Online Supplement

Testing Computer Models Predicting Human Responses to a High Salt Diet: Implications for Understanding Mechanisms of Salt Sensitive Hypertension

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METHODS

The data supporting the findings of this study are available within the article and its online-only Data Supplement, and from the corresponding author on appropriate request.

Definitions and Study Design

For purposes of the current studies, “normal” subjects were defined as individuals with normal blood pressure (< 140 mmHg systolic/90 mmHg diastolic, or < 106 mmHg mean arterial pressure) who are salt resistant according to dietary salt loading test criteria similar to those recommended by experts of the American Heart Association and also by other scientists for classifying subjects with respect to salt sensitivity or salt resistance.[1, 2] Clinically realistic increases in salt intake were defined as increases in salt intake occurring within the real life range of dietary salt intake in humans (a range of approximately 10 mmol/day to approximately 350 mmol/day).[3]

Validation Testing of Contemporary Models

For validation testing of contemporary models of integrative physiology, we used QCP 2005 (build date November 24, 2004) and HumMod version 3.0.4. QCP 2005 was the first “extension” of the 1972 Guyton model developed by investigators at the University of Mississippi to run on personal computers with the Windows operating system, and is reported to contain ~ 4000 distinct variables and several hundred mathematical functions.[4, 5] According to the model developers, “HumMod is an extension of QCP” [5] and version 3.0.4 of HumMod is reported to contain over 8000 independent variables and ~ 2000 parameters and mathematical relationships.[6] Both HumMod 3.0.4 and QCP 2005 were downloaded from www.humod.org (downloaded 1/11/2018) and run on a desktop computer with a 64 bit Windows 7 operating system.

In the model validation studies, the results of published salt loading studies in normotensive salt resistant humans were compared to the predictions of the models that were programmed to simulate, as closely as possible, the experimental conditions of the published human studies. Our primary objective was to test the accuracy of the contemporary models to predict the daily changes in sodium balance and cardiac output that occur over the first 5 to 7 days of physiologic salt loading in normal humans. This period was selected because it corresponds to the time when clinically realistic degrees of salt loading initiate increases in blood pressure in salt sensitive subjects.

In identifying the human studies to serve as benchmarks for the validation testing, we selected only rigorous studies involving: 1) normal subjects (salt resistant subjects with normal blood pressure); 2) performance on a metabolic unit or inpatient ward in which daily sodium intake and food intake were strictly controlled, with the entire fixed amount of prescribed NaCl ingested each day as required per protocol; 3) water intake ad libitum; 4) limitation of exercise activity to minimize sodium losses through sweat; 5) careful collection of 24 hour urine samples on a daily basis, and 6) determination of daily sodium balances throughout the study. Based on these criteria, 2 benchmark human studies were identified for the model validation testing of salt-

induced changes in sodium balance in response to salt loading.[7, 8] One of the benchmark studies of salt loading and sodium balance also included serial measurements of cardiac output and was used for validation testing of the models with respect to their accuracy in predicting serial changes in cardiac output during clinically realistic, short-term increases in salt intake.[7] Because nearly all of the subjects in the benchmark studies were males, and the QCP model does not provide separate settings for simulations in females, the validation testing was performed with data generated mainly in male subjects. Details of the computer settings for these simulations are described further below.

Testing Consistency Between Predictions of the Contemporary Models

An additional objective was to test the consistency between the contemporary models with respect to the changes in metabolic and hemodynamic variables predicted to occur during both short-term and long-term salt loading. Specifically, we determined whether the salt loading study results predicted by HumMod 3.0.4 were consistent with those predicted by QCP 2005. Because HumMod 3.0.4 allows for simulations in male or female subjects, we also compared the results of HumMod simulations of long-term salt loading in males to those in females. Details of the computer settings for these simulations are described further below.

Testing Consistency Between Predictions of the Original 1972 Guyton Model and HumMod 3.0.4

Given that HumMod 3.0.4 is the most recent extension of the original 1972 Guyton model, our final objective was to compare the predictions of HumMod 3.0.4 to those of the original 1972 Guyton model. Specifically, we tested the agreement between HumMod 3.0.4 and the 1972 Guyton model with respect to the changes in mean arterial pressure, sodium balance, cardiac output, and systemic vascular resistance predicted to occur in response to increases in salt intake.

For testing of the 1972 Guyton model, we used the Fortran code from the original version of the 1972 model that is documented in a detailed technical report[9] prepared for the National Aeronautics and Space Administration (NASA) by Dr. Ronald J. White, one of the scientists who worked in Dr. Guyton's laboratory and was involved in organizing the Fortran code of Dr. Guyton. To compile the code and run the working Fortran model,[10] we used the following computer software and hardware: Ubuntu 16.04 LTS (Xenial Xerus) 64 bit Linux operating system (downloaded from http://releases.ubuntu.com/16.04/ubuntu-16.04.5-desktop-amd64.iso), gfortran 5.4.0 compiler (installed from the Ubuntu repository), and a Dell Precision M4600 computer (Intel Core I7-2720QM processor, 16GB RAM). The gfortran compiler version 5.4.0 was installed with the command line command: *sudo apt-get install gfortran* in Ubuntu 16.04. Details for running the Fortran model are described in the instructions provided with the model,[10] including instructions for using the Windows operating system instead of Linux. Details of the salt loading protocols used in the simulations are described further below.

Statistical Analysis

For validation testing, the predictions of the computer models were plotted against the human experimental data. A model was considered to fail validation testing when the salt-induced change predicted by the model fell outside the 95% confidence limits of the mean of the salt-induced changes observed in human studies. When comparing predictions between models, we applied criteria of +/-25% to the *changes* that occur with salt loading. Specifically, the predictions of HumMod 3.0.4 were considered to agree with those of another model when the changes predicted by the HumMod simulation were within ± 25% of those predicted by the other model.

Method Details of Simulations With QCP 2005 and HumMod 3.0.4 of Short-Term Salt Loading Studies in Humans

To perform validation testing of model predictions of the effects of short-term increases in salt intake, we used HumMod 3.0.4 and QCP 2005 to simulate the salt loading studies of Schmidlin and colleagues[7] and Ishii and colleagues[8] that were originally performed in salt resistant subjects with normal blood pressure. The simulations were initiated with a two-week baseline stabilization period using the default model settings for all variables including caloric intakes, electrolyte intakes, and ad lib fluid intakes for 37 year old normal male subjects. The details of these settings are shown in supplemental Tables 1 and 2 (Tables S1 and S2). After the two week stabilization period on the default settings, the model settings were switched to follow the low salt and high salt protocols described in the studies of Schmidlin et al or Ishii et al.[7, 8] The levels of salt intake in the humans studies and in the model simulations are shown in supplemental Tables 1 and 2. In HumMod, the setting for the control of food intake was always fixed to equal 100% of the goal food intake to insure that 100% of the prescribed amount of dietary salt was ingested as specified in the protocols.

In the benchmark studies of Schmidlin et al,[7] hemodynamic data were obtained at 4-hour intervals around the clock. Daily mean arterial pressure (MAP) was determined from the average of blood measurements obtained with an oscillometric device (Dinamap, Criticon Inc. Tampa, Florida) programmed to take 5 readings within 5 minutes after the patient had rested supine for 5 minutes. Daily cardiac output (CO) was determined with impedance cardiography (BioZ ICG monitor, Cardiodynamics, San Diego) from the average of measurements obtained at 4-hour intervals immediately after the blood pressure measurements. Systemic vascular resistance was calculated as (MAP-CVP)/CO where CVP was the central venous pressure, assumed to be 6 mmHg. The CO measurements were based on the average values obtained over 30-beat intervals for 5 minutes. Details on the reproducibility and accuracy of the impedance cardiography technique for measuring CO have been previously reported by Bellardinelli et al.[11] In non-acutely ill subjects, the impedance cardiography method shows very good agreement with measurements of CO obtained using thermodilution and direct Fick methods.[11] In the studies by Schmidlin et al,[7] the method appeared capable of detecting increases in CO as small as 5% and has also been reported to be capable of detecting large increases in CO (up to 50% or more).[11] In the benchmark studies of Ishii et al, daily blood pressure values were calculated from the average of blood pressure measurements obtained using a mercury manometer 3 times per day (before each meal) with the patient supine.[8] In the simulations, data were sampled at 6

hour intervals in the short-term salt loading studies and at weekly intervals in the long-term salt loading studies.

Data Reporting

In the model simulations, the hemodynamic changes induced by switching from the low salt diet to the high salt diet were calculated by comparing the results obtained on each day of the high salt diet to the results obtained on the last day of the low salt diet. The changes in the variables which occurred during salt loading are displayed in the figures as percentage changes from the results on the last day of the low salt diet. Absolute values for the variables from the simulations used for validation testing are contained within the spreadsheet files labeled “Simulations_of_Schmidlin_short_term_study.xls” and “Simulations_of_Ishii_short_term_study.xls” in the online-only Data Supplement.

Estimates of Cumulative Na^+ Balance and Impact of Non-Renal Losses of Sodium

According to measurements made by Heer and colleagues, normal humans consuming less than 400 mmol of NaCl per day lose a total of approximately 7 - 8 mmol of sodium per day through the skin and gastrointestinal tract (combined).[12] In the benchmark human studies used for model validation testing, the sodium balance calculations are assumed to mainly reflect the effects of renal losses of sodium and do not incorporate the effects of sodium losses that may occur through sweating or through the gastrointestinal (GI) tract. In contrast, the sodium balance calculations reported in the HumMod and QCP model simulations reflect the effect of sodium losses through sweating and through the GI tract in addition to renal losses of sodium. Thus, to the extent that non-renal losses of sodium occur in the model simulations, the sodium balance results in the model simulations will tend to underestimate those reported in the human studies. However, the impact of non-renal losses of sodium in the model simulations was small because the models reported no sodium losses through sweating and only small losses of sodium through the GI tract. In addition, to prevent GI tract losses of sodium in the model simulations from causing the models to underestimate the sodium balance results reported in the human studies, we removed the GI tract losses of sodium from the model estimates of sodium balance presented in Figures 1 and 2. In the online-only Data Supplement, the sodium balance results from the model simulations, with and without removal of GI losses of sodium, are shown in the spreadsheets that compare the model simulations to the human studies. The interpretation of the results is not affected by the GI tract losses of sodium reported in the model simulations.

Method Details of Simulations with QCP 2005 and HumMod 3.0.4 of Long-Term Salt Loading

To compare the predictions made by HumMod 3.0.4 and QCP 2005 with respect to hemodynamic and sodium balance responses to long-term salt loading, the effects of switching normal subjects from a very low salt diet (~30 mmol NaCl/70 kg body weight/day) to a high salt diet (~250 mmol NaCl/70 kg body weight/day) were simulated for 16 weeks. Except for the duration of the high salt intake period, the details for the settings used in simulations with this long-term salt loading protocol were identical to those shown in the Schmidlin protocol in Table S1. The only modification of the Schmidlin protocol shown in Table S1 was to extend the high NaCl diet phase from 1 week to 16 weeks. The raw data values are in the file labeled

"HumMod_and_QCP_hemodynamic_simulations_long_term.xls" in the online-only supplemental materials.

