Supplemental Material

Table S1. Baseline characteristics of study subjects.

Parameter	Bumetanide (*n* = 14)	Dapagliflozin (*n* = 14)	Bumetanide plus dapagliflozin (*n* = 14)
Age, years			
Mean	30 (7)	30 (7)	31 (7)
Men (*n*)	11	10	11
Race (*n*)			
White	8	9	11
Black	6	5	3
Body weight, kg			
Mean	79.7 (12.0)	76.9 (13.9)	78.5 (10.6)
Body mass index, kg/m^2			
Mean	26.9 (3.4)	26.3 (3.2)	26.3 (3.1)

Mean ± standard deviation.

Table S2. Pharmacokinetic parameters.

	Bumetanide		
	Bumetanide (1 mg)	**Bumetanide (1 mg) plus dapagliflozin (10 mg)**	**Point estimate (90% CI) of ratio of adjusted geometric mean**
C_{max}, adjusted geometric mean, ng/ml	55.0 ± 3.1	62.3 ± 3.6	1.13 (0.98–1.31)
AUC_{tau}, adjusted geometric mean, ng·h/ml	121 ± 10	138 ± 9	1.13 (0.99–1.30)
Median T_{max}, h (min, max)	1.50 (0.50, 2.00)	1.53 (0.50, 2.00)	

	Dapagliflozin		
	Dapagliflozin (10 mg)	**Dapagliflozin plus bumetanide (1mg) (10 mg)**	**Point estimate (90% CI) of ratio of adjusted geometric mean**
C_{max}, adjusted geometric mean, ng/ml	192 ± 17	208 ± 18	1.08 (0.95–1.22)
AUC_{tau} , adjusted geometric mean, ng·h/ml	723 ± 39	757 ± 37	1.05 (0.99–1.11)
Median T_{max}, h (min, max)	0.78 (0.75, 1.50)	0.75 (0.5, 4.00)	

Mean ± SEM values

Table S3. Blood pressure and heart rate.

Parameter	Before (D1)	Dapagliflozin (D8)	Before (D1)	Bumetanide (D8)	Before (D1)	Dapagliflozin + Bumentanide (D8)	Dapagliflozin + Bumentanide (D14)
Systolic BP (mmg)	110 ± 3	104 ± 3	114 ± 3	110 ± 4	109 ± 3	110 ± 3	112 ± 2
Diastolic BP (mmHg)	72 ± 3	72 ± 2	74 ± 2	75 ± 2	73 ± 2	75 ± 2	70 ± 2
Mean BP (mmHg)	84 ± 2	83 ± 3	87 ± 2	87 ± 2	85 ± 2	87 ± 2	84 ± 1
Heart rate (min^{-1})	65 ± 3	78 ± 3*	67 ± 4	77 ± 4*	62 ± 2	77 ± 2***	77 ± 2***

Mean ± SEM values (n-14 per group). Mean BP: Diastolic BP + 1/3 pulse pressure. Comparing D8 or D14 with D1: *p<0.05; ***p<0.005

Table S4. Renal Excretion during First Day of Administration of Dapagliflozin and After One Week of Adaptation to Bumetanide.

Parameter (excretion)	Before (D-1)	Dapagliflozin Alone (D1)	Difference (D-1 to D1)	Before Bumetanide (D7)	Dapagliflozin Added to Bumetanide (D8)	Difference (D7 to D8)	Difference in Response to Dapagliflozin (D1 and D8)
Volume ($ml \cdot 24\ h^{-1}$)	2127 ±264	2928 ±516	+800 * ±327*	2071 ±232	2757 ±247	+686 ±120***	NS
Osmoles ($osm \cdot kg^{-1}$)	248 ±22	325 ±36	+78 ±26*	254 ±18	314 ±22	+60 ±17***	NS
Glucose ($g \cdot 24\ hr^{-1}$)	0.1 ±0.01	37.1 ±2.9	+37.0 ±2.9***	0 ±0.01	31.3 ±2.9	+31.3 ± 2.9***	NS
Na^+ ($mmol \cdot 24\ h^{-1}$)	65 ±5	87 ±8	+22 ±6***	81 ±4	146 ±6	+64 ±6***	P<0.005
K^+ ($mmol \cdot 24\ h^{-1}$)	43 ±3	62 ±4	+19 ±3***	67 ±5	69 ±4	+3 ±4	NS
Urate ($mg \cdot 24\ h^{-1}$)	415 ±22	626 ±38	+210 ±23***	306 ±27	567 ±41	+261 ±24***	NS

Mean ± SEM values during the day before, and the first day of administration of dapagliflozin (10 mg) on day 1 (D1), or day 8 D (D8) when given on a background of 8 days of bumetanide (1mg) administration.

