Effects of the Dual Endothelin Receptor Antagonist Aprocitentan on Body Weight and Fluid Homeostasis in Healthy Subjects on a High Sodium Diet

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Aprocitentan is a novel, oral, dual endothelin receptor antagonist (ERA) in development in difficult-to-control hypertension. As fluid retention and edema are concerns with ERAs, we investigated whether aprocitentan causes weight gain in healthy subjects on a high sodium diet and explored potential mechanisms if occurring. This double-blind, randomized, placebo-controlled, crossover study enrolled 28 subjects. Three doses of aprocitentan (10, 25, or 50 mg/day for 9 days) were compared with placebo. Increases in body weight were observed with aprocitentan (placebo-corrected mean weight gains [90% confidence interval]) of 0.43 [0.05–0.80], 0.77 [0.03–1.51], and 0.83 [0.33–1.32] kg at 10 mg, 25 mg, and 50 mg, respectively. Decreases in hemoglobin and uric acid were observed. Plasma volume increased at most by 5.5% without dose-response relationship. Urinary sodium excretion decreased at 10 mg and 25 mg but not at 50 mg. Therefore, aprocitentan produced moderate weight increases in healthy subjects on high sodium diet, without obvious sodium retention.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Aprocitentan is a new dual endothelin (ET) A/ET B receptor antagonist developed for the treatment of difficult-to-control (resistant hypertension).

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ We evaluated the impact of aprocitentan on body weight, body fluid, and electrolyte homeostasis in healthy subjects on a high salt diet.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Aprocitentan induces a moderate increase in body weight in healthy subjects on a high sodium intake, at doses between 10 mg and 50 mg. No dose-dependent sodium retention was observed.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Aprocitentan might be an effective and well-tolerated new treatment of difficult-to-control (resistant) hypertension.

The endothelin (ET) system plays an important role in the regulation of systemic vascular tone.¹ In the kidney, ET-1 and its receptors (ET A and ET B) are present in the vasculature and in almost all renal cell types. The ET system is not only a key regulator of renal blood flow but also a modulator of glomerular hemodynamics² and sodium and water homeostasis.³ An activated ET system has been shown to exacerbate proteinuria, increase glomerular capillary permeability, and presumably lead to glomerular hypertension.³ Through multiple renal effects, the ET system could contribute to the development of various forms of salt-sensitive hypertension.¹,³ The ET system has also been recognized as a key pathogenic mechanism in the progression of chronic kidney diseases (CKD).³ Thus, recent developments in our understanding of the role of the ET system in regulating blood pressure (BP) and renal functions have provided strong arguments supporting the clinical development of endothelin receptor antagonists (ERAs) for the treatment of hypertension⁴ and/or the prevention of diabetic and nondiabetic nephropathies.⁴,⁷

Several clinical studies have demonstrated that blockade of the ET system lowers BP in patients with mild to moderate hypertension.⁸,⁹ ET receptor blockade was also effective in patients with resistant hypertension.¹⁰,¹¹ Injection of exogenous ET-1 caused natriuresis via ET B receptors.¹² However, this apparent protective effect of ET B receptor activation was not confirmed,
as ET_A-selective antagonists were found to induce sodium retention. Furthermore, studies using exogenous ET-1 injection are difficult to interpret, as they are confounded by the unnatural location of injection (endogenous ET-1 is mostly produced in a polarized fashion toward the tissue and not the blood). Moreover, studies using ET_A-selective ERAs are confounded by the fact that ET_B selective antagonists increase circulating ET-1 levels, which can then cause ET_A receptor activation.

