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Effects of pregnancy and delivery on the availability of plasma tryptophan to the brain: relationships to delivery-induced immune activation and early post-partum anxiety and depression

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ABSTRACT

Background. There is now evidence that the availability of plasma tryptophan is decreased during pregnancy and the puerperium and also in patients with major depression and inflammation. The aims of the present study were to examine: (*i*) the effects of pregnancy and delivery on plasma tryptophan and the amino acids known to compete for the same cerebral uptake mechanism (CAAs), valine, leucine, tyrosine, phenylalanine and isoleucine; (*ii*) the relationships between the availability of plasma tryptophan and postpartum depression or anxiety; and (*iii*) the relationships between the availability of plasma tryptophan to the brain and inflammatory markers, such as serum interleukin-6 (IL-6), interleukin-1 receptor-antagonist (IL-1RA) and the leukaemia inhibitory factor receptor (LIF-R).

Methods. The above variables were measured in 13 healthy non-pregnant and in 98 pregnant women 3 to 6 days before delivery and 1 and 3 days after delivery. On each occasion the parturient women completed the state version of Spielberger State–Trait Anxiety Inventory (STAI) and the Zung Depression Rating Scale (ZDS).

Results. Plasma tryptophan and the tryptophan/CAA ratio were significantly lower at the end of term and after delivery than in the plasma of non-pregnant, healthy women. The tryptophan/CAA ratio was significantly lower in the early puerperium than at the end of term. There were no significant relationships between the availability of plasma tryptophan and either post-partum depression or changes in the STAI or ZDS scores in the early puerperium. The changes in the tryptophan/CAA ratio from the end of term to the early puerperium were significantly and inversely related to serum IL-6, IL-1RA and LIF-R.

Conclusions. The results show that the reduction in the availability of plasma tryptophan from the end of term to the early puerperium is related to immune activation; and that the lowered availability of plasma tryptophan is not related either to depressive or anxiety symptoms in the early puerperium or to post-partum depression ensuing some months later.

INTRODUCTION

Major depression, post-partum blues and pregnancy may be accompanied by lowered plasma concentrations of tryptophan, the precursor of serotonin (5-HT). Plasma tryptophan is related to brain tryptophan and 5-HT contents (Moir & Eccleston, 1968). Total and free plasma tryptophan and the ratio of tryptophan to amino acids known to compete for the same cerebral uptake mechanism, i.e. the competing amino

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acids (CAA), valine, leucine, isoleucine, phenylalanine and tyrosine, determine the availability of tryptophan to the brain (Curzon & Knott, 1975; Pardridge, 1979; Curzon & Sarna, 1984; Fernstrom, 1984; Fernstrom *et al*. 1987; Smith *et al*. 1990.) One of the most consistently reported abnormal findings in the metabolism of 5-HT in major depression is the reduction in plasma tryptophan or the plasma tryptophan/CAA ratio (DeMyer *et al*. 1981; Maes *et al*. 1990; 1993*a*; 1996; Maes & Meltzer, 1995).

There are a few papers reporting that post-partum blues or depression may be accompanied by lowered plasma tryptophan (Handley *et al*. 1980; Gard *et al*. 1986). A significant inverse correlation between the plasma tryptophan/CAA ratio and post-partum depressive mood was reported (Maes *et al*. 1992*a*; Abou-Saleh *et al*. 1999). In healthy pregnant women, plasma tryptophan concentrations decrease with the duration of pregnancy and normalize in the puerperium (Schrocksnadel *et al*. 1996). Pregnant women show significantly lower tryptophan concentrations compared with non-pregnant controls (Fuchs *et al*. 1996).

It is thought that activation of the inflammatory response system (IRS) in major depression may, in part, explain the lowered availability of plasma tryptophan (Maes, 1999). Thus, major depression is accompanied by signs of IRS activation, such as an increased production of: (*a*) pro-inflammatory cytokines, e.g. interleukin-1 (IL-1), IL-6, interferon-γ (IFNγ) and tumour necrosis factor-α (TNFα) (Maes *et al*. 1993*b*, *c*, 1994; Seidel *et al*. 1995; Sluzewska *et al*. 1995, 1996*a*; Maes, 1999; Lanquillon *et al*. 2000); (*b*) an acute phase response (Joyce *et al*. 1992; Maes *et al*. 1992*b*; Seidel *et al*. 1995; Sluzewska *et al*. 1996*b*); and (*c*) increased production of neopterin (Duch *et al*. 1984; Dunbar *et al*. 1992; Maes *et al*. 1994); which is a product of cytokine-stimulated (e.g. IFNγ) monocytes and macrophages (Wachter *et al*. 1992). In depression, highly significant inverse relationships are found between plasma tryptophan and increased production of IL-6, positive acute phase proteins and neopterin (Maes *et al*. 1993*c*, 1994, 1996).

