221 obtained contrasting results and found that the rate at which leptin concentrations increased was greater
222 in women with a normal weight vs. overweight or obesity (Misra and Trudeau, 2011). They hypothesized
223 that the lower rate in leptin increase in women with obesity could be explained by a lower weight gain.
224 Still, in the present study, a lower weight gain in women with obesity did not translate into a lower rate
225 in leptin increase. It is possible that in a physiological context like pregnancy, in which energy and
226 nutrient intakes need to be increased and transferred to the fetus, leptin production is regulated by other
227 mechanisms than those predicted by ppBMI and GWG (Brunton and Russell, 2010, Hauguel-de Mouzon
228 et al., 2006). Moreover, any given circulating leptin concentration is hard to interpret without knowing
229 the extent of leptin resistance, which occurs during pregnancy and also varies with obesity (Augustine et
230 al., 2008). Furthermore, other factors like placental leptin production, efficacy of leptin production by
231 the adipose tissue as well as pre-pregnancy levels of adipose tissue may differ between ppBMI categories,
232 which might partly explain our results (Tessier et al., 2013, Franco-Sena et al., 2015). Additional studies

are necessary in order to better understand the complex physiology between circulating leptin concentrations and GWG across ppBMI categories.

Secondly, we found that circulating adiponectin concentrations decreased across trimesters, similarly to what was reported in previous studies (Catalano et al., 2006, Lekva et al., 2017). The decrease in adiponectin was significant in all ppBMI categories, which differs from the results of Andersson-Hall et al., who found that pregnant women of lower ppBMI had a more pronounced decrease in circulating adiponectin concentrations (Andersson-Hall et al., 2020). It could be hypothesized that since an increase in fat mass leads to the downregulation of adiponectin, the decrease in circulating adiponectin concentrations may be more pronounced in women of lower ppBMI, as they are required to gain more weight (Mazaki-Tovi et al., 2005, National Research Council, 2009b). In the present study, the decrease in adiponectin also appears to be more pronounced, although not significantly, among women with a ppBMI $<25 \text{ kg/m}^2$ (**Figure 2**). However, we found no associations between total GWG and circulating adiponectin concentrations. Similarly to what we observed with leptin concentrations and GWG, it is possible that the new adipose tissue gained during pregnancy becomes less and less functional as pregnancy progresses. This dysfunction could be reflected in an altered production or downregulation of adiponectin (Simjak et al., 2018, Svensson et al., 2016). These hypotheses should however be confirmed in future studies. It is also important to note that total GWG comprises several components other than the gain in adipose tissue, namely the development of the placenta, an increase in total body water, fat-free mass, blood, amniotic fluid, etc. (National Research Council, 2009a). These tissues are not all implicated in the regulation of adiponectin. Nevertheless, the associations between GWG, ppBMI and circulating adiponectin concentrations should be further investigated.

Thirdly, circulating IL-6 concentrations increased across trimesters, but only in women with a ppBMI $<25 \text{ kg/m}^2$, whereas circulating CRP concentrations remained stable for all ppBMI categories. The patterns of circulating IL-6 concentrations in pregnancy are still unclear, as both increases and

decreases have been reported (Stokkeland et al., 2019, Holtan et al., 2015, Vassiliadis et al., 1998). Those conflicting results might be due to differences in sample size and measurement methods as well as by the fact that, in the present study, women who developed pregnancy complications such as gestational diabetes (n=10) and hypertension (n=8) were not excluded. Such complications are generally diagnosed later in pregnancy and are associated with a greater inflammatory state, which could explain the increase in circulating IL-6 concentrations we observed (Lekva et al., 2016, Villar et al., 2006, Walker, 2011). In fact, Stokkeland et al., who observed a decrease in IL-6 concentrations, excluded women with previous or current disease from their analyses (Stokkeland et al., 2019). Still, in the present study, the overall increase in circulating IL-6 concentrations remained significant even after the exclusion of women who developed gestational diabetes and/or hypertension (data not shown). The evolution of CRP concentrations throughout pregnancy also remains unclear in the current literature. Belo et al. observed, similarly to the present study, a stability in CRP concentrations, in their prospective cohort of 23 Portuguese pregnant women (Belo et al., 2005). Furthermore, in the present study, advancing trimesters of pregnancy were significantly associated with all inflammatory markers' concentrations, except for CRP, which is in line with the stability of that marker across trimesters. This stability may however be explained by ours and Belo et al.'s smaller samples. Indeed, other studies with greater samples observed an increase in circulating CRP concentrations, from the 10th to the 24th week of pregnancy (Stokkeland et al., 2019) as well as from the first to the third trimester (Larsson et al., 2008, Skarzynska et al., 2018). Other important factors like physical activity, anxiety and depression were reported to be associated with inflammation in non pregnant cohorts, and it is possible that it might have affected our sample's circulating CRP and IL-6 concentrations (Werneck et al., 2020, Naude et al., 2018, Ostrowski et al., 1998, Wedell-Neergaard et al., 2019). Future studies should consider those factors in the assessment of inflammation during pregnancy.

