Human adrenal glands secrete vitamin C in response to adrenocorticotrophic hormone[1–5]

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ABSTRACT

Background: When vitamin C intake is from foods, fasting plasma concentrations do not exceed 80 μmol/L. We postulated that such tight control permits a paracrine function of vitamin C.

Objective: The purpose of this study was to determine whether paracrine secretion of vitamin C from the adrenal glands occurs.

Design: During diagnostic evaluation of 26 patients with hyperaldosteronism, we administered adrenocorticotrophic hormone intravenously and measured vitamin C and cortisol in adrenal and peripheral veins.

Results: Adrenal vein vitamin C concentrations increased in all cases and reached a peak of 176 ± 71 μmol/L at 1–4 min, whereas the corresponding peripheral vein vitamin C concentrations were 35 ± 15 μmol/L ($P < 0.0001$). Mean adrenal vein vitamin C increased from 39 ± 15 μmol/L at 0 min, rose to 162 ± 101 μmol/L at 2 min, and returned to 55 ± 16 μmol/L at 15 min. Adrenal vein vitamin C release preceded the release of adrenal vein cortisol, which increased from 1923 ± 2806 nmol/L at 0 min to 27 191 ± 16 161 nmol/L at 15 min ($P < 0.0001$). Peripheral plasma cortisol increased from 250 ± 119 nmol/L at 0 min to 506 ± 189 nmol/L at 15 min ($P < 0.0001$).

Conclusions: Adrenocorticotrophic hormone stimulation increases adrenal vein but not peripheral vein vitamin C concentrations. These data are the first in humans showing that hormone-regulated vitamin secretion occurs and that adrenal vitamin C paracrine secretion is part of the stress response. Tight control of peripheral vitamin C concentration is permissive of higher local concentrations that may have paracrine functions. *Am J Clin Nutr* 2007;86:145–9.

KEY WORDS Vitamin C, adrenal gland, stress response, cortisol, paracrine secretion

INTRODUCTION

The physiologic response to stress is coordinated by the pituitary gland, which secretes trophic hormones in response to central nervous system input from the hypothalamus. The essential adrenocorticotrophic hormone (ACTH) secreted by the pituitary gland stimulates adrenal glands to synthesize and secrete cortisol. In animals, ACTH also causes vitamin C loss from adrenals (1–3). Adrenal glands are rich in vitamin C, with concentrations as high as 10 mmol/L (4). For these reasons, vitamin C and stress in humans have long been associated, despite a lack of direct evidence for such a link. There are no human data, and animal evidence is inconsistent regarding utilization within adrenals or release from adrenals that could increase vitamin C concentrations in either local or systemic veins (4).

Humans, unlike most animals, cannot synthesize vitamin C and instead must obtain it from diet. Healthy humans consuming 200–300 mg vitamin C/d, an amount obtainable from foods such as fruit and vegetables in which the vitamin is abundant, maintain steady-state fasting plasma concentrations of 70 to 80 μmol/L (5, 6). Tightly controlled plasma vitamin C concentrations are exceeded transiently with oral doses of ≥1 g in amounts obtainable only from supplements and not from foods. Concentrations produced by supplement doses of ≥500 mg would not occur in nature (7). In tissues other than red blood cells, vitamin C intracellular concentrations are usually maintained in the millimolar range, in contrast to the micromolar range in plasma (8, 9). The observed tight control of vitamin C plasma and tissue concentrations is mediated by gastrointestinal absorption, cellular transport, and renal reabsorption and excretion. The especially tight control of plasma concentrations resulting from ingestion of vitamin C amounts found in foods (5–7) could facilitate paracrine actions of the vitamin, if local concentrations were higher. We hypothesized that the adrenal glands secrete vitamin C after simulated stress and that tight control of plasma vitamin C concentrations would permit intraadrenal vitamin C concentrations to be far higher than those in peripheral veins. To test this, we studied patients with hyperaldosteronism who underwent adrenal vein sampling for specific diagnosis. In these patients, we measured adrenal and peripheral vein vitamin C and cortisol concentrations after ACTH administration.

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SUBJECTS AND METHODS

Subjects

We studied 16 men and 10 women aged 52.3 ± 8.6 y (range: 32–68 y) who underwent bilateral adrenal vein sampling at the National Institutes of Health. Subjects had clinical and biochemical features of hyperaldosteronism and were referred for adrenal vein sampling to differentiate between aldosterone-secreting adrenal adenoma and hyperplasia as the cause of hyperaldosteronism.

