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Effects of free fatty acids found increased in women who develop pre-eclampsia on the ability of endothelial cells to produce prostacyclin, cGMP and inhibit platelet aggregation

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Recently, we showed that levels of circulating free fatty acids are increased in women who later develop pre-eclampsia long before the clinical onset of the disease. Among the serum free fatty acids, oleic-, linoleic-, and palmitic acid were found to be increased by 37, 25 and 25%, respectively. In the present study we asked if these free fatty acids can interfere with endothelial cell functions.

Cultured endothelial cells were exposed to linoleic-, oleic- and palmitic acid in concentrations ranging from 0.016 to 0.133 μmol ml^{-1}, resulting in molar ratios of free fatty acids to albumin of 0.2–1.6.

We found that among these fatty acids, linoleic acid reduced the thrombin-stimulated prostacyclin release by 30–60%, oleic acid by 10–30%, whereas palmitic acid had no effect. Endothelial cells incubated in presence of linoleic acid showed a concentration-dependent reduction in prostacyclin release in response to thrombin, and cells incubated with linoleic acid for up to 28 h, showed a reduced thrombin-induced prostacyclin release at every time point. Endothelial level of cGMP mainly reflected the synthesis of endothelium-derived relaxing factor/nitrogen monoxide (EDRF/NO), since blocking of the endogenous production of EDRF/NO with N-omega-nitro-L-arginine, resulted in about 90% reduction in cGMP-content of the endothelial cells. Incubation with linoleic acid reduced the endothelial cGMP level by 70%. Linoleic acid reduced the endothelial cells ability to inhibit platelet aggregation by 10–45%, ($p = 0.0019$).

It was concluded that linoleic acid impedes the ability of the endothelial cells to produce prostacyclin and cGMP, and to inhibit platelet aggregation.

Key words: endothelium; endothelium-derived relaxing factor; free fatty acids; linoleic acid; platelet aggregation; pre-eclampsia; prostacyclin

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Disturbance of the vascular endothelium seems to play a role in the pathogenesis of many diseases, like atherosclerosis, diabetes and pre-eclampsia [1–3]. In pre-eclampsia there are several indications that altered endothelial functions is a central pathophysiological event [3]. These include activated haemostasis, altered response to vasoactive substances, decreased prostacyclin synthesis, and increased level of plasma fibronectin [4–6].

Recently, we provided evidence that alterations in the circulating lipids may be involved in inducing disturbance of the endothelial cells [7, 8]. More specifically, we have shown that sera of women with pre-eclampsia induce accumulation of triglycerides in cultured endothelial cells, with a concomitant reduction in prostacyclin release [8]. The endothelial triglyceride accumulation is due to an increased content of free fatty acids (FFA) in pre-eclamptic sera, resulting in enhanced uptake and intracellular re-esterification of FFA by cultured endothelial cells [7]. In addition, sera of pre-eclamptic women contain an elevated lysophospholipase activity, which further provides FFA for the cells during the incubations [9]. We have suggested that the elevated level of FFA may interfere with the endothelial cell functions during development of pre-eclampsia [7, 10]. A possible role of FFA as a pathogenetic factor is further supported by our finding that the total serum FFA level is increased 15–20 weeks before the women show clinical signs of pre-eclampsia [10]. Linoleic- (18:2n-6), oleic-(18:1n-9) and palmitic acid (16:0) were increased by 25, 37 and 25%, respectively, early in pregnancy in women who later developed pre-eclampsia, compared to women with uneventful pregnancies (B. Lorentzen *et al.*, unpublished observations). Given these observations, we wanted to investigate the effect of these three fatty acids on cultured endothelial cells. We have chosen three different physiological properties of endothelial cells which are assumed to play a role in the pathophysiology of pre-eclampsia: Endothelial prostacyclin release, endothelial EDRF/NO-production, and the ability of endothelial cells to inhibit platelet aggregation.