Compared to before:

***, p<0.05; **, p<0.01; ***, p<0.005**

Table S5. Renal Excretion during First Day of Administration of Bumetanide and After One Week of Adaptation to Dapagliflozin.

Parameter (excretion)	Before (D-1)	Bumetanide Alone (D1)	Difference(D-1 to D1)	Dapagliflozin (D7)	Bumetanide Added to Dapagliflozin (D8)	Difference (D7 to D8)	Difference in Response to Bumetanide (D1 to D8)
Volume	2687	3510	+823	2344	3339	+995	NS
(ml·24 h^{-1})	±344	+1-395	±294*	+391	±528	±217***	
Osmoles	224	221	-4	358	331	-27	P<0.05
(osm·kg^{-1})	±29	±21	±24	±31	±43	±46	
Glucose	0.1	0.1	0	29.5	27.0	-2.4	P<0.005
(g·24 hr^{-1})	±0.01	±0.001	± 0.01	±2.9	±2.9	±1.4	
Na^+	70	143	+74	60	195	+101	P<0.05
(mmol·24 h^{-1})	±8	±9	±7***	±4	±6	±8***	
K^+	47	69	+21	60	71	+11	NS
(mmol·24 h^{-1})	±4	±6	±5***	±4	±5	±5*	
Urate	406	336	-66	404	397	-7	NS
(mg·24 h^{-1})	±19	±24	±30*	±25	±55	±60	

Mean ± SEM values during the day before (D-1), and the first day (D1) of administration of bumetanide (1 mg) on day 1 (D1) or day 8 (D8) when given on a background of 8 days of dapagliflozin (10mg) administration. Compared to before: *, p<0.05; **, p<0.01; ***, p<0.005

Table S6. Renal Excretion during First Day of Administration of Dapagliflozin + Bumetanide and After One Week of Adaptation to the Combined Diuretics.

Parameter (excretion)	Before (D-1)	Both Diuretics Together (D1)	Difference (D-1 to D1)	Before Second Week of combined therapy (D7)	Both Diuretics Together (D8)	Difference (D7 to D8)	Difference in Response to Diuretic (D1 to D8)
Volume ($ml \cdot 24\ h^{-1}$)	2317 ±234	3504 ±357	+1187 ±297***	2861 ±318	2882 ±376	+21 ±123	-622 ±321 NS
Osmoles ($osm \cdot kg^{-1}$)	250 ±27	279 ±25	+17 ±23	295 ±33	302 ±29	+7 ±24	NS
Glucose ($g \cdot 24\ hr^{-1}$)	0.1 ±0.05	27.1 ±2.6	+27 ±3***	21.8 ±3.3	21.8 ±3.0	0 ±2	-5.1 ±2.1
Na^+ ($mmol \cdot 24\ h^{-1}$)	66 ±7	150 ±11	+80 ±5***	95 ±4	111 ±5	+16 ±7*	-43 ±11***
K^+ ($mmol \cdot 24\ h^{-1}$)	56 ±3	75 ±6	+20 ±5***	76 ±5	69 ±5	-7 ±3	-5 ±4 NS
Urate ($mg \cdot 24\ h^{-1}$)	424 ±23	522 ±55	+87 ±51	392 ±28	368 ±29	-25 ±18	-139 ±48*

Mean ± SEM values during the day before (D-1), or day of (D1) the first day of administration of bumetanide (1 mg) plus dapagliflozin (10 mg) and at the beginning of the second week (D7 and D8) of combined therapy. Compared to before: *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$