Fluid retention leading to peripheral edema is a common and well-recognized side effect of ERAs. Fluid retention has often impaired the development of ERAs in clinical conditions other than pulmonary arterial hypertension, or essential arterial hypertension. In healthy subjects on a high sodium diet (HSD), avosentan, an ET_A-selective receptor antagonist, caused significant, dose-dependent sodium and water retention leading to weight gain after repeated administration. Additionally, the phase III ASCEND study investigating avosentan in patients with diabetic nephropathy was prematurely terminated due to an excess of cardiovascular events with avosentan (mainly congestive heart failure and fluid overload) and a trend toward an increased mortality. A more recent study using another ET_A-selective ERA, atrasentan, demonstrated beneficial effects on the progression of diabetic kidney disease, however, with a moderate increase in the rate of hospitalization for heart failure. The intensity of fluid retention and its clinical impact vary among ERAs as some led to worsening of heart failure and excess death, whereas others did not. A recent meta-analysis reported that the occurrence and severity of fluid retention depend on the ET_A/ET_B receptor selectivity as well as on the presence or absence of concomitant diseases favoring the development of fluid retention, such as congestive heart failure or CKD.

Aprocitentan (ACT-132577) is a dual ERA that potently inhibits the binding of ET-1 to both ET_A and ET_B receptors. The tolerability, safety, pharmacokinetics, and pharmacodynamics of aprocitentan were investigated in a first-in-human study. Aprocitentan administered once a day was well-tolerated up to 600 mg as single dose and 100 mg as multiple doses. The pharmacokinetic profile of aprocitentan was dose-proportional with a half-life of ~44 hours. The efficacy, safety, and tolerability of aprocitentan were then investigated as monotherapy in patients with essential hypertension in a multicenter phase II dose finding study (ClinicalTrials.gov identifier: NCT02603809). Aprocitentan (5–50 mg) decreased BP in a dose-dependent fashion with a maximal effect of 9.9 ± 2.5 mmHg on systolic BP at 25 mg. Aprocitentan produced dose-dependent decreases in hemoglobin, hematocrit, albumin, and uric acid, and an increase in estimated plasma volume, but no change in weight vs. placebo. The overall frequency of adverse events (AEs) was similar between aprocitentan and placebo. No deleterious sign of fluid retention was observed and very few patients gained significant weight under treatment.

The primary aim of the present study was to evaluate the impact of aprocitentan on body weight in healthy subjects on an HSD, in accordance with a setting previously used to uncover fluid retention induced by avosentan. Three doses (10 mg, 25 mg, and 50 mg) of aprocitentan corresponding to the higher doses tested in a phase II study were administered to normotensive male subjects on an HSD in a double-blind, randomized, placebo-controlled, two-way crossover study design. In addition, acute and sustained renal and hormonal responses to aprocitentan were explored to identify any potential mechanisms that could explain fluid retention.

**Subjects and Methods**

**Subjects**

Healthy male subjects (18–45 years, body mass index between 20.0 and 25.0 kg/m²) were enrolled in this study. They were considered healthy based on medical history, physical examination, cardiovascular assessments, and hematology, clinical chemistry, and urinalysis tests, all assessed at a screening visit. The study protocol was reviewed and approved by the investigational review board (Ethics Committee of the Canton de Vaud, Lausanne, Switzerland) and by Swissmedic (Swiss Health Authority). The study was registered with the EudraCT number 2016-000138-24 and ClinicalTrials.gov identifier NCT02708004. Written consent was obtained from each subject after the nature, purpose, and potential risks of the study were explained. The trial was performed in accordance with the Declaration of Helsinki.

**Study design**

This was a single-center, double-blind, randomized, placebo-controlled, two-way crossover study conducted in the Service of Nephrology and Hypertension at the University Hospital of Lausanne, Switzerland. Figure S1 shows a schematic representation of the study design.

Each subject received two treatments (i.e., aprocitentan 10 mg, 25 mg, or 50 mg once daily and placebo (P)) according to a crossover design. Thus, subjects were randomized to one of the following 6 sequences: 10 mg/P, 10 mg/P, 25 mg/25 mg/P, and 50 mg/P. The aprocitentan and placebo phases were separated by 10–12 days without treatment until the end of each study period and a washout period of at least 7 days. From day 1 to day 9, subjects had to visit the investigational site under fasted condition every morning to receive the study treatment and sodium tablets to maintain the HSD. They were discharged after the assessments planned for each visit had been performed (see below).