MATERIALS AND METHODS

Materials

1(3)^{3}H-glycerol, 99,9 Gbq mmol^{-1} was obtained from Amersham (Arlington Heights, IL, USA). Tissue culture equipment was supplied by Costar (Cambridge, MA, USA) and Falcon (NJ, USA). Ham's F-10 medium, bovine calf serum, horse serum and penicillin-streptomycn solution were purchased from Gibco (Grand Island, NY). Linoleic-(18:2n-6), oleic-(18:1n-9), palmitic acid (16:0), thrombin, bradykinin, N-omega-nitro-L-arginine and platelet-activating factor were obtained from Sigma Chemical Company (St. Louis, MO, USA). The fatty acids were stored at 4 °C under an atmosphere of 100% nitrogen. All organic solutions were of reagent grade. Hepes buffer containing mmol l^{-1} Ca^{2+} and 3 mmol l^{-1} Hepes, pH 7.4. Tris-buffer was phosphate buffered saline without Ca^{2+} containing 23.8 mmol l^{-1} Tris and 9.6 mmol l^{-1} glucose, pH 7.4.

Blood samples

Blood samples were drawn from women with normal pregnancies after 8–10 h of fasting. Informed consent was obtained from each woman. The blood was allowed to coagulate for 90–120 min at room temperature. Sera were then obtained by centrifugation at 800 *g* for 10 min and immediately placed at −70 °C. These sera were the control sera (C-sera).

Cultures of endothelial cells

Endothelial cells were isolated from the veins of umbilical cords obtained from women with normal pregnancies. The cells were seeded in 2 or 10 cm^2 wells in 1 or 2 ml, respectively, of culture medium consisting of Ham's F-10 medium supplemented with 2.5% (v/v) bovine calf serum, 7.5% (v/v) horse serum, penicillin (100 IU mol^{-1}), streptomycin (100 μg ml^{-1}), and amphotericin (2.5 μg ml^{-1}). This medium will be referred to as medium A. For each experiment primary cultures were obtained from a new umbilical cord, and the cells were used 2–4 days after seeding. Then, medium A was removed, and the cells were washed twice in serum-free Ham's F-10. Thereafter, the cells received Ham's F-10 medium supplemented with 20% (v/v) C-sera with or without additional fatty acid. This medium will be referred to as medium B. Addition of free fatty acids to C-sera was performed as previously described [7]. Briefly, fatty acids were neutralized by

adding equimolar amounts of 0.1 mol l^{-1} NaOH. Then small aliquots of fatty acids were added to heat inactivated C-sera (56 °C for 1 h). The final concentration of added FFA in the medium ranged between 0.016 and 0.133 μmol ml^{-1}, as specified in legends to figures. This resulted in molar ratios of free fatty acids/albumin between 0.2 and 1.6.

Cell counting in a Coulter counter

At the end of each experiment medium B was removed, the cells were washed once with Ham's F-10 serum-free medium. Trypsin-EDTA (100 μl, trypsin 0.25% and EDTA 0.02%) was then added to each well and after 3–4 min all cells were detached. Medium A (100 μl) was added to inactivate the enzyme. Of this cell suspension 200 μl was mixed with 8 ml Diluid 'Azide free' (J. T. Baker, Deventer, The Netherlands) and Lyzerglobin (J. T. Baker), and after 2–3 min the number of cells was determined by counting the nuclei in a Coulter counter.

Trypan blue exlusion

At the end of an experiment the medium B was removed and the cells were washed once with Ham's F-10 serum-free medium. Trypsin-EDTA (100 μl) was then added to each well, and after detachment 100 μl medium A was added. From this cell suspension a volume of 100 μl was mixed with 200 μl trypan blue (0.05% in 0.15 mol l^{-1} NaCl). After 5 min at room temperature, the percentage of Trypan blue positive cells were determined in a haematocytometer.