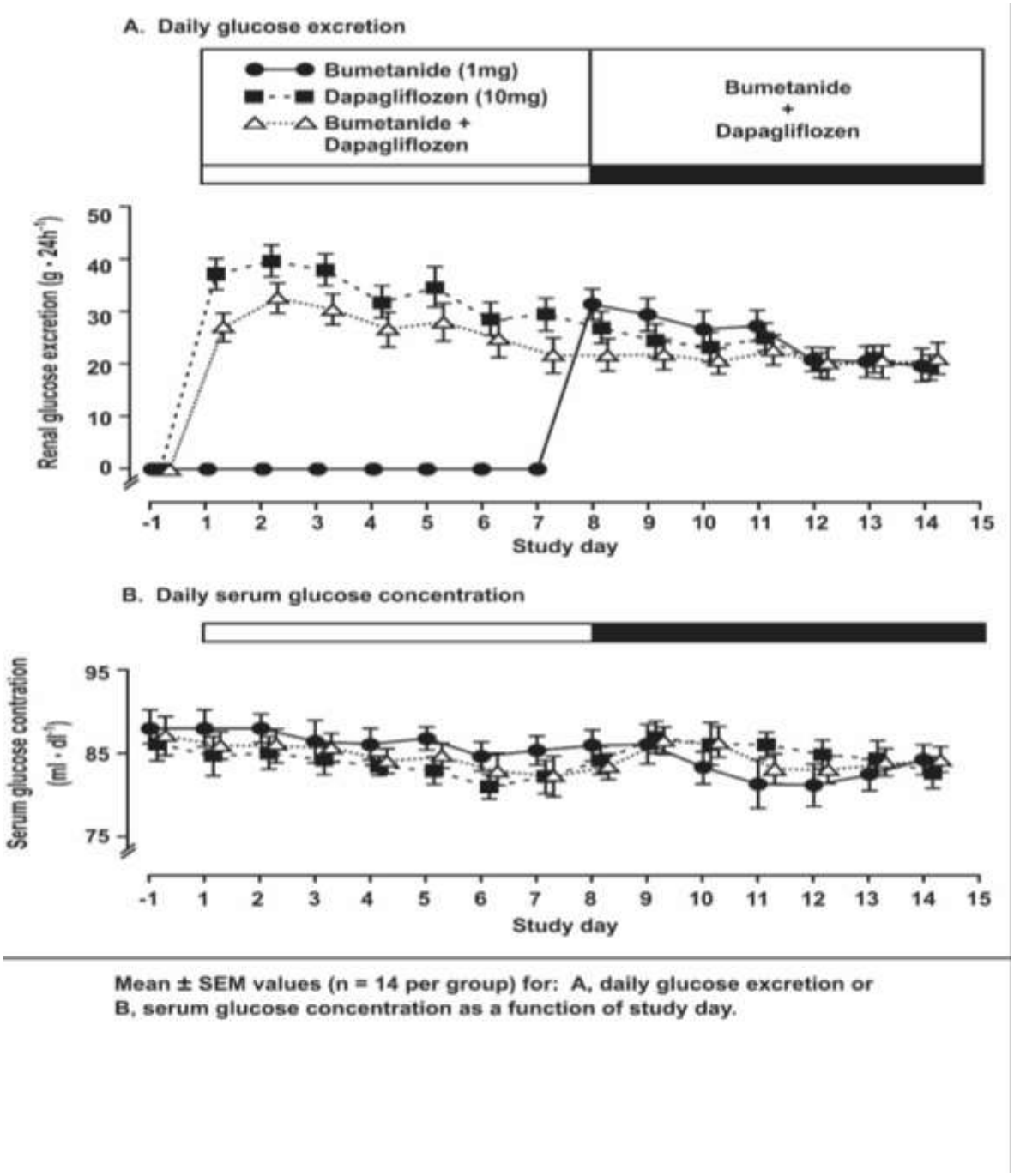
Table S7. Summary of adverse events.

