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F₂-isoprostanes are correlated with *trans* fatty acids in the plasma of pregnant women

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Abstract

We hypothesized that the mild physiological oxidative stress present during pregnancy could increase both, plasma F₂-isoprostanes (F₂-isoPs) by lipid oxidation and *trans* fatty acids (TFA) through *cis-trans* isomerization respectively. Plasma samples collected at 12–18 weeks (MIROS cohort; n=65) and 38–41 weeks of pregnancy (CHUL cohort; n = 21) were subjected to alkaline hydrolysis followed by liquid-liquid extraction in order to extract total F₂-isoPs for quantification by HPLC-MS/MS. Several positive correlations were found between F₂-isoPs and TFA, measured by GC-FID in plasma phospholipids, such as 6*t*-18:1, 9*t*-18:1 and 9*t*,12*c*-18:2 (*r* > 0.306; *p* < 0.045). Despite its low level, the 9*t*,12*c*-18:2 *trans* isomer, known to be associated to cardiovascular diseases, showed the most significant correlations with F₂-isoPs. No correlation was observed between F₂-isoPs and 9*t*-16:1 or 11*t*-18:1. In summary, this study suggests either a concomitant phenomenon or a competition between lipid peroxidation and *cis-trans* isomerisation of the *cis* precursor fatty acid *in vivo* during pregnancy.

Keywords

elaidic acid; oxidative stress; pregnancy; mass spectrometry; vaccenic acid; 8-iso-PGF_{2α}

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Conflict of interest

None.

1. Introduction

Trans fatty acids (TFA) are unsaturated fatty acids containing at least one non-conjugated carbon-carbon double bond in the *trans* configuration. Unsaturated fatty acids are synthesized only in the *cis* configuration in the human but a small amount of TFA are provided naturally from the diet. Indeed, TFA are produced by bacteria in the rumen of ruminants and account for 2–5% of total fatty acids content of their meat and milk [1]. The distribution of TFA isomers from ruminants (rTFA) differs clearly from TFA of industrial sources (iTFA). The iTFA are mainly represented by 9*t*-18:1 (elaidic acid) [2], whereas 11*t*-18:1 (vaccenic acid) is the predominant rTFA [3].

Consumption of iTFA has adverse effects on cardiovascular health by altering blood lipid profile (increase of total to HDL cholesterol ratio, increase in LDL-cholesterol, decrease in HDL-cholesterol), by modifying apolipoprotein levels, and by promoting systemic inflammation as well as endothelial dysfunction [4–10]. The various isomers of TFA may have different biological and physiological effects as a function of the length of the carbon chain, the number and the position of the carbon-carbon double bonds. The *trans*-18:2 isomers seem more detrimental to health than the *trans*-18:1 isomers while the *trans*-16:1 isomers appeared to have no effect on risk factors for cardiovascular diseases [5, 10, 11].

Beside ingested TFA, there is also a possibility for TFA to be generated indirectly from oxidative stress as shown by liposomes exposed to γ -radiation or UV photolysis [12, 13]. The mechanism proposed for *cis-trans* isomerisation is the action of either the thiyl or the nitrogen dioxide radicals on *cis* fatty acids [14–16]. The latter phenomenon was shown to occur in rats that were fed only with *cis*-fatty acids, then were exposed to carbon tetrachloride, an agent causing an oxidative stress [17]. However, there is no clear evidence of this *cis-trans* isomerisation in humans under physiological or pathological oxidative stress.

F₂-isoprostanes (F₂-isoPs) are reliable markers of oxidative stress known to be associated to coronary heart diseases [18, 19], hypercholesterolemia [20], inflammatory diseases [21], endothelial dysfunctions [22, 23] and even pregnancy [24–27]. The 8-iso-PGF_{2 α} has been the most studied isomer so far [28]. However, more than 64 isomers of F₂-isoPs divided into four classes of regioisomers can be generated from the oxidation of arachidonic acid esterified to phospholipids [29]. F₂-isoPs must be freed from phospholipids by the action of phospholipases A₂ (PLA₂) in order to induce vasoconstriction in blood vessels [30, 31]. The concomitant assessment of several F₂-isoPs can provide information on the balance between generation and elimination according to the physiological state or the disorder studied [32–35].

The exact link between oxidative stress and TFA remains to be elucidated. However, we know that TFA may elicit inflammation known to involve reactive oxygen species (ROS) [10, 11]. Few controlled dietary trials have been conducted in order to understand the link between TFA intake and levels of urinary 8-iso-PGF_{2 α} . Generally, consumption of TFA was shown to increase excretion of the latter [36–40]. Urinary level of F₂-isoPs is a good indicator of oxidative stress but relies on other factors such as the rate of hydrolysis of F₂-

isoPs from phospholipids, their metabolism as well as their excretion [41]. However, valuable information could be obtained from the simultaneous analysis of the plasma concentrations of several isomers of F₂-isoP.