In the maternal immune system there is an increased potential rather than increased activity of peripheral blood mononuclear cells (PBMCs) pointing toward a non-reactive status in the IRS (MacLean *et al*. 1992). Serum IL-6 and the IL-1 receptor antagonist (IL-1RA), which is produced by activated monocytes/macrophages, are increased in pregnant women (Laham *et al*. 1993; Austgulen *et al*. 1994; Vassiliadis *et al*. 1998; Maes *et al*. 2000). The early puerpurium is characterized by an increased inflammatory capacity in the serum as compared with the antenatal period. For example, serum IL-6 and the IL-1RA are higher in the early puerperium than in the pre-partum period (De Jongh *et al*. 1998; Maes *et al*. 2000). In healthy pregnant women, there are significant inverse correlations between the decrease in plasma tryptophan and neopterin concentrations, suggesting that IRS activation is the cause of enhanced tryptophan degradation during pregnancy (Fuchs *et al*. 1996; Schrocksnadel *et al*. 1996). Parturients whose anxiety and depression ratings increase in the early puerperium have signs of IRS activation, such as increased serum IL-6 and IL-1RA and lower serum concentrations of the leukemia inhibitory factor receptor (LIF-R) (Maes *et al*. 2000). Serum concentration of the LIF-R may block the pro-inflammatory activities of LIF (Layton *et al*. 1992).

The aims of the present study are to examine: (*a*) serum tryptophan, the CAAs and the tryptophan/CAA ratio at the end of pregnancy and in the early puerperium compared with non-pregnant healthy women; (*b*) whether plasma tryptophan and the tryptophan/CAA ratio are significantly lower in puerperial women who develop post-partum depression or whose anxiety and depression ratings increase in the early puerperium; and (*c*) whether the alterations in plasma tryptophan and the tryptophan/CAA ratio at the end of term and in the early puerperium are significantly related to signs of IRS activation, as measured by serum IL-6, IL-1RA and LIF-R.

METHOD

Subjects

The participants were 13 non-pregnant, healthy females who were not using contraceptive drugs and 98 healthy females admitted to the hospital for delivery. The latter were consecutively admitted to the St Jan Hospital, ZOL, Campus St. Jan, Genk, Belgium. There was no selection based on previous miscarriages, parity or grav-

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ity. Pregnant women were excluded as follows: (*a*) had signs of infection before delivery, such as fever or leukocytosis; (*b*) had ruptured membranes for more than 12 hours; (*c*) went into labour prematurely (< 37 weeks); (*d*) had a secondary Caesarean section after labour; (*e*) had any medical disorders; (*f*) had a past or present axis-I psychiatric disorder, except depression, as assessed by means of DSM-IV criteria using the Semi-structured Interview for the DSM-III-R (SCID) (Spitzer *et al*. 1990); (*g*) were depressed antenatally as assessed by means of DSM-IV criteria using the SCID; and (*h*) had ever used major psychotropic drugs including antidepressants and antipsychotics.

The non-pregnant controls were females with a normal, regular menstrual cycles of 28–30 days. They were recruited by advertisement. Controls were excluded for the following criteria: (*a*) women who were not free of any drugs, including anticontraceptive drugs, for at least 6 months prior to blood sampling; (*b*) regular drinkers; (*c*) women who ever had been taking psychotropic drugs; and (*d*) women with a present, past and family history of mental disorders as assessed by means of the SCID.

Inclusion criteria for non-pregnant and pregnant females were: (*a*) medically healthy; (*b*) normal on physical examination, normal values for blood tests, such as SGOT, SGPT, γGT, haematocrit, serum electrolytes and renal tests (blood urea and creatinine); (*c*) free of any drugs known to interfere with immune or endocrine functions; and (*d*) free of chronic illnesses known to affect the immune system and of acute infectious or inflammatory reactions for at least 2 weeks prior to the study. The study protocol was approved by the Institutional Review Board of the ZOL, Genk, Belgium. All subjects gave written informed consent after the study design was fully explained.

Procedure

In the non-pregnant controls, fasting plasma for the assay of the amino acids was sampled at 08.00 h ($\pm$30 min) at four different time points during their menstrual cycle, i.e. days 7, 14, 21 and 28. The plasma amino acid values obtained at these four time points were averaged and the mean values were employed in the statistical analyses. In the pregnant women, fasting blood samples for the assays of amino acids and IRS variables were collected at 08.00 h ($\pm$30 min) 3 to 6 days before the anticipated date of delivery (baseline condition) and 1 and 3 days after delivery. Day 1 post-partum was timed such that at least 24 h evolved from delivery to blood sampling. Day 3 post-partum was determined 2 days later. The prepartum sample was obtained during the last visit at the Antenatal Clinic and the two post-partum blood samples were collected in the maternity hospital. Women completed, during each of these three sessions, the Spielberger State–Trait Anxiety Inventory (STAI) (Spielberger *et al*. 1987) and the Zung Depression Scale (ZDS) (Zung, 1995). The STAI, state version was employed to measure state anxiety, whereas the ZDS is one of the most commonly used self-rating scales to measure severity of depression. The parturients were divided into groups on the basis of changes in the STAI and ZDS scores from baseline (the prepartum) to the third day post-partum, as defined by the q75 values, i.e. the value whereby 25% and 75% of the subjects have higher *versus* lower values, respectively, of the residualized STAI or ZDS scores (computed by means of regression analyses with the post-partum scores 3 days after delivery as dependent variable and the prepartum scores as explanatory variable). Subjects with residualized STAI or ZDS scores greater or equal to the q75 values are called STAI and ZDS responders, respectively.