280 Fourthly, contrary to our hypothesis, dietary scores were very poorly associated with inflammatory
281 markers. As such, the only significant association observed in our multivariable regression analyses was
282 between the MDS and circulating leptin concentrations. The literature is limited regarding the potential
283 effect of the Mediterranean diet on circulating leptin concentrations, but some studies observed lower
284 leptin concentrations among pregnant women with lower intakes or lower blood levels of saturated fatty
285 acids and with higher intakes of fibers (Lepsch et al., 2016, Vähämiko et al., 2013). Although those
286 studies did not assess their participants' adherence to the Mediterranean diet, the latter is associated with
287 lower intakes of foods that are high in saturated fatty acids and with higher intakes of foods rich in fibers.
288 Finally, a recent Brazilian study by Alves-Santos et al. found that a greater adherence to a 'Western'
289 dietary pattern, characterized by higher intakes of fast food, snacks and processed meats, for example,
290 was associated with higher circulating leptin concentrations in 173 pregnant women (Alves-Santos et al.,
291 2018). Their results are comparable to ours, since the 'Western' dietary pattern that they identified could
292 be considered as the Mediterranean diet's opposite.

293 Fifthly, the DII was not associated with any of the four inflammatory markers. Our results are in
294 contrast with previous studies among pregnant women (Moore et al., 2018, Sen et al., 2016, Shivappa et
295 al., 2017) and with the fact that a pro-inflammatory diet should be associated with higher concentrations
296 of inflammatory markers. Still, other studies found few or no association between the DII and
297 concentrations of CRP and IL-6 among pregnant women (McCullough et al., 2017, Pieczynska et al.,
298 2020). Pregnancy-induced inflammation most certainly differs from the systemic inflammation of non
299 pregnant populations. Therefore, the associations between the DII and inflammatory markers observed
300 in the general population may not always be replicated in pregnancy. Furthermore, according to the
301 regression analyses of the present study, pregnancy-induced inflammation appears to be mostly
302 associated with ppBMI and the progression of pregnancy itself. Overall, our results regarding
303 associations between diet and inflammation are inconsistent, which is in line with the observations of a

recent systematic review (Yeh et al., 2021). Most of the 17 studies included in that review showed associations between pro-inflammatory markers and dietary patterns like the pro-/anti-inflammatory diet and the Mediterranean diet. However, the three studies with a more rigorous methodology that were reviewed, including one cross-sectional and two intervention studies, found no significant associations.

Finally, the present study has various strengths, namely the assessment of trimester-specific dietary intakes and the measurement of four distinct inflammatory markers. The use of a validated Web-based 24h recall provided detailed information on our sample's dietary intakes. It is also worth mentioning that the DII may have some limitations regarding pregnancy, since the DII's global database is not based on intakes of pregnant populations. Moreover, no information was found regarding the inclusion of supplemental intakes in the calculation of the DII. Most of our sample were taking prenatal supplements (Savard et al., 2018b), thus, those additional nutrient intakes may have altered some of our analyses. We did not collect dietary data prior to pregnancy, thus we were not able to, for example, explore the associations between pre-pregnancy diet and inflammatory status in the first trimester. In a similar way, our study does not include fetal and neonatal data, which would have been interesting to study in association with concentrations of inflammatory markers. Furthermore, our small sample size certainly impacted the statistical power of some of our analyses. Still, posteriori analyses revealed that our sample size of $n=79$ provided 65% power to detect a correlation of $r=-0.26$ at $p<0.05$ between the MDS and circulating leptin concentrations in the second trimester. As previously mentioned, the present study used data from an existing cohort whose primary objective was not to study associations between diet and inflammation. Our study sample was fairly homogenous in terms of ethnicity and socioeconomic status, which limits the generalizability of our findings.

In conclusion, the gradual increase in circulating leptin and decrease in adiponectin concentrations are in line with the physiological inflammation settling in during pregnancy, but other studies are necessary to understand how ppBMI influences their evolution. Further prospective studies are also

needed to better understand the fluctuations of IL-6 and CRP in pregnancy. Very few associations were observed between diet and inflammation, and the impact of ppBMI and pregnancy itself appeared to surpass the effect that diet can have on inflammatory markers during that period. Our results should however be confirmed in larger samples with the consideration of other factors that can influence inflammation (physical activity level, stress, etc.). Still, diet remains an important factor to consider in the prenatal period, as a greater diet quality is associated with a healthier pre-pregnancy weight status, which can positively influence maternal and neonatal outcomes. It is also possible that prenatal anti-inflammatory dietary patterns, through pathways other than inflammation, are beneficial for various health outcomes.

ACKNOWLEDGEMENTS

We would like to acknowledge the help of Marie-Frederique Gauthier, research assistant, for performing the adiponectin and interleukin-6 assays.

COMPETING INTERESTS STATEMENT

André Tchernof received research funding from Johnson & Johnson, Medtronic and Bdynov for studies unrelated to this manuscript, in addition to consulting fees from Novo Nordisk and Bausch Health. All the remaining authors declare they have no competing interests.

AUTHORS' CONTRIBUTIONS

A.-S.M., A.V., A.T., S.L. and C.-S. designed research; A.-S.M., C.S., and A.-S.P. conducted research; C.S., A.-S.P., M.G. and N.L. analyzed data; and C.S. wrote the first draft of the manuscript. A.-S.M. had primary responsibility for final content. All authors made substantial contributions to the conception and

351 design of the manuscript, and all critically revised a first draft of the manuscript for important intellectual
352 content. Finally, all authors read and approved the final manuscript.