Dietary intake of TFA can be estimated based on the TFA levels in plasma phospholipids [40, 42–45]. Besides, the impact of a mild oxidative event like a typical pregnancy [24–27] on TFA generation remains to be determined. Thus, the aim of this study was to assess the relationship between the plasma concentrations of TFA in phospholipids and the total (esterified + free) levels of several F₂-isoP isomers from women at the first and the third trimester of pregnancy.

2. Patients and methods

2.1 Materials

The F₂-isoprostanes (8-iso-15(R)-PGF_{2α}, 8-iso-PGF_{2α}, 15(R)-PGF_{2α}, iPF_{2α}-IV, (±)5-iPF_{2α}-VI, (±)5-8,12-iso-iPF_{2α}-VI) and their deuterated counterparts 8-iso-PGF_{2α}-d4, iPF_{2α}-IV-d4, iPF_{2α}-VI-d4, (±)5-iPF_{2α}-VI-d11, and (±)5-8,12-iso-iPF_{2α}-VI-d11 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Butylated hydroxytoluene (BHT) was bought from Sigma-Aldrich (Oakville, ON, Canada). The standards for fatty acids, FAME 37 mix, 18:2 cis/trans mix 18:3 cis/trans mix, 22:5, 9*t*-14:1, 9*t*-C16:1 and several 18:1 fatty acids (6*c*-, 11*c*-, 12*c*-, 13*c*-, 6*t*-, 11*t*-18:1) were all purchased from Supelco Inc (Bellefonte, PA, USA). A mixture of 31 fatty acids (GLC-411) was obtained from NuChek Prep Inc (Elysian, MN, USA). The 9*c*,12*t*-18:2 was bought from Larodan (AB Malmö, Sweden) whereas the C17:0 isobranched fatty acid was obtained from Matreya, (Pleasant Gap, PA, USA). All other reagents and solvents were HPLC grade and were purchased from VWR International (Ville Mont-Royal, QC, Canada).

2.2 Patient recruitment

Plasma from sixty-five women with uncomplicated pregnancies were recruited prospectively early between the 12th to the 18th weeks of pregnancy and were provided by biobank. This comprised a subset of the 665 possible uncomplicated pregnancies recruited in 17 Canadian centers over a 26-month period. The patients' data for the Maternal and Infant Research on Oxidative Stress (MIROS cohort) have been published previously [46]. Plasma samples from twenty-one normotensive (< 140/90 mm Hg) pregnant women were collected at the Centre Mère-Enfant du Centre Hospitalier Universitaire de Québec (CHUL cohort) within a one-year period. The respective institutions approved the protocol and informed consents were obtained from all pregnant women. Exclusion factors were age (<18 years old or >40 years old), intake of anticoagulant drugs or drugs affecting lipid metabolism, chronic hypertension, diabetes mellitus, obesity (body mass index > 30 prior to pregnancy), kidney diseases, inflammatory intestinal diseases, blood clotting disorders and gestational diabetes.

2.3 Blood collection and processing

Twenty mL of blood were collected in EDTA-tubes within 4 hours before the active phase of labor and was centrifuged at 180 × *g* for 10 minutes as described previously [47]. The resulting plasma was recentrifuged at 1300 × *g* for 25 min to remove platelets and, was

frozen and kept at -80°C until analyzed. The 0.1% BHT was added just before aliquoting for the two cohorts.

2.4 F₂-isoPs analysis by HPLC-MS/MS

The total F₂-isoPs (free + esterified) were extracted from plasma after alkaline hydrolysis using a liquid-liquid extraction described in details before for method I and II [47]. Method I (CHUL cohort): The HPLC-MS/MS analysis of seven F₂-isoPs was described and fully validated by us recently [47]. Briefly, the chromatography was carried out using a Shimadzu Prominence system (Columbia, MD, USA) coupled to a 3200 QTRAP[®] LC/MS/MS system from AB Sciex (Concord, ON, Canada) operated in the negative mode. A Kinetex XB-C18 100 Å column (100 × 3.0 mm, 2.6 µm) was used from Phenomenex (Torrance, CA, USA). The injection volume was 40 µL. The separation was done using a gradient of three solvents (water, acetonitrile and methanol, each containing 0.01 % (v/v) acetic acid) at a flow rate of 0.45 mL/min as described before [47].