The three STAI and ZDS scores in the prepartum and post-partum conditions were complete in 98 (94·9%) and 89 (90·8%) of the 98 parturients, respectively. Within 6 to 10 months after delivery, women had a telephone interview by one of the authors, a resident in psychiatry trained in the DSM interview techniques. The diagnosis of post-partum depression was made according to DSM-IV criteria using the follow-up SCID interview. Although the criteria for the post-partum onset specifier in the DSM-IV includes that the onset of the episode occurs within 4 weeks post-partum, we decided to use 3 months as onset specifier, since a review of the literature shows that a considerable number of post-partum depressions may occur after the first month (Claes *et al*. 1997). Telephone interviews to assess the history of major depression according to DSM criteria are commonly used in epidemiological studies (Kendler & Prescott, 1999). The SCID interview to assess

post-partum depression was complete in 67 (75·2%) of the 98 parturients. Only four females refused to complete the diagnostic interviews. The number of missing values is caused by a large number of females who had moved after delivery or who did not give any information either on their (new) addresses or telephone numbers.

We have adjusted the results (see Statistics section) for the following maternal and labour variables: (*a*) type of delivery, i.e. normal vaginal delivery; vaginal delivery by means of a forceps or ventouse; and elective Caesarean section under epidural anaesthesia (indications for Caesarean section included cephalopelvic disproportion, placenta previa and breech presentation); (*b*) parity (nulliparae *versus* multiparae); (*c*) duration of pregnancy and labour; (*d*) labour induction with oxytocin i.v. and/or prostaglandin intravaginally (yes or no); (*e*) amniotomy (spontaneous *versus* induced); (*f*) breast feeding (yes or no); (*g*) post-partum complications, such as malleolar oedema and use of antibiotics for possible infection (yes or no); (*h*) type of analgesia, i.e. without epidural analgesia (with pentazocine 30 mg i.m.) and with epidural analgesia. All subjects undergoing vaginal delivery received oxytocin, at a rate determined by the obstetrician (Syntocinon, Sandoz, Switzerland, 10 u in 1000 ml dextrose 5%). All subjects received methylergometrine-maleate (Methergin, Sandoz) 0·25 mg i.v. or i.m. and oxytocin infusion after delivery of the placenta. Post-partum pain was treated with piritramide (Dipidolor, Janssen, Belgium) 15 to 20 mg i.m. in all subjects.

All blood specimens for the assay of plasma amino acids in patients and healthy volunteers were assayed in a single run with a single lot number of reagents and consumables employed by a single operator. The amino acids were assessed by means of HPLC as previously explained by us (Maes *et al.* 1996). The intra-assay coefficient of variation (CV) values obtained in our laboratory are: tryptophan 3·3%; tyrosine 3·8%; valine 3·0%; phenylalanine 3·2%; isoleucine 3·4%; and leucine 3·7%. The tryptophan/valine + leucine + isoleucine + tyrosine + phenylalanine (CAA) ratio was computed and multiplied by 100. The analytical intra-assay CV value for the L-TRP/CAA ratio was 4·4%. Serum IL-6, IL-1RA and LIF-R were quantified by means of ELISA methods (Eurogenetics, Tessenderlo, Belgium) based on appropriate and validated sets of monoclonal antibodies. All assays of IL-6, IL-IRA, and LIF-R were carried out at the same time, in one and the same run with a single lot number of reagents and consumables employed by a single operator. The intra-assay CV values $< 8\%$ for all assays.

Statistics

We used repeated measure (RM) design analyses of variance (ANOVAs) in order to examine: (*a*) the within-subject variability with the prepartum and two post-partum conditions as time factor; (*b*) the between-subject variability with effects of post-partum depression; STAI and ZDS responder status, and the maternal/labour variables; and (*c*) two way interactions between time × post-partum depression; time × STAI or ZDS responder status; and time × the maternal/labour variables. Results of RM design ANOVAs were corrected for sphericity. Tests on simple effects were carried out in order to explore significant main effects or significant interaction patterns. A simple effect is defined as the effect of one variable at one level of the other variable (Howell, 1982). *A priori* comparisons among treatment means were assessed with the Dunn test (Howell, 1982). Relationships between variables were checked by means of Pearson's product moment correlation coefficients or through multiple regression analyses. Group mean differences were assessed by means of ANOVA.

RESULTS

Effects of pregnancy and delivery

Table 1 shows the plasma concentrations of the amino acids and the sum of the CAAs and the tryptophan/CAA ratio in non-pregnant women and in pregnant women before and 1 and 3 days after delivery. There were no significant differences in age between non-pregnant women (mean = 27·3 ± 6·2 years) and pregnant women (mean = 27·8 ± 3·5 years) ($F = 0{\cdot}2$, df = 1/109, $P = 0{\cdot}7$). Table 2 shows that all plasma amino acids, the sum of the CAAs, as well as the tryptophan/CAA ratio were significantly different between non-pregnant women and pregnant women (before and 1 and 3 days after

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Table 1. *Measurements of the plasma amino acids in non-pregnant healthy women, and in women at the end of delivery and* 1 *and* 3 *days after delivery*

	Women		Post-delivery	
Amino acids	Non-pregnant	End of term	1 day	3 days
Tryptophan	72·1 (11·9)	47·3 (8·2)	52·1 (11·1)	60·2 (11·2)
Tyrosine	73·5 (13·2)	49·6 (11·6)	62·6 (17·9)	74·4 (18·5)
Phenylalanine	66·5 (7·3)	86·1 (16·8)	101·9 (24·9)	110·0 (22·9)
Valine	234·8 (33·9)	170·1 (31·7)	230·4 (54·2)	255·7 (47·4)
Leucine	137·2 (31·9)	113·1 (24·9)	154·1 (41·6)	167·7 (33·7)
Isoleucine	74·5 (13·9)	55·2 (11·7)	80·9 (22·8)	88·1 (18·9)
CAA	586·5 (87·7)	474·2 (87·1)	629·8 (144·6)	695·6 (126·6)
Tryptophan/CAA × 100	12·39 (1·70)	10·13 (1·60)	8·44 (1·59)	8·75 (1·57)

All results are shown as mean (S.D.).
All amino acids are expressed as μmol/l.
CAA, sum of the competing amino acids, i.e. tyrosine, phenylalanine, valine, leucine and isoleucine.

