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The influence of dietary sodium restriction on renal and ovarian renin and prorenin production during ovarian stimulation

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In a prospective study, the effect of dietary sodium restriction on plasma and follicular fluid renin and prorenin concentrations and on fertilization measures was investigated during ovarian stimulation. In all, 18 women undergoing ovarian stimulation for in-vitro fertilization and embryo transfer were randomly divided into groups with and without sodium restriction. Plasma renin and prorenin concentrations were higher in the low sodium than in the normal sodium group. Plasma renin concentrations showed a mid-luteal rise. Plasma prorenin concentrations increased 4-fold on the day of oocyte retrieval, followed by a gradual decline to basal values. The low sodium group had more follicles than the normal sodium group. Neither follicular fluid renin and prorenin concentrations, nor the total amount of follicular fluid renin and prorenin per ovary differed significantly between the two groups. Oocyte yield and fertilization rates were similar in both groups. These rates were correlated with neither renin nor prorenin concentrations in follicular fluid. We conclude that sodium restriction did not influence cyclic plasma variations of renin or prorenin or follicular fluid renin and prorenin concentrations. In addition, fertilization rates were not affected by sodium restriction.

Key words: follicular fluid/IVF and embryo transfer/ovarian renin–angiotensin system/ovarian stimulation/sodium

Introduction

Renin is essential for the regulation of a series of reactions leading to the formation of angiotensin II. Angiotensin II is a key hormone in blood pressure regulation and in water, sodium and potassium homeostasis (Sealey and Laragh, 1995). The kidney is the main, if not the only, source of plasma renin and is also a major source of plasma prorenin, the inactive precursor of renin (Derkx *et al.*, 1976; Sealey *et al.*, 1977). In plasma, ~90% of the total renin concentration circulates as prorenin (Derkx *et al.*, 1983).

Extrarenal synthesis and release of prorenin into the circulation has been demonstrated in various organs, including the ovary (Glorioso *et al.*, 1986; Derkx *et al.*, 1987; Itskovitz *et al.*, 1987). In response to stimulation by gonadotrophic hormones, prorenin is produced and released into the circulation by the theca cells (Do *et al.*, 1988) at the time of ovulation (Sealey *et al.*, 1987) and during pregnancy (Derkx *et al.*, 1986).

Both ovulation induction and ovarian stimulation may result in ovarian hyperstimulation syndrome (OHSS; Navot *et al.*, 1992). In its most pronounced form, this iatrogenic syndrome is characterized by massive ovarian enlargement, ascites, pleural effusion, oliguria, hypercoagulability, disturbances in serum electrolytes and stimulation of the renin–angiotensin system (Navot *et al.*, 1987; Ong *et al.*, 1991). Therefore it is conceivable that the ovarian renin–angiotensin system may play a role in the pathogenesis of this syndrome.

Dietary sodium restriction has been advocated as a treatment modality for OHSS (Shapiro *et al.*, 1977; Haning *et al.*, 1985; Balasch *et al.*, 1991). The primary purpose of this pilot study was to examine the effect of dietary sodium restriction on renal and ovarian renin and prorenin production during ovarian stimulation.

Materials and methods

Patients and stimulation protocol

A total of 18 healthy Caucasian women, participating in a programme for in-vitro fertilization (IVF) and embryo transfer, were included in this study. The study was approved by the hospital's ethical committee and written consent was obtained from each patient. Patients were randomly divided into two groups: those receiving an *ad libitum* dietary intake of ~100–150 mmol Na^+/day (normal sodium diet) (n = 10) and those who were given a sodium-restricted diet of 10–20 mg Na^+/day (low sodium diet) (n = 8). Dietary instructions were given by dieticians. The low sodium diet started 10 days before the onset of the ovarian stimulation and was maintained until the end of the IVF cycle (pregnancy test or menstruation).

The mean ± SD age of the patients in the normal sodium group was similar to that of patients in the low sodium group: 33.8 ± 3.7 and 33.6 ± 2.8 years respectively.

The indications for IVF in the normal sodium group (primary infertility n = 6) were: tubal disease (n = 4), severe endometriosis (n = 1), male factor (n = 2), unexplained (n = 2) and combined factors (n = 1). Indications for IVF in the low sodium group (primary infertility n = 3) were: tubal disease (n = 5), severe endometriosis (n = 2) and male factor (n = 1). In both groups there was one patient with only one ovary.