353

354 **FUNDING STATEMENT**

355 The ANGE project is funded by the Danone Institute of Canada (grant number: FO115961) and by startup
356 funds (*Fonds de recherche du Québec-Santé et Fondation du CHU de Québec*). C.S. received a graduate
357 student award from the Canadian Institutes of Health Research (FRN: GSD-167043). All funding
358 allowed the collection, analysis, and interpretation of data, but played no role in the writing of this
359 manuscript.

360

361 **DATA AVAILABILITY STATEMENT**

362 The datasets generated and/or analyzed during the present study are available from the corresponding
363 author upon reasonable request.

REFERENCES

- ALVES-SANTOS, N. H., COCATE, P. G., ESHRIQUI, I., BENAÏM, C., BARROS, E. G., EMMETT, P. M. & KAC, G. 2018. Dietary patterns and their association with adiponectin and leptin concentrations throughout pregnancy: a prospective cohort. *Br J Nutr*, 119, 320-329. <https://doi.org/10.1017/S0007114517003580>
- ANDERSSON-HALL, U., SVEDIN, P., SVENSSON, H., LONN, M., MALLARD, C. & HOLMANG, A. 2020. Longitudinal changes in adipokines and free leptin index during and after pregnancy in women with obesity. *Int J Obes (Lond)*, 44, 675-683. <https://doi.org/10.1038/s41366-019-0452-7>
- AUGUSTINE, R. A., LADYMAN, S. R. & GRATAN, D. R. 2008. From feeding one to feeding many: hormone-induced changes in bodyweight homeostasis during pregnancy. *J. Physiol*, 586, 387-397. <https://doi.org/10.1113/jphysiol.2007.146316>
- BELO, L., SANTOS-SILVA, A., ROCHA, S., CASLAKE, M., COONEY, J., PEREIRA-LEITE, L., QUINTANILHA, A. & REBELO, I. 2005. Fluctuations in C-reactive protein concentration and neutrophil activation during normal human pregnancy. *Eur J Obstet Gynecol Reprod Biol*, 123, 46-51. <https://doi.org/10.1016/j.ejogrb.2005.02.022>
- BRUNTON, P. J. & RUSSELL, J. A. 2010. Endocrine induced changes in brain function during pregnancy. *Brain Res*, 1364, 198-215. <https://doi.org/10.1016/j.brainres.2010.09.062>
- CATALANO, P. M., HOEGH, M., MINIUM, J., HUSTON-PRESLEY, L., BERNARD, S., KALHAN, S. & HAUGUEL-DE MOUZON, S. 2006. Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. *Diabetologia*, 49, 1677-85. <https://doi.org/10.1007/s00125-006-0264-x>
- CHRYSOHOOU, C., PANAGIOTAKOS, D. B., PITSAVOS, C., DAS, U. N. & STEFANADIS, C. 2004. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults: The ATTICA Study. *J Am Coll Cardiol*, 44, 152-8. <https://doi.org/10.1016/j.jacc.2004.03.039>
- ESMAILZADEH, A., KIMIAGAR, M., MEHRABI, Y., AZADBAKHT, L., HU, F. B. & WILLETT, W. C. 2006. Fruit and vegetable intakes, C-reactive protein, and the metabolic syndrome. *Am J Clin Nutr*, 84, 1489-97. <https://doi.org/10.1093/ajcn/84.6.1489>
- FRANCO-SENA, A. B., DE OLIVEIRA, L. C., DE JESUS PEREIRA PINTO, T., FARIAS, D. R., VAZ JDOS, S. & KAC, G. 2015. Factors associated with prospective leptin concentrations throughout pregnancy in pregestational normal weight, overweight and obese women. *Clin Endocrinol (Oxf)*, 82, 127-35. <https://doi.org/10.1111/cen.12487>
- GALLAND, L. 2010. Diet and inflammation. *Nutr Clin Pract*, 25, 634-40. <https://doi.org/10.1177/0884533610385703>
- GOULET, J., LAMARCHE, B., NADEAU, G. & LEMIEUX, S. 2003. Effect of a nutritional intervention promoting the Mediterranean food pattern on plasma lipids, lipoproteins and body weight in healthy French-Canadian women. *Atherosclerosis*, 170, 115-24. [https://doi.org/10.1016/s0021-9150\(03\)00243-0](https://doi.org/10.1016/s0021-9150(03)00243-0)
- HAUGUEL-DE MOUZON, S., LEPERCQ, J. & CATALANO, P. 2006. The known and unknown of leptin in pregnancy. *Am J Obstet Gynecol*, 194, 1537-45. <https://doi.org/10.1016/j.ajog.2005.06.064>
- HEALTH CANADA. 2015. *Canadian Nutrient File* [Online]. Available: <https://food-nutrition.canada.ca/cnf-fce/index-eng.jsp> [Accessed May 2021].
- HIGHMAN, T. J., FRIEDMAN, J. E., HUSTON, L. P., WONG, W. W. & CATALANO, P. M. 1998. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting

- metabolic rate in pregnancy. *Am J Obstet Gynecol*, 178, 1010-5. [https://doi.org/10.1016/s0002-9378\(98\)70540-x](https://doi.org/10.1016/s0002-9378(98)70540-x)
- HOLTAN, S. G., CHEN, Y., KAIMAL, R., CREEDON, D. J., ENNINGA, E. A., NEVALA, W. K. & MARKOVIC, S. N. 2015. Growth modeling of the maternal cytokine milieu throughout normal pregnancy: macrophage-derived chemokine decreases as inflammation/counterregulation increases. *J Immunol Res*, 2015, 952571. <https://doi.org/10.1155/2015/952571>
- JACQUES, S., LEMIEUX, S., LAMARCHE, B., LARAMEE, C., CORNEAU, L., LAPOINTE, A., TESSIER-GRENIER, M. & ROBITAILLE, J. 2016. Development of a web-based 24-h dietary recall for a French-Canadian population. *Nutrients*, 8. <https://doi.org/10.3390/nu8110724>
- KAC, G., VAZ, J. S., SCHLUSSEL, M. M. & MOURA, A. S. 2011. C-reactive protein and hormones but not IL-6 are associated to body mass index in first trimester of pregnancy. *Arch Gynecol Obstet*, 284, 567-73. <https://doi.org/10.1007/s00404-010-1573-3>
- LAFRENIERE, J., LARAMEE, C., ROBITAILLE, J., LAMARCHE, B. & LEMIEUX, S. 2018. Assessing the relative validity of a new, web-based, self-administered 24 h dietary recall in a French-Canadian population. *Public Health Nutr*, 1-9. <https://doi.org/10.1017/S1368980018001611>
- LARSSON, A., PALM, M., HANSSON, L. O., BASU, S. & AXELSSON, O. 2008. Reference values for alpha1-acid glycoprotein, alpha1-antitrypsin, albumin, haptoglobin, C-reactive protein, IgA, IgG and IgM during pregnancy. *Acta Obstet Gynecol Scand*, 87, 1084-8. <https://doi.org/10.1080/00016340802428146>
- LEKVA, T., NORWITZ, E. R., AUKRUST, P. & UELAND, T. 2016. Impact of Systemic Inflammation on the Progression of Gestational Diabetes Mellitus. *Curr Diab Rep*, 16, 26. <https://doi.org/10.1007/s11892-016-0715-9>
- LEKVA, T., ROLAND, M. C. P., MICHELSEN, A. E., FRIIS, C. M., AUKRUST, P., BOLLERSLEV, J., HENRIKSEN, T. & UELAND, T. 2017. Large Reduction in Adiponectin During Pregnancy Is Associated With Large-for-Gestational-Age Newborns. *J Clin Endocrinol Metab*, 102, 2552-2559. <https://doi.org/10.1210/jc.2017-00289>
- LEPSCH, J., FARIAS, D. R., VAZ JDOS, S., DE JESUS PEREIRA PINTO, T., DA SILVA LIMA, N., FREITAS VILELA, A. A., CUNHA, M., FACTOR-LITVAK, P. & KAC, G. 2016. Serum saturated fatty acid decreases plasma adiponectin and increases leptin throughout pregnancy independently of BMI. *Nutrition*, 32, 740-7. <https://doi.org/10.1016/j.nut.2016.01.016>
- MASUZAKI, H., OGAWA, Y., SAGAWA, N., HOSODA, K., MATSUMOTO, T., MISE, H., NISHIMURA, H., YOSHIMASA, Y., TANAKA, I., MORI, T. & NAKAO, K. 1997. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med*, 3, 1029-33. <https://doi.org/10.1038/nm0997-1029>
- MAZAKI-TOVI, S., KANETY, H. & SIVAN, E. 2005. Adiponectin and human pregnancy. *Curr Diab Rep*, 5, 278-81. <https://doi.org/10.1007/s11892-005-0023-2>
- MCCULLOUGH, L. E., MILLER, E. E., CALDERWOOD, L. E., SHIVAPPA, N., STECK, S. E., FORMAN, M. R., M, A. M., MAGUIRE, R., FUEMMELER, B. F., KOLLINS, S. H., S, D. B., HUANG, Z., MURTHA, A. P., MURPHY, S. K., HEBERT, J. R. & HOYO, C. 2017. Maternal inflammatory diet and adverse pregnancy outcomes: Circulating cytokines and genomic imprinting as potential regulators? *Epigenetics*, 12, 688-697. <https://doi.org/10.1080/15592294.2017.1347241>
- MCGOWAN, C. A. & MCAULIFFE, F. M. 2013. Maternal dietary patterns and associated nutrient intakes during each trimester of pregnancy. *Public Health Nutr*, 16, 97-107. <https://doi.org/10.1017/S1368980012000997>
- MOORE, B. F., SAUDER, K. A., STARLING, A. P., HEBERT, J. R., SHIVAPPA, N., RINGHAM, B. M., GLUECK, D. H. & DABELEA, D. 2018. Proinflammatory Diets during Pregnancy and