All patients gave written informed consent. The study protocol was approved by the institutional review board of the National Institutes of Health.

Adrenal vein sampling

Adrenal vein sampling was performed in the morning after an overnight fast. Patients received 2 mg midazolam given intravenously at the beginning of the procedure. Two peripheral venous cannulae were inserted, one for blood sampling and the other for drug infusions. Both adrenal veins were cannulated via the femoral vein, and cannulation was guided by digital subtraction angiography (10). Blood samples were drawn from each adrenal vein and the peripheral vein at time 0. A 250-μg bolus of ACTH was given intravenously and then 250 μg ACTH in 250 mL normal saline was infused intravenously at a rate of 200 mL/h. Blood samples were collected at 0, 2, 4, 6, 8, 10, and 15 min.

Assays

All samples were assayed for vitamin C and cortisol concentrations. The blood samples were kept on ice until sampling ended. Plasma was processed at 4 °C for vitamin C and cortisol analyses as described previously (5, 11). Briefly, 1–5 mL heparinized whole blood was centrifuged at 1000 × *g* for 10 min at 4 °C. Plasma (supernatant) was removed, diluted 1-in-5 with 90% methanol/water containing 1 mmol EDTA/L, and vigorously mixed by vortex for 10 s. Precipitated protein was removed by centrifugation at 25 000 × *g* for 20 min at 4 °C. Supernatants were stored at −80 °C until they were analyzed. For ascorbic acid, all samples from the same patient were assayed together by using HPLC with coulometric electrochemical detection (12, 13). The intraassay and interassay CVs were <1% and <3%, respectively. Plasma cortisol was measured by using Immulite 2000 Cortisol Immunoassay. The intraassay and interassay CVs were 6% and 9%, respectively.

Statistical analysis

Results were compared by using paired *t* tests or repeated-measures analysis of variance (ANOVA) with Bonferroni's post test when appropriate, and 2-tailed *P* values were calculated. Adrenal vein samples taken from the right and left adrenal glands were related because they were from the same patient. However, there were variations between the right and left values because of differences in catheter position, venous anatomy, or possibly other local factors. Because not all of the 47 available vitamin C and cortisol measurement pairs were statistically independent (they came from 26 subjects, most of whom contributed 2 measurement pairs), we used an adjusted calculation to compute the significance of the observed sample correlation. This calculation takes into account this clustering (observations are independent among clusters but may be dependent within a cluster) and uses a robust variance calculation (the Huber-White sandwich estimator of variance) in STATA statistical software (release 8.2; Stata Corporation, College Station, TX).

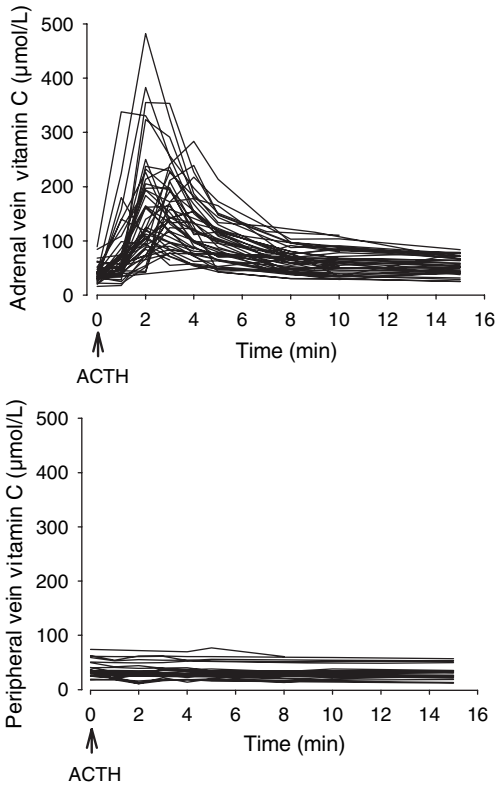

FIGURE 1. Vitamin C concentrations in the adrenal and peripheral veins in 26 patients with primary hyperaldosteronism. Under radiographic guidance, catheters were placed in both adrenal veins, and blood samples were taken after stimulation with adrenocorticotrophic hormone. Vitamin C concentrations in each of the adrenal (n = 47) and peripheral (n = 26) veins sampled are shown. In 5 patients, blood samples were obtained from only one adrenal vein because of unusual venous anatomy or technical difficulties with adrenal vein catheterization. In the adrenal veins, peak vitamin C concentrations ($\bar{x}$ ± SD: 176 ± 71 μmol/L) were reached between 1 and 4 min, and these were significantly ($P < 0.0001$, paired *t* test) higher than corresponding peripheral plasma vitamin C concentrations (35 ± 15 μmol/L). In those patients in whom adrenal vein vitamin C concentration could be measured in only one adrenal gland, that single value was used in the calculation. In those in whom both adrenals were successfully sampled, the mean of the 2 adrenal vein vitamin C concentrations was used for statistical calculation, but all values are shown in the figure.