Patients were treated with a gonadotrophin-releasing hormone (GnRH) agonist in a flare-up protocol. From day –13, leuprolide acetate, a short-acting GnRH agonist (Lucrin; Abbott, Amstelveen, The Netherlands), was given 1 mg s.c. daily and continued until

ovulation induction. From day –12, two ampoules (=150 IU) human menopausal gonadotrophin (HMG) i.m. (Humegon; Organon, Oss, The Netherlands) were administered daily. Stimulation was monitored by serum oestradiol concentrations and transvaginal, ultrasonographical follicle measuring. Ovulation was induced by the administration of 10 000 IU human chorionic gonadotrophin (HCG) i.m. (Pregnyl; Organon) 6 days after serum oestradiol concentrations reached 1.0 nmol/l. Oocyte retrieval (day 0) was performed 34–35 h later. The luteal phase was supported by 1500 IU HCG i.m. (Pregnyl; Organon) on days +3 (the day of embryo transfer), +6, +9 and +12 after oocyte retrieval.

Physiological parameters

Body weight was assessed in kg to one decimal place (SECA-alpha, Modell 770; Wigro, Nieuwegein, The Netherlands) with the patient wearing clothing but no shoes.

After 5 min of rest, while the patient was in a sitting position, blood pressure was measured with an automatic device (BOSO-prestige automatic; Bosch + Sohn, Juningen, Germany). The results of three measurements were averaged.

Body weight and blood pressure were measured at the start of the cycle and on days –4, 0 (oocyte retrieval), +3, +6, +9 and +12.

Sampling of follicular fluid, blood and urine

Follicular fluid

The number of follicles and the follicular diameter (mean of three directions) were measured with an ATL Ultramark 4 (Advanced Technology Laboratories, Scientific Medical Systems, Dordrecht, The Netherlands) just before oocyte retrieval. Follicles were divided into three groups according to their mean diameter: 'small' with a mean diameter of ≤9 mm, 'medium' with a mean diameter of 10–15 mm inclusive, and 'large' with a mean diameter of >15 mm.

Oocyte retrieval was performed with an ultrasound-guided single lumen follicle aspiration needle (Cook, 17 gauge, length 35 cm; William A.Cook Australia, Queensland, Australia). Follicular fluid was collected in scaled tubes and the volume was measured separately for each follicle. After separation of the oocyte, follicular fluid was centrifuged within 5 min at 2000 *g* for 10 min, and then stored in polystyrene tubes at –70°C.

Blood sampling

Blood was obtained from an antecubital vein, between 08:00 and 12:00 h, after the patient had been recumbent for at least 30 min. Blood sampling was performed on the same occasion as the measurement of body weight and blood pressure and at the end of the cycle. Vacutainers containing disodium ethylenediamine-tetraacetate (final concentration 10 mmol/l) were used and kept at room temperature for no longer than 10 min until plasma was separated by centrifugation at 2000 *g* for 10 min. Plasma samples were stored at –70°C in polystyrene tubes until further analysis. On the day of oocyte retrieval, blood was collected for assessing the oestradiol concentration.

Urine sampling

Compliance with the diet was checked by measuring the sodium:creatinine ratio in urine samples three times during the cycle: days –4, 0 (day of oocyte retrieval) and +9.

Renin, prorenin and oestradiol assays

Renin and prorenin assays

Plasma and follicular fluid prorenin were converted to renin by the addition of trypsin (Derkx *et al.*, 1983). Sepharose-bound trypsin was used at a final concentration of 500 mg/l for 24 h at 4°C. Trypsin was then removed by centrifugation. With this trypsin concentration, maximal conversion of the prorenin to renin was obtained without further breakdown of the renin or prorenin. The recovery of added purified kidney renin was >95%.

Both trypsin-activated prorenin and the native renin were measured using an immunoradiometric assay (Diagnostics Pasteur, Marnes La Coquette, France; Simon *et al.*, 1992). The primary monoclonal antibody recognizing prorenin and renin was bound to magnetizable beads. The secondary monoclonal antibody, which reacted equally well with kidney renin, plasma renin and trypsin-activated prorenin but not with prorenin, was iodinated. The results of this assay were expressed in pg/ml. The lower limit of detection was 3.5 pg/ml. Intra- and interassay coefficients of variation were <6 and <9% respectively. The renin standard used in this kit was calibrated against the World Health Organization renin standard 68/356 (Bangham *et al.*, 1975). One pg of this standard equals 1.6×10^{-6} IU.