The HSD started 3 days before the first study treatment administration and was maintained during the study. For this purpose, subjects received salt tablets to be taken with breakfast (2 g) and lunch (2 g). This resulted in an additional daily salt load of 4 g sodium chloride (i.e., ~66.7 mmol sodium). Diet compliance was evaluated by means of repeated 24-hour urine collections on days 3, 5, and 8. Smoking, consumption of grapefruit or grapefruit juice, alcohol-containing and xanthine-containing beverages, and concomitant drugs were forbidden during the study. The detailed procedures of investigations and the methods used to calculate urinary parameters and hormones are presented in the Supplemental Material (Methods and Results).

**Statistical plan**

The primary end point on which the power of the study was calculated was the change in body weight from baseline to day 9. It was to be demonstrated that at least one aprocitentan dose was noninferior to placebo with respect to change from baseline to day 9 in body weight, defined as the upper limit of the 90% confidence interval (CI) for the difference excluding 1 kg. To protect the overall type I error (0.05, one-sided) in the presence of multiple testing, a hierarchical approach was used for statistical inference of the primary end point. A separate model was created for each dose (10 mg, 25 mg, and 50 mg), with the testing done in the following order: 10 mg, followed by 25 mg, and then 50 mg as the last group. If for a given dose noninferiority compared with placebo could...
not be demonstrated, then noninferiority was not concluded for higher dose(s). The analysis set comprised all subjects who did not deviate from the protocol in a way that might affect the evaluation of the study drug on the primary end point. Baseline to day 9 changes in body weight between placebo and aprocitentan were evaluated using mixed-effects models, including treatment, period, and sequence as fixed effects, and subject as random effect for each dose level separately. Based on the mixed-effects model, least square means difference was derived, including its 90% CI. If the upper limit of least square means CI (aprocitentan − placebo) was lower than one, noninferiority was concluded for the dose under consideration. With 8 subjects per dose level, the power of the study was > 80%, assuming a true mean delta = 0 for all 3 dose levels.

All other measured parameters were analyzed using descriptive statistics, including mean, median, SD, SE, and 95% CI minimum/maximum.

RESULTS
Twenty-eight male subjects were randomized: one subject withdrew during the study, two were withdrawn due to AEs, and two were excluded because of nonadherence to the protocol. Thus, 23 subjects completed the study (8 in the 10 mg, 7 in the 25 mg, and 8 in the 50 mg group) and were included in the per-protocol analysis set. Their mean age was 28.9 years (range 21–45 years) and mean weight was 72.7 kg (range 57.5–87.0 kg). Mean body mass index was 22.7 kg/m² (range 20.0–25.0 kg/m²).

Effect of aprocitentan on body weight
The primary end point was the change from baseline to day 9 in body weight. It was to be demonstrated that at least one tested dose of aprocitentan was noninferior to placebo with respect to weight gain. Noninferiority was met for the 10 mg dose. Noninferiority was not concluded for the 25 mg and 50 mg aprocitentan doses as the upper level of the 90% CI exceeded 1 kg for both the 25 mg and 50 mg doses. From day 1 to day 9, mean placebo-corrected weight gains were 0.43 kg (90% CI: 0.05–0.80) for the 10 mg, 0.77 kg (90% CI: 0.03–1.51) for the 25 mg, and 0.83 kg (90% CI: 0.33–1.32) for the 50 mg dose (Figure 1). The number of subjects with a weight gain > 1 kg was 2, 2, and 3 in the 10 mg, 25 mg, and 50 mg aprocitentan groups, respectively, during periods on active treatment, and 2 in the 50 mg aprocitentan group during the period on placebo.