Radioimmunoassay

Determination of 6 keto-$PGF_{1\alpha}$ and cGMP by radioimmunoassay (RIA) were performed according to the procedure accompanying the kits from Amersham (Arlington Heights IL, USA), codes no. RPA 515 and RPA 525.

EXPERIMENTAL DESIGN

Cytotoxicity study

Endothelial cells were exposed to medium B with or without linoleic-, oleic- or palmitic acid. After 2 days medium B was removed, the cells washed twice and then either counted in a Coulter counter for total cell number, or in a haemocytometer after incubation with Trypan blue to determine the percentage of Trypan blue positive cells.

Prostacyclin release study

Endothelial cells were preincubated in medium B with the various fatty acids at concentrations and times indicated in legends to figures. After the preincubation the medium was removed, the cells were washed carefully twice with 1 ml of prewarmed (37 °C) Hepes buffer. The cultures then received 0.7 ml prewarmed (37 °C) Hepes buffer, with or without thrombin (3 NIH ml^{-1}, final concentration). After 15 min at 37 °C, the cell tray was placed on ice and the supernatants were immediately transferred to tubes. After 10 min at room temperature the tubes were placed at −20 °C until RIA of 6-keto-$PGF_{1\alpha}$ was performed.

Measurement of tritiated glycerol incorporation into cellular triglycerides after incubation with linoleic acid

Endothelial cells were preincubated in medium B with or without additional linoleic acid in various concentrations as indicated in legends to figures. Six hours prior to the end of the experiment 10 μCi ml^{-1} tritiated glycerol and 0.1 μmol ml^{-1} unlabelled glycerol (both final concentrations) were added in a volume of 50 μl per well with 1 ml medium B with or without linoleic acid. At the end of the experiment, the supernatant was removed and the cells were washed four times with Hepes-buffer. Cellular lipids were extracted *in situ* with hexane (1 ml per well for 2 h). Previously, we have shown that 85% of hexane extractable lipids were found in the triglyceride fraction [7]. The radioactivity was measured in a Rackßeta 1217 (LKB, Pharmacia, Oslo).

EDRF/NO-production study

Preincubation of endothelial cells with N-omega-nitro-L-arginine and cGMP production. Endothelial cells were seeded in 6-well trays as described above. After 2–3 days the cultures had reached confluence and the experiment started. Then medium A was removed and 1 ml N-omega-nitro-L-arginine in Hepes (10^{-4}

$mol\,l^{-1}$, final concentration), or only Hepes as control was added to each well. After 30 min incubation at 37 °C, the medium was removed. The cells were then incubated in presence of bradykinin in Hepes ($10^{-6}\,mol\,l^{-1}$, final concentration) for 40 s, or acetate ($10^{-6}\,mol\,l^{-1}$) as control. The experiment were ended by rapidly removing the Hepes buffer followed by immediate addition of 1 ml 0.1 $mol\,l^{-1}$ HCl per well to extract the cellular cGMP. After 30 min the HCl-extracts were transferred to tubes and stored in −20 °C until RIA of cGMP was performed.

Preincubation of endothelial cells with linoleic acid and cGMP-production. Endothelial cells were seeded in 6-well trays as described above. After 2–3 days the cultures had reached confluence and the experiments started. Then medium A was removed and the cells received medium B with or without linoleic acid. Final concentration of added linoleic acid was 0.08 $\mu mol\,ml^{-1}$. The linoleic acid-containing medium was prepared as described above. After 24 h incubation at 37 °C, the medium was removed and the cellular cGMP content with or without prior bradykinin-stimulation was determined as described above.