All samples from each patient were always run in the same assay. The concentration of prorenin in plasma or follicular fluid was calculated by subtracting the results obtained before trypsin activation from those after activation (= renin + prorenin).

Oestradiol assay

Serum samples were assayed with a commercially available oestradiol radioimmunoassay kit (Sorin Biomedica, Amsterdam, The Netherlands). Intra- and interassay coefficients of variation were 2.9% (mean oestradiol concentration 260 pmol/l) and 10.8% (mean oestradiol concentration 290 pmol/l) respectively.

Statistical analysis

To obtain approximately normal distributions, plasma and follicular renin and prorenin, follicular fluid volume and the sodium:creatinine ratio were transformed logarithmically. Therefore outcomes were expressed as geometric means with a 95% confidence interval (CI) or as otherwise indicated. Differences in plasma concentrations of renin and prorenin during the ovarian stimulation cycle in the groups with the low and normal sodium diets were analysed with repeated measures of analysis of variance (ANOVA; Dixon, 1990). The effect of the low sodium diet on the plasma concentrations of renin and prorenin was expressed as a percentage increase (with the 95% CI of this increase) compared with the plasma concentrations during a normal sodium diet.

The effects of a low sodium diet on follicular fluid renin and prorenin concentrations were analysed using a mixed-model ANOVA. For other data, *t*-tests or non-parametric tests were used when appropriate. Spearman's correlation coefficients were calculated. Proportions were compared with Fisher's exact test. *P* values <0.05 (two-sided) were considered statistically significant.

Results

Clinical parameters of IVF outcome

In the group with the normal sodium diet the results of one patient were not included because her IVF cycle was cancelled due to inadequate ovarian response. In both groups there was one patient who did not have an embryo transfer because of failed fertilization. The groups were comparable with regard to the duration of ovarian stimulation, i.e. 11.8 (SD 1.9) days for the women with a normal sodium diet and 11.8 (SD 1.9) days for the women with a low sodium diet. In addition, the dose of HMG was similar in both groups: 2.30 (SD 0.70) and 2.37 (SD 0.41) IU HMG/kg/day respectively.

Serum oestradiol concentrations on the day of oocyte retrieval were 3.2 (SD 1.4) nmol/l in the group with the normal

Table I. Urinary sodium:creatinine ratios in subjects with normal and low sodium intakes

Day	Sodium diet		*P* value
	Normal	Low	
–4	9.39 (3.02–29.14)	0.79 (0.14–4.50)	<0.01
0	14.87 (3.36–65.73)	2.29 (0.31–16.77)	<0.01
+9	9.42 (2.04–43.42)	2.70 (0.48–15.19)	0.02

The sodium:creatinine ratio was calculated from the data expressed in mmol/l. Day 0 was the day of oocyte retrieval. Results are expressed as geometric means and 95% confidence intervals. The analysis was performed with the unpaired *t*-test.

sodium diet and 4.6 (SD 2.1) nmol/l in the group with the low sodium diet (not significant).

In the normal sodium group, 99 follicles were aspirated, and in 77 aspirates (78%) there was enough follicular fluid for the determination of renin and prorenin. For the low sodium group, these data were 135 and 114 (84%) respectively.

Oocyte yield was 47% in the normal sodium group and 39% in the low sodium group (not significant). The fertilization rates were 76 and 73% respectively (not significant). Unfortunately, none of the patients became pregnant during the study.

Effect of a low sodium diet on body weight and blood pressure

The urinary sodium excretion expressed as the sodium:creatinine ratio in urine was significantly lower in the low sodium group than in the normal sodium group (Table I).

At the start of the cycle, body weight did not differ significantly between the two groups. Mean body weight in the normal sodium group increased from 62.9 (SD 10.7) kg at the start of the study to 63.5 (SD 11.3) kg on the day of oocyte retrieval (not significant); it did not change significantly until the late luteal phase. Women in the low sodium group started their sodium-restricted diet 10 days before the start of their cycle so as to reach a steady low sodium state. During this period they lost 1.0 kg (SE of difference 0.2; $P < 0.01$), but during the whole cycle their weights remained stable. The mean values at the start of the ovarian stimulation and at oocyte retrieval were 57.1 (SD 7.1) and 57.3 (SD 6.7) kg respectively.