Effect of aprocitentan on plasma volume
From day 1 (baseline) to day 9 (predose), significant decreases in mean hemoglobin were observed with the 3 doses of aprocitentan. The mean placebo-corrected changes in hemoglobin were −3.1 g/L with 10 mg, −3.3 g/L with 25 mg, and −5.5 g/L with 50 mg of aprocitentan (Figure 2). Placebo-corrected changes in mean hematocrit were small and not dose-dependent, (i.e., −0.75, −1.57, and −0.25%) with the 10 mg, 25 mg, and 50 mg aprocitentan doses, respectively. The changes in hemoglobin and hematocrit likely represent hemodilution (and not a loss of red blood cells). A calculation of estimated mean changes in plasma volume based on hemoglobin and hematocrit changes, according to Strauss’ formula, resulted in estimated placebo-corrected increases in plasma volume from baseline to day 9 of 3.6, 5.5, and 4.7% for aprocitentan 10 mg, 25 mg, and 50 mg, respectively. These increases were not dose-dependent.

Effect of aprocitentan on renal electrolytes and water excretion
On days 1 and 9, the changes in plasma electrolytes and the drug-induced changes in urinary excretion of sodium, chloride, potassium, and lithium were explored during the 2 hours preceding the drug/placebo administration and for 10 hours thereafter. Administration of aprocitentan did not affect plasma electrolytes. In particular, plasma sodium concentrations remained stable over the duration of the study (Table S1). The urinary excretion rate of sodium in µmol/min (UNaV) over 10 hours was high due to the HSD, with a large variability between subjects. Compared with placebo, nonsignificant decreases in UNaV were observed on day 1 and day 9 after 10 mg and the 25 mg doses of aprocitentan (Table 1). In contrast, 50 mg aprocitentan increased urinary sodium excretion during the first 4 hours (Table 1). When cumulative 10-hour urinary sodium excretion was calculated, a trend to a retention of sodium and chloride was found after 10 mg and 25 mg aprocitentan, without a clear association with duration of treatment. In contrast, there was no evidence of sodium retention after 50 mg aprocitentan, neither on day 1 nor on day 9 (Table 1). No significant changes in urinary potassium were observed. No decrease in lithium clearance suggesting sodium retention at the renal proximal tubule was found at any dose (Table S2).

We also assessed the effect of aprocitentan on urinary water excretion on days 1 and 9 by calculating the total volume of urine eliminated from time 0 to 10 hours. Under placebo, the mean 10-hour urinary volume was relatively stable, ranging between 1,700 and 2,000 mL/10 hours in subjects randomized to the 3 groups. No significant difference between placebo and aprocitentan was found after 10 mg and 25 mg aprocitentan. At 50 mg aprocitentan, urinary volume excretion increased by 200 mL/10 hours on day 1 and by 300 mL/10 hours on day 9 as compared with the placebo phase (Figure 3). This increase in urinary volume was in accordance with a significant decrease in urinary osmolality (Figure S2) and an increase in calculated free water clearance (Figure S3). This excretion of diluted urine on 50 mg aprocitentan was in line with a decrease in plasma copeptin revealing a reduced secretion of antidiuretic hormone (see below).
Effect of aprocitentan on hormonal markers

Due to the HSD and the hydration protocol, baseline plasma renin activity (PRA) and plasma aldosterone levels were low, means between 0.17 and 0.55 ng/mL/h for PRA and 20 to 38 pg/mL for aldosterone. Overall, no clear dose-related or treatment-related pattern of changes in PRA and aldosterone was observed after aprocitentan on day 1 and day 9 (Figure 4). At the dose level of 50 mg, aprocitentan decreased plasma aldosterone and copeptin compared with placebo, but variability was high. The decrease in copeptin indicates a reduction in antidiuretic hormone and is consistent with the excretion of diluted urine. There was no significant change in plasma renin activity, aldosterone, and copeptin on day 1 and day 9 are shown in Table S3.