For the MIROS cohort, we used a slightly different HPLC method that allowed separation of five F₂-isoPs. Method II: a Shim-Pack XR-ODS column (3.0 × 100 mm, 2.2 µm) from Shimadzu was used. The column temperature was controlled at 30°C and the injection volume was 40 µL. The chromatographic separation was done using a gradient of three solvents at 0.4 mL/min. The mobile phase A was 0.01% acetic acid in water, B was 0.01% acetic acid in acetonitrile and C was 0.01% acetic acid in methanol. First, solvents B and C were both hold at 5% for 1 min while solvent A was at 90%. This was followed by a linear gradient for 1 min to 25% of B and C. The next step was a linear gradient over 8 min to 35% B and C followed by a gradient to 45% B and C within 0.5 min. The last condition was maintained for 0.5 min and the solvents B and C were decreased to 5% in 0.1 min. This last condition was maintained for the remaining 3.9 min to complete 15 min run.

For method I and II, the F₂-isoPs and their corresponding internal standard, were monitored in the multiple-reaction monitoring (MRM) mode at the transitions 353.3 → 193.2 and 357.3 → 197.2 m/z respectively for class III, and at the transitions 353.3 → 127.0 and 357.0 → 127.0 m/z for class IV. Lastly, class VI were analyzed at the transitions 353.0 → 115.0 and 364.6 → 115.0 m/z respectively. Quantification of F₂-isoPs was performed using the Analyst[®] 1.4.2 Software (AB Sciex) [47].

2.5 Determination of plasma fatty acid profile by gas chromatography

Plasma fatty acids were isolated according to a method previously described [48]. Briefly, a solution of chloroform:methanol (2:1, by volume) was used to extract lipids from plasma. Then, phospholipids were separated by thin layer chromatography using a mix of isopropyl ether:acetic acid (96:4) as elutant and phospholipid fatty acids were methylated following a transesterification reaction using a mix of methanol:benzene (4:1) and acetyl chloride at 95°C for 1.5 hour. Methylated fatty acids were finally analysed by gas chromatography coupled with a flame ionisation detector (GC-FID) as explained elsewhere [48]. The peroxidation index (PI) and the unsaturation index (UI) were calculated as described elsewhere [49].

2.6 Statistical analyses

Statistical analyses were performed with GraphPad Prism 6.0e (GraphPad Software Inc., 2013, San Diego, USA). Normality was verified using the D'Agostino & Pearson omnibus test. Since normality was not achieved for most of the data, the Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparisons test was used to compare levels of both, F₂-isoPs and fatty acids. The Spearman's rank correlation coefficient was used to study relationship between variables. A *p*-value lower than 0.05 (two-tailed) was considered statistically significant.

3. Results

3.1 F₂-isoPs in the plasma of early and late pregnancies

The plasma F₂-isoPs levels from sixty-five women (65, MIROS cohort) early in pregnancy and twenty-one women (21, CHUL cohort) at the end of their pregnancies before the active phase of labor, were measured by HPLC-MS/MS (see Fig. 1 for typical MRM chromatograms). All were uneventful pregnancies from two independent cohorts. The plasma median levels for 8-iso-15(R)-PGF_{2α}, 8-iso-PGF_{2α}, 15(R)-PGF_{2α}, iPF_{2α}-IV, iPF_{2α}-VI, 5-iPF_{2α}-VI and 5-8,12-iso-iPF_{2α}-VI are reported in table 1. Since the measurement of F₂-isoPs from the CHUL cohort was performed after the MIROS cohort, the HPLC method used was more selective for the separation of iPF_{2α}-VI and 5-iPF_{2α}-VI that co-eluted in the MIROS cohort (Fig. 1C). Thus, the sum of these two isomers was calculated for the CHUL cohort. In early pregnancies, the isomers of F₂-isoPs were in equal amounts compared to iPF_{2α}-VI + 5-iPF_{2α}-VI, a blend of two isomers. The (±)5-8,12-iso-iPF_{2α}-VI is the most abundant of all isomers measured in the CHUL cohort (*p* < 0.05, Table 1).

3.2 Analysis of plasma fatty acids in pregnancy

Determination of plasma phospholipid fatty acids profile was done by GC-FID (see profile in Fig. 2). This method allows for the determination of a large number of fatty acids. The mean concentrations of *trans* fatty acids of interest and of their corresponding *cis* isomer are presented in Table 2 for both cohorts. The calculated PI and UI index is also reported. The *cis* isomers are generally found in greater quantities than *trans* isomers except for the 6*c*-18:1 + 7*c*-18:1, which level was about the same as the 6*t*-18:1 (*p* > 0.05) in the CHUL cohort only. The 9*c*,12*c*-18:2 (linoleic acid) is the most abundant of all unsaturated fatty acids measured while 9*c*-18:1 is the most abundant of all monounsaturated fatty acids, representing respectively about 19 and 9 % of total fatty acids. Monounsaturated *trans* fatty acids (6*t*-18:1, 9*t*-18:1 and 11*t*-18:1) are present in greater amounts than the *trans* isomers of linoleic acid (9*c*,12*t*-18:2, 9*t*,12*c*-18:2 and 9*t*,12*t*-18:2). Of note, 6*t*-18:1 was only present at the end of pregnancy (CHUL cohort).