Aggregation study

Preincubation of endothelial cells with linoleic acid. Endothelial cells were incubated with 20% C-sera with or without linoleic acid (0.133 $\mu mol\,ml^{-1}$ serum) for 2 days. Then the medium was renewed and the incubation continued for another day. After this preincubation the cells were stimulated to prostacyclin release as follows: the preincubation medium was removed, the cells washed once with Hepes buffer (2 ml per well) and once with Tris-buffer without Ca^{2+} (1 ml per well). The cells were then incubated in Tris-buffer (0.5 ml per well) with either 10 μl bradykinin ($10^{-6}\,mmol\,l^{-1}$, final concentration) or 10 μl acetate as control ($10^{-6}\,mmol\,l^{-1}$, final concentration). After 5 min at 37 °C, the cell tray was placed on ice and the supernatant was instantly transferred to polypropylene tubes kept in isopentan/dry-ice, resulting in immediate freezing of the samples. The supernatants were kept at −70 °C until used in the aggregation studies.

Preparation of platelets. Platelets were prepared from citrate plasma within 2 h before they were used. The procedure was as follows: platelet-rich plasma (PRP) was prepared by centrifugation at 250 *g* for 1·0 min. After removing the platelet-rich supernatant, platelet-poor plasma was prepared by another centrifugation at 1700 *g*. To standardize the procedure the same platelet donor (healthy, female) was used throughout the study. For the aggregation studies the platelet count in PRP was adjusted to $3 \times 10^{11}\,ml^{-1}$ using autologous platelet-poor plasma.

Platelet activation. Aliquots of PRP (300 μl) were placed in a glass aggregometer cuvette with a metal stirrer bar and prewarmed to 37 °C. The cuvette was then transferred to an aggregometer (Chrono-Log, Dual Channel, Chrono-Log Corp., Plesner Farmasøytiske, Norway) and the content were stirred at 300 rpm. Platelet aggregation was induced by adding platelet-activating factor (PAF) in 50 μl Tris-buffer, final concentration 0.125 $\mu mol\,l^{-1}$. The aggregation was recorded as changes in light absorbance. When the effects of endothelial supernatants were investigated, a sample of 100 μl endothelial supernatant was added to the cuvette 24 s before the PAF-agonist. One hundred microlitres of Tris-buffer was used as control.

Care was taken to keep the endothelial supernatants frozen on ice and to thaw each sample immediately before transferring the 100 μl to the cuvette. Initial studies demonstrated that all inhibitory activity of the endothelial supernatant disappeared within 5 min after thawing.

Calculation of inhibition of platelet aggregation was performed as follows: the optical density 2 min after addition of PAF was considered as full aggregation (0% inhibition). The optical density after 2 min in absence of aggregation was set to 100% inhibition. Difference in inhibiting capacity was calculated as difference in per cent inhibition.

RESULTS

Fatty acids are potentially cytotoxic. Therefore, we incubated the endothelial cells with 18:1n-9, 18:2n-6 and 16:0 to test if cell growth was affected. We found no significant difference in cell number between cultures exposed to the various fatty acids (Table I). In addition, more

TABLE I. Effect of linoleic-, oleic- and palmitic acid on growth of endothelial cells. The cells were incubated for 2 days in presence of the various fatty acids at final concentrations of 0.133 μmol ml^{-1}. Control incubations were without fatty acids (C). Values are the mean ±SD of triplicates.

	Increase in cell number ± SD during the incubation (%)
C	45 ± 10
Oleic acid	38 ± 16
Linoleic acid	55 ± 5
Palmitic acid	46 ± 9

than 95% of the endothelial cells excluded Trypan blue, irrespective of whether they were exposed to fatty acids or not (data not shown).

Endothelial release of prostacyclin was investigated after exposure of the cells to 16:0, 18:1n-9 and 18:2n-6. Linoleic acid reduced the thrombin-induced prostacyclin release by 30–60% ($p < 0.05$), oleic acid by 10–30% ($p < 0.05$), whereas palmitic acid was without effect (Fig. 1). The basal release of prostacyclin, however, seems to be unaffected by these fatty acids (Fig. 1). Since 18:2n-6 had the most