- Neonatal Adiposity in the Healthy Start Study. *J Pediatr*, 195, 121-127 e2. <https://doi.org/10.1016/j.jpeds.2017.10.030>
- MOR, G., CARDENAS, I., ABRAHAMS, V. & GULLER, S. 2011. Inflammation and pregnancy: the role of the immune system at the implantation site. *Ann N Y Acad Sci*, 1221, 80-7. <https://doi.org/10.1111/j.1749-6632.2010.05938.x>
- MORAN, L. J., SUI, Z., CRAMP, C. S. & DODD, J. M. 2013. A decrease in diet quality occurs during pregnancy in overweight and obese women which is maintained post-partum. *Int J Obes (Lond)*, 37, 704-11. <https://doi.org/10.1038/ijo.2012.129>
- NATIONAL RESEARCH COUNCIL 2009a. Composition and Components of Gestational Weight Gain: Physiology and Metabolism. *Weight gain during pregnancy: Reexamining the guidelines*. Washington, DC: The National Academies Press. <https://doi.org/https://doi.org/10.17226/12584>
- NATIONAL RESEARCH COUNCIL 2009b. *Weight gain during pregnancy: Reexamining the guidelines*, Washington, DC, The National Academies Press. <https://doi.org/https://doi.org/10.17226/12584>
- NAUDE, P. J. W., ROEST, A. M., STEIN, D. J., DE JONGE, P. & DOORNBOS, B. 2018. Anxiety disorders and CRP in a population cohort study with 54,326 participants: The LifeLines study. *World J Biol Psychiatry*, 19, 461-470. <https://doi.org/10.1080/15622975.2018.1433325>
- OSTROWSKI, K., ROHDE, T., ZACHO, M., ASP, S. & PEDERSEN, B. K. 1998. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol*, 508 (Pt 3), 949-53. <https://doi.org/10.1111/j.1469-7793.1998.949bp.x>
- PANAGIOTAKOS, D. B., DIMAKOPOULOU, K., KATSOUYANNI, K., BELLANDER, T., GRAU, M., KOENIG, W., LANKI, T., PISTELLI, R., SCHNEIDER, A., PETERS, A. & GROUP, A. S. 2009. Mediterranean diet and inflammatory response in myocardial infarction survivors. *Int J Epidemiol*, 38, 856-66. <https://doi.org/10.1093/ije/dyp142>
- PEREZ-PEREZ, A., TORO, A., VILARINO-GARCIA, T., MAYMO, J., GUADIX, P., DUENAS, J. L., FERNANDEZ-SANCHEZ, M., VARONE, C. & SANCHEZ-MARGALET, V. 2018. Leptin action in normal and pathological pregnancies. *J Cell Mol Med*, 22, 716-727. <https://doi.org/10.1111/jcmm.13369>
- PIECZYNSKA, J., PLACZKOWSKA, S., PAWLIK-SOBECKA, L., KOKOT, I., SOZANSKI, R. & GRAJETA, H. 2020. Association of Dietary Inflammatory Index with Serum IL-6, IL-10, and CRP Concentration during Pregnancy. *Nutrients*, 12. <https://doi.org/10.3390/nu12092789>
- POSTON, L., IGOSHEVA, N., MISTRY, H. D., SEED, P. T., SHENNAN, A. H., RANA, S., KARUMANCHI, S. A. & CHAPPELL, L. C. 2011. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. *Am J Clin Nutr*, 94, 1980S-1985S. <https://doi.org/10.3945/ajcn.110.001156>
- RAMSAY, J. E., FERRELL, W. R., CRAWFORD, L., WALLACE, A. M., GREER, I. A. & SATTAR, N. 2002. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. *J Clin Endocrinol Metab*, 87, 4231-7. <https://doi.org/10.1210/jc.2002-020311>
- ROMERO, R., GOTSCH, F., PINELES, B. & KUSANOVIC, J. P. 2007. Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. *Nutr Rev*, 65, S194-202. <https://doi.org/10.1111/j.1753-4887.2007.tb00362.x>
- SALAS-SALVADO, J., GARCIA-ARELLANO, A., ESTRUCH, R., MARQUEZ-SANDOVAL, F., CORELLA, D., FIOL, M., GOMEZ-GRACIA, E., VINOLES, E., AROS, F., HERRERA, C., LAHOZ, C., LAPETRA, J., PERONA, J. S., MUNOZ-AGUADO, D., MARTINEZ-GONZALEZ, M. A., ROS, E. & INVESTIGATORS, P. 2008. Components of the Mediterranean-type food pattern and serum inflammatory markers among patients at high risk for cardiovascular disease. *Eur J Clin Nutr*, 62, 651-9. <https://doi.org/10.1038/sj.ejcn.1602762>