Table 2. *Results of ANOVAs comparing the plasma amino acids between non-pregnant healthy women* (*HW*) *and either pregnant women at the end of term* (*end*) *and parturients* 1 *and* 3 *days after delivery. The mean* (*S.D.*) *values of the amino acids are shown in Table* 1

				HW *v.* end		HW *v.* 1 day		HW *v.* 3 days	
Amino acids	*F**	df	*P*	*t*	*p*	*t*	*p*	*t*	*p*
Tryptophan	38·7	3/276	$< 10^{-5}$	8·22	$< 10^{-5}$	6·56	$< 10^{-5}$	3·92	0·0003
Tyrosine	38·9	3/276	$< 10^{-5}$	5·07	0·00002	2·29	0·02	0·2	0·8
Phenylalanine	29·5	3/276	$< 10^{-5}$	3·12	0·002	5·59	0·00001	6·86	$< 10^{-5}$
Valine	60·3	3/275	$< 10^{-5}$	4·92	0·00003	0·3	0·7	1·58	0·1
Leucine	43·8	3/275	$< 10^{-5}$	2·42	0·01	1·68	0·09	3·05	0·003
Isoleucine	56·5	3/275	$< 10^{-5}$	3·62	0·0006	1·18	0·2	2·53	0·01
CAA	55·7	3/275	$< 10^{-5}$	3·19	0·002	1·21	0·2	3·08	0·003
Tryptophan/CAA × 100	36·5	3/275	$< 10^{-5}$	4·78	0·00004	8·31	$< 10^{-5}$	7·63	$< 10^{-5}$

* All results of ANOVAs with the four study groups listed in Table 1 as study groups. Dunn's tests (at $P = 0{\cdot}0167$) were used to examine multiple comparisons among the treatment means, i.e. comparisons with the values in the non-pregnant women.
CAA, sum of the competing amino acids, i.e. tyrosine, phenylalanine, valine, leucine and isoleucine.

Table 3. *Results of repeated measure* (*RM*) *design ANOVAs comparing the plasma amino acids between the pregnant women at the end of term* (*end*) *and the parturients* 1 *and* 3 *days after delivery. The mean* (*S.D.*) *values of the amino acids are shown in Table* 1

				End *v.* 1 day		End *v.* 3 days		1 day *v.* 3 days	
Amino acids	*F**	df	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Tryptophan	51·7	2/156	$< 10^{-5}$	3·15	0·002	9·95	$< 10^{-5}$	6·8	$< 10^{-5}$
Tyrosine	54·8	2/156	$< 10^{-5}$	5·54	0·00001	10·47	$< 10^{-5}$	4·92	0·00003
Phenylalanine	31·1	2/156	$< 10^{-5}$	5·22	0·00002	7·72	$< 10^{-5}$	2·51	0·012
Valine	105·6	2/154	$< 10^{-5}$	10·24	$< 10^{-5}$	14·05	$< 10^{-5}$	3·81	0·0004
Leucine	77·7	2/154	$< 10^{-5}$	9·29	$< 10^{-5}$	11·85	$< 10^{-5}$	2·56	0·01
Isoleucine	86·6	2/154	$< 10^{-5}$	10	$< 10^{-5}$	12·41	$< 10^{-5}$	2·42	0·016
CAA	87·2	2/154	$< 10^{-5}$	9·27	$< 10^{-5}$	12·78	$< 10^{-5}$	3·51	0·0009
Tryptophan/CAA × 100	49·5	2/154	$< 10^{-5}$	9·56	$< 10^{-5}$	7·18	$< 10^{-5}$	2·37	0·018

* All results of RM design ANOVAs with the values at the end of term and one and three days after delivery as time factor. Dunn's tests (at $P = 0{\cdot}0167$) were used to examine multiple comparisons among the three time points.
CAA, sum of the competing amino acids, i.e. tyrosine, phenylalanine, valine, leucine and isoleucine.

delivery). Dunn's test (tested at $P = 0{\cdot}016$ after P correction) showed that: (*a*) plasma tryptophan was significantly lower in pregnant women before and 1 and 3 days after delivery than in non-pregnant women; (*b*) plasma tyrosine was significantly lower in pregnant women