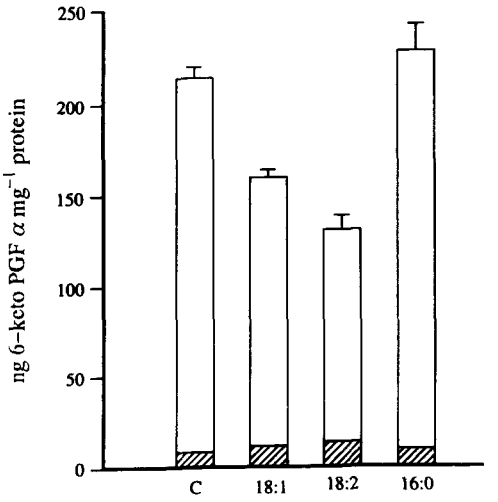

FIG 1. Endothelial release of prostacyclin after exposure to oleic- linoleic- and palmitic acid for 3 days. The final concentration of the various fatty acids in the incubation medium was 0.133 μmol ml^{-1}. For prostacyclin release, endothelial cells were stimulated with 3 NIH ml^{-1} thrombin (final concentration). Open bars show the stimulated values (mean of triplicates ± SD), whereas the basal values are indicated as hatched parts of the bars.

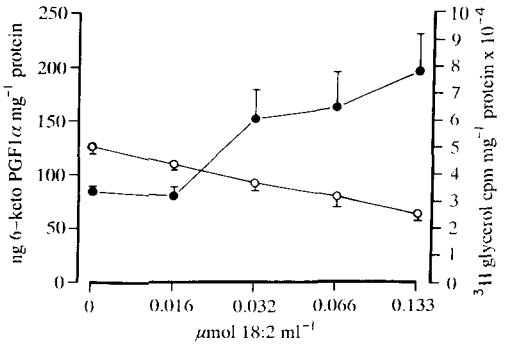

FIG 2. Endothelial, thrombin-induced (3 NIH ml^{-1}) release of prostacyclin (○) and triglyceride synthesis (●) following 24 h incubation with various concentrations of linoleic acid. Values are the mean of triplicates ± SD.

marked effect, we decided to investigate in more detail the effect of this fatty acid on prostacyclin release, triglyceride synthesis, cGMP-production, and platelet aggregation.

The prostacyclin release decreased linearly as the linoleic acid-concentration increased (Fig. 2). The reduction in prostacyclin release became significant at a 18:2n-6-concentration of 0.032 μmol ml^{-1} ($p < 0.05$). At this 18:2n-6-level, there was a concomitant and significant increase ($p < 0.05$) in the endothelial triglyceride synthesis (Fig. 2). In addition, endothelial cells incubated with 18:2n-6 for up to 28 h showed reduced thrombin-induced prostacyclin release at every time point, compared to controls (Fig. 3).

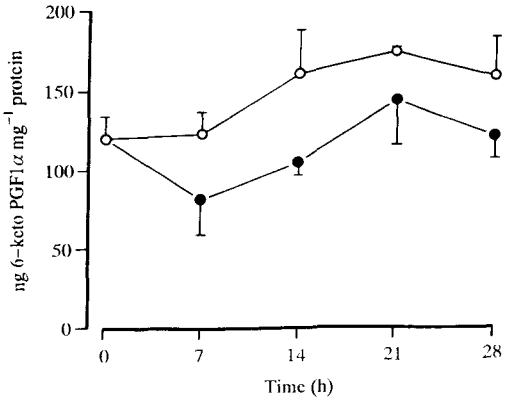

FIG 3. Endothelial thrombin-stimulated (3 NIH ml^{-1}) release of prostacyclin after incubations with (●) or without (○) linoleic acid (0.08 μmol ml^{-1}, final concentration) for the times indicated. Values represent the mean of triplicates ± SD.