Tolerability profile

In this study, all tested doses of aprocitentan (10 mg, 25 mg, and 50 mg) were well-tolerated. No serious AEs were reported. The most frequently reported AE was headache (6 of 8 subjects (75.0%) on 50 mg aprocitentan), which was reported more frequently with increasing dose of aprocitentan. However, headache was also mentioned in about one-third of subjects on placebo. Two subjects withdrew. No AEs related to fluid retention were reported during treatment with aprocitentan. Of note, no subject developed peripheral edema or any other sign or symptom of fluid overload. Beyond the decrease in hemoglobin described above, no treatment-related patterns were observed for hematology, clinical laboratory, vital signs, or electrocardiogram variables for any treatment. Aprocitentan decreased serum uric acid from day 1 to day 9 (Supplementary Text Material). No consistent change in blood pressure and creatinine clearance were observed (Table S4).

DISCUSSION

The results of this mechanistic study in healthy subjects on an HSD show that aprocitentan induces a moderate (i.e., less than 1 kg) but statistically significant increase in body weight, which could be suggestive of fluid retention. The weight gain was associated with signs of hemodilution, such as a dose-dependent decrease in hemoglobin and a modest decrease in hematocrit. However, we did not find evidence of a marked increased reabsorption of sodium with aprocitentan. After administration of 50 mg

Table 1 Arithmetic mean (SD) UNaV and cumulative 10-hour sodium excretion in healthy normotensive subjects on a high sodium diet before and after administration of 10 mg, 25 mg, and 50 mg aprocitentan

|            | Aprocitentan 10 mg (n = 8) | Placebo (n = 8) | Aprocitentan 25 mg (n = 7) | Placebo (n = 7) | Aprocitentan 50 mg (n = 8) | Placebo (n = 8) |
|------------|-----------------------------|-----------------|-----------------------------|-----------------|-----------------------------|-----------------|
| UNaV, μmol/min |                             |                 |                             |                 |                             |                 |
| Day 1      |                             |                 |                             |                 |                             |                 |
| Baseline   | 316 (220)                   | 275 (142)       | 326 (162)                   | 227 (94)        | 262 (144)                   | 200 (102)       |
| 4 hours    | 284 (125)                   | 277 (116)       | 300 (110)                   | 238 (62)        | 325 (102)                   | 209 (60)        |
| 10 hours   | 243 (93)                    | 261 (112)       | 243 (99)                    | 244 (80)        | 225 (75)                    | 184 (46)        |
| Day 9      |                             |                 |                             |                 |                             |                 |
| Baseline   | 293 (166)                   | 265 (82)        | 281 (159)                   | 284 (96)        | 269 (135)                   | 255 (152)       |
| 4 hours    | 307 (126)                   | 389 (99)        | 319 (81)                    | 277 (126)       | 304 (111)                   | 239 (140)       |
| 10 hours   | 282 (97)                    | 297 (104)       | 255 (66)                    | 298 (78)        | 237 (65)                    | 240 (69)        |
| Cumulative 10-hour sodium excretion, mmoles |                             |                 |                             |                 |                             |                 |
| Day 1      | −27.8 (109)                 | 6.4 (44)        | −16.9 (58)                  | 22 (30)         | 2.42 (72)                   | −0.18 (41)      |
|            | NS vs. placebo              |                 | P = 0.07 vs. placebo        |                 | NS vs. placebo              |                 |
| Day 9      | −16.8 (122)                 | 34.6 (77)       | −23.6 (75)                  | 42 (83)         | 6.20 (104)                  | 17.8 (68)       |
|            | P = 0.09 vs. placebo        |                 | NS vs. placebo              |                 | NS vs. placebo              |                 |

NS, not significant; UNaV, urinary sodium excretion rate.
aprocitentan, increases in water excretion and free water clearance associated with decreases in plasma aldosterone and copeptin levels were observed, particularly on day 9. In this short-lasting study, the tolerability profile of aprocitentan was good, mild to moderate headache being the only consistent AE reported. No subject developed peripheral edema.