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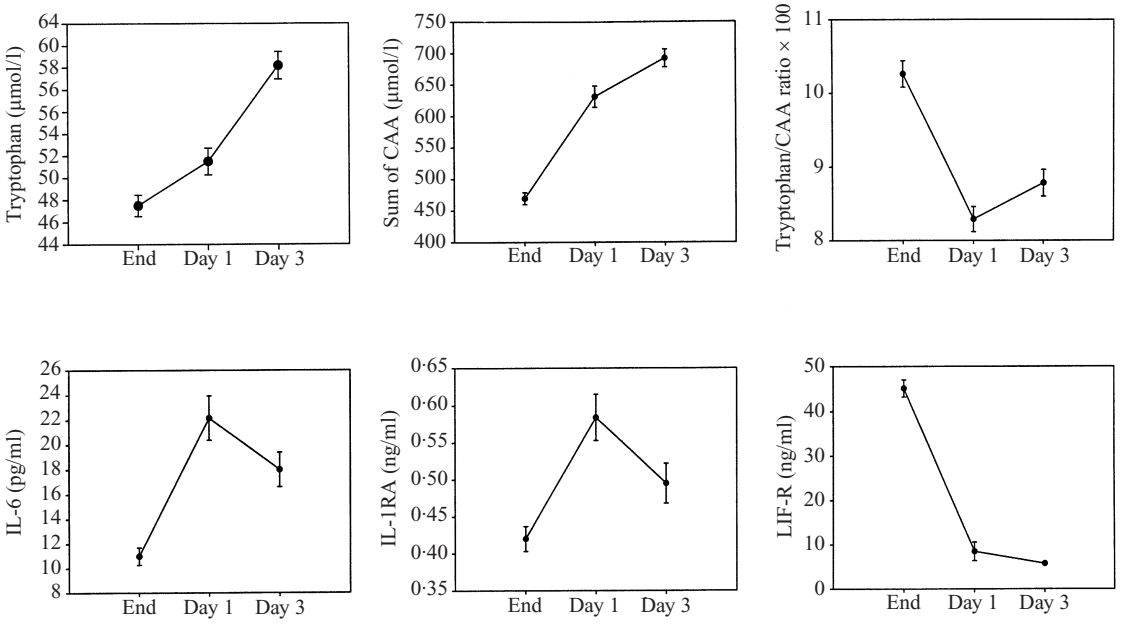

FIG. 1. Mean (S.E.M.) values of plasma tryptophan, the sum of the competing amino-acids (CAA), the tryptophan/CAA ratio, and the serum concentrations of interleukin-6 (IL-6), IL-1 receptor antagonist (IL-1RA) and leukemia inhibitory factor receptor (LIF-R) in pregnant females at the end of term (end) and 1 (day 1) and 3 (day 3) days after delivery.

at the end of term than in non-pregnant women; (*c*) plasma phenylalanine was significantly higher in pregnant women before and 1 and 3 days after delivery than in non-pregnant women; (*d*) plasma valine was significantly lower in pregnant women at the end of term than in non-pregnant women; (*e*) plasma leucine and isoleucine and the sum of the CAAs were significantly lower at the end of term and significantly higher 3 days after delivery than in the controls; and (*f*) the tryptophan/CAA ratio was significantly lower in pregnant women before delivery as well as 1 and 3 days after delivery than in non-pregnant women.

Table 3 shows the results of RM design ANOVAs with the plasma amino acids as dependent variables and with the prepartum and two post-partum conditions as time factor (within subject variability). RM design ANOVAs showed significant time effects on all amino acids. Dunn's tests (tested at $P = 0{\cdot}0167$ after P correction) showed that: (*a*) the post-partum plasma tryptophan and tyrosine values were significantly higher 1 and 3 days after delivery than at the end of term and significantly higher 3 days than 1 day after delivery; (*b*) plasma concentrations of phenylalanine, valine, leucine, and isoleucine and the sum of the CAAs significantly increased from the prepartum condition to the first and third day after delivery; and (*c*) the two post-partum tryptophan/CAA ratios were significantly lower that before delivery. Fig. 1 shows plasma tryptophan, the CAAs and the tryptophan/CAA ratio before and 1 and 3 days after delivery.

Associations of STAI and ZDS scores in the puerperium and post-partum depression at follow-up

There were 17 (25·4%) women who had suffered from post-partum depression at follow-up. By means of RM design ANOVAs no significant interaction patterns were found between time (pre-delivery and first and third day after delivery) and post-partum depression at follow-up (present or not according to DSM-IV criteria) for the plasma amino acids, the sum of the CAAs and the tryptophan/CAA ratio.

Fig. 2 shows the STAI and ZDS scores in STAI and ZDS responders and non-responders. There were 21 and 22 STAI and ZDS responders, respectively. There were no significant

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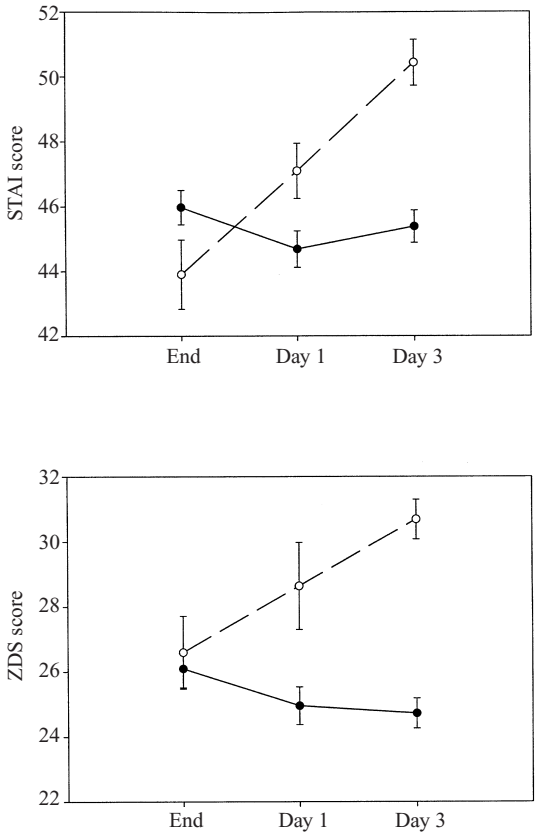