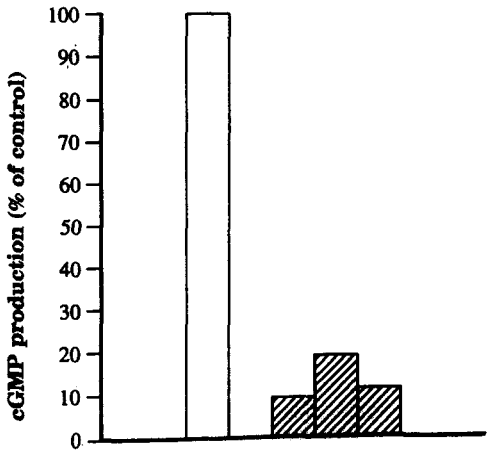

FIG 4. Non-stimulated endothelial cGMP-production following preincubation with N-omega-nitro-L-arginine (10^{-4} mol l^{-1}, final concentration). After 30 min preincubation, cellular cGMP was extracted by 0.1 mol l^{-1} HCL for 30 min. The open bar represents the controls, whereas the three hatched bars show the cGMP-level after preincubation with N-omega-nitro-L-arginine in three individual experiments.

Cultured endothelial cells had a basal (no specific agonist added) production of cGMP. The level of cGMP in the endothelial cells can be used as an indicator of the EDRF/NO synthesis. This is demonstrated in Fig. 4 where the endothelial EDRF/NO-production is blocked by N-omega-nitro-L-arginine, resulting in close to 90% reduction in cGMP-production.

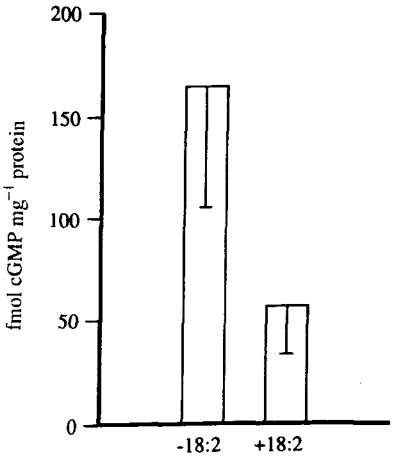

FIG 5. Non-stimulated endothelial cGMP-production following exposure to linoleic acid (0.08 μmol ml^{-1}, final concentration) for 24 h. The values are the mean ±SD of four individual experiments, ($p < 0.05$).

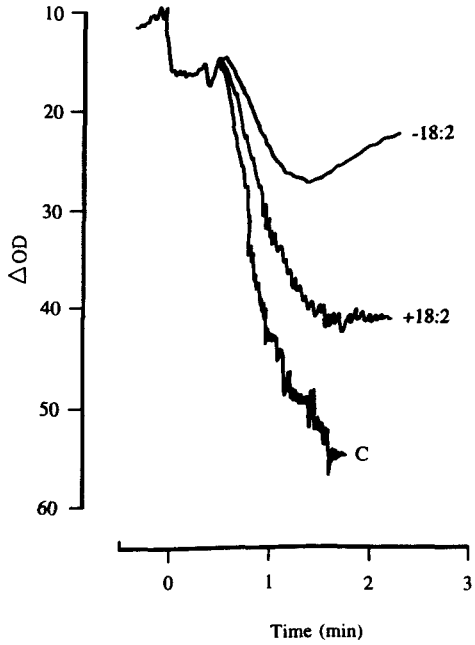

FIG 6. Effect of linoleic acid on the ability of endothelial cells to inhibit platelet aggregation. Endothelial cells were incubated for 3 days with or without additional linoleic acid (0.133 μmol ml^{-1}, final concentration). The endothelial cells were stimulated with bradykinin or acetate as control, both 10^{-6} mol l^{-1}, final concentrations, for 5 min, as described in the text. Platelet aggregation was tested in an aggregometer, as changes in light absorbance. Platelet activating factor (PAF) (0.25 μmol l^{-1}, final concentration), was used for inducing platelet aggregation. When the endothelial supernatants were tested, 100 μl was added to the cuvette 24 s before the PAF-agonist. Control was 100 μl Tris-buffer (= C). The Figure shows one typical experiment out of seven. In bradykinin-stimulated endothelial cells, linoleic acid significantly ($p = 0.0019$) reduced their ability to inhibit platelet aggregation by 10–45% (range of differences between the pairs with or without linoleic acid, $p = 0.0019$). In non-stimulated endothelial cells there was no significant difference.