Method Details of Simulations with HumMod 3.0.4 of the Responses to Salt Loading in Male Versus Female Subjects

The simulations of long-term salt loading in male versus female subjects were performed using HumMod 3.0.4. This version of HumMod permits the user to specify either male or female study subjects. These simulations employed a modified longer version of the protocol of Schmidlin and colleagues that was used to compare the HumMod and QCP simulations of long-term salt loading in males. These HumMod simulations were initiated with a two-week baseline stabilization period using the default settings for all variables including caloric intakes, electrolyte intakes, and ad lib fluid intakes. The details for those settings are shown in supplemental Table 3 (Table S3). The raw data from the simulation in the male subject and the female subject are contained within the spreadsheet file labeled "HumMod_simulations_male_and_female_subjects.xls" in the online-only supplemental materials.

Method Details of Simulations with the 1972 Guyton Model and HumMod 3.0.4 of the Responses to Salt Loading in Normal Subjects

Simulations of short-term and long-term salt loading with the 1972 Guyton model were compared to simulations performed with HumMod 3.0.4. Simulations were initiated with a two week baseline stabilization period with the salt intake set at 180 mmol/day (0.125 mmol/min). In each model, the default settings of each model were used for all other variables with one exception. In HumMod, the default setting for the control of food intake was increased to equal 100% of the goal food intake to ensure that 100% of the prescribed amount of dietary salt was ingested as specified in the protocols.

After the two-week stabilization period, the model settings were switched to provide a low NaCl intake of 0.0208 mmol/min (30 mmol/day) for 7 days followed by a high NaCl intake of 0.1875 mmol/min (270 mmol/day) for 7 days. These salt intakes were chosen to match closely with the levels of salt intake used in the human studies by Schmidlin and colleagues.[7] To model the effects of long-term salt loading, the simulation of short-term salt loading for one week with 270 mmol NaCl/day was extended for an additional 15 weeks (total duration of high salt diet of 16 weeks as shown in Table S4). The hemodynamic changes induced by switching from the low salt diet to the high salt diet were calculated by comparing the results obtained during the high salt diet to the results obtained on the last day of the low salt diet. The raw data from the simulations comparing the 1972 Guyton model with HumMod 3.0.4 are in the file labeled "Guyton_1972_Fortran_vs_Humod 304.xls" in the online-only supplemental materials. The data from the 1972 Guyton model simulations that were used for comparison with the results from HumMod 3.0.4 were also used for comparison with the results of studies in humans by Schmidlin and colleagues[7] (Figures S8 to S11).

RESULTS

Contemporary Computer Models Fail to Accurately Predict Changes in Hematocrit and Circulating Protein Concentrations Induced by Short-Term Salt Loading in Normal Humans

In addition to failing to accurately predict the usual magnitude of salt-induced changes in cardiac output and vascular resistance (see main text), both HumMod and QCP failed to accurately predict the extent to which circulating protein concentrations normally decrease in response to increased salt intake (Figure S1). QCP also failed validation testing with respect to predicting the usual extent to which hematocrit normally decreases in response to increased salt intake (Figure S2). Thus, despite being extensions of the same 1972 Guyton model, the HumMod and QCP models generate strikingly different results from each other with respect to the magnitude of the changes in hematocrit, cardiac output, and systemic vascular resistance predicted to normally occur with clinically realistic degrees of salt loading (Figure S2, Figures 4 and 5 in main article).

Computer Simulations of Cardiac Output, Systemic Vascular Resistance, and Blood Pressure Responses to Long-Term Salt Loading: Conflicting Results Between Contemporary Models

There are no published studies in humans of the hemodynamic effects of switching from a low salt diet to a high salt diet in which sodium intake has been strictly controlled on a daily basis, and sodium balance, cardiac output, and systemic vascular resistance carefully monitored over an extended period (several weeks to months). Thus, it was not possible to perform validation studies to rigorously test whether computer models accurately predict the changes in sodium balance, cardiac output and systemic vascular resistance that usually occur over long periods of time after switching from a low salt diet to a high salt diet in normal subjects. However, it is possible to compare the predictions made by different computer models (hypotheses) about the usual effects of long-term salt loading on these particular variables. Therefore, we compared the predictions made by HumMod and QCP of the metabolic and hemodynamic effects of switching normal subjects from a very low salt diet to a high salt diet for 16 weeks.

QCP predicts a small increase in mean arterial pressure above baseline (≤ 3%) within the first few weeks of salt loading, followed by a slight decrease in blood pressure below baseline (~ -1%) that is sustained with long-term administration of the high salt diet (Figure S3). In the HumMod simulation, blood pressure is moderately increased (~5 - 7%) during long-term salt loading (Figure S3). Despite being extensions of the same 1972 Guyton model, the HumMod and QCP models generate substantially different results from each other with respect to the changes in cardiac output and systemic vascular resistance predicted to occur with long-term salt loading. HumMod predicts the chronic occurrence of much greater salt-induced increases cardiac output (~ 25%), and much greater salt-induced decreases in systemic vascular resistance (~ -17%) than those predicted by the QCP model (~ 5% for cardiac output and ~ -6% for systemic vascular resistance) (Figure S3). The results with HumMod 3.0.4 also differ substantially from those with QCP when the simulations involve increasing salt intake from 30

mmol/day to 180 mmol/day (unpublished observations). A salt intake of 180 mmol/day approximates the average amount of salt consumed by individuals worldwide.[13, 14]

With respect to the effects of long-term salt loading on systemic vascular resistance [also termed total peripheral resistance (TPR)], the principal developers of the HumMod program contend that in the salt-loading simulations they conducted with HumMod 3.0.4, "there is no sustained decrease in TPR in salt resistant subjects."[6] In those simulation studies by Clemmer and colleagues, the subjects were deemed to be salt sensitive if mean arterial pressure increased > 30 mmHg in response to a clinically unrealistic, extreme degree of salt loading, ie, in response to increasing salt intake over 30-fold from 30 mmol/day to 1000 mmol/day.[6] We are not aware of any studies in humans that have ever employed such an extreme blood pressure change or massive salt load to define salt sensitivity. Furthermore, Clemmer and colleagues did not discuss the long-term effects on TPR of switching from a low salt diet of 30 mmol NaCl/day to a high salt diet containing clinically realistic amounts of salt. In the present simulations with both HumMod 3.0.4 and QCP, substantial reductions in total peripheral resistance (systemic vascular resistance) were sustained for at least 16 weeks after switching from a low salt diet of ~ 30 mmol NaCl/day to a high salt diet with a clinically realistic amount of salt (~270 mmol/day) (Figure S3); the sustained reductions in vascular resistance being twice as great in the HumMod simulation than in the QCP simulation.

Careful scrutiny of the study by Clemmer et al reveals that in their HumMod simulations, the level of TPR in a normal subject given a moderately high salt diet (180 mmol NaCl/day) for 4 weeks is lower than the TPR of a normal subject given a very low salt diet (30 mmol NaCl/day) for 4 weeks.[6] This is consistent with our findings in HumMod and QCP showing that in normal subjects, switching from 30 mmol NaCl/day to 270 mmol NaCl/day results in a sustained decrease in TPR (with the TPR reductions being much greater in HumMod than with QCP).

Computer Simulations of Sodium Balance, Fluid Volume, Hematocrit, and Protein Responses to Long-Term Salt Loading: Conflicting Results Between Contemporary Models

In subjects switched from a very low salt diet to a high salt diet for approximately 4 months, HumMod predicts the chronic occurrence of salt-induced increases in sodium balance (~ 135 mmol) and extracellular fluid volume (ECFV) (~ 6%) that are much smaller than those predicted to occur by the QCP model (> 200 mmol for sodium balance and ~ 10% for ECFV) (Figure S4). Although HumMod predicts smaller salt-induced increases in sodium balance and ECFV than QCP (Figure S4), HumMod predicts much greater salt-induced increases in blood volume (Figure S4) and cardiac output (Figure S3) than the QCP model. The reason for these incongruous results is that HumMod makes the surprising prediction that practically all of the increase in extracellular fluid volume induced by salt-loading is confined to the intravascular space and very little, if any, is distributed to the interstitial space (Figure S4). In contrast, QCP makes the surprising prediction that practically all of the increase in extracellular fluid volume induced by salt-loading is confined to the interstitial space and very little, if any, is distributed to the intravascular space (Figure S4).

HumMod also makes the surprising prediction that salt-loading causes large chronic increases in plasma protein concentration and plasma protein mass while simultaneously causing large chronic increases in plasma volume and large decreases in hematocrit (Figure S5). If anything, with retention of water and expansion of plasma volume, one might expect decreases in both plasma protein concentration and hematocrit. QCP predicts that salt-loading causes moderate increases in plasma volume and moderate decreases in hematocrit together with moderate decreases in plasma protein concentration (Figure S5).

Surprising Differences Between HumMod Simulations of the Responses to Salt Loading in Normal Males Versus Normal Females

HumMod 3.0.4 provides the option of performing simulations in male subjects or female subjects whereas QCP does not provide for separate simulations in female subjects. We compared HumMod simulations of long-term salt loading studies in males to those in females in which subjects were switched from a very low NaCl intake of ~ 30 mmol/70 kg body weight/day to a high NaCl intake of ~ 250 mmol NaCl/70 kg body weight/day. The HumMod simulations of female subjects yielded striking differences from those of male subjects (Figure S6). For example, HumMod predicts that long-term salt loading causes sustained increases in cardiac output and plasma volume in males but not in females, while causing substantial decreases in hematocrit in males and substantial increases in females (Figure S6). In simulations of long-term salt loading for 12 months in females, HumMod predicted that hematocrit and hemoglobin levels reach 57% and 19 g/dL, respectively (unpublished observations). In adult females, polycythemia is diagnosed when hematocrit is >48% or when hemoglobin is > 16 g/dL. We are unaware of any evidence showing that a chronic high salt diet causes substantial polycythemia in females.

Effects of Salt Loading on Total Peripheral Resistance and Autoregulation: Comparing the 1972 Guyton Model to Its Contemporary Derivative, HumMod 3.0.4

As discussed in the primary manuscript, the greater salt-induced increases in blood pressure in the 1972 Guyton model than in HumMod 3.0.4 are initiated and sustained by greater levels of total peripheral resistance in the 1972 Guyton model than in HumMod, not by greater levels of sodium retention and cardiac output than in HumMod (Figure S7). In HumMod, clinically realistic degrees of salt loading induce very large increases in cardiac output, acutely and chronically, without inducing substantial increases in blood pressure because salt loading also induces very large, sustained reductions in systemic vascular resistance [total peripheral resistance (TPR)] (Figure S7). These results from HumMod 3.0.4, which was developed by Coleman, Hester, and colleagues, are at odds with their contention that “reductions in TPR after increases in salt intake” have “little importance in long-term BP regulation.”[6] Without the sustained reductions in TPR that occur with clinically realistic degrees of salt loading in the HumMod simulations of normal subjects, the sustained salt-induced increases in cardiac output would cause sustained hypertension (similar to the results observed with long-term salt loading in simulations with the 1972 Guyton model (Figure S7). In normal,

salt resistant humans, the actual effects of long-term salt loading on TPR remain to be determined.[15]

As defined by Guyton, "the phenomenon of autoregulation means the ability of the body's tissues to control their own blood flows in relation to their local needs. When the cardiac output is too high, excess blood flows through the tissues, and the local intrinsic blood flow regulatory mechanisms then cause marked increase in total peripheral resistance in an attempt to return the tissue blood flow back to normal."[16] According to Coleman, Guyton, and Granger, "increased cardiac output, if it is maintained for an adequate length of time, causes an autoregulatory response that increases total peripheral resistance and arterial pressure."[17] However, in the present studies with HumMod 3.0.4 (Figure S7) which was developed by Coleman and others,[6, 18] very large increases in cardiac output induced by clinically realistic degrees of salt loading do not cause an "autoregulatory response that increases total peripheral resistance and arterial pressure."[17] Rather, in HumMod 3.0.4, very large, salt-induced increases in cardiac output were accompanied by *decreases* in total peripheral resistance, not increases in total peripheral resistance (Figure S7).