3.3 Relation between F₂-isoPs and *trans* fatty acids

Spearman's rank correlation coefficient was used to study associations between F₂-isoPs and fatty acids in plasma of healthy women at the early stage (12–18 weeks, Table 3) and at the late stage (38–41 weeks, Table 4) of pregnancy. Many positive and significant correlations were found between F₂-isoP isomers and *trans* fatty acids but no correlation was found

between F₂-isoPs and *cis* fatty acids, the 11 ϵ -18:1. In early pregnancy, three highly significant correlations were observed ($p < 0.009$), the first between the well-known 8-iso-PGF_{2 α} and the 9 ϵ -18:1. With the same *trans* fatty acid, the correlation with (iPF_{2 α} -VI + 5-iPF_{2 α} -VI) was also highly significant ($p = 0.006$). The (iPF_{2 α} -VI + 5-iPF_{2 α} -VI) was also correlated with the 9 ϵ ,12 c -18:2 ($p = 0.0003$).

At the end of pregnancy (Table 4), a correlation was found between the 6 ϵ -18:1 and the 8-iso-PGF_{2 α} ($r = 0.471$, $p = 0.038$) and strong tendencies for similar correlations were also observed for this fatty acid with the 5-iPF_{2 α} -VI ($r = 0.412$, $p = 0.058$) and with the ($\pm$)5-8,12-iso-iPF_{2 α} -VI ($r = 0.458$, $p = 0.054$) respectively. The 9 ϵ -18:1 correlated positively with both, the 5-iPF_{2 α} -VI ($r = 0.306$, $p = 0.036$) and the ($\pm$)5-8,12-iso-iPF_{2 α} -VI ($r = 0.351$, $p = 0.027$). The 9 c ,12 ϵ -18:2 correlated with 15(R)-PGF_{2 α} ($r = 0.506$, $p = 0.006$), iPF_{2 α} -IV ($r = 0.421$, $p = 0.045$) and iPF_{2 α} -VI ($r = 0.391$, $p = 0.032$) and the sum of iPF_{2 α} -VI and 5-iPF_{2 α} -VI ($r = 0.440$, $p = 0.020$) respectively. Of note, F₂-isoPs were not correlated with UI and PI indexes in the two independent cohorts.

4. Discussion and conclusions

In this work, the median level of 8-iso-PGF_{2 α} , the most studied isomer of F₂-isoPs, was respectively 131 pg/mL of plasma, early in pregnancy, and 184 pg/mL at the end of the third trimester. Previous studies reported values for total level of 8-iso-PGF_{2 α} between 40 and 170 pg/mL in plasma from healthy non-pregnant individuals [50, 51]. Data from these independent cohorts suggest a 40% increase in 8-iso-PGF_{2 α} levels at the end of pregnancy. However, it was previously shown that plasma 8-iso-PGF_{2 α} levels are significantly increased by about two-fold from 15–20 weeks to the 37–41 weeks of pregnancy [52]. In the present study, levels of the various F₂-isoPs varied from 0 (iPF_{2 α} -IV) to 2.4-fold (iPF_{2 α} -VI + 5-iPF_{2 α} -VI) at the end of pregnancy compared to early pregnancy.

Fatty acids associated with plasma phospholipids were also examined in each of the pregnant women. The plasma fatty acid profile is known to be influenced by dietary habits [43]. The 9 ϵ -18:1/11 ϵ -18:1 index can be used to evaluate whether the diet is high in iTFA or in rTFA. This ratio is known to be reduced by high dairy fat intake [53]. A previous study in lactating women demonstrated the effect of a change in the diet on the fatty acid composition in different plasma lipid fractions [44]. Thus, when women increased their consumption of butter, the phospholipid 9 ϵ -18:1/11 ϵ -18:1 index indicated a ratio of 0.5. On the other hand, if they consumed low-iTFA margarine, this ratio was lowered at 0.23. However, if they consumed regular margarine (high-iTFA), this ratio rose to 0.78. In the MIROS and CHUL cohorts, the 9 ϵ -18:1/11 ϵ -18:1 index was 0.67 and 0.39 respectively. Taken together, these results suggest that the pregnant women in the CHUL cohort had a diet richer in rTFA, and/or low in iTFA than the MIROS cohort [44]. Though, it would have been of interest to know the exact diet in TFA for the pregnant women of the two cohorts.