Fluid retention, which is considered as a classic side effect of ERAs, has been reported to occur with ETA-selective and to a lesser extent with dual ETₐ/ETₐ receptor antagonists. However, no head-to-head study has been performed. In some patients, fluid retention after ETA-selective antagonists has led to peripheral edema and/or pulmonary edema and heart failure. At high doses, severe hyponatremia has been reported with atrasentan. Although the mechanisms of this fluid retention are not completely understood, two mechanisms appear to predominate. The first is water and sodium retention via activation of the renin-angiotensin-aldosterone and vasopressin systems, and the second is an increased vascular permeability leading to a redistribution of body volumes. Preclinical studies in healthy animals showed that moderate but significant vasodilation induced by ETA-selective blockade could trigger a BP decrease and a neuro-hormonal activation associated with increased aldosterone and vasopressin release leading to fluid retention. This was not observed with dual ETₐ/ETₐ blockade, and, in contrast, aldosterone and vasopressin were decreased by dual antagonism. In man, aldosterone was decreased by the ERA bosentan in patients with heart failure. Because of the complex involvement of the ET system in vascular function and sodium homeostasis, and the pharmacology of ETA and ETₐ receptors, we have comprehensively investigated the dose-related effects of aprocitentan on fluid regulation.

In the present study, the administration of aprocitentan, a dual ETₐ/ETₐ receptor antagonist, was associated with an increase in body weight without edema. Mean body weights at baseline were 70 kg, 74 kg, and 79 kg for the 10 mg, 25 mg, and 50 mg groups, respectively. The mean gain in body weight with 25 mg or 50 mg aprocitentan was about 0.8 kg (i.e., 1.0–1.1% overstimulation of the unblocked endothelial ETₐ receptor by endogenous ET-1. Therefore, ETA-selective blockade could favor fluid retention and vascular leakage, and hence increase the risk of edema. However, up to this study, no human mechanistic study had yet been done with a dual ERA.

Direct effects of ET-1 on the renal tubular reabsorption of sodium and water have also been described. ET-1 inhibits water reabsorption through the renal collecting duct leading to an increased diuresis. This effect appears to be mediated by the ETₐ receptor. Regarding the impact on sodium transport, ET-1 induces sodium excretion in the collecting duct under physiological conditions, whereas in the proximal tubule it may either increase or decrease sodium reabsorption depending on the experimental conditions. However, despite these data suggesting a natriuretic role of the ETₐ receptor, clinical studies, including recent results from the SONAR clinical trial, indicate that ETA-selective antagonism is clearly associated with fluid retention. Because of the complex involvement of the ET system in vascular function and sodium homeostasis, and the pharmacology of ETA and ETₐ receptors, we have comprehensively investigated the dose-related effects of aprocitentan on fluid regulation.

Figure 3 Mean cumulative 10 h urinary volume excretion (mL; ± SE) on days 1 and 9 in healthy normotensive subjects on a high sodium diet after administration of 10 mg, 25 mg, and 50 mg aprocitentan.
of body weight). It should be noted that, according to the protocol, 24-hour sodium excretion was assessed on days 3, 5, and 8 to ascertain adherence to the HSD, but subjects’ food intake and sodium diet between days 1 and 9 were not fully controlled except for the addition of salt tablets. Some modest renal sodium retention was observed with the 10 mg and 25 mg doses of aprocitentan, but surprisingly an increase in renal sodium elimination was observed at the highest dose of 50 mg. There is, therefore, no correlation between the increase in body weight and renal sodium excretion. On the contrary, the observed increases in body weight correspond to the estimated increases in plasma volume that were observed in the study, suggesting an increase in the extracellular water and sodium content of 5%. Interestingly, a comparable increase in plasma volume was estimated in the aprocitentan phase II study in hypertension, based on the decreases in hemoglobin and hematocrit induced by aprocitentan.22 A moderate increase of 5% extracellular fluid, representing fluid retention, can be observed with typical antihypertensive vasodilators, such as prazosin, for which it represents a redistribution of fluids due to expansion of the intravascular volume and the decrease in Starling forces in the microcirculation, followed by enough sodium retention to replenish the vascular tree. This increase is usually insufficient to produce marked peripheral edema, but it is recommended to use this alpha-blocker preferentially with diuretics.29 A similar approach may be taken for endothelin antagonists.