In the 1972 Guyton model, clinically realistic degrees of salt-loading induced substantial increases in cardiac output and blood pressure with little or no change in total peripheral resistance (systemic vascular resistance) (Figure S7). As discussed in the main text, switching from a low salt diet to a high salt diet induces a substantial increase in the ARM variable which represents the "vasoconstrictor effect" of autoregulation (see data in supplemental file "Guyton_1972_Fortran_vs_Humod 304.xls"). In the 1972 Guyton model, this vasoconstrictor effect of autoregulation may be contributing to the failure of systemic vascular resistance to normally decrease in response to salt loading. In response to short-term and long-term salt loading in subjects with "normal" renal function in the 1972 Guyton model, renal vascular resistance is increased, skeletal muscle vascular resistance is decreased, and resistance in non-renal, non-muscle vascular beds undergoes little or no change (data not shown). Further analysis will be required to determine the extent to which various factors contribute to the abnormal renal vascular resistance responses to salt loading observed in simulations of subjects with "normal" renal function in the 1972 Guyton model. In normal humans, salt loading induces decreases in renal vascular resistance, not increases in renal vascular resistance.[19]

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Table S1. Model settings in HumMod and QCP for simulations of the short-term salt loading protocol of Schmidlin et al.

	HumMod simulation settings				QCP simulation settings		
Diet Phase	Baseline	Low NaCl	High NaCl		Baseline	Low NaCl	High NaCl
Duration (d)	14	7	7		14	7	7
NaCl (mmol/d)	180	30	270		180	30	280
K+ (mmol/d)	70	50	50		70	50	50
CHO % of calories	40.9%	49.8%	49.8%		40.9%	50.0%	50.0%
Fat % of calories	36.4%	34.2%	34.2%		36.4%	33.6%	33.6%
Protein % of calories	22.7%	16.0%	16.0%		22.7%	16.4%	16.4%
Total kcal/day	2250	2250	2250		2200	2200	2200
Fluid intake	ad lib	ad lib	ad lib		ad lib	ad lib	ad lib

Schmidlin et al studied 16 African American males and 2 African American females with a mean age of 44 years and weight of 82 kg.[7] HumMod 3.0.4 sets the default normal male subject in the model to be 37 years of age with a body weight of 75 kg (race not specified). QCP 2005 sets the default normal male subject in the model to be 37 years of age with a body weight of 77.5 kg (race not specified). The intakes of salt and potassium during the low NaCl and high NaCl diet phases of the simulations closely match those used by Schmidlin et al. The distribution of calories during the low NaCl and high NaCl diet phases of the simulations also closely match those used by Schmidlin et al. During the low NaCl diet phase, intake of salt was set at approximately 30 mmol/70 kg body weight per day. During the high NaCl diet phase, intake of NaCl was set at approximately 250 mmol/70 kg body weight per day. Dietary intakes during the baseline phases represent the default model settings for 37 year-old male subjects.

Table S2. Model settings in HumMod and QCP for the simulations of the short-term salt loading protocol of Ishii et al.

	HumMod simulation settings				QCP simulation settings		
Diet Phase	Baseline	Low NaCl	High NaCl		Baseline	Low NaCl	High NaCl
Duration (d)	14	9	5		14	9	5
NaCl (mmol/d)	180	100	280		180	100	280
K+ (mmol/d)	70	70	70		70	70	70
CHO % of calories	40.9%	40.9%	40.9%		40.9%	40.9%	40.9%
Fat % of calories	36.4%	36.4%	36.4%		36.4%	36.4%	36.4%
Protein % of calories	22.7%	22.7%	22.7%		22.7%	22.7%	22.7%
Total kcal/day	2250	2250	2250		2200	2200	2200
Fluid intake	ad lib	ad lib	ad lib		ad lib	ad lib	ad lib

Ishii et al studied 8 Japanese males and 3 Japanese females with a mean age of 32 years.[8] The body weights and caloric intakes in the study of Ishii et al. were not specified. HumMod 3.0.4 sets the default normal male subject in the model to be 37 years of age with a body weight of 75 kg (race not specified). QCP 2005 sets the default normal male subject in the model to be 37 years of age with a body weight of 77.5 kg (race not specified). All dietary intakes during the baseline phases represent the default model settings for 37 year-old male subjects. The intakes of salt and potassium during the low NaCl and high NaCl diet phases of the study simulations matched closely with those of the study of Ishii et al in which subjects were switched from a low salt diet of ~100 mmmol/day to a high salt diet of ~ 275 mmol/day. Caloric intakes during the low NaCl and high NaCl phases represent the default model settings for 37 year-old male subjects.

Table S3. Model settings in HumMod for the simulations of the long-term salt loading protocol in males versus females.

	HumMod settings in males				HumMod settings for females		
Diet Phase	Baseline	Low NaCl	High NaCl		Baseline	Low NaCl	High NaCl
Duration (d)	14	7	112		14	7	112
NaCl (mmol/d)	180	30	270		180	30	220
K+ (mmol/d)	70	50	50		70	50	50
CHO % of calories	40.9%	49.8%	49.8%		41.0%	50.0%	50.0%
Fat % of calories	36.4%	34.2%	34.2%		37.0%	34.0%	34.0%
Protein % of calories	22.7%	16.0%	16.0%		22.0%	16.0%	16.0%
Total kcal/day	2250	2250	2250		1780	1780	1780
Fluid intake	ad lib	ad lib	ad lib		ad lib	ad lib	ad lib

The default settings in HumMod 3.0.4 specify a 37 year-old normal male subject weighing 75 kg and a 37 year-old female subject weighing 61 kg. Dietary intakes during the baseline phase represent the default model settings for normal male or female subjects. The intakes of salt and potassium and the distribution of calories during the low NaCl and high NaCl diet phases approximately match those used by Schmidlin et al.[7] During the high NaCl diet phase, intake of NaCl was ~ 250 mmol/70 kg body weight per day.

Table S4. Model settings in HumMod 3.0.4 and the 1972 Guyton model for the salt-loading simulations

	HumMod settings				1972 Guyton model settings		
Diet Phase	Baseline	Low NaCl	High NaCl		Baseline	Low NaCl	High NaCl
Duration (d)	14	7	112		14	7	112
NaCl (mmol/d)	180	30	270		180	30	270
Fluid intake ad lib	ad lib	ad lib	ad lib		ad lib	ad lib	ad lib

The settings for all other variables in each model were the default settings with 1 exception. In HumMod, the setting for the control of food intake was fixed to equal 100% of the goal food intake to ensure that 100% of the prescribed amount of dietary salt was ingested as specified in the protocols.

Supplemental Figure Legends

Figure S1. Experimental and predicted changes in circulating protein concentrations induced by switching from a very low salt diet to a high salt diet in normal males. In the study of Schmidlin et al,[7] the protein concentrations (means ± 95% confidence intervals) were determined in serum. In HumMod, the protein concentrations are reported for plasma and from QCP, the concentrations are reported for blood.

Figure S2. Experimental and predicted changes in hematocrit induced by switching from a very low salt diet to a high salt diet in normal males. Observed results are from Schmidlin et al[7] and are shown as means ± 95% confidence intervals.

Figure S3. Model predictions of long-term hemodynamic changes induced by switching from a very low salt diet to a high salt diet. This figure shows long-term percent changes in mean arterial pressure, cardiac output, and systemic vascular resistance predicted to occur by HumMod 3.0.4 and QCP 2005 in normal adult male subjects switched from a NaCl intake of ~ 30 mmol/70 kg body weight/day to ~ 250 mmol/70 kg body weight/day while adhering to the dietary protocol of Schmidlin et al. [7] The chronic changes predicted to occur by HumMod differ substantially from those predicted by QCP.

Figure S4. Model predictions of long-term changes in sodium balance and percent changes in body fluid volumes induced by switching from a very low salt diet to a high salt diet. This figure shows changes in cumulative sodium balance and percent changes in extracellular fluid volume, blood volume, and interstitial fluid volume predicted to occur by HumMod 3.0.4 and QCP 2005 in normal adult male subjects switched from a NaCl intake of ~ 30 mmol/70 kg body weight/day to ~ 250 mmol/70 kg body weight/day while adhering to the dietary protocol of Schmidlin et al.[7] The chronic changes predicted to occur by HumMod differ substantially from those predicted to occur by QCP 2005.

Figure S5. Percent changes in plasma volume, plasma protein concentration, plasma protein mass, and hematocrit predicted to occur by HumMod 3.0.4 and QCP in normal males switched from a low NaCl intake of ~ 30 mmol/70 kg body weight/day to a high NaCl intake of ~ 250 mmol/70 kg body weight/day. The changes predicted by the QCP model differ substantially from those predicted by HumMod 3.0.4, particularly with respect to plasma volume, plasma protein concentration, and plasma protein mass.

Figure S6. Percent changes in mean arterial pressure, plasma volume, cardiac output and hematocrit predicted to occur by HumMod 3.0.4 in normal males versus normal females when all subjects are switched from a low NaCl intake of ~30 mmol NaCl/kg body weight/day to a high NaCl intake of ~ 250 mmol/70 kg body weight/day. Changes predicted by HumMod 3.0.4 to occur in females differ substantially from those predicted in males, particularly with respect to plasma volume, cardiac output, and hematocrit.

Figure S7. Long-term hemodynamic and sodium balance responses to salt loading predicted to occur by HumMod 3.0.4 and the 1972 Guyton model in subjects with normal renal function. This figure shows the percent changes in mean arterial pressure, cardiac output, and total peripheral resistance (systemic vascular resistance), and the change in sodium balance, predicted to occur in response to switching from a low NaCl intake of 30 mmol per day to a high NaCl intake of 270 mmol per day for 1 to 16 weeks.

The predictions of HumMod 3.0.4 differ substantially from the predictions of the 1972 Guyton model with respect to hemodynamic and sodium balance responses to both short-term (Figure 6) and long-term salt loading (Figure S7).

Figure S8. Comparison of predictions from the 1972 Guyton model to the results from human studies with respect to short-term changes in blood pressure induced by switching from a very low salt diet to a high salt diet in subjects with normal renal function. This figure shows model predictions for the changes (%) in mean arterial pressure induced by switching NaCl intake from 30 mmol NaCl/ day to 270 mmol NaCl/day for one week. The results predicted by the 1972 Guyton model are compared with those observed in studies conducted by Schmidlin and colleagues in normotensive salt resistant humans.[7] Results of studies in humans are presented as means and 95% confidence intervals.