We studied in depth the relation between the five or seven plasma F₂-isoPs isomers and the fatty acids composition of plasma phospholipids in two independent cohorts. Several significant and positive correlations were found, demonstrating a clear link between oxidative stress and TFA *in vivo*. However, not all F₂-isoP isomers correlated with a specific

TFA. This shows the importance of measuring various F₂-isoP isomers. Furthermore, this finding indicates that *in vivo* F₂-isoPs formation and elimination may vary from one isomer to another. Many controlled dietary trials have shown a relation between urinary 8-iso-PGF_{2α} and higher consumption of TFA [36, 38, 39]. Indeed, one study showed a 20% increase in the level of urinary 8-iso-PGF_{2α} in healthy subjects after three weeks of diet rich in iTFA in comparison to the control diet [36]. In contrast, another report showed no effect of a 16-week diet containing iTFA on the level of urinary 8-iso-PGF_{2α} in fifty-two healthy overweight postmenopausal women [37]. However, only a single urinary 8-iso-PGF_{2α} was used as a biomarker of oxidative stress in the aforementioned studies. Its level may depend on other factors than oxidative stress such as the rate of hydrolysis of 8-iso-PGF_{2α} from phospholipids, its metabolism and its excretion.

In the present study all *cis* fatty acids were not associated with markers of oxidative stress. Similarly, 11*Δ*-18:1 was not found to be associated with oxidative stress in the present report. A dietary intervention study designed to understand the effects of 11*Δ*-18:1 showed no differences on urinary 8-iso-PGF_{2α} level among twenty-two young men receiving a test diet for 5 weeks compared to twenty young men receiving the control diet [40]. However, two other studies using a 1:1 mixture of 11*Δ*-18:1 (rTFA) and 12*Δ*-18:1 to supplement the daily diet over a 6-week or a 9-day period showed an increased level of urinary 8-iso-PGF_{2α} in healthy subjects. Nevertheless, it was not clear which TFA isomer was responsible for the observed increase in 8-iso-PGF_{2α} [38, 39]. Therefore, the relationship between 11*Δ*-18:1 and oxidative stress is not yet clearly established.

Our study has shown that each TFA is differentially linked to oxidative stress. Both, the 6*Δ*-18:1 and the 9*Δ*-18:1 are iTFA related to oxidative stress in our cohort. This is in agreement with results obtained by most of the dietary trials discussed earlier. The 9*Δ*, 12*Δ*-18:2, a *trans* isomer of linoleic acid, is the TFA that presents the highest degree of correlation with F₂-isoP isomers, especially iPF_{2α}-VI + 5-iPF_{2α}-VI. Thus, among all TFA considered in our study, the 9*Δ*, 12*Δ*-18:2 is clearly associated with oxidative stress despite its very low level in plasma phospholipids. The *trans*-18:2 isomers have generally been associated with a higher relative risk of cardiovascular diseases than *trans*-18:1 isomers [5, 54] but its association with oxidative stress in pregnancy was unknown before the present study.

Several studies have shown that high consumption of both industrial and natural TFA causes oxidative stress and induces the formation of F₂-isoPs as discussed earlier, but discrepancies remain. The present study suggests that free radicals formed during a typical pregnancy are able to induce the formation of TFA *in vivo* via *cis-trans* isomerization of their *cis* precursor as early as 12–18 weeks post-conception. This phenomenon of oxidative stress mediated increase of TFA *in vivo* was first demonstrated in liver, heart, kidney, adipose tissue, and erythrocyte membranes phospholipids of young adult rats fed with a diet free of *trans* isomers exposed to γ -irradiation [17]. This work was further confirmed in erythrocyte membranes and kidney of aged rats fed with food containing no TFA and exposed to carbon tetrachloride, as an oxidative stress inducer [55]. Beside, the thiyl radical, it has also been demonstrated that some isomers of *trans*-arachidonic acid may originate from the NO₂-mediated AA isomerization in human [14]. An *in vitro* study has shown the competition

between lipid peroxidation and isomerization [12]. This competition could explain, at least in part, why correlations are observed between F₂-isoPs and 9*t*-18:1 generated from the high level 9*c*-18:1 but not with 11*t*-18:1 derived from the low level 11*c*-18:1. Thus the *cis* precursor of 9*t*-18:1 is the most abundant monounsaturated fatty acid measured in plasma phospholipids during pregnancy. It accounts for 8.1–9.5 % of total fatty acid content while 11*c*-18:1 accounts for less than 1.5 %. Thus, the 9*c*-18:1 fatty acid is more likely to be isomerized by free radicals than 11*c*-18:1 fatty acid. Similar observation can be made concerning 9*c*,12*t*-18:2, whose precursor, linoleic acid (9*c*,12*c*-18:2), is the most abundant of all unsaturated fatty acids in our cohort, accounting for 19.2 % of total fatty acids. Knowing that polyunsaturated fatty acids are more easily isomerized than monounsaturated [56], a stronger link between 9*c*,12*t*-18:2 and F₂-isoPs was expected. This was also in agreement with an *in vitro* study that investigated the competition between lipid peroxidation and isomerization. Indeed, the major *trans* isomer of 9*c*,12*c*-18:2 formed in liposomes was 9*t*,12*c*-18:2 [12]. We detected only this specific isomer in our cohorts but not the 9*t*,12*t*-18:2 or 9*c*,12*t*-18:2.