Number of events (%)	Bumetanide (*n* = 14)	Dapagliflozin (*n* = 14)	Bumetanide plus dapagliflozin* (*n* = 41)	All subjects (*n* = 42)
Subjects with an adverse event	6 (43)	7 (50)	22 (54)	29 (69)
Total adverse events	23	13	50	81
Most common adverse events (>10% in any group)				
Abdominal pain	4 (29)	3 (21)	3 (7)	10 (24)
Nausea	3 (21)	2 (14)	7 (17)	10 (24)
Vomiting	2 (14)	1 (7)	1 (2)	4 (10)
Asthenia	4 (29)	2 (14)	5 (12)	10 (24)
Headache	2 (14)	1 (7)	4 (10)	7 (17)
Dizziness	1 (7)	1 (7)	5 (12)	6 (14)

*Includes all subjects who received bumetanide plus dapagliflozin at any time during the study.

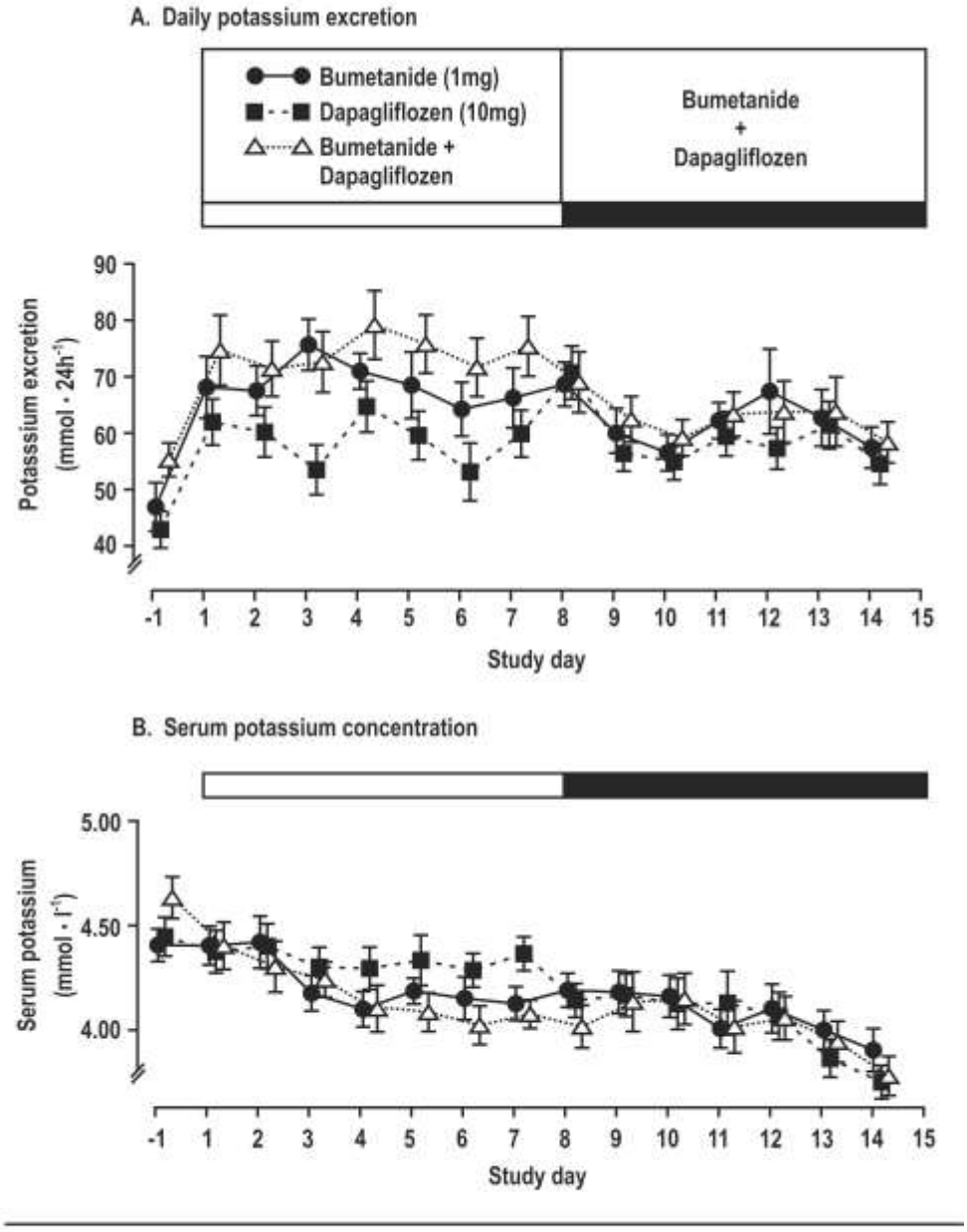
Number (%)

Figure S1. Daily glucose excretion and serum glucose concentration.