RESULTS

On adrenal vein catheterization, blood samples were successfully obtained from both adrenal glands in 21 patients and from one adrenal gland in 5 patients. Twenty-one patients had unilateral adrenal adenoma and 5 had bilateral adrenal hyperplasia. For this study, samples were assayed for vitamin C and cortisol. After ACTH stimulation, adrenal vein vitamin C concentrations increased in every adrenal vein sampled (**Figure 1**). The highest values, mean ± SD concentrations of 176 ± 71 μmol/L, were reached between 1 and 4 min, and they were significantly ($P <$ 0.0001) higher than corresponding peripheral plasma vitamin C concentrations of 35 ± 15 μmol/L. Repeated-measures ANOVA of adrenal vein vitamin C concentrations gave a Huynh-Feldt–corrected *P* value of <0.0001. Mean adrenal vein vitamin C

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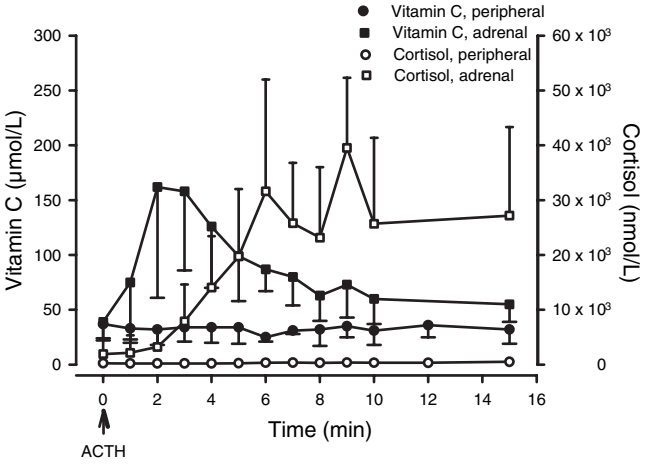

FIGURE 2. Mean (±SD) vitamin C and cortisol concentrations in the peripheral and adrenal veins of all patients studied ($n = 26$). Adrenal vitamin C concentrations were measured in the right and left adrenals in most but not all subjects. When adrenal vitamin C concentrations were available from only one side, values from that side were used for statistical calculations, but the mean of the right and left adrenal vein vitamin C concentrations was used when both were available. Repeated-measures ANOVA of adrenal vein vitamin C concentrations gave a Huynh-Feldt–corrected P value of $<$ 0.0001. Mean adrenal vein vitamin C increased from 39 ± 15 μmol/L at 0 min to 162 ± 101 μmol/L at 2 min and returned to 55 ± 16 μmol/L at 15 min. Bonferroni-adjusted adrenal vein vitamin C concentrations at 2 min were significantly greater than those at 0, 6, 8, 10, and 15 min. The mean highest value was 4.1-fold the mean lowest value. Peripheral vein vitamin C concentrations were obtained for all patients. Repeated-measures ANOVA gave a Huynh-Feldt–corrected P value of 0.002 for peripheral vein vitamin C concentrations, but values varied by only 8–16%, and the direction of change was inconsistent. Because we studied these patients for only 15 min, whereas peripheral vein cortisol concentrations would continue to increase for much longer, we compared 0- and 15-min cortisol values. Adrenal vein cortisol increased from 1923 ± 2806 nmol/L at 0 min to 27 191 ± 16 161 nmol/L at 15 min ($P < 0.0001$ paired t test). Peripheral plasma cortisol increased from 250 ± 119 nmol/L at 0 min to 506 ± 189 nmol/L at 15 min ($P < 0.0001$, paired t test).