In summary, this is the first *in vivo* demonstration of a link between the levels of TFA and oxidative stress markers, F₂-isoPs, in the plasma of pregnant women, a condition involving a mild but physiological oxidative stress. The dietary TFA found in biological membranes can induce oxidative stress. Additionally, TFA can be formed *in vivo* by oxidative stress since ROS can react with unsaturated fatty acids to cause either a *cis-trans* isomerization [16], or a peroxidation reaction yielding to F₂-isoPs. These two pathways most likely explain the link between TFA and F₂-isoPs in the present report. In perspective, since F₂-isoPs are derived from the oxidation of arachidonic acid, it would be of interest to investigate the *trans*-arachidonic isomers in pregnancy. Similarly, links between TFAs and F₂-isoPs isomers in pathophysiological conditions involving much higher oxidative challenge, such as hypertension, preeclampsia and diabetes, should also be investigated.

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Abbreviations

9<i>t</i>-16:1	palmitelaidic acid
9<i>c</i>,12<i>c</i>-18:2	linoleic acid
9<i>c</i>,12<i>t</i>-18:2	<i>cis</i> -9, <i>trans</i> -12 octadecadienoic acid
9<i>t</i>,12<i>t</i>-18:2	linolenelaidic acid
9<i>t</i>-18:1	elaidic acid
11<i>t</i>-18:1	vaccenic acid

F₂-isoP	F ₂ -isoprostane
GC-FID	gas chromatography coupled to a flame ionisation detector
IS	internal standard
iTFA	industrial <i>trans</i> fatty acids
PGF_{2α}	prostaglandin F _{2α}
PI	peroxidation index
PLA₂	phospholipase A ₂
ROS	Reactive oxygen species
rTFA	ruminant <i>trans</i> fatty acids
TFA	<i>Trans</i> fatty acids
UI	unsaturation index

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Fig. 1.

Mass chromatograms of a representative plasma samples spiked with 50 pg of deuterated internal standards (- - -) using method I (A and C) and II (B and D). See material and methods for chromatographic details. Peak identification: 1 = 8-iso-15(R)-PGF_{2α}; 2 = 8-iso-PGF_{2α}; 3 = 15(R)-PGF_{2α}; 4 = PGF_{2α}; 5 = 8-iso-PGF_{2α}-d₄; 6 = PGF_{2α}-d₄; 7 = iPF_{2α}-VI + 5-iPF_{2α}-VI; 8 = iPF_{2α}-VI-d₁₁ + 5-iPF_{2α}-VI-d₁₁; 9 = (±)5-8,12-iso-iPF_{2α}-VI-d₁₁; 10 = iPF_{2α}-VI; 11 = 5-iPF_{2α}-VI; 12 = iPF_{2α}-VI-d₁₁; 13 = 5-iPF_{2α}-VI-d₁₁; 14 = (±)5-8,12-iso-iPF_{2α}-VI.

Fig. 2.

GC chromatograms of measured 18:1 and 18:2 fatty acids. Standards alone (A–B) and a typical plasma sample of the MIROS cohort (C–D) are shown. Peak identification (% CV): 1 = 6*t*-18:1 (7.9); 2 = 9*t*-18:1 (2.0); 3 = 11*t*-18:1 (5.0); 4 = 6*c*-18:1/7*c*-18:1 (2.4); 5 = 9*c*-18:1 (1.0); 6 = 11*c*-18:1 (1.4); 7 = 12*c*-18:1 (1.9); 8 = 13*c*-18:1 (2.7); 9 = 9*tl*2*t*-18:2 (2.1); 10 = 9*c*12*t*-18:2 (11.1); 11 = 9*tl*12*c*-18:2 (10.3); 12 = 9*c*12*c*-18:2 (2.9); ? = undetermined fatty acid in plasma.

TABLE 1

F₂-isoprostane levels in the plasma during pregnancy.