Interestingly, the weight gain was not homogenously distributed among all subjects. Indeed, some subjects exhibited a marked increase in body weight, by no more than 2 kg, whereas others showed no change in body weight during the study. There is no clear explanation for this heterogeneous response to aprocitentan, at least in healthy subjects. In the phase II trial in patients with hypertension, very few patients developed a significant weight gain during the 8 weeks of treatment.22 In patients with a high risk of developing peripheral edema, such as patients with left ventricular dysfunction, congestive heart failure, or CKDs, the occurrence of fluid retention and peripheral edema upon administration of ERAs appears to be more frequent,15 even with compounds inducing little if any peripheral edema in clinical trials, such as macitentan.19 Phase III studies will provide more information on the incidence and clinical impact of the weight gain in patients. This is true also for the incidence of headaches. Although the mean increase in body weight with aprocitentan was similar to the increase observed under almost identical experimental conditions with the ET₃ selective receptor antagonist avosentan at 50 mg, a major difference between the 2 studies is the absence of a clear dose-dependent sodium retention effect with aprocitentan, contrary to avosentan.13

Figure 4 Change between predose day 9 and predose day 1 (± SD) for aldosterone, copeptin, and plasma renin activity in healthy normotensive subjects on a high sodium diet after administration of 10 mg, 25 mg, and 50 mg aprocitentan.
As reported previously in clinical studies performed with ERAs, hemoglobin decreased upon administration of aprocitentan. The changes in hemoglobin were evident mainly on day 9 and with the 2 highest doses. The magnitude of the changes induced by 50 mg aprocitentan (−6.9 g/L, not placebo-corrected) was comparable to that found with 50 mg avosentan. For this parameter, the change was consistent among subjects. Decreases in hematocrit were also observed but, as reported with avosentan, changes in hematocrit were less pronounced and not dose-dependent. The mechanism(s) leading to the observed decrease in hemoglobin during ERA treatment are unclear. Today, it is thought to be, at least in part, secondary to increased fluid retention as it develops rapidly (within a week). Alternatively, it could be due to redistribution of fluids between venous and arterial compartments, as suggested in a study in rats. Moreover, so far, no evidence of hemolysis or toxic effect on erythrocyte production has been demonstrated with ERAs. In accordance with previous observations in pulmonary arterial hypertension with bosentan and with the results of the phase II study in hypertension with aprocitentan and the data in healthy subjects with avosentan, a significant dose-dependent decrease in serum uric acid levels was observed in our subjects on day 9. In our study, this decrease was associated with a dose-dependent increase in cumulative 10-hour urinary uric acid excretion as calculated on day 1 and day 9 and thus may be due to a tubular effect of aprocitentan and a decreased reabsorption of uric acid.

As mentioned previously, fluid retention without any changes in plasma sodium implies that aprocitentan, as other ERAs, induced an isotonic fluid retention. This may occur in response to several mechanisms, including a decrease in BP leading to a reduction in renal perfusion, a decrease in GFR, or an increased tubular reabsorption of sodium and water promoted by blockade of renal ET receptors. Renal sodium and water retention could also be the consequence of a marked exsuscitation of fluid, as has been suspected to happen with ERAs. However, in this study, we observed neither a marked reduction of BP nor a significant decrease in GFR that could trigger a significant sodium and water reabsorption.

The ability of ERAs to block more or less specifically ETA and/or ETB receptors appears to have a major impact on the development of fluid retention and fluid redistribution. Thus, in the rat, significant differences in fluid retention and redistribution of fluid between arterial and venous compartments have been shown between an ETA-selective receptor antagonist and a dual antagonist. The ability to redistribute volume in the various body compartments and different effects on sodium handling could explain why fluid retention may be more important with some ERAs than others despite comparable decreases in hemoglobin and hematocrit. In contrast to previous finding with avosentan, there was no clear evidence of a dose-dependent retention of sodium in the subjects receiving aprocitentan when analyzing the urinary sodium excretion rate or the fractional excretion of sodium. Therefore, these two studies conducted with either an ETA-selective or a dual ERA indicate that for a similar body weight gain, differential effects on sodium retention can be observed depending on the ERA selectivity profile. Strikingly, there was a complete absence of any signal of sodium retention after administration of 50 mg aprocitentan despite an almost 1 kg increase in body weight.