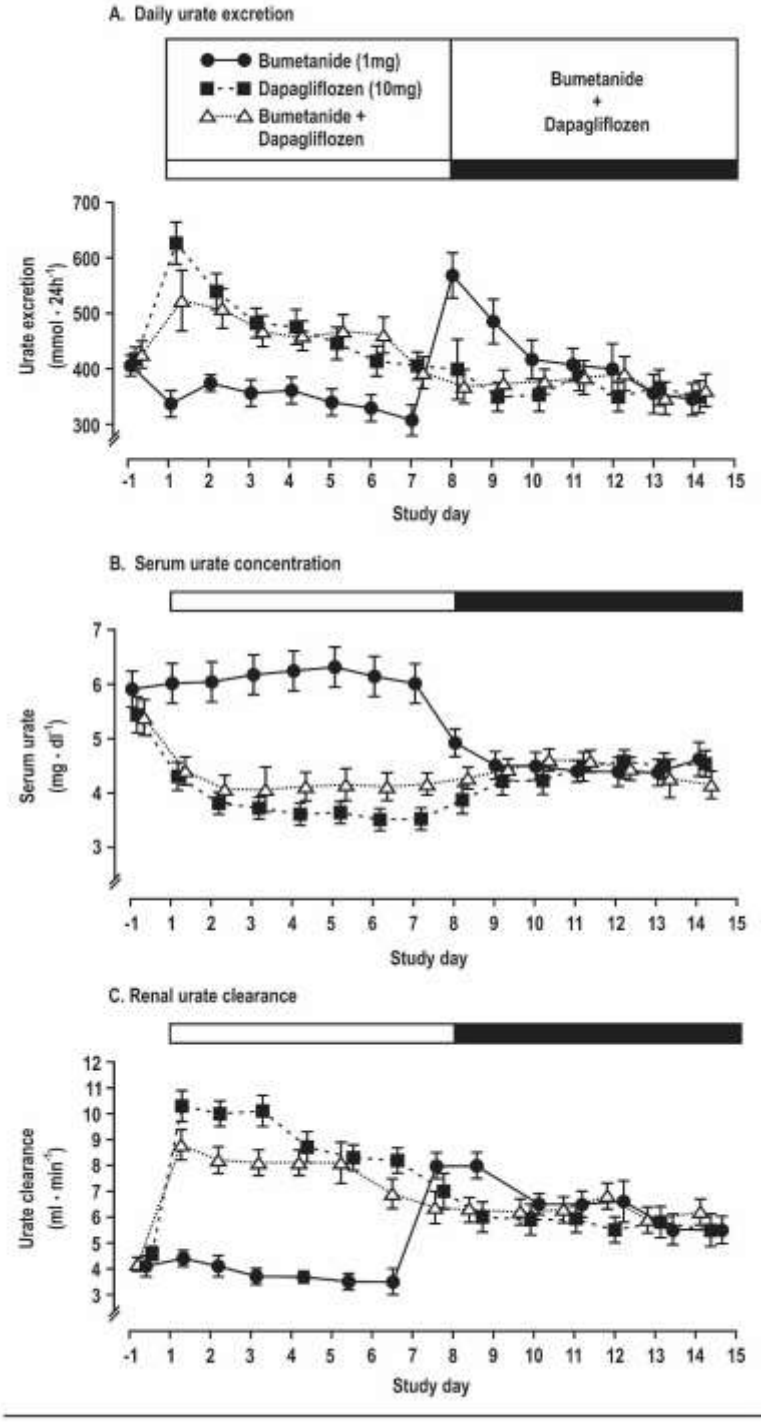
Mean ± SEM values (n = 14 per group) for: A, daily glucose excretion or B, serum glucose concentration as a function of study day.

Figure S2. Daily Potassium excretion and serum potassium concentration.