- SAVARD, C., LEMIEUX, S., LAFRENIERE, J., LARAMEE, C., ROBITAILLE, J. & MORISSET, A. S. 2018a. Validation of a self-administered web-based 24-hour dietary recall among pregnant women. *BMC Pregnancy Childbirth*, 18, 112. <https://doi.org/10.1186/s12884-018-1741-1>
- SAVARD, C., LEMIEUX, S., WEISNAGEL, S. J., FONTAINE-BISSON, B., GAGNON, C., ROBITAILLE, J. & MORISSET, A. S. 2018b. Trimester-specific dietary intakes in a sample of French-Canadian pregnant women in comparison with national nutritional guidelines. *Nutrients*, 10. <https://doi.org/10.3390/nu10060768>
- SEN, S., RIFAS-SHIMAN, S. L., SHIVAPPA, N., WIRTH, M. D., HÉBERT, J. R., GOLD, D. R., GILLMAN, M. W. & OKEN, E. 2016. Dietary Inflammatory Potential during Pregnancy Is Associated with Lower Fetal Growth and Breastfeeding Failure: Results from Project Viva. *J Nutr*, 146, 728-36. <https://doi.org/10.3945/jn.115.225581>
- SHIN, D., HUR, J., CHO, E. H., CHUNG, H. K., SHIVAPPA, N., WIRTH, M. D., HEBERT, J. R. & LEE, K. W. 2017. Pre-Pregnancy Body Mass Index Is Associated with Dietary Inflammatory Index and C-Reactive Protein Concentrations during Pregnancy. *Nutrients*, 9. <https://doi.org/10.3390/nu9040351>
- SHIN, D., LEE, K. W., BRANN, L., SHIVAPPA, N. & HEBERT, J. R. 2019. Dietary inflammatory index is positively associated with serum high-sensitivity C-reactive protein in a Korean adult population. *Nutrition*, 63-64, 155-161. <https://doi.org/10.1016/j.nut.2018.11.016>
- SHIVAPPA, N., HEBERT, J. R., AKHOUNDAN, M., MIRMIRAN, P. & RASHIDKHANI, B. 2019. Association between inflammatory potential of diet and odds of gestational diabetes mellitus among Iranian women. *J Matern Fetal Neonatal Med*, 32, 3552-3558. <https://doi.org/10.1080/14767058.2018.1466275>
- SHIVAPPA, N., HEBERT, J. R., MARCOS, A., DIAZ, L. E., GOMEZ, S., NOVA, E., MICHELS, N., AROUCA, A., GONZALEZ-GIL, E., FREDERIC, G., GONZALEZ-GROSS, M., CASTILLO, M. J., MANIOS, Y., KERSTING, M., GUNTER, M. J., DE HENAUW, S., ANTONIOS, K., WIDHALM, K., MOLNAR, D., MORENO, L. & HUYBRECHTS, I. 2017. Association between dietary inflammatory index and inflammatory markers in the HELENA study. *Mol Nutr Food Res*, 61. <https://doi.org/10.1002/mnfr.201600707>
- SHIVAPPA, N., STECK, S. E., HURLEY, T. G., HUSSEY, J. R. & HEBERT, J. R. 2014. Designing and developing a literature-derived, population-based dietary inflammatory index. *Public Health Nutr*, 17, 1689-96. <https://doi.org/10.1017/S1368980013002115>
- SIMJAK, P., CINKAJZLOVA, A., ANDERLOVA, K., PARIZEK, A., MRAZ, M., KRSEK, M. & HALUZIK, M. 2018. The role of obesity and adipose tissue dysfunction in gestational diabetes mellitus. *J Endocrinol*, 238, R63-R77. <https://doi.org/10.1530/JOE-18-0032>
- SKARZYNSKA, E., ZBOROWSKA, H., JAKIMIUK, A. J., KARLINSKA, M. & LISOWSKA-MYJAK, B. 2018. Variations in serum concentrations of C-reactive protein, ceruloplasmin, lactoferrin and myeloperoxidase and their interactions during normal human pregnancy and postpartum period. *J Trace Elem Med Biol*, 46, 83-87. <https://doi.org/10.1016/j.jtemb.2017.11.015>
- SPADAFRANCA, A., PIURI, G., BULFONI, C., LIGUORI, I., BATTEZZATI, A., BERTOLI, S., SPECIANI, A. F. & FERRAZZI, E. 2018. Adherence to the Mediterranean Diet and Serum Adiponectin Levels in Pregnancy: Results from a Cohort Study in Normal Weight Caucasian Women. *Nutrients*, 10. <https://doi.org/10.3390/nu10070928>
- STOKKELAND, L. M. T., GISKEODEGARD, G. F., STRIDSKLEV, S., RYAN, L., STEINKJER, B., TANGERAS, L. H., VANKY, E. & IVERSEN, A. C. 2019. Serum cytokine patterns in first half of pregnancy. *Cytokine*, 119, 188-196. <https://doi.org/10.1016/j.cyto.2019.03.013>
- SVENSSON, H., WETTERLING, L., BOSAEUS, M., ODEN, B., ODEN, A., JENNISCHE, E., EDEN, S., HOLMANG, A. & LONN, M. 2016. Body fat mass and the proportion of very large