Endothelial cells exposed to linoleic acid showed a 70% decrease in basal cGMP-production (Fig. 5). In bradykinin-stimulated endothelial cells (resulting in two to four-fold increase in cGMP) there was a 20% non-significant reduction in cGMP content following exposure to 18:2n-6 (data not shown).

Since endothelial cells incubated with 18:2n-6 showed reduced prostacyclin release and EDRF/NO-production, we tested whether exposure of endothelial cells to 18:2n-6 altered their ability

to inhibit platelet aggregation. In bradykinin-stimulated endothelial cells, 18:2n-6 reduced the capacity of endothelial cells to inhibit platelet aggregation by 10–45%, ($p = 0.0019$) (Fig. 6), whereas in non-stimulated endothelial cells the effect of linoleic acid was not significant.

DISCUSSION

The present study demonstrates that one of the free fatty acids increased in the circulation of women with pre-eclampsia, can affect properties of cultured endothelial cells. Fatty acids are potentially cytotoxic to cultured endothelial cells [12]. Therefore, we first adressed the question of whether observations made in this study could be explained by a non-specific cytotoxic effect of the fatty acids. A good criterion of cytotoxicity is inhibited cell growth. The proliferation of the endothelial cells in the present study was not affected by any of the fatty acids investigated. In addition, there was no increase in the number of Trypan blue positive cells following exposure to the fatty acids. Therefore, we do not attribute the present findings to an unspecific cytotoxic effect of the fatty acids.

It has been reported previously that thrombin may be cytotoxic to cultured endothelial cells [12]. However, we found no indications of endothelial damage at the thrombin concentration used in this study. In the previous studies of thrombin induced 'injury' to cultured endothelial cells, serum was present during the incubations [12]. We exposed the cells to thrombin in a protein-free buffer solution. No 'contractions' of the endothelial cells were observed. Thus, the reported injurious effect of thrombin may be due to an effect dependent on the presence of serum proteins. Furthermore, untreated endothelial cells responded to thrombin by increasing their prostacyclin synthesis, compared to unstimulated cells. In contrast, when the cells were pretreated with linoleic acid they showed a reduced production of prostacyclin in response to thrombin. Thus, the effect of thrombin on the prostacyclin production is differentiated and therefore, cannot easily be explained as a cytotoxic effect.

Linoleic acid was subjected to a more detailed study in the present investigation for several reasons. First, linoleic acid (18:2n-6) was one of three fatty acids that was increased in women with pre-eclampsia [B. Lorentzen *et al.*, unpublished observations]. Second, among these fatty acids, 18:2n-6 had the most pronounced effect on prostacyclin release. Third, the metabolism of linoleic acid seems to be altered at an early stage in pregnancies of women who develop pre-eclampsia [B. Lorentzen *et al.*, unpublished observations]. Endothelial-derived prostacyclin and EDRF/NO are important mediators in the regulation of vasotonicity and haemostasis [13]. Since disturbance of these functions is central in the pathophysiology of pre-eclampsia, the effects of 18:2n-6 on the ability of endothelial cells to produce prostacyclin, EDRF/NO and to inhibit platelet aggregation were investigated. The endothelial cells have a basal production of both prostacyclin and EDRF/NO [14]. The present study provides evidence that 18:2n-6 influences prostacyclin and EDRF/NO production in different ways. In the prostacyclin studies 18:2n-6 affected mainly the stimulated release of prostacyclin. On the other hand, only the basal cGMP-production was significantly affected by 18:2n-6. *In vivo*, the basal as well as the stimulated release of vascular compounds like prostacyclin and EDRF/NO appears to be of physiological importance [13].