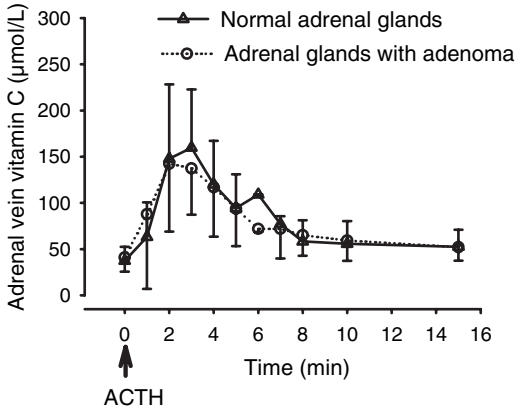

FIGURE 3. Mean (±SD) adrenal vein vitamin C concentrations for all patients ($n = 21$) on the side with the normal adrenal gland and the side with an adrenal adenoma. The area under the curve of adrenal vein vitamin C concentrations in these 2 groups did not differ significantly ($P = 0.57$, unpaired t test).

increased from 39 ± 15 μmol/L at 0 min to 162 ± 101 μmol/L at 2 min and returned to 55 ± 16 μmol/L at 15 min (**Figure 2**). Bonferroni-adjusted adrenal vein vitamin C concentrations at 2 min were significantly greater than those at 0, 6, 8, 10, and 15 min. Peripheral vein vitamin C concentrations showed a significant change ($P = 0.002$ by repeated-measures ANOVA with Huynh-Feldt correction), but the direction of change was inconsistent and its magnitude was small (range: 32–37 μmol/L). Adrenal vein cortisol concentrations increased from 1923 ± 2806 nmol/L at 0 min to 27 191 ± 16 161 nmol/L at 15 min ($P < 0.0001$). Peripheral plasma cortisol increased from 250 ± 119 nmol/L at 0 min to 506 ± 189 nmol/L at 15 min ($P < 0.0001$) (**Figure 3**).

Because adrenal vein catheterization is an invasive procedure with a small risk of serious complications, vitamin C secretion in healthy persons was not studied. To address whether normal adrenal glands secrete vitamin C and, if so, to determine whether it differs from abnormal adrenal glands with respect to vitamin C secretion, we compared ascorbic acid secretion from the 21 patients with unilateral adrenal adenomas with the contralateral normal adrenals in the same patients (Figure 3). The data show that ascorbic acid secretion did not differ significantly according to whether adrenal adenoma was present or not. Peak adrenal vein vitamin C and cortisol concentrations were strongly correlated in all adrenals ($r^2 = 0.35$, $P < 0.001$; **Figure 4**). The correlations between peak adrenal vein vitamin C and cortisol for normal adrenal glands, for adrenal glands with adenoma, and for hyperplastic adrenal glands are shown. Analysis of covariance with interaction to test whether the 3 slopes were equal found no significant difference between the 3 slopes.

DISCUSSION

These data are the first description in any species of simultaneous adrenal vein and peripheral vitamin C concentrations after ACTH stimulation and the first to indicate that the putative function of secreted vitamin C must be local rather than systemic. After ACTH stimulation, peak adrenal vitamin C concentrations (176 ± 71 μmol/L) were significantly ($P < 0.0001$) higher than matched peripheral vein vitamin C concentrations (35 ± 15

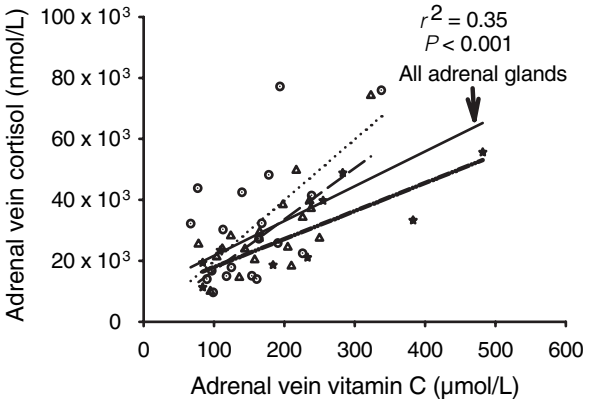

FIGURE 4. Correlation between the highest vitamin C and cortisol concentration reached in each of the sampled adrenal veins for all the adrenal glands sampled (—). Correlation between peak vitamin C and cortisol for each of the adrenal glands is also shown for normal adrenal glands (△), for adrenal glands with adenoma (◎), and for hyperplastic adrenal glands (★). The relation between peak adrenal vein vitamin C and cortisol for normal adrenal glands (– –), for adrenal glands harboring an adenoma (···), and for hyperplasatic adrenal glands (— · · —) are shown. ANCOVA with interaction to test whether the 3 slopes were equal showed no significant difference between the 3 slopes.

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μmol/L). This was not a sustained increase but rather a secretory peak, and the highest mean value at 2 min was significantly greater than the values at 0, 6, 8, 10, and 15 min. Such a peak did not occur in the peripheral vein and could have only a local action in the adrenal gland. Cortisol release was clearly preceded by vitamin C release, which was waning as cortisol release increased. Small variations seen in the peripheral vein vitamin C concentrations were inconsistent in direction and much smaller in magnitude than those that follow normal meals. Hence, these variations are unlikely to have any physiologic significance.