	Isoprostane levels ^I (pg/mL plasma)	
	MIROS cohort (12–18 weeks) n = 65	CHUL cohort (38–41 weeks) n = 21
Class III		
8-iso-15(R)-PGF _{2α}	110 [74, 183] ^a	173 [143, 760] ^{a,b}
8-iso-PGF _{2α}	131 [0, 209] ^a	184 [149, 537] ^a
15(R)-PGF _{2α}	128 [107, 142] ^a	322 [250, 1724] ^{a,b}
Class IV		
iPF _{2α} -IV	131 [66, 180] ^a	132 [98, 957] ^a
Class VI		
iPF _{2α} -VI	n/a	201 [165, 1942] ^a
5-iPF _{2α} -VI	n/a	168 [120, 2797] ^{a,b}
iPF _{2α} -VI + 5-iPF _{2α} -VI	154 [124, 182] ^b	375 [287, 4619] ^b
(±)5-8,12-iso-iPF _{2α} -VI	n/a	766 [480, 2440] ^b

^I Values are medians and quartiles [Q1, Q3]. The data were not normally distributed. Medians with different superscript letters (a–e) within a cohort are statistically different (Kruskal-Wallis ANOVA followed by Dunn's multicomparison test, $p < 0.05$).

n/a = non available

TABLE 2

Fatty acids levels of interest in the plasma of pregnant women.

	Fatty acids levels ¹			
	MIROS cohort (12–18 weeks) n = 65		CHUL cohort (38–41 weeks) n = 21	
	(µg/mL plasma)	(% of total ²)	(µg/mL plasma)	(% of total ²)
6 <i>t</i> -18:1	0 ^a	0	4.2 ± 2.3 ^{a,b,e}	0.24
9 <i>t</i> -18:1	5.3 ± 2.3 ^{b,e}	0.38	2.1 ± 1.0 ^{a,e}	0.12
11 <i>t</i> -18:1	7.5 ± 2.8 ^{b,d}	0.53	5.2 ± 1.5 ^{b,f,e}	0.29
9 <i>t</i> ,12 <i>t</i> -18:2	0.2 ± 0.6 ^a	0.01	0.1 ± 0.2 ^b	<0.01
9 <i>c</i> ,12 <i>t</i> -18:2	0.1 ± 0.4 ^a	<0.01	0.1 ± 0.3 ^b	<0.01
9 <i>t</i> ,12 <i>c</i> -18:2	1.4 ± 1.3 ^c	0.10	1.6 ± 0.8 ^{a,e}	0.09
Total <i>trans</i> fatty acids³	14.6 ± 5.4	1.03	12.5 ± 5.6	0.70
9 <i>c</i> -16:1	9.2 ± 3.5 ^d	0.65	11.7 ± 4.8 ^{b,d,g}	0.65
6 <i>c</i> -18:1 + 7 <i>c</i> -18:1	4.3 ± 1.2 ^e	0.30	4.1 ± 1.3 ^{b,e}	0.23
9 <i>c</i> -18:1	118.4 ± 24.9 ^{f,g}	8.37	167.5 ± 23.5 ^{c,d}	9.37
11 <i>c</i> -18:1	21.0 ± 3.8 ^f	1.48	23.9 ± 4.1 ^{c,f,g}	1.34
9 <i>c</i> ,12 <i>c</i> -18:2	273.9 ± 48.5 ^g	19.37	351.7 ± 64.2 ^c	19.68
Total <i>cis</i> fatty acids⁴	768.5 ± 114.5	54.35	949.2 ± 118.9	53.12
UI	143.9 ± 5.8		136.5 ± 6.0	
PI	115.6 ± 9.6		107.3 ± 9.1	

¹ Values are means ± SD.² from the total of all fatty acids measured by the method.Means for fatty acids with different superscript letters (a–h) are statistically different (Kruskal-Wallis ANOVA followed by Dunn's multicomparison test, $p < 0.05$).³ Total *trans* fatty acids = 9*t*-14:1 + 6*t*-18:1 + 9*t*-18:1 + 11*t*-18:1 + 9*t*,12*t*-18:2 + 9*t*,12*c*-18:2 + 9*c*,12*t*-18:2 + 9*t*,12*t*,15*t*-18:3⁴ Total *cis* fatty acids = 9*c*-14:1 + 9*c*-16:1 + (7*c*-18:1 + 6*c*-18:1; coelution) + 9*c*-18:1 + 11*c*-18:1 + 12*c*-18:1 + 13*c*-18:1 + 9*c*,12*c*-18:2 + 6*c*,9*c*,12*c*-18:3 + 9*c*,12*c*,15*c*-18:3 + 8*c*-20:1 + 11*c*-20:1 + 6*c*,9*c*,12*c*,15*c*-18:4 + 11*c*,14*c*-20:2 + 8*c*,11*c*,14*c*-20:3 + 11*c*,14*c*,17*c*-20:3 + 5*c*,8*c*,11*c*,14*c*-20:4 + 13*c*-22:1 + 8*c*,11*c*,14*c*,17*c*-20:4 + 13*c*,16*c*-22:2 + 5*c*,8*c*,11*c*,14*c*,17*c*-20:5 + 13*c*,16*c*,19*c*-22:3 + 15*c*-24:1 + 7*c*,10*c*,13*c*,16*c*-22:4 + 4*c*,7*c*,10*c*,13*c*,16*c*-22:5 + 7*c*,10*c*,13*c*,16*c*,19*c*-22:5 + 4*c*,7*c*,10*c*,13*c*,16*c*,19*c*-22:6.