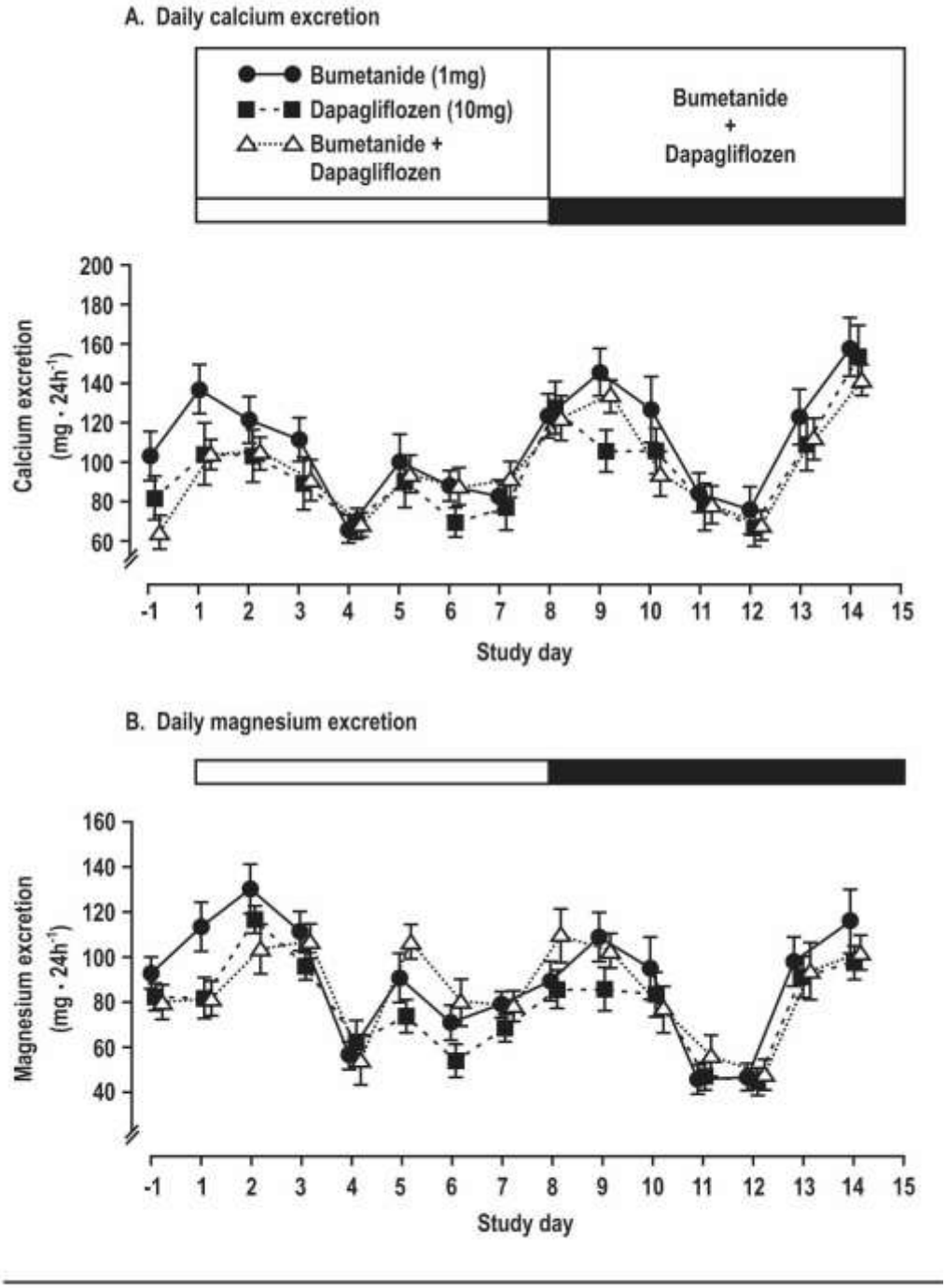
Mean ± SEM values (n = 14 per group) for: A, potassium excretion and B, serum potassium concentration

Figure S3. Daily Urate Excretion, Serum Urate Concentration and and Renal Urate Clearance.