Figure S9. Comparison of predictions from the 1972 Guyton model to the results from human studies with respect to short-term changes in cumulative sodium retention induced by switching from a very low salt diet to a high salt diet in subjects with normal renal function. This figure shows model predictions for the changes in cumulative sodium balance induced by switching NaCl intake from 30 mmol NaCl/ day to 270 mmol NaCl/day for one week. The results predicted by the 1972 Guyton model are compared with those observed in studies conducted by Schmidlin and colleagues in normotensive salt resistant humans.[7] Results of studies in humans are presented as means and 95% confidence intervals.

Figure S10. Comparison of predictions from the 1972 Guyton model to the results from human studies with respect to short-term changes in cardiac output induced by switching from a very low salt diet to a high salt diet in subjects with normal renal function. This figure shows model predictions for the changes (%) in cardiac output induced by switching NaCl intake from 30 mmol NaCl/ day to 270 mmol NaCl/day for one week. The results predicted by the 1972 Guyton model are compared with those observed in studies conducted by Schmidlin and colleagues in normotensive salt resistant humans.[7] Results of studies in humans are presented as means and 95% confidence intervals.

Figure S11. Comparison of predictions from the 1972 Guyton model to the results from human studies with respect to short-term changes in systemic vascular resistance (total peripheral resistance) induced by switching from a very low salt diet to a high salt diet in subjects with normal renal function. This figure shows model predictions for the changes (%) in systemic vascular resistance induced by switching NaCl intake from 30 mmol NaCl/ day to 270 mmol NaCl/day for one week. The results predicted by the 1972 Guyton model are compared with those observed in studies conducted by Schmidlin and colleagues in normotensive salt resistant humans.[7] Results of studies in humans are presented as means and 95% confidence intervals.

Figure S1

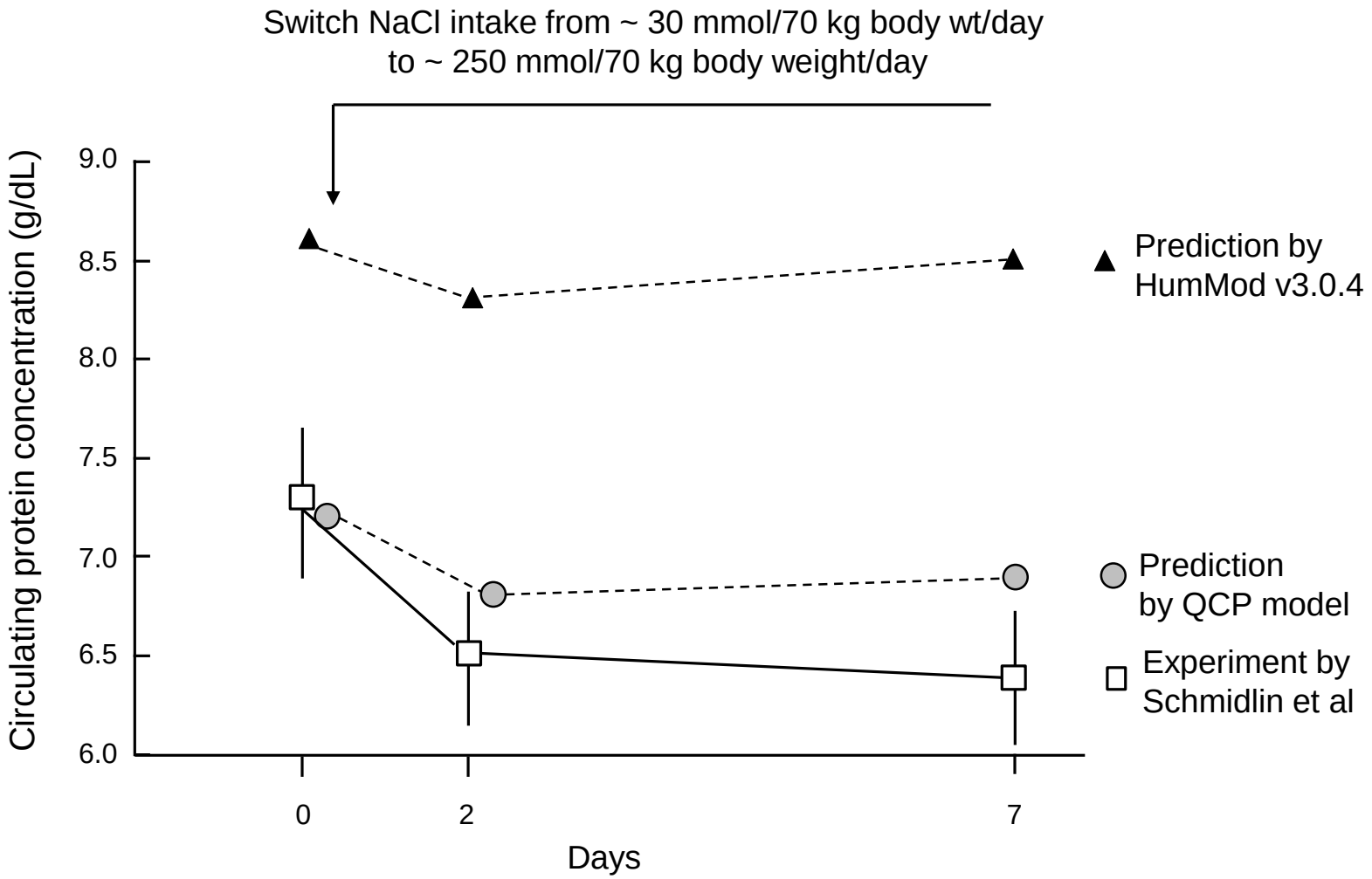

Switch NaCl intake from ~ 30 mmol/70 kg body wt/day
to ~ 250 mmol/70 kg body weight/day
Circulating protein concentration (g/dL)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
0
2
7
Days
Prediction by HumMod v3.0.4
Prediction by QCP model
Experiment by Schmidlin et al

Figure S2

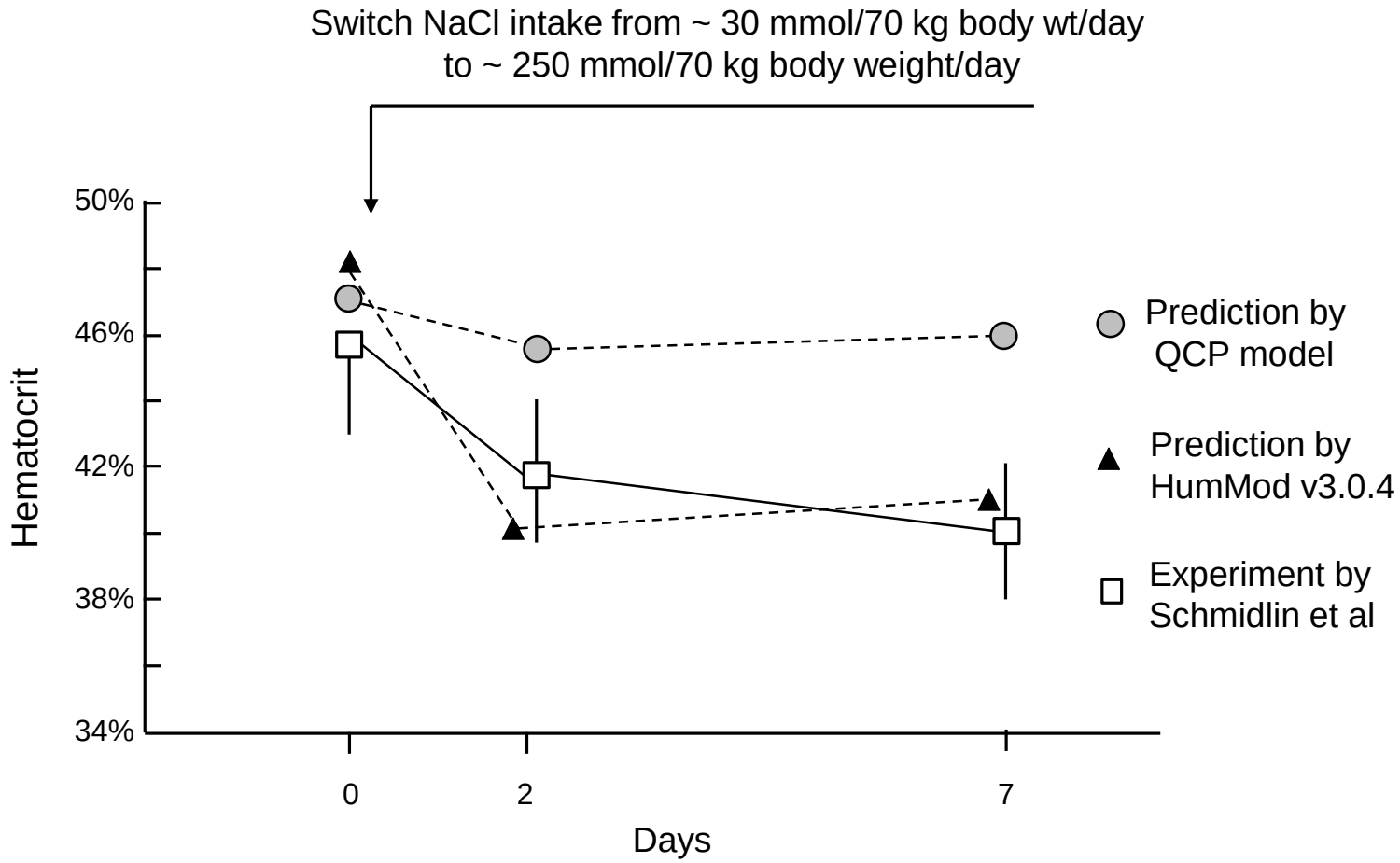
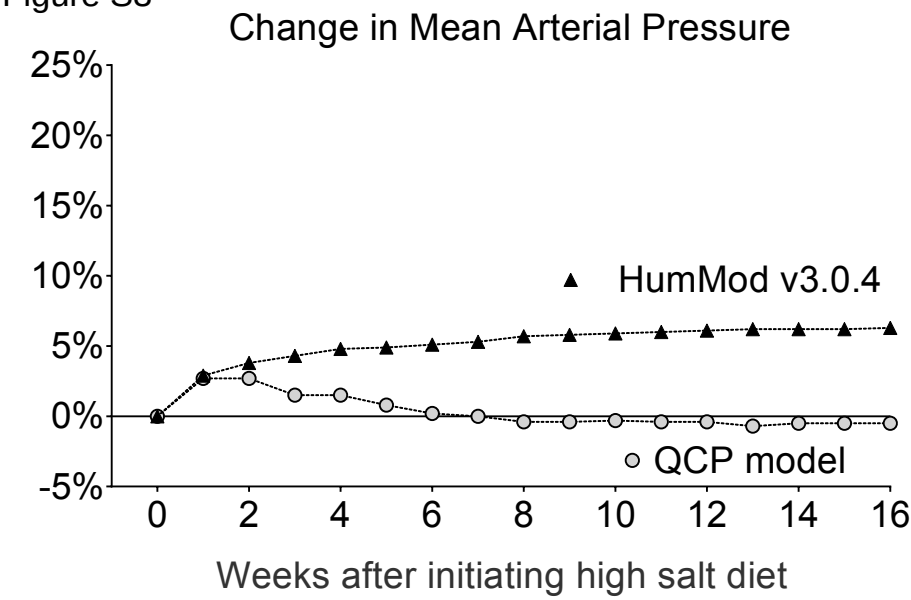
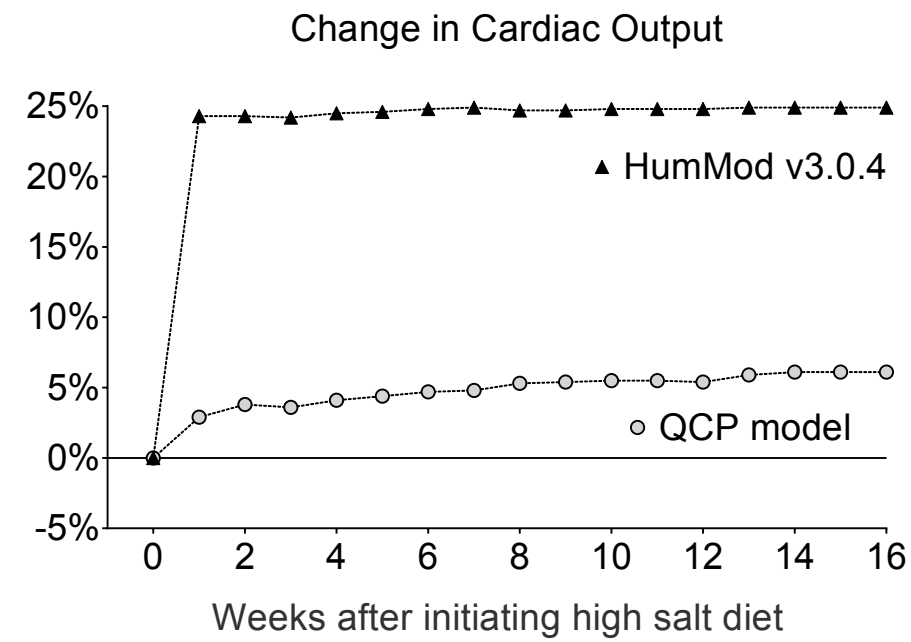