A rapid increase in adrenal vein but not peripheral vein vitamin C concentrations provides several novel insights. One insight is that, in humans, adrenal vitamin C secretion is an integral part of the stress response. The function of released vitamin C in the stress response is unknown, but it may include the quenching of oxidants released during steroidogenesis (14); nitric oxide protection or synthesis to promote cortisol release (15) or local vasodilation, which may increase cortisol delivery to the medulla, the vena cava, or both; or the modification of ACTH receptor sensitivity. In addition, part of medullary blood originates in the adrenal cortex and is enriched with cortisol and vitamin C secreted by the adrenal cortex. Vitamin C is a cofactor necessary for the synthesis of norepinephrine localized to the adrenal medulla, whereas cortisol increases epinephrine biosynthesis from norepinephrine in adrenal medulla by up-regulating phenylethanolamine-*N*-methyltransferase. The local medullary vitamin C concentrations resulting from ACTH-induced vitamin C release may ensure that norepinephrine synthesis always proceeds at maximum velocity ($\dot{V}_{max}$) (16, 17). Because norepinephrine is the substrate for epinephrine synthesis, and because local cortisol may up-regulate phenylethanolamine-*N*-methyltransferase, the combined effects of vitamin C– and cortisol-enriched blood from adrenal cortex could also ensure that epinephrine synthesis proceeds at $\dot{V}_{max}$ in the adrenal medulla (4, 18).

Another insight, supported by the new data presented here, is the concept that one purpose of tight control of plasma vitamin C concentrations is to allow much higher local intraadrenal concentrations to occur transiently. When vitamin C is obtained from foods, despite varied dietary intakes, fasting steady-state plasma vitamin C concentrations do not exceed 70–80 μmol/L in humans (5–7). In another insight, as shown here, the function of released vitamin C must be local, within adrenals, rather than systemic. Furthermore, because of blood sampling limitations, the measured concentrations very likely underestimate true intraadrenal concentrations. Sampled blood reflects the dilution of adrenal vein outflow that is due to catheter placement. Ascorbate released within adrenal is diluted in an increasing venous blood volume before reaching the catheter. Thus, tight control of peripheral plasma vitamin C concentrations may permit the occurrence of much higher concentrations of locally released vitamin, and such concentrations may have special functions. As a corollary and as another novel insight, we show, for the first time in humans, hormone-stimulated secretion of any vitamin, not just vitamin C. These data indicate that a substance that is an essential nutrient may also have unanticipated paracrine or local hormone-like properties.

Adrenal vein catheterization is a technically challenging procedure, further complicated by variations in adrenal vein drainage. It is often unclear whether low cortisol concentrations in the adrenal vein blood result from catheter displacement or some other reason. Measurement of adrenal vein vitamin C concentration is useful as an additional measure of catheter placement and is so used at our institution now. Efforts are underway to develop a rapid vitamin C assay that will give an answer while the patient is still on the table—ie, before catheterization has ended.

If adrenal vitamin C secretion has physiologic consequences, consideration should be given to vitamin C intake above that possible from foods alone. Vitamin C supplements of 1 g, taken twice daily as a supplement, can produce transient peak plasma concentrations of ≈140 μmol/L. Higher doses taken more frequently—eg, every 4–6 h—may produce transient peak plasma concentrations approaching 200 μmol/L, and the average concentrations would be only slightly lower (7) These concentrations are possible only from either oral supplements or intravenous injection; would be expected to be distributed uniformly in plasma, including adrenal veins; and simulate some concentrations measured in adrenal vein samples in this study. However, these concentrations do not reflect the higher intraadrenal concentrations expected with ACTH-induced vitamin C release. It is not known whether such concentrations produced by supplements will have inadvertent paracrine signaling consequences. Finally, we cannot determine from the data presented here whether vitamin C secretion occurs during episodic ACTH secretion by the pituitary gland.

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The authors' responsibilities are as follows—JLD, JG, and ML: study concept and design; SJP, JLD, RC, WY, DAP, and ML: data collection and analysis and interpretation of results; SJP and ML: writing of the manuscript; and all authors (except JLD, who is deceased) reviewed the final manuscript. The funding source had no role in study design, collection, analysis and interpretation of data, or in writing or in submitting the paper for publication. None of the authors had a personal or financial conflict of interest.

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