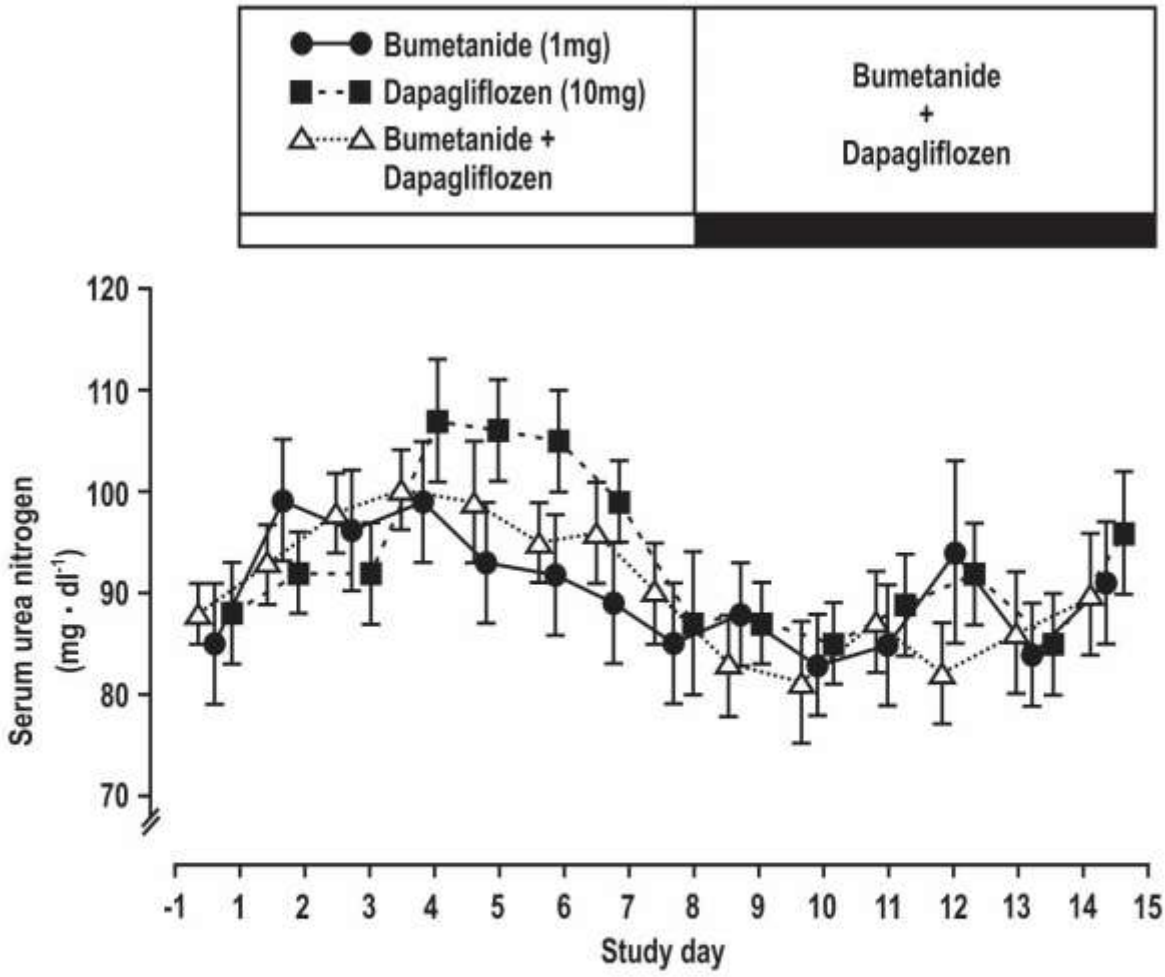
Mean ± SEM values (n = 14 per group) for: A, urate excretion; B, serum urate concentration and C, renal urate clearance as a function of study day

Figure S4. Daily Calcium and Magnesium Excretion.



Mean ± SEM values (n = 14 per group) for: A, calcium excretion and B, magnesium excretion as a function of study day

Figure S5. Creatinine Clearance.



Mean ± SEM values (n = 14 per group) for creatinine clearance as a function of study day

Figure S6. Plasma Retin Activity.