On the day of oocyte retrieval the mean systolic blood pressure in the normal sodium group was higher than in the low sodium group [121 (SD 16) and 111 (SD 13) mm Hg respectively], but this difference was not significant. In addition, no significant difference in the mean diastolic blood pressure was demonstrated on the day of oocyte retrieval: 70 (SD 11) and 67 (SD 8) mm Hg respectively.

Changes in the plasma concentrations of renin and prorenin

Figure 1 depicts the time-course of the changes in the plasma concentrations of renin and prorenin during the ovarian stimulation cycle. The effect of the phase of the cycle on the circulating plasma concentrations of renin and prorenin was highly significant ($P < 0.001$).

The changes in the plasma concentrations of renin and prorenin after ovarian stimulation did not run in parallel. On the day of oocyte retrieval there was a 3- to 4-fold increase

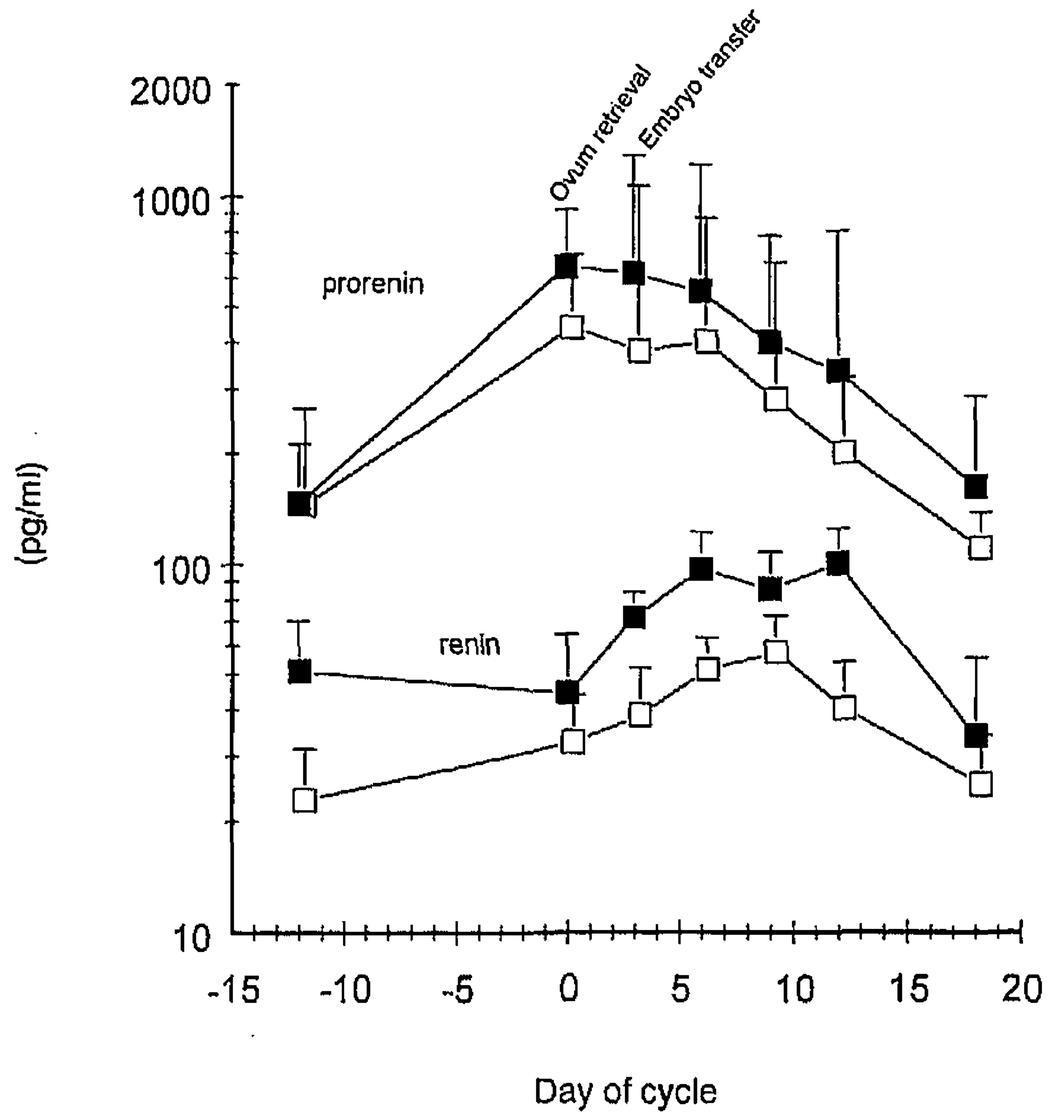

Figure 1. Plasma renin and prorenin concentrations in patients during a cycle with ovarian stimulation. (□) Patients with a normal sodium intake (100–150 mmol Na^+/day); (■) patients with a sodium-restricted diet (10–20 mmol Na^+/day). Results are expressed as geometric means + SD. The analysis was performed with a multiple analysis of variance. The effect of a low sodium diet on renin and prorenin concentrations was significant ($P = 0.03$). The effect of the phase of the cycle on renin and prorenin concentrations was also significant ($P < 0.001$).

in plasma prorenin concentrations in both groups. However, the plasma renin concentration did not change at this stage of the cycle. In the luteal phase, plasma prorenin concentrations gradually decreased to basal values. In contrast, plasma renin concentrations gradually increased 2-fold by day +9 of the cycle and then returned to basal values.

In both phases of the ovarian stimulation cycle plasma renin and prorenin concentrations were higher in the patients with the low sodium diet compared with those with the normal sodium diet. The geometric mean plasma renin concentrations in the low sodium group were on average 60% (95% CI 33–92%) higher compared with the normal sodium group. Moreover, the differences between both groups appeared to depend significantly ($P < 0.001$) on the day of the luteal phase. However, the deviations from parallelism did not appear major.

In general, plasma prorenin concentrations were significantly ($P = 0.03$) higher (mean difference 28% with 95% CI 2–95%) in the low sodium group compared with the normal sodium group. In contrast to renin, the differences for prorenin between the groups did not vary significantly during the cycle.