FIG. 2. Mean (S.E.M.) scores of the Spielberger State–Trait Anxiety Inventory (STAI) and the Zung Depression Scale (ZDS) in pregnant women, divided in STAI or ZDS responders (○—○) *versus* non-responders (●—●), at the end of term (end) and 1 (day 1) and 3 (day 3) days after delivery.

associations between post-partum depression and either STAI ($\chi^2 = 1{\cdot}1$, df = 1, $P = 0{\cdot}3$) or ZDS ($\chi^2 = 0{\cdot}0$, df = 1, $P = 0{\cdot}9$) responder status. For the STAI score, there was a significant effect of time ($F = 23{\cdot}6$, df = 2/161, $P < 10^{-5}$) and a significant time × group (STAI responders *versus* non-responders) interaction ($F = 33{\cdot}3$, df = 2/161, $P < 10^{-5}$). For the ZDS score, there was a significant effect of time ($F = 3{\cdot}9$, df = 2/147, $P = 0{\cdot}02$) and a significant time × group (ZDS responders *versus* non-responders) interaction ($F = 15{\cdot}4$, df = 2/147, $P = 0{\cdot}00002$). No significant interactions between time × STAI responder status were found for any of the plasma amino acids, the sum of the CAAs, and the tryptophan/CAA ratio. No significant interaction patterns between time × ZDS responder status were found for any of the amino acids, the sum of the CAAs and the tryptophan/CAA ratio.

By means of intraclass regression analyses (pooled over the subjects) no significant time-relationship could be found between the changes in STAI scores and the changes in plasma tryptophan, tyrosine, isoleucine, leucine, valine, the sum of the CAAs, and the tryptophan/CAA ratio. There was a significant correlation (without P correction) between the changes in the STAI and those in plasma phenylalanine ($r = 0{\cdot}16$, $P = 0{\cdot}04$). By means of intraclass correlation analysis (pooled over the subjects) no significant time-relationships were found between the changes in the ZDS and the plasma amino acids, the sum of the CAAs and the tryptophan/CAA ratio.

Relationships to IRS variables

Fig. 1 shows the serum IL-6, IL-1RA and LIF-R concentrations before delivery and 1 and 3 days after delivery. RM design ANOVA with IL-6 as dependent variable and with the pre-partum and two post-partum values as time factor showed significant effects of time ($F = 22{\cdot}9$, df = 2/123, $P < 10^{-5}$). Dunn's test performed at $P = 0{\cdot}0167$ (after P correction) showed that serum IL-6 was significantly higher 1 ($t = 6{\cdot}69$, $P < 10^{-5}$) and 3 ($t = 4{\cdot}19$, $P = 0{\cdot}0002$) days after delivery than at the end of term, and significantly higher 1 day after delivery than 3 days after delivery ($t = 2{\cdot}50$, $P = 0{\cdot}013$). RM design ANOVA showed that there were also significant time effects for IL-1RA ($F = 18{\cdot}3$, df = 2/135, $P = 0{\cdot}00001$). Dunn's test performed at $P = 0{\cdot}0167$ (after P correction) showed significantly higher serum IL-1RA values 1 ($t = 6{\cdot}05$, $P < 10^{-5}$) and 3 ($t = 2{\cdot}78$, $P = 0{\cdot}006$) days after delivery than before, and significantly higher serum IL-1RA 1 day after delivery than after 3 days ($t = 3{\cdot}27$, $P = 0{\cdot}002$). RM design ANOVA showed a significant effect of time on serum LIF-R ($F = 451$, df = 1/74, $P < 10^{-5}$). Dunn's test showed that serum LIF-R significantly decreased from the end of term to the first ($t = 25{\cdot}04$, $P < 10^{-5}$) and third ($t = 26{\cdot}95$, $P < 10^{-5}$) day after delivery. There were no significant differences in serum LIF-R between the first and third day after delivery ($t = 1{\cdot}87$, $P = 0{\cdot}06$). Intraclass regression analyses (pooled over the subjects) showed significant positive time-relationships between plasma tryptophan

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and serum IL-6 ($r = 0{\cdot}26$, $P = 0{\cdot}002$) and between the sum of the CAAs and serum IL-6 ($r = 0{\cdot}47$, $P < 10^{-5}$) and IL-1RA ($r = 0{\cdot}29$, $P = 0{\cdot}0005$). There were significant and inverse time relationships between serum LIF-R and plasma tryptophan ($r = 0{\cdot}58$, $P < 10^{-5}$) and the sum of the CAAs ($r = -0{\cdot}74$, $P < 10^{-5}$). There were significant time-correlations between the plasma tryptophan/CAA ratio and serum IL-6 ($r = -0{\cdot}44, P < 10^{-5}$), IL-1RA ($r = -0{\cdot}44, P < 10^{-5}$) and LIF-R ($r = 0{\cdot}51$, $P < 10^{-5}$). Intraclass, multiple regression analyses showed that 36·7% of the variance in the tryptophan/CAA ratio ($F = 41{\cdot}1$, df = 2/142, $P < 10^{-5}$) could be explained by the regression on serum IL-1RA ($F = 25{\cdot}5$, $P < 10^{-5}$) and LIF-R ($F = 40{\cdot}7$, $P < 10^{-5}$) (IL-1RA was negatively loaded and LIF-R positively).