Whether aprocitentan induced an extravasation of fluid leading to fluid retention is difficult to conclude from our data. The lack of a marked decrease in hematocrit would be compatible with such mechanisms. However, the vasodilatory effect of aprocitentan in healthy subjects seems to be less marked than that observed with avosentan, that induces a significant decrease in BP in healthy subjects on an HSD. Moreover, in contrast to avosentan, we did not observe any peripheral edema in our subjects. This might be due to a more balanced blockade of ETA and ETB receptors with aprocitentan that prevented an activation by endogenous ET-1 of unblocked ETB receptors, known to trigger vascular leakage via nitric oxide and VEGF release.

In conclusion, our data show that aprocitentan induces a moderate weight gain in healthy subjects associated with signs of hemodilution, decreases in aldosterone and copeptin, and a decrease in uric acid. Considering that aprocitentan is developed for the treatment of difficult-to-control (resistant) hypertension, which implies the administration of an adequate dose of a diuretic, the modest increase in body weight observed in our subjects should not represent a major challenge. However, this remains to be demonstrated in additional prospective studies involving patients with hypertension with comorbidities, such as renal impairment.

SUPPORTING INFORMATION
Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

ACKNOWLEDGMENTS
The intellectual property rights for aprocitentan passed from Actelion Pharmaceuticals Ltd. to Idorsia Pharmaceuticals Ltd. on June 15, 2017. Actelion remained the sponsor of this study after this date, but agreements were put in place for study-related sponsor activities to be transferred to and executed by Idorsia later on. Aprova, Czech Republic, carried out statistical analyses. Authors were responsible for critical revisions of the manuscript and for important intellectual content. The authors wish to thank the members of Idorsia Pharmaceuticals Ltd.; Susanne Globig (bioanalytical work), Alexandre Kupferberg (project management), Pascale Gasser (project management), Denis Boutin (data management), and Adil Aouboukdir (clinical data monitoring). We would like to thank Martine Clozel, Chief Scientific Officer at Idorsia Pharmaceuticals Ltd., for her critical review and pertinent comments of the paper.

FUNDING
This study was supported financially by Idorsia Pharmaceuticals and realized at the Centre Hospitalier Universitaire Vaudois in Lausanne, Switzerland. This work was supported by a research grant from Actelion Pharmaceuticals Ltd., Allschwil, Switzerland.

CONFLICT OF INTEREST
M.B. has received the research grant and consultant fees from Actelion Pharmaceuticals Ltd. and Idorsia Pharmaceuticals Ltd. P.N.S. is an employee of Actelion Pharmaceuticals Ltd. and current employee of Idorsia Pharmaceuticals Ltd. N.G. is an employee of Actelion Pharmaceuticals Ltd. and current employee of Idorsia Pharmaceuticals Ltd. M.S.M. is an employee of Actelion Pharmaceuticals Ltd. and current employee of Idorsia Pharmaceuticals Ltd. M.I. is an employee of Actelion Pharmaceuticals Ltd. and current employee of Idorsia Pharmaceuticals Ltd. L.J. is an employee of Actelion Pharmaceuticals Ltd. and current employee of Idorsia Pharmaceuticals Ltd. All other authors declared no competing interests for this work.
AUTHOR CONTRIBUTIONS

M.B., P.G.M., P.N.S., G.W., M.I., B.F., and J.D. wrote the manuscript. P.N.S., J.D., and M.B. designed the research. P.G.M., G.W., M.P.M., and M.B. performed the research. B.F., M.I., N.G., M.P.M., J.D., P.N.S., and M.B. analyzed the data. M.P.M. contributed new reagentsanalytical tools.

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