Switch NaCl intake from ~ 30 mmol/70 kg body wt/day
to ~ 250 mmol/70 kg body weight/day
50%
46%
42%
38%
34%
Hematocrit
0
2
7
Days
Prediction by QCP model
Prediction by HumMod v3.0.4
Experiment by Schmidlin et al

Figure S3


Change in Mean Arterial Pressure
25%
20%
15%
10%
5%
0%
-5%
HumMod v3.0.4
QCP model
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Cardiac Output
25%
20%
15%
10%
5%
0%
-5%
HumMod v3.0.4
QCP model
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

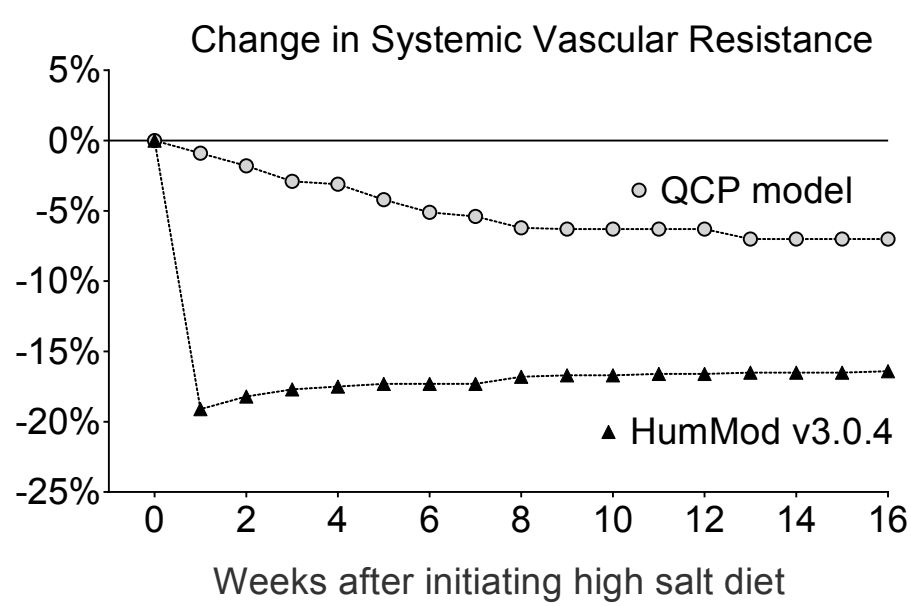
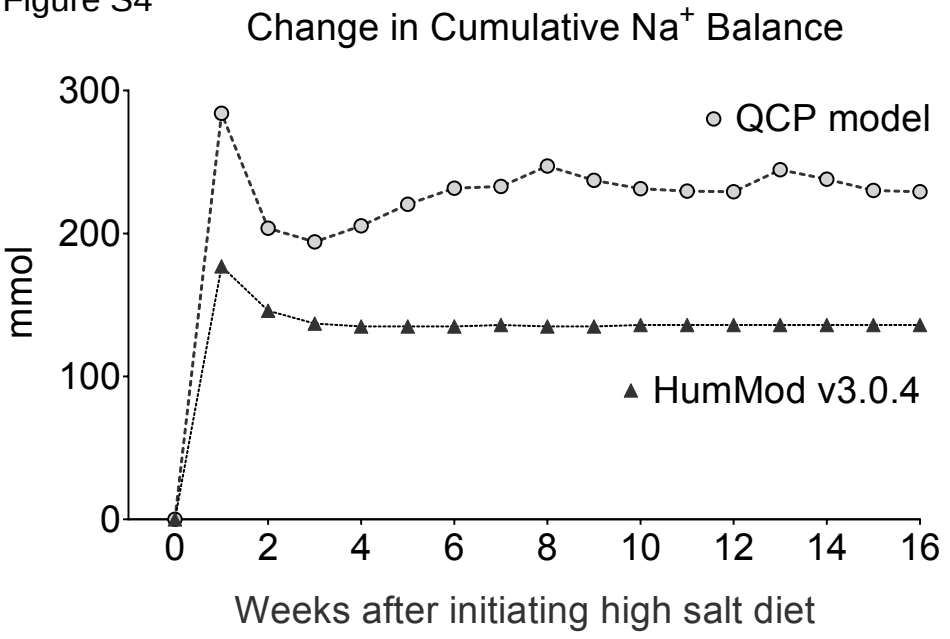
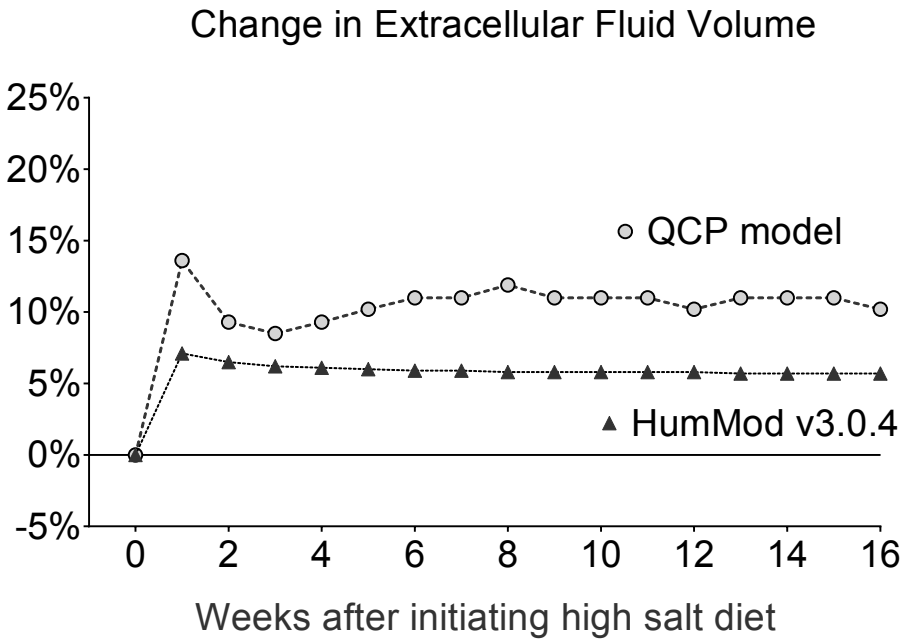
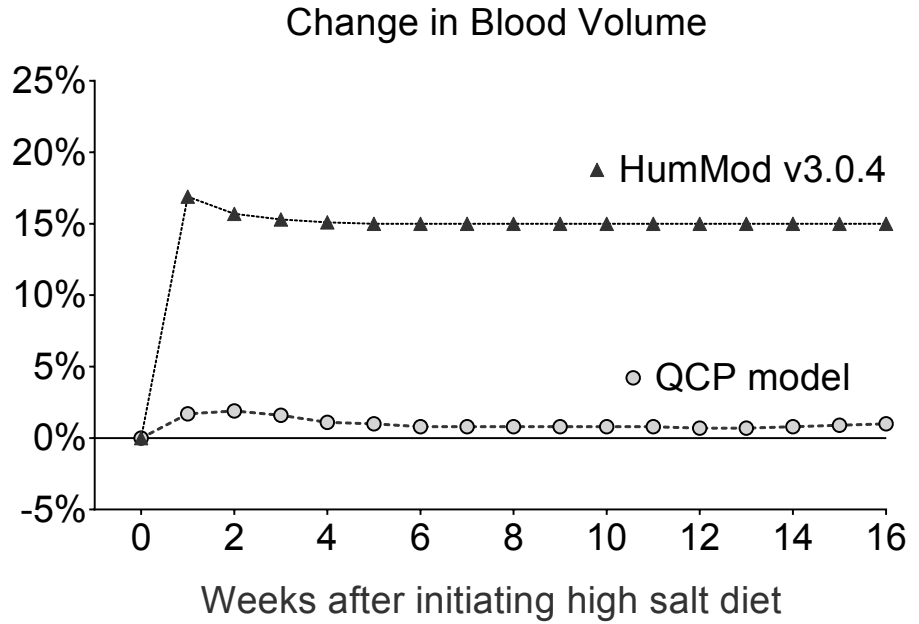

Change in Systemic Vascular Resistance
5%
0%
-5%
-10%
-15%
-20%
-25%
QCP model
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

Figure S4


Change in Cumulative Na+ Balance
300
200
100
0
mmol
QCP model
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Extracellular Fluid Volume
25%
20%
15%
10%
5%
0%
-5%
QCP model
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Blood Volume
25%
20%
15%
10%
5%
0%
-5%
HumMod v3.0.4
QCP model
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