Unsaturation index (UI) = (%Monoenoic × 1) + (%Dienoic × 2) + (%Trienoic × 3) + (%Tetraenoic × 4) + (%Pentaenoic × 5) + (%Hexaenoic × 6).

Peroxidation index (PI) = (%Monoenoic × 0.025) + (%Dienoic × 1) + (%Trienoic × 2) + (%Tetraenoic × 4) + (%Pentaenoic × 6) + (%Hexaenoic × 8).

TABLE 3

Correlations between F₂-isoprostanes and *trans* fatty acids in the plasma of first trimester pregnant women using the MIROS cohort.

	11<i>t</i>-18:1	9<i>t</i>-18:1	9<i>t</i>,12<i>c</i>-18:2
8-iso-15(R)-PGF _{2α}	$r = 0.182$ ($p = 0.146$)	$r = 0.238$ ($p = 0.056$)	$r = 0.203$ ($p = 0.104$)
8-iso-PGF _{2α}	$r = 0.081$ ($p = 0.522$)	$r = 0.320$ ($p = 0.009$ *)	$r = 0.241$ ($p = 0.053$)
15(R)-PGF _{2α}	$r = 0.166$ ($p = 0.186$)	$r = 0.242$ ($p = 0.052$)	$r = 0.214$ ($p = 0.087$)
iPF _{2α} -IV	$r = 0.153$ ($p = 0.224$)	$r = 0.225$ ($p = 0.071$)	$r = 0.165$ ($p = 0.189$)
iPF _{2α} -VI + 5-iPF _{2α} -VI	$r = 0.155$ ($p = 0.218$)	$r = 0.340$ ($p = 0.006$ *)	$r = 0.438$ ($p = 0.0003$ *)

Values (r) are Spearman correlation coefficients (n=65).

* $p < 0.05$ is considered significant.

TABLE 4

Correlations between F₂-isoprostanes and *trans* fatty acids in the plasma of third trimester pregnant women using the CHUL cohort.

	11 <i>t</i> -18:1	6 <i>t</i> -18:1	9 <i>t</i> -18:1	9 <i>t</i> ,12 <i>c</i> -18:2
8-iso-15(R)-PGF _{2α}	$r = 0.166$ ($p = 0.540$)	$r = 0.291$ ($p = 0.225$)	$r = 0.131$ ($p = 0.185$)	$r = 0.356$ ($p = 0.077$)
8-iso-PGF _{2α}	$r = 0.230$ ($p = 0.345$)	$r = \mathbf{0.471}$ ($p = \mathbf{0.038}^*$)	$r = 0.229$ ($p = 0.103$)	$r = 0.347$ ($p = 0.069$)
15(R)-PGF _{2α}	$r = 0.110$ ($p = 0.461$)	$r = 0.377$ ($p = 0.076$)	$r = 0.075$ ($p = 0.217$)	$r = \mathbf{0.506}$ ($p = \mathbf{0.006}^*$)
iPF _{2α} -IV	$r = 0.204$ ($p = 0.430$)	$r = 0.382$ ($p = 0.109$)	$r = 0.214$ ($p = 0.108$)	$r = \mathbf{0.421}$ ($p = \mathbf{0.045}^*$)
iPF _{2α} -VI	$r = 0.151$ ($p = 0.394$)	$r = 0.317$ ($p = 0.125$)	$r = 0.042$ ($p = 0.298$)	$r = \mathbf{0.391}$ ($p = \mathbf{0.032}^*$)
5-iPF _{2α} -VI	$r = 0.266$ ($p = 0.203$)	$r = 0.412$ ($p = 0.058$)	$r = \mathbf{0.306}$ ($p = \mathbf{0.036}^*$)	$r = 0.372$ ($p = 0.059$)
iPF _{2α} -VI + 5-iPF _{2α} -VI	$r = 0.252$ ($p = 0.192$)	$r = 0.339$ ($p = 0.088$)	$r = 0.185$ ($p = 0.081$)	$r = \mathbf{0.440}$ ($p = \mathbf{0.020}^*$)
(±)5-8,12-iso-iPF _{2α} -VI	$r = 0.317$ ($p = 0.173$)	$r = 0.458$ ($p = 0.054$)	$r = \mathbf{0.351}$ ($p = \mathbf{0.027}^*$)	$r = 0.381$ ($p = 0.075$)

Values (r) are Spearman correlation coefficients (n=21).

* $p < 0.05$ is considered significant.