- adipocytes in pregnant women are associated with gestational insulin resistance. *Int J Obes (Lond)*, 40, 646-53. <https://doi.org/10.1038/ijo.2015.232>
- TESSIER, D. R., FERRARO, Z. M. & GRUSLIN, A. 2013. Role of leptin in pregnancy: consequences of maternal obesity. *Placenta*, 34, 205-11. <https://doi.org/10.1016/j.placenta.2012.11.035>
- VÄHÄMIKO, S., ISOLAURI, E. & LAITINEN, K. 2013. Weight status and dietary intake determine serum leptin concentrations in pregnant and lactating women and their infants. *Br J Nutr*, 110, 1098-106. <https://doi.org/10.1017/s0007114513000214>
- VASSILIADIS, S., RANELLA, A., PAPADIMITRIOU, L., MAKRYGIANNAKIS, A. & ATHANASSAKIS, I. 1998. Serum levels of pro- and anti-inflammatory cytokines in non-pregnant women, during pregnancy, labour and abortion. *Mediators Inflamm*, 7, 69-72. <https://doi.org/10.1080/09629359891199>
- VILLAR, J., CARROLI, G., WOJDYLA, D., ABALOS, E., GIORDANO, D., BA'AQEEL, H., FARNOT, U., BERGSJO, P., BAKKETEIG, L., LUMBIGANON, P., CAMPODONICO, L., AL-MAZROU, Y., LINDHEIMER, M., KRAMER, M. & WORLD HEALTH ORGANIZATION ANTENATAL CARE TRIAL RESEARCH, G. 2006. Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? *Am J Obstet Gynecol*, 194, 921-31. <https://doi.org/10.1016/j.ajog.2005.10.813>
- WALKER, J. J. 2011. Inflammation and preeclampsia. *Pregnancy Hypertens*, 1, 43-7. <https://doi.org/10.1016/j.preghy.2010.10.004>
- WEDELL-NEERGAARD, A. S., LANG LEHRSKOV, L., CHRISTENSEN, R. H., LEGAARD, G. E., DORPH, E., LARSEN, M. K., LAUNBO, N., FAGERLIND, S. R., SEIDE, S. K., NYMAND, S., BALL, M., VINUM, N., DAHL, C. N., HENNEBERG, M., RIED-LARSEN, M., NYBING, J. D., CHRISTENSEN, R., ROSENMEIER, J. B., KARSTOFT, K., PEDERSEN, B. K., ELLINGSGAARD, H. & KROGH-MADSEN, R. 2019. Exercise-Induced Changes in Visceral Adipose Tissue Mass Are Regulated by IL-6 Signaling: A Randomized Controlled Trial. *Cell Metab*, 29, 844-855 e3. <https://doi.org/10.1016/j.cmet.2018.12.007>
- WERNECK, A. O., CHRISTOFARO, D. G. D., RITTI-DIAS, R. M., CUCATO, G. G., STUBBS, B., OYEYEMI, A. L., CONCEICAO, R. D. O., SANTOS, R. D. & BITTENCOURT, M. S. 2020. Does physical activity influence the association between depressive symptoms and low-grade inflammation in adults? A study of 8,048 adults. *Physiol Behav*, 223, 112967. <https://doi.org/10.1016/j.physbeh.2020.112967>
- WILLETT, W. C., SACKS, F., TRICHOPOULOU, A., DRESCHER, G., FERRO-LUZZI, A., HELSING, E. & TRICHOPOULOS, D. 1995. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr*, 61, 1402S-1406S. <https://doi.org/10.1093/ajcn/61.6.1402S>
- YANG, Y., KAN, H., YU, X., YANG, Y., LI, L. & ZHAO, M. 2020. Relationship between dietary inflammatory index, hs-CRP level in the second trimester and neonatal birth weight: a cohort study. *J Clin Biochem Nutr*, 66, 163-167. <https://doi.org/10.3164/jcbtn.19-100>
- YEH, K. L., KAUTZ, A., LOHSE, B. & GROTH, S. W. 2021. Associations between Dietary Patterns and Inflammatory Markers during Pregnancy: A Systematic Review. *Nutrients*, 13. <https://doi.org/10.3390/nu13030834>

Table 1. Baseline characteristics of pregnant women in the ANGE cohort (n=79).

	Mean ± SD or n (%)
Age, years	32.1 ± 3.7
Gestational age at enrollment, weeks	9.3 ± 0.7
Parity ^a	
0	28 (35.4)
≥1	51 (64.6)
Pre-pregnancy body mass index, kg/m ²	25.7 ± 5.8
Underweight	2 (2.5)
Normal weight	43 (54.4)
Overweight	19 (24.1)
Obesity	15 (19.0)
Total gestational weight gain, kg ^b	15.2 ± 4.9
Ethnicity	
Caucasian	77 (97.5)
Hispanic	1 (1.3)
Arab	1 (1.3)
Education	
High school	4 (5.0)
College ^c	13 (16.5)
University	62 (78.5)
Annual household income	
< 60,000 CAD	15 (19.0)
60,000-79,999 CAD	13 (16.5)
80,000-99,999 CAD	17 (21.5)
≥ 100,000 CAD	33 (41.8)
Income not disclosed	1 (1.2)

^aRefers to the number of children previously born, excluding the current pregnancy. ^bFor n=63, the other 16 participants' medical records had no documented body weight value ≥37 weeks. ^cIn Québec, college refers to a degree obtained after high school and before university.

Table 2. Correlations between pre-pregnancy BMI or total GWG and trimester-specific circulating concentrations of leptin, adiponectin, interleukin-6 and C-reactive protein.

	First trimester	Second trimester	Third trimester
<i>Pre-pregnancy BMI</i>			
Leptin ^a	0,75***	0,75***	0,74***
Adiponectin ^b	-0,33**	-0,35**	-0,18
IL-6 ^c	0,48***	0,28*	0,31**
CRP ^d	0,30**	0,56***	0,41**
<i>Total GWG</i>			
Leptin ^e	0.20	0.45**	0.30*
Adiponectin ^f	0.01	0.11	-0.01
IL-6 ^g	-0.11	-0.27*	0.09
CRP ^h	-0.14	-0.30*	0.01

Values are presented as Pearson correlation coefficients (r). For the total GWG correlations, all coefficients are adjusted for ppBMI. ^aFor n=78. ^bFor n=76. ^cFor n=73. ^dFor n=74. ^eFor n=63. ^fFor n=61. ^gFor n=57. ^hFor n=59 *** $p < 0.0001$, ** $p < 0.01$, * $p < 0.05$. CRP, C-reactive protein; GWG, gestational weight gain; IL-6, interleukin-6; ppBMI, pre-pregnancy body mass index.