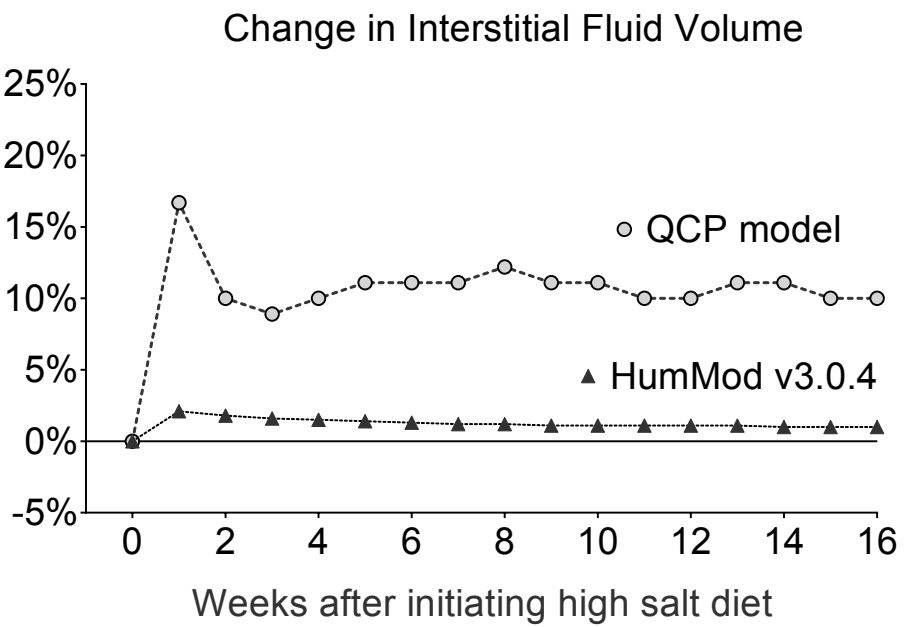
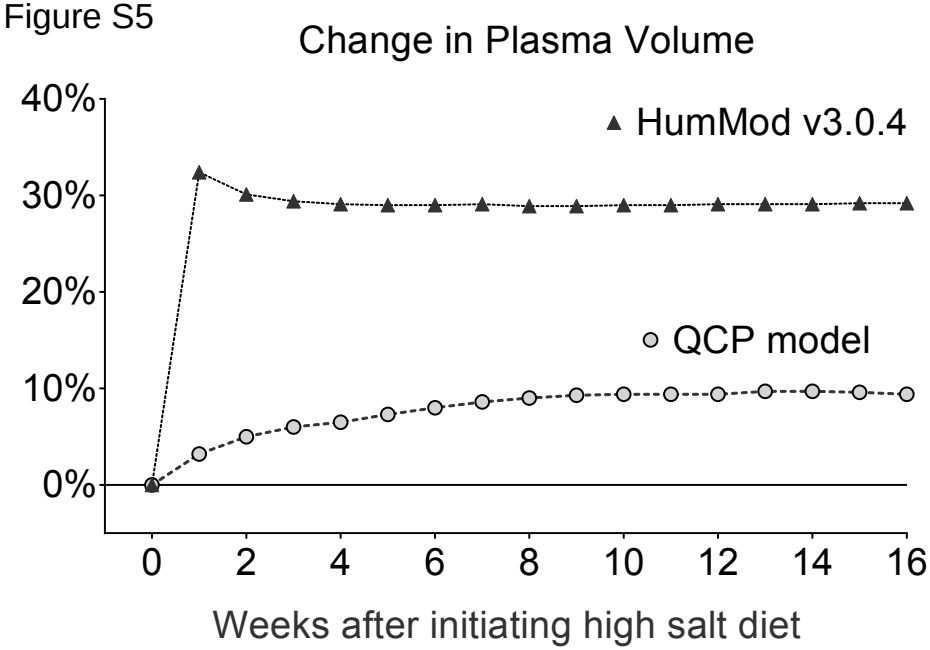
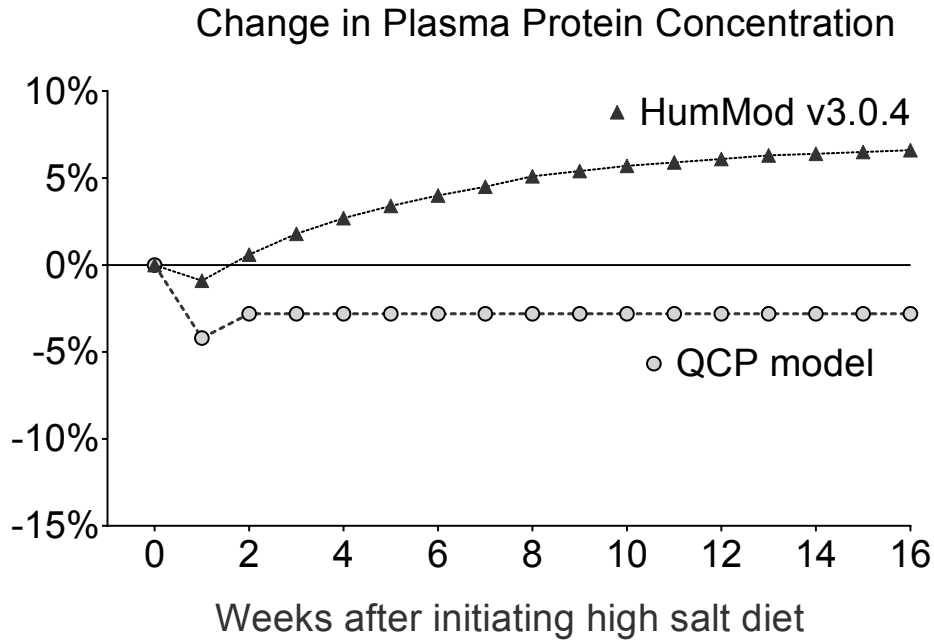
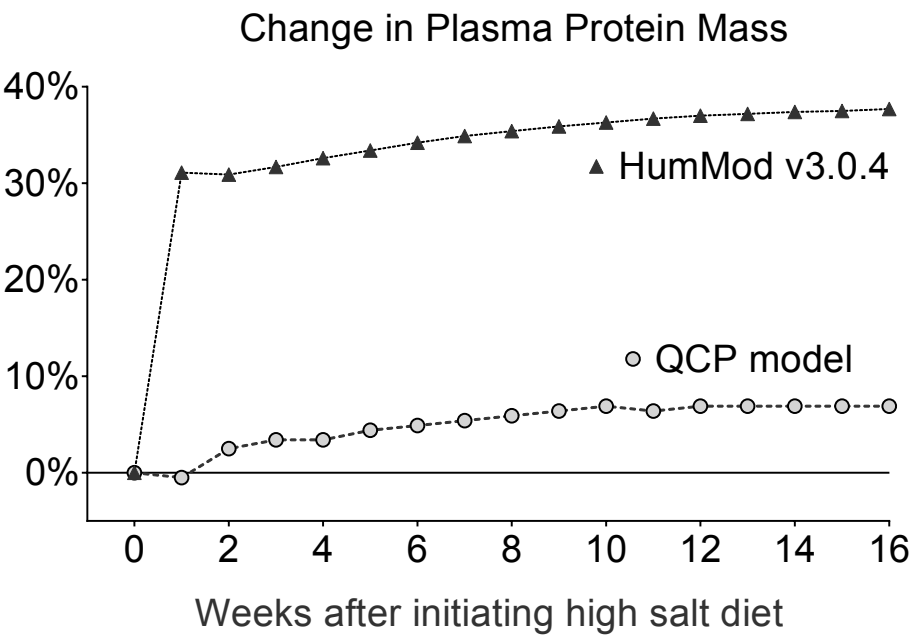

Change in Interstitial Fluid Volume
25%
20%
15%
10%
5%
0%
-5%
QCP model
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

Figure S5


Change in Plasma Volume
40%
30%
20%
10%
0%
HumMod v3.0.4
QCP model
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Plasma Protein Concentration
10%
5%
0%
-5%
-10%
-15%
HumMod v3.0.4
QCP model
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Plasma Protein Mass
40%
30%
20%
10%
0%
HumMod v3.0.4
QCP model
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