There were no significant correlations between plasma renin or prorenin concentrations on the one hand and blood pressure, changes in body weight and urinary sodium:creatinine ratios on the other.

Follicular fluid prorenin and renin

Follicular development

The number of follicles per ovary was significantly ($P = 0.02$) lower in the normal sodium group than in the low sodium

Table II. Follicular fluid renin and prorenin concentrations (pg/ml) in various sizes of follicle

Follicle size category (mm)	Sodium diet[a]			
	n	Normal	*n*	Low
Renin				
All sizes	77	253 (71–898)	114	209 (58–757)
<9 mm	20	300 (107–842)	29	194 (30–1257)
9–15 mm	30	297 (89–988)	44	219 (84–571)
>15 mm	27	187 (50–699)	41	209 (69–632)
Prorenin				
All sizes	77	10 762 (2788–41 545)	114	8752 (2548–30 058)
<9 mm	20	13 833 (5769–33 172)	29	9888 (2997–32 621)
9–15 mm	30	13 303 (4170–42 441)	44	8400 (2561–27 554)
>15 mm	27	7060 (1614–30 891)	41	8415 (2258–31 362)

Results are expressed as geometric means and 95% confidence intervals. The analysis was performed with a mixed analysis of variance.
[a]The differences in renin and prorenin concentrations between the normal sodium diet and the low sodium diet were not significant for all various sizes of follicles. Neither were there significant differences in renin and/or prorenin concentrations between the various sizes of follicles within the normal sodium diet group or the low sodium diet group.

group: 4 (1–14) and 9 (5–15) [median (range)] respectively. No significant differences were found between the groups concerning the distribution of follicles over the three ultrasound-defined size categories: 35% small, 34% medium and 31% large. The mean (95% CI) fluid content per follicle was 2.5 (0.4–14.2) in the normal sodium group and 2.3 (0.4–12.9) in the low sodium group (not significant). The amounts of follicular fluid per follicle were significantly correlated with the ultrasound-defined size categories ($P < 0.01$) in the groups.

Follicular fluid:plasma ratios

The follicular fluid:plasma ratios for renin and prorenin concentrations did not differ significantly between the normal and low sodium groups. Mean renin and prorenin concentrations in follicular fluid at the time of oocyte retrieval were on average six and 20 times higher than in plasma respectively. In both the normal and low sodium groups, follicular fluid total renin was on average 97% prorenin, whereas in plasma, 92% of total renin was prorenin.

Follicular fluid renin and prorenin concentrations

Between groups. Table II shows that both follicular fluid renin and prorenin concentrations in the low sodium group were not significantly different from the normal sodium group. In addition, after splitting up data into the various follicular size categories, no significant difference could be demonstrated for any of the size categories.