Table 3. Correlations between trimester-specific circulating concentrations of leptin, adiponectin, IL-6 and CRP and dietary scores.

	Leptin ^a		Adiponectin ^b		IL-6 ^c		CRP ^d	
	r	r _{adj}	r	r _{adj}	r	r _{adj}	r	r _{adj}
<i>First trimester</i>								
MDS	-0.26*	-0.09	0.10	0.02	-0.05	0.04	-0.10	0.06
DII	0.01	0.21	-0.02	-0.04	-0.22	-0.20	-0.02	0.06
<i>Second trimester</i>								
MDS	-0.19	-0.26*	-0.12	-0.16	-0.10	-0.09	-0.05	-0.004
DII	-0.01	-0.07	0.05	0.08	-0.05	-0.06	0.17	0.15
<i>Third trimester</i>								
MDS	-0.12	-0.20	-0.12	-0.11	0.22	0.21	0.03	0.02
DII	0.07	0.10	0.10	0.11	-0.03	-0.03	0.06	0.05

Values are presented as Pearson correlation coefficients, raw (r) or adjusted for ppBMI (r_{adj}). Correlation analyses were performed between circulating concentrations and dietary scores measured in the same trimesters (e.g., first trimester leptin concentrations with first trimester MDS). ^aFor n=78. ^bFor n=76. ^cFor n=73. ^dFor n=74. Mean scores were calculated using the mean intakes from the nine dietary recalls from the three trimesters. ***p*<0.01, **p*<0.05. CRP, C-reactive protein; DII, dietary inflammatory index; IL-6, interleukin-6; MDS, Mediterranean diet score; ppBMI, pre-pregnancy body mass index.

FIGURE CAPTIONS

Figure 1. Circulating concentrations of leptin, adiponectin, IL-6 and CRP across trimesters, according to ppBMI categories

Legend. *p*-values refer to the mixed models for repeated measures used to examine the evolution of trimester-specific circulating concentrations of (A) leptin (n=78), (B) adiponectin (n=76), (C) IL-6 (n=73) and (D) CRP (n=74) according to ppBMI. CRP, C-reactive protein; IL-6, interleukin-6; ppBMI, pre-pregnancy body mass index.

Figure 2. Standardized regression coefficients of independent predictors of circulating concentrations of (A) leptin, (B), adiponectin, (C), IL-6, (D) CRP and overall inflammation status.

Legend. Trimester-specific circulating concentrations of (A) leptin (n=78), (B), adiponectin (n=76), (C), IL-6 (n=73), (D) CRP (n=74) and (E) overall inflammation status (n=66) were entered as dependent variables. Trimester, ppBMI, weight gain (kg) and MDS were entered as independent variables. The overall inflammation status corresponds to the averaged Z-scores $[(X - \text{mean}) / \text{standard deviation}]$ computed independently for each inflammatory marker. Confidence intervals not crossing the zero line indicate statistical significance ($p < 0.05$). CRP, C-reactive protein; IL-6, interleukin-6; MDS, Mediterranean diet score; ppBMI, pre-pregnancy body mass index.

Draft

FIGURES

Figure 1. Circulating concentrations of leptin, adiponectin, IL-6 and CRP across trimesters, according to ppBMI categories

Figure 2. Standardized regression coefficients of independent predictors of circulating concentrations of (A) leptin, (B), adiponectin, (C), IL-6, (D) CRP and overall inflammation status (E)

Figure 1. Circulating concentrations of leptin, adiponectin, IL-6 and CRP across trimesters, according to ppBMI categories

Legend. p-values refer to the mixed models for repeated measures used to examine the evolution of trimester-specific circulating concentrations of (A) leptin (n=78), (B) adiponectin (n=76), (C) IL-6 (n=73) and (D) CRP (n=74) according to ppBMI. CRP, C-reactive protein; IL-6, interleukin-6; ppBMI, pre-pregnancy body mass index.

169x100mm (600 x 600 DPI)

Figure 2. Standardized regression coefficients of independent predictors of circulating concentrations of (A) leptin, (B), adiponectin, (C), IL-6, (D) CRP and overall inflammation status. Legend. Trimester-specific circulating concentrations of (A) leptin (n=78), (B), adiponectin (n=76), (C), IL-6 (n=73), (D) CRP (n=74) and (E) overall inflammation status (n=66) were entered as dependent variables. Trimester, ppBMI, weight gain (kg) and MDS were entered as independent variables. The overall inflammation status corresponds to the averaged Z-scores $[(X - \text{mean}) / \text{standard deviation}]$ computed independently for each inflammatory marker. Confidence intervals not crossing the zero line indicate statistical significance ($p < 0.05$). CRP, C-reactive protein; IL-6, interleukin-6; MDS, Mediterranean diet score; ppBMI, pre-pregnancy body mass index.

149x95mm (600 x 600 DPI)