Effects of background variables

ANOVAs performed on plasma tryptophan, the sum of CAAs, and the tryptophan/CAA ratio showed no significant differences in post-partum tryptophan, the CAAs or tryptophan/CAA values between post-partum women without analgesia, i.m. analgesia and epidural analgesia; women with and without induction; women with a vaginal delivery, a Caesarian section and delivery with the forceps or ventouse; women with and without breast feeding; women with a spontaneous *versus* induced amniotomy; women with or without post-partum complications; and nulliparae *versus* multiparae. There were no significant correlations between plasma tryptophan, the sum of the CAAs and the tryptophan/CAA ratio and duration of pregnancy.

RM design ANOVAs performed on plasma tryptophan, the sum of the CAAs and the tryptophan/CAA ratio did not show any significant interaction patterns between time × type of analgesia, time × induction (except for the tryptophan/CAA ratio), time × type of delivery (except for the tryptophan/CAA ratio), time × breast feeding, time × induced amniotomy, time × post-partum complications, and time × parity. There was a significant interaction between time × induction for the tryptophan/CAA ratio ($F = 6{\cdot}5$, df = 2/142, $P = 0{\cdot}002$). Analyses of simple effects showed a significantly lower tryptophan/CAA ratio ($F = 6{\cdot}5$, df = 1/141, $P = 0{\cdot}002$) in women who were induced. There was a significant interaction between time × type of delivery ($F = 2{\cdot}5$, df = 4/146, $P = 0{\cdot}04$). Analyses on simple effects showed a significantly lower tryptophan/CAA ratio ($F = 5{\cdot}4$, df = 2/129, $P = 0{\cdot}005$) in women with a Caesarian section *versus* those with a normal vaginal delivery or delivery by the ventouse or forceps.

DISCUSSION

A first major finding of this study is that plasma tryptophan is significantly lower in pregnant women at the end of term than in non-pregnant healthy women. Previous studies have shown lower plasma tryptophan concentrations in pregnant women compared with non-pregnant controls (Handley *et al*. 1980; Fuchs *et al*. 1996). In healthy women, plasma tryptophan concentrations decreased with the duration of pregnancy (Schrocksnadel *et al*. 1996). These authors found plasma tryptophan concentrations of 72 μmol/L in the first, 51 μmol/L in the second, and 46 μmol/L in the third trimester (Schrocksnadel *et al*. 1996). In our study plasma tryptophan concentrations in the third trimester were 47·3 μmol/L, i.e. 65·6% of the normal control values. However, not all authors were able to find significant changes in plasma tryptophan during pregnancy (Okatani *et al*. 1990). Another finding of our study is that also the CAAs, i.e. tyrosine, valine, leucine and isoleucine, but not phenylalanine, were significantly lower at the end of term compared with the control values. Previously, it was found that pregnancy lowered the plasma CAA concentrations of valine, leucine and isoleucine as compared to their concentrations in non-pregnant controls (Sastry *et al*. 1993). Notwithstanding, the decreased plasma CAA concentrations at the end of term, we found that the tryptophan/CAA ratio was significantly lower in pregnant women than in non-pregnant females.

The second major finding of this study is that the early puerperium is characterized by increases in the plasma concentrations of all amino acids, including tryptophan and the CAAs, as compared with their prepartum values. Schrocksnadel *et al*. (1996) report that the lowered plasma tryptophan concentrations at the end of pregnancy normalized in the puer-

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perium. Handley *et al.* (1977, 1980) found a rapid rise in plasma total and free tryptophan on the first to the fifth day post-partum. Nevertheless, in the present study we found that plasma tryptophan was still significantly lower in puerperal women than in non-pregnant healthy controls. Previously, it was shown that post-partum women have significantly lowered plasma tryptophan concentrations (Maes *et al.* 1992*a*; Abou-Saleh *et al.* 1999). Most importantly, the present study showed that the tryptophan/CAA ratio was significantly lower in the early puerperium than at the end of term and than in non-pregnant healthy females. Thus, the relative increase in CAAs after delivery is greater than that in plasma tryptophan. This study found that women who underwent an elective Caesarian section and whose labour was induced had a significantly lower plasma tryptophan/CAA ratio than those who did not. The umbilical blood total and free tryptophan concentrations are lower in newborn infants after elective Caesarian section than after a spontaneous delivery (Zanardo *et al.* 1985; Kazda *et al.* 1998).

The third major finding of this study is that there are no significant relationships between the availability of plasma tryptophan (plasma tryptophan or the tryptophan/CAA ratio) and either the increase in anxiety and depressive symptoms in the early puerperium or the occurrence of post-partum depression. Previous research concerning tryptophan in relation to post-partum depression/blues has revealed mixed results. Thus, no alterations in plasma total and free tryptophan were detected in women with post-partum blues (Garnier *et al.* 1985). Gard *et al.* (1986) on the other hand, report reduced plasma total tryptophan concentrations in puerperal blues. The initial peak in the rapid rise in plasma tryptophan on day 1 and 2 post-partum was absent in a considerable number of parturients with post-partum blues and in those who developed complaints of post-partum depression in the ensuing 6 months (Handley *et al.* 1980). Low plasma tryptophan shows a significant contribution to increased ratings on the STAI and ZDS scores and the Edinburgh Postnatal Depression Scale in puerperal women (Maes *et al.* 1992*a*; Abou-Saleh *et al.* 1999). However, while most previous studies examined plasma tryptophan, the present study showed that the tryptophan/CAA ratio, which best reflects the availability of tryptophan to the brain, decreased in the early puerperium and was not related either to symptoms of depression or anxiety in the early puerperium or to post-partum depression ensuing within the first 3 months after delivery.