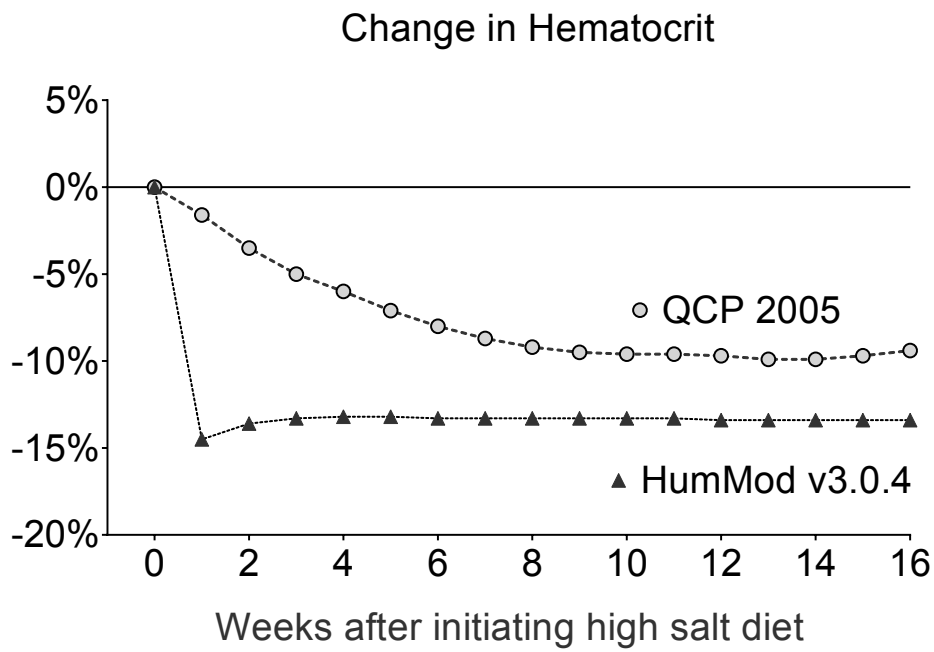
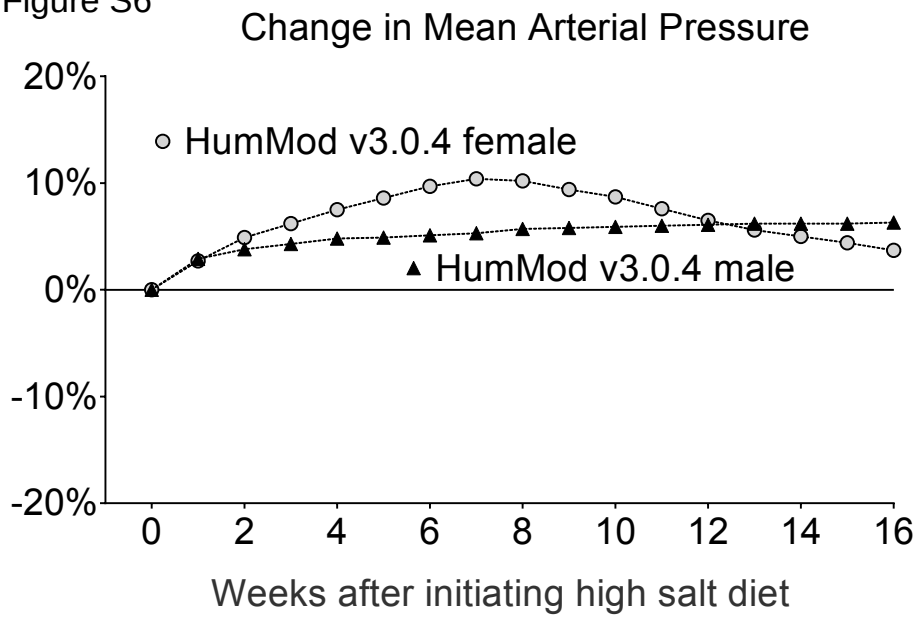
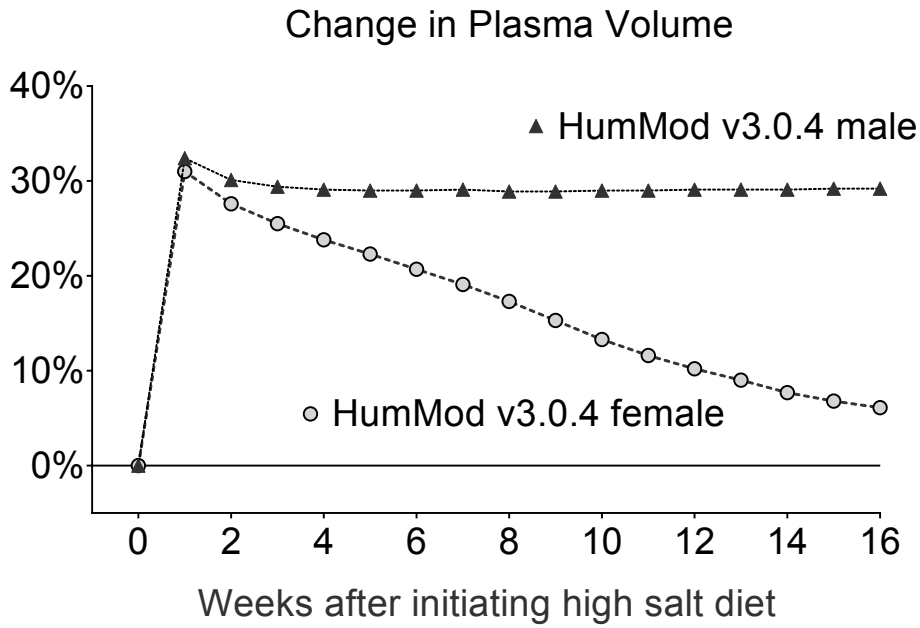
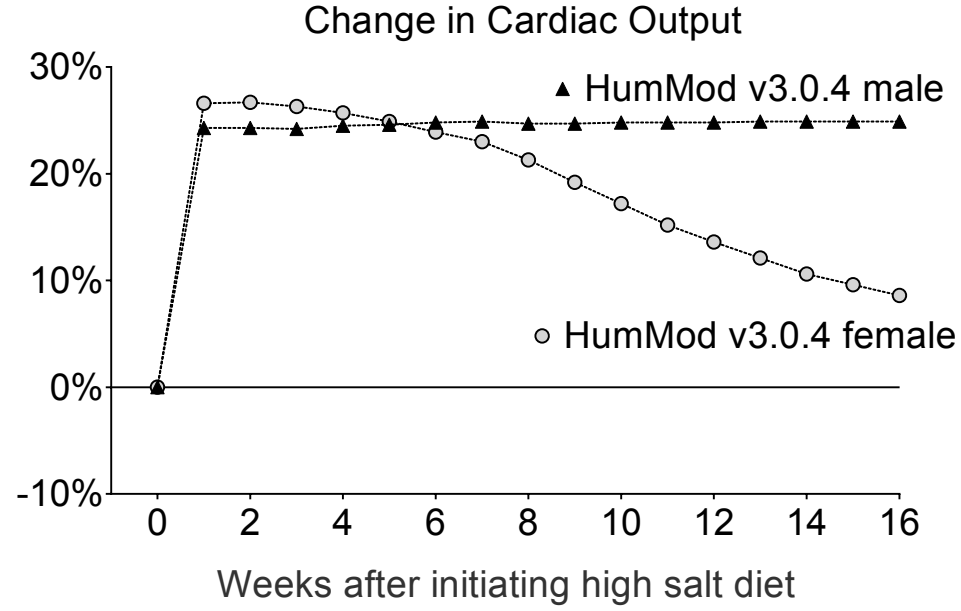

Change in Hematocrit
5%
0%
-5%
-10%
-15%
-20%
QCP 2005
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

Figure S6


Change in Mean Arterial Pressure
20%
10%
0%
-10%
-20%
HumMod v3.0.4 female
HumMod v3.0.4 male
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Plasma Volume
40%
30%
20%
10%
0%
HumMod v3.0.4 male
HumMod v3.0.4 female
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Cardiac Output
30%
20%
10%
0%
-10%
HumMod v3.0.4 male
HumMod v3.0.4 female
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

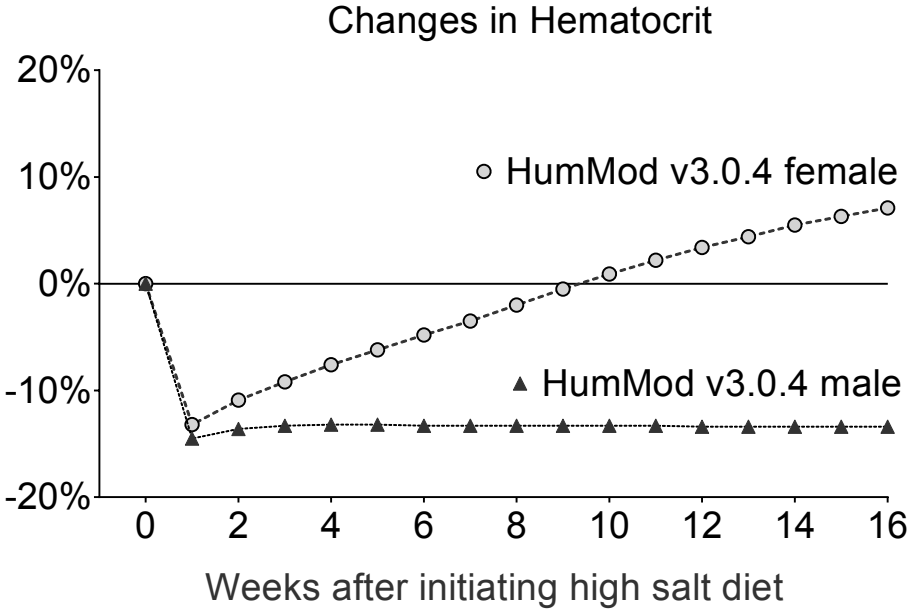
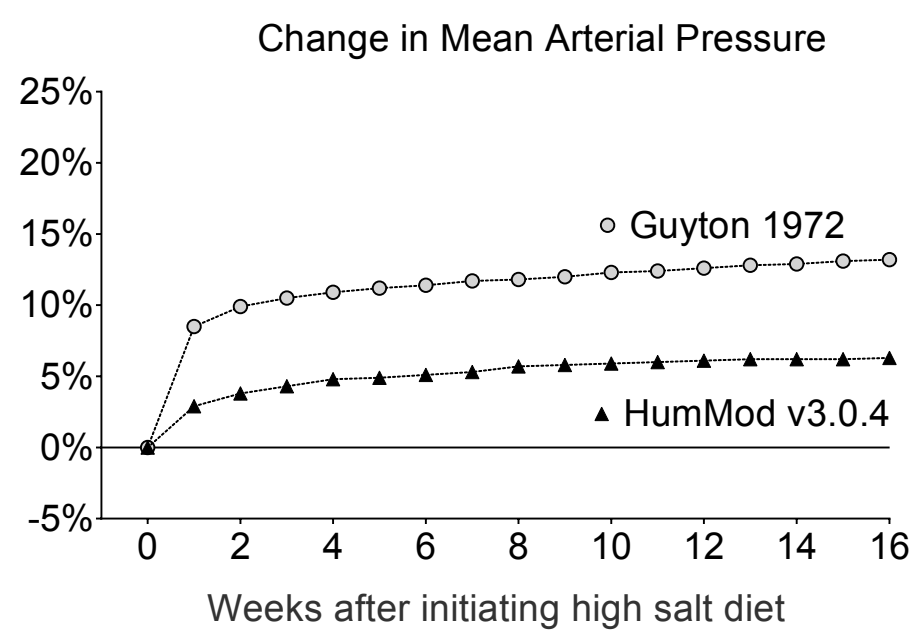
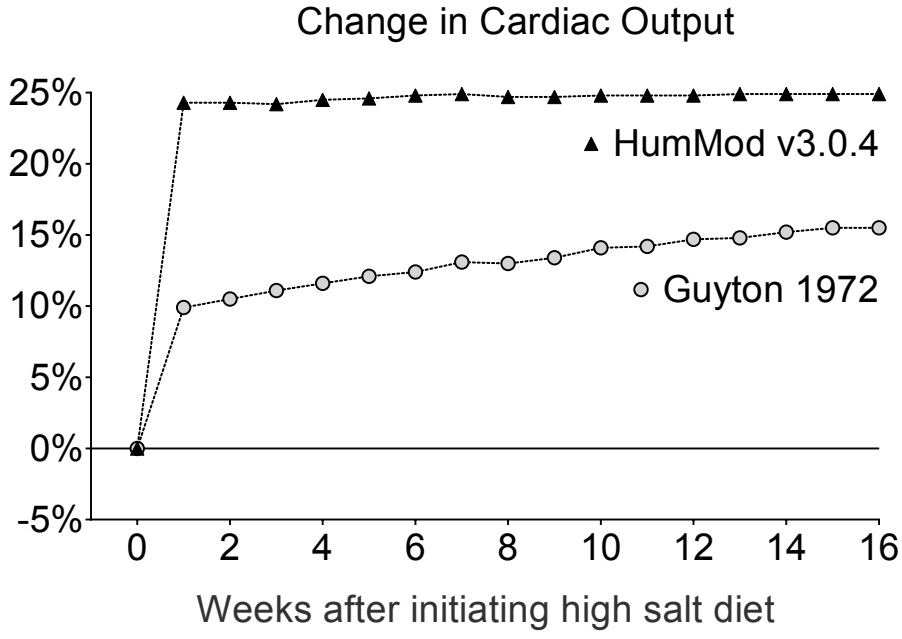
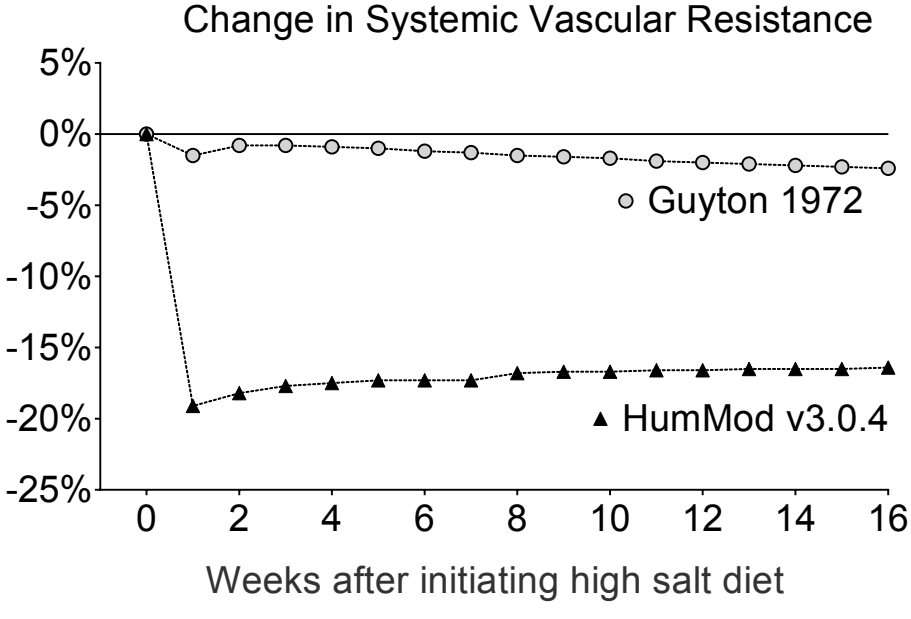