The observed effect of 18:2n-6 on prostacyclin release is in accordance with the previous report by Spector *et al.* [15]. They elaborate on the possible mechanism for the inhibitory effect of 18:2n-6 on the endothelial prostacyclin release, and suggest a competitive interaction between linoleic and arachidonic acid in the synthesis of prostacyclin. The competition could be at the level of membrane phospholipid fatty acid composition, because endothelial cells exposed to linoleic acid have increased content of linoleic acid in their membranes [15]. If linoleic acid rather than arachidonic acid is released upon activation of membrane phospholipase A_2, the prostacyclin precursor will not be formed. However, in the present study, 18:2n-6 inhibited mainly the stimulated prostacyclin release, i.e. when the cyclo-oxygenase expectedly was provided with increased amounts of substrate. This interpretation is in accordance those of Brox & Nordøy who found inhibitory effect of linoleic acid on arachidonic acid stimulated endothelial prostacyclin production [16]. If the competition hypothesis is correct, there must be two pools of arachidonic acid available for the prostacyclin synthesis. One of the pools is not influenced by linoleic acid and the other is. The former must

be the one that is used for the basal prostacyclin production.

The effect of linoleic acid on the endothelial cGMP-production, to our knowledge, has not been previously reported. We found evidence that the endothelial cGMP-production is mainly mediated by EDRF/NO, since a specific inhibitor of the endogenous EDRF/NO-production reduced the endothelial cGMP content by close to 90%. Thus, 18:2n-6 seems to interfere with a endothelial EDRF/NO-mediated signal system. The present work, however, is not conclusive with regard to the mechanism whereby 18:2n-6 inhibits the endothelial cGMP-production. Oxidized LDL, which contains peroxidized linoleic acid, may inactivate EDRF/NO released by endothelial cells [1, 17]. If oxidized derivatives of linoleic acid are formed within the endothelial cells, as has been reported [18], intracellular EDRF/NO could be inactivated.

The results of the platelet aggregation studies demonstrated that 18:2n-6 not only impaired the production of the platelet inhibiting compounds, prostacyclin and EDRF/NO, but also the actual ability of endothelial cells to inhibit platelet aggregation. The reduced capacity of the endothelial cells to inhibit platelet aggregation after exposure to 18:2n-6 is of special relevance in understanding the activated haemostasis in pre-eclampsia, where consumption of platelets is a predominant feature [4]. The effect of various fatty acids on platelet aggregation have previously been extensively studied by Brox & Nordøy [16]. These authors did not find any effect of 18:2n-6 on the ability of endothelial cells to inhibit platelet aggregation. Their studies differed from ours in terms of experimental procedure. First, they used fetal calf serum on human cells whereas we employed human serum. Second, the observed effect of 18:2n-6 on the endothelial ability to inhibit platelet aggregation in our study was seen mainly in the cells stimulated with bradykinin. In the work of Brox & Nordøy only unstimulated cells were used [16].

Linoleic acid is easily oxidized, both spontanously and enzymatically [19]. Even though linoleic acid is stored under anti-oxidizing conditions it may be oxidized during the time it takes to prepare linoleic acid for the use in the experiments. Therefore, the possibility that oxidized forms of linoleic acid may have been present in the culture medium in our work cannot be excluded. This question is relevant also for the reason that oxidized forms of linoleic acid, such as 9- and 13-hydroxy linoleic acid, also are biologically active [20]. More extensive studies are needed to clarify whether linoleic acid in its native form or as oxidized derivatives are the most active compounds at the cellular level.

We have previously hypothesized that alterations in the circulating lipids are important in inducing endothelial disturbance during development of pre-eclampsia. This hypothesis is given support by our recent finding that free fatty acids are increased 15–20 weeks before clinical onset of the disease [10]. The present study supports the possibility that one of these fatty acids (or derivatives thereoff) can interfere with endothelial properties that are assumed to be of major importance in the development of pre-eclampsia.

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