Within groups. In the normal sodium group, both follicular fluid renin and prorenin concentrations tended to be lower in large follicles compared with small and medium-sized ones; however this difference was not significant. No significant differences were found between small and medium-sized follicles. In the low sodium group, both follicular fluid renin and prorenin concentrations did not differ significantly between follicular size categories (Table II).

Table III. Total ovarian amount of follicular fluid renin and prorenin (pg per ovary)

	Sodium diet		*P* value
	Normal	Low	
Renin	3466 (359–33 472)	5874 (2457–14 045)	NS
Prorenin	132 059 (11 379–1 532 685)	236 893 (90 516–619 981)	NS

Results are expressed as geometric means and 95% confidence intervals. The analysis was performed with the unpaired *t*-test. NS = not significant.

Total amount of follicular fluid renin and prorenin per ovary

The total amount of follicular fluid renin and prorenin per ovary was estimated by the addition of the total amount of (pro)renin of each follicle, as calculated by the volume of follicular fluid×(pro)renin concentration per follicle. Neither the total follicular fluid renin content nor the total follicular fluid prorenin content per ovary differed significantly between the two groups (Table III).

Table IV. Renin and prorenin concentrations (pg/ml) in follicular fluid related to oocyte yield and the presence or absence of fertilization

	n	Renin	Prorenin
Oocyte			
Present	84	209 (54–812)	9493 (2448–36 816)
Absent	107	240 (71–817)	9536 (2731–33 302)
P value		NS	NS
Fertilization			
Positive	63	216 (62–750)	9806 (2638–36 454)
Negative	21	187 (35–998)	8612 (1936–38 316)
P value		NS	NS

Results are expressed as geometric means and 95% confidence intervals. The analysis was performed with the unpaired *t*-test. NS = not significant.

Oocytes and fertilization

Follicular fluid renin and prorenin concentrations in follicles yielding an oocyte or not did not differ significantly between the two groups. The same applied to whether or not the oocyte was fertilized. For this reason the data from both groups were combined and are presented in Table IV. No significant differences were found in mean follicular fluid renin or prorenin concentrations between follicles yielding an oocyte or not. The presence or absence of fertilization appeared to make no significant difference in the follicular fluid renin or prorenin concentrations of follicles yielding an oocyte.

Correlations between plasma (pro)renin concentrations and follicular parameters

The plasma prorenin concentration on the day of oocyte retrieval was correlated with the number of follicles ($r = 0.56$, $P < 0.02$), the serum oestradiol concentration ($r = 0.72$, $P < 0.01$) and the total amount of ovarian follicular fluid prorenin per person ($r = 0.57$, $P < 0.02$). Plasma renin concentration was not significantly correlated with the aforementioned parameters.

Discussion

Effect of a low sodium diet on plasma concentrations of renin and prorenin during ovarian stimulation cycles

Renin is formed within the juxtaglomerular cells in the kidney. Sodium depletion is a classic stimulus of the synthesis of renin (Sealey and Laragh, 1995). When the renal formation of renin is enhanced for prolonged periods, the rate of synthesis of the renin precursor prorenin is also increased (Derkx *et al.*, 1983; Derkx and Schalekamp, 1988). Therefore chronic dietary sodium restriction results in an elevation of the plasma concentrations of renin and prorenin (Derkx and Schalekamp, 1988).

Our study shows that women undergoing ovarian stimulation also respond with a rise in plasma renin and prorenin concentration when the sodium content of the diet is reduced. Moreover, the increase in plasma renin and prorenin concentration persisted throughout both phases of the ovarian stimulation cycle.

A mid-luteal rise in plasma renin concentration has been described previously during ovarian stimulation cycles as well as during the normal menstrual cycle (Brown *et al.*, 1964; Sealey *et al.*, 1987). In our study, this mid-luteal increase in plasma renin concentration was present not only in the women with the normal sodium diet but also in those with the low sodium intake. This rise in renin concentration is considered to be caused by the natriuretic effect of progesterone and is of renal origin (Brown *et al.*, 1964).