The fourth major finding of the present study is that the changes in plasma tryptophan, the CAAs and the tryptophan/CAA ratio from the prepartum period to the early puerperium are significantly related to signs of IRS activation. Thus, the changes in the amino acid concentrations (including tryptophan and the CAAs) were positively related to signs of IRS activation (increased serum IL-6 and IL-1RA and lower LIF-R), whereas the changes in the tryptophan/CAA ratio were inversely related to IRS activation. These findings extend those of previous reports which showed that the lowered plasma tryptophan concentrations at the end of term were significantly and inversely related to neopterin (Fuchs *et al.* 1996; Schrocksnadel *et al.* 1996). However, the results of the present study suggest that at least two different IRS-related mechanisms may modulate the plasma amino acids *versus* the tryptophan/CAA ratio. First, the relationships between the increased plasma concentrations of the amino acids in the early puerperium and IRS activation may be explained by accelerated muscle proteolysis induced by IRS activation (Moldawer *et al.* 1987). Pro-inflammatory cytokines, such as IL-6, IL-1 and TNFα, induce muscle proteolysis (Romette *et al.* 1986; Fong *et al.* 1989; Wolvekamp & Marquet, 1990), which is accompanied by a net flux of amino acids out of the muscle to other tissues, such as the liver where they are reutilized for synthesis of acute phase proteins (Blackburn *et al.* 1979; Hasselgren *et al.* 1986, 1988; Heinrich *et al.* 1990; Pacitti *et al.* 1993). Secondly, the inverse relationships between the lowered tryptophan/CAA ratio and IRS activation from the end of term to the early puerperium may be explained by the effects of pro-inflammatory cytokines on indoleamine 2, 3 dioxygenase (IDO). IDO, a major tryptophan catabolizing enzyme in the brain, kidney, lung, spleen, duodenum and immune cells, is induced during immune stimulation by a number of pro-inflammatory cytokines, such as IFNγ, IL-2 and IL-1 (Takikawa

et al. 1984; Brown *et al.* 1989; Moroni *et al.* 1991; Saito *et al.* 1993). Thus, inflammation and infection may elicit pro-inflammatory cytokines, which stimulate the catabolism of tryptophan by inducing IDO, which in turn inhibits T cell proliferation, owing to reduced access of tryptophan (Mellor & Munn, 1999). This mechanism may play an important role in antimicrobial defences and in the protection from attacks by autoreactive T cells (Mellor & Munn, 1999). IDO is also expressed at the maternal-foetal interface, e.g. by trophoblasts and macrophages (Munn *et al.* 1998). In pregnancy, IDO-induced catabolism of tryptophan prevents the immunological rejection of foetal allografts through suppression of T cell activity by lowered tryptophan (Munn *et al.* 1998; Kudo & Boyd, 2000). The lowered plasma tryptophan/CAA ratio in parturients with a Caesarian section may be explained by the finding that operative deliveries induce a significant activation of IDO in the human placenta (Iwamoto & Kido, 1995).

Thus, while we found significant positive correlations between IRS activation (e.g. increased serum IL-6 and IL-1RA) and post-natal depressive and anxiety symptoms (Maes *et al.* 2000), no significant correlations between the latter and the lowered tryptophan/CAA ratio could be found, although there was an inverse correlation between IRS activation and the tryptophan/CAA ratio. Previously, it has been argued that there may be a causal relationship between IRS activation and the occurrence of depression and post-partum blues/depression (Maes, 1999; Maes *et al.* 2000). Indeed, in humans and experimental animals, administration of pro-inflammatory cytokines and induction of IRS activation may induce depression, anhedonia, depressive and anxiety symptoms and 'sickness' behaviour (Bluthé *et al.* 1992; Sakic *et al.* 1997; Yirmiya, 1997; Connor *et al.* 1998; Maier & Watkins, 1998; Spath-Schwalbe *et al.* 1998). It is thought that the 'depressogenic' effects of IRS activation are related to their modulatory effects on the function of the hypothalamic–pituitary–adrenal axis, the central turnover of catecholamines and 5-HT, including lowered availability of plasma tryptophan (Yirmiya, 1997; Maes, 1999). However, the results of the present study suggest that the reduced availability of plasma tryptophan, which may be caused by IRS activation, is probably not related to the aetiology of depressive or anxiety symptoms in the early puerperium or to post-partum depression.

A first limitation of the present study is that the results relating to presence or not of post-natal depression and plasma tryptophan will be affected by the fact that not all those included in this study had been assessed for post-natal depression using the SCID. Nevertheless, we examined 67 parturients of whom 25·4% developed post-partum depression. Secondly 13 non-pregnant normal volunteers may not be a sufficient number to assess normal tryptophan levels, although the plasma levels and the tryptophan/CAA ratio established here are of the same order as those found previously in younger controls. Moreover, the normal plasma amino acid values used here are the mean values of four time points during the menstrual cycle, i.e. days 7, 14, 21 and 28.

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