Ovarian prorenin but not renin seems to be produced and released into the circulation in response to luteinizing hormone and HCG, as demonstrated by observations during spontaneous menstrual cycles (Sealey *et al.*, 1987; Blankestijn *et al.*, 1990), during pregnancy (Derkx *et al.*, 1986; Sealey *et al.*, 1986) and in women undergoing ovarian stimulation (Derkx *et al.*, 1987; Itskovitz *et al.*, 1987). In our study we demonstrated that in patients with the normal sodium diet, as well as in patients with the low sodium diet, the plasma prorenin concentration was positively correlated with the number of follicles, the serum oestradiol concentration and the ovarian prorenin concentration on the day of the oocyte retrieval. In contrast to prorenin, renin was not correlated with the aforementioned parameters.

Plasma prorenin concentrations remained high in the early luteal phase and then gradually declined in both groups of patients.

Thus, in conclusion, sodium intake *per se* does not seem to affect the time-course of the changes in the plasma concentration of renin and prorenin during the ovarian stimulation cycle. The higher plasma concentrations of renin and prorenin in the women with the low sodium diet can be explained by an increased renal release of these proteins.

Effect of a low sodium diet on follicular fluid renin and prorenin concentrations during ovarian stimulation cycles

Mean follicular fluid renin-like activity has been found to be much higher in stimulated than in unstimulated cycles (Derkx *et al.*, 1987). The prorenin concentration in follicular fluid during ovarian stimulation cycles is 10–40 times higher than in plasma (Glorioso *et al.*, 1986; Derkx *et al.*, 1987; Itskovitz *et al.*, 1987).

The question as to whether a low sodium intake may influence the follicular fluid concentrations of renin and prorenin during ovarian stimulation can be answered negatively. We found no differences in the concentrations of renin and prorenin in the various size categories of follicles, and no differences in the amount of total renin or prorenin per ovary. This finding was not unexpected because a sodium-sensing system, such as the macula densa in the kidney, is not present in the ovary.

It is generally accepted that the ovary secretes only prorenin and not renin into the circulation (Paulson *et al.*, 1989). However, the renin concentration in follicular fluid is higher than in plasma (Glorioso *et al.*, 1986; Derkx *et al.*, 1987; Itskovitz *et al.*, 1987). It is possible that *in vivo* proteolytic enzymes in the follicular fluid may convert prorenin into renin. However, this discrepancy may also be explained by methodological problems of the renin assay. In our study we measured renin and trypsin-activated prorenin by using an immunoradiometric assay (Simon *et al.*, 1992). This assay uses a monoclonal antibody that reacts with renin but does not cross-react with prorenin. It is possible that this monoclonal antibody does cross-react with truncated forms of prorenin which are present in follicular fluid. This may lead to falsely elevated values for renin, especially when the prorenin concentrations are very high, as is the case in follicular fluid.

Prorenin may also be inadvertently activated at low temperature (cryoactivation) during the storage and handling of plasma or follicular fluid samples (Pitarresi *et al.*, 1992).

On theoretical grounds one can argue that the ovary may have a mechanism for the selective uptake of kidney-derived plasma renin and prorenin. However, such a mechanism is unlikely for the ovary because here we have shown that the total ovarian renin and prorenin content in women with a low sodium diet was not increased despite an increase in the circulating plasma renin and prorenin concentrations.

Effect of low sodium intake on follicular development, the oocyte and fertilization

We do not have an explanation for the differences in the number of follicles between the women with the normal sodium diet and those with the low sodium diet. An ongoing study including more patients is necessary to confirm this finding. Because the numbers in the two groups of patients were small, chance is as yet the only explanation.

Sodium intake does not seem to affect oocyte yield and/or fertilization during ovarian stimulation, as demonstrated by our results.

In accordance with the results of Paulson *et al.* (1989), our data did not show a correlation between follicular fluid renin and prorenin concentrations, oocyte yield and/or fertilization. Itskovitz *et al.* (1991) suggested a relationship between follicular fluid prorenin concentration and fertilization that resulted in pregnancy, but the follicular fluid prorenin concentration did not discriminate between oocytes that were fertilized and oocytes that were not fertilized or were fertilized abnormally.

In conclusion, this is the first report describing the effect of sodium restriction on plasma and follicular fluid concentrations of renin and its precursor prorenin during ovarian stimulation. Plasma renin and prorenin concentrations were higher in women with a low sodium diet. This rise can be explained by an increased renal release of renin and its precursor. However, the cyclic changes in plasma renin and prorenin concentration in the women undergoing ovarian stimulation were not influenced by the low sodium diet. In addition, sodium deprivation did not influence follicular fluid renin and prorenin concentrations. Finally, sodium restriction does not seem to impair the objectives of ovarian stimulation.

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