Changes in Hematocrit
20%
10%
0%
-10%
-20%
HumMod v3.0.4 female
HumMod v3.0.4 male
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

Figure S7


Change in Mean Arterial Pressure
25%
20%
15%
10%
5%
0%
-5%
Guyton 1972
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Cardiac Output
25%
20%
15%
10%
5%
0%
-5%
HumMod v3.0.4
Guyton 1972
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet


Change in Systemic Vascular Resistance
5%
0%
-5%
-10%
-15%
-20%
-25%
Guyton 1972
HumMod v3.0.4
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

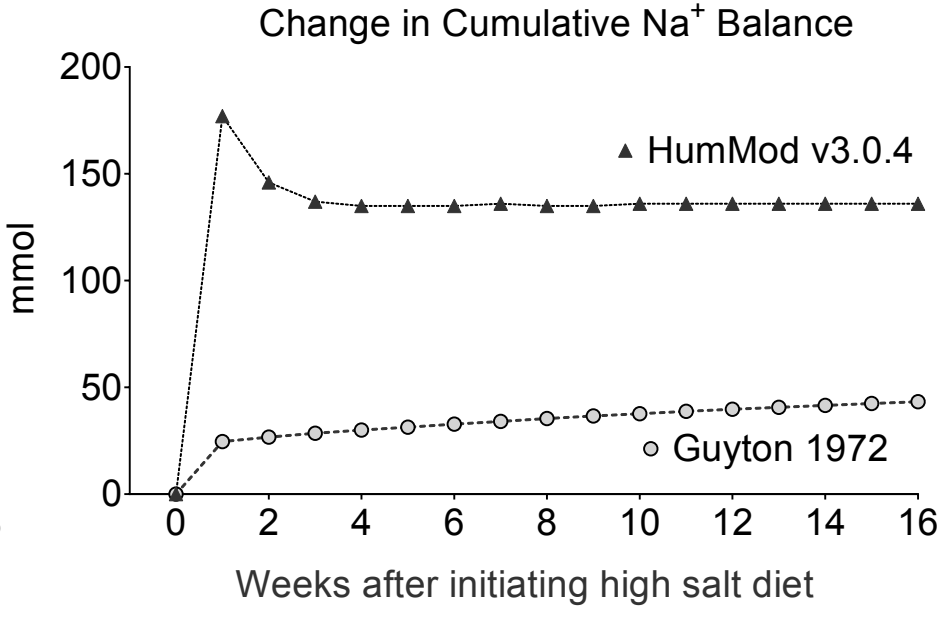

Change in Cumulative Na+ Balance
mmol
200
150
100
50
0
HumMod v3.0.4
Guyton 1972
0
2
4
6
8
10
12
14
16
Weeks after initiating high salt diet

Figure S8

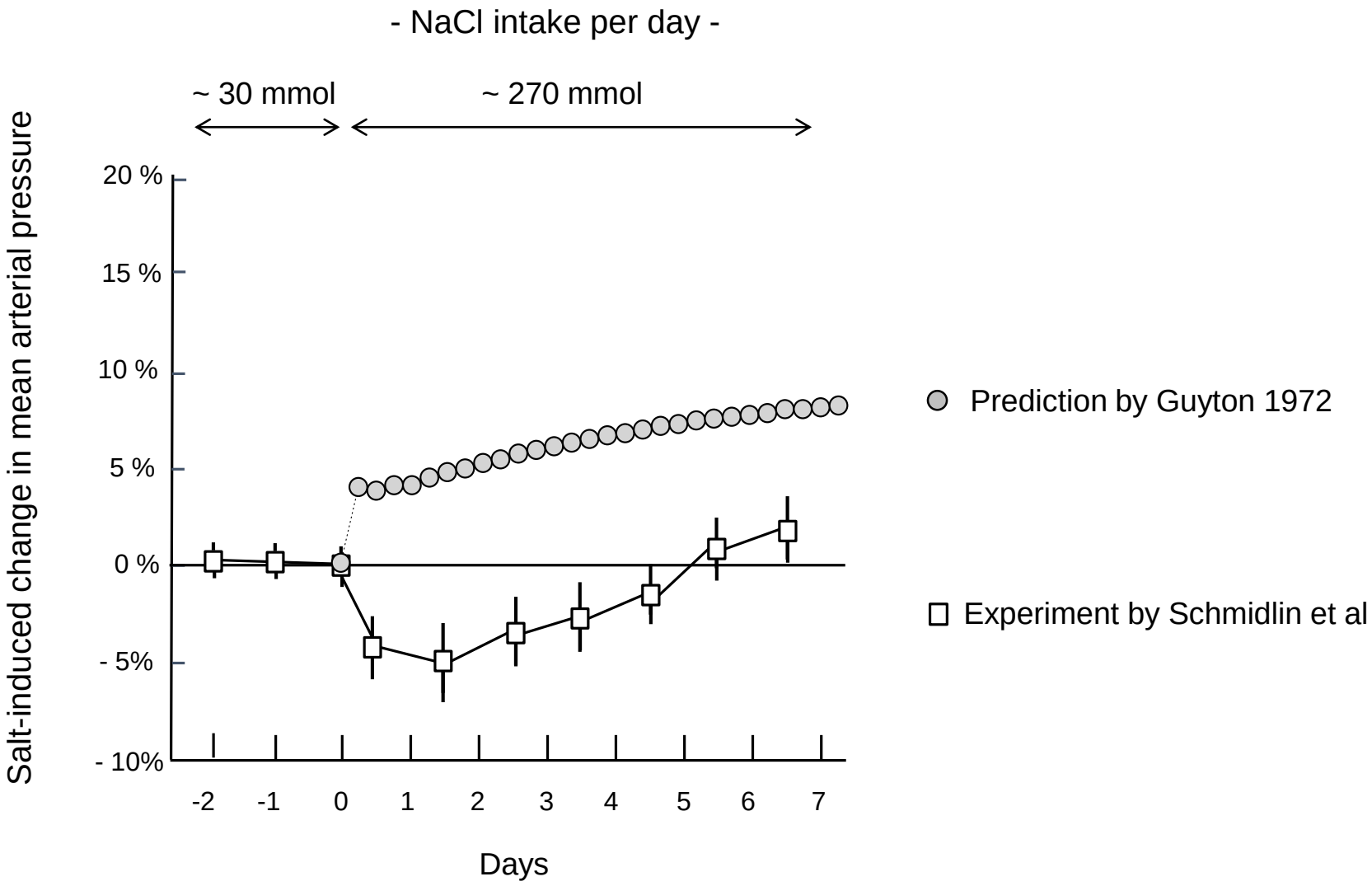

- NaCl intake per day -
~ 30 mmol
~ 270 mmol
Salt-induced change in mean arterial pressure
20 %
15 %
10 %
5 %
0 %
- 5%
- 10%
-2
-1
0
1
2
3
4
5
6
7
Days
Prediction by Guyton 1972
Experiment by Schmidlin et al

Figure S9

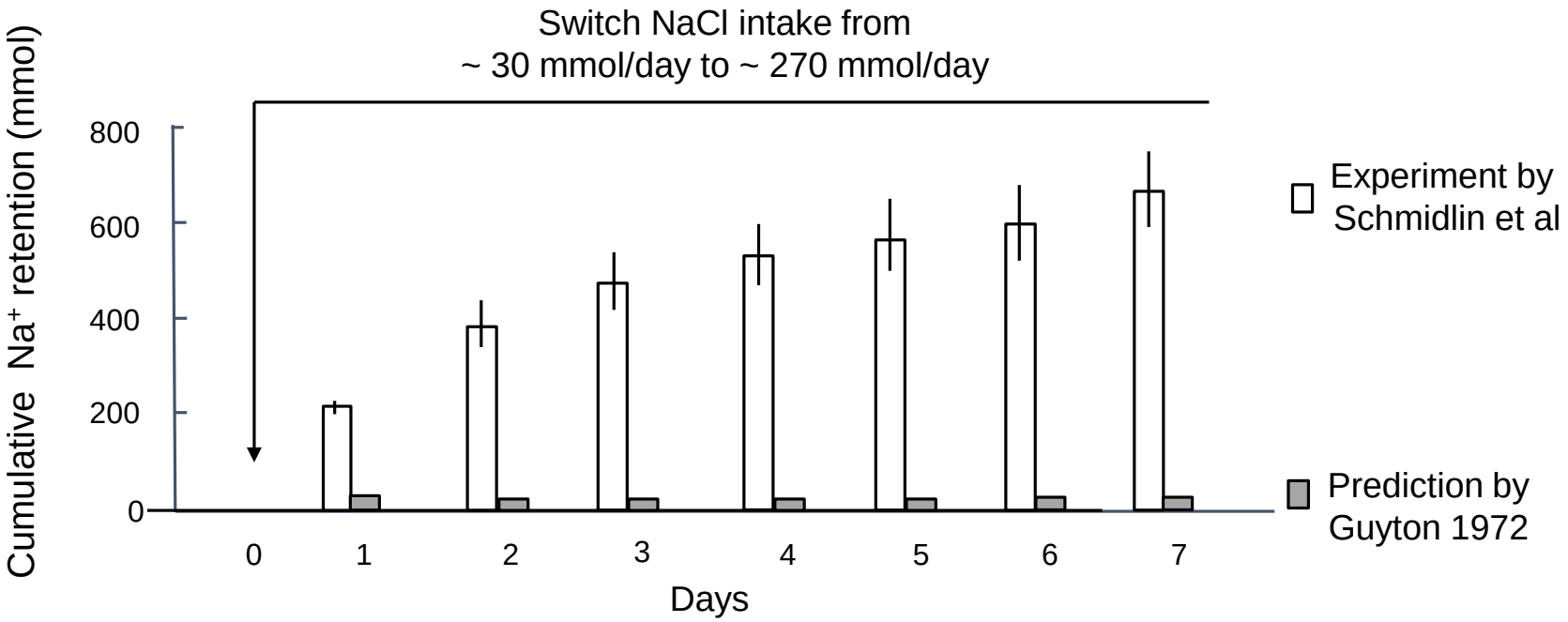

Switch NaCl intake from
~ 30 mmol/day to ~ 270 mmol/day
Cumulative Na+ retention (mmol)
800
600
400
200
0
0
1
2
3
4
5
6
7
Days
Experiment by
Schmidlin et al
Prediction by
Guyton 1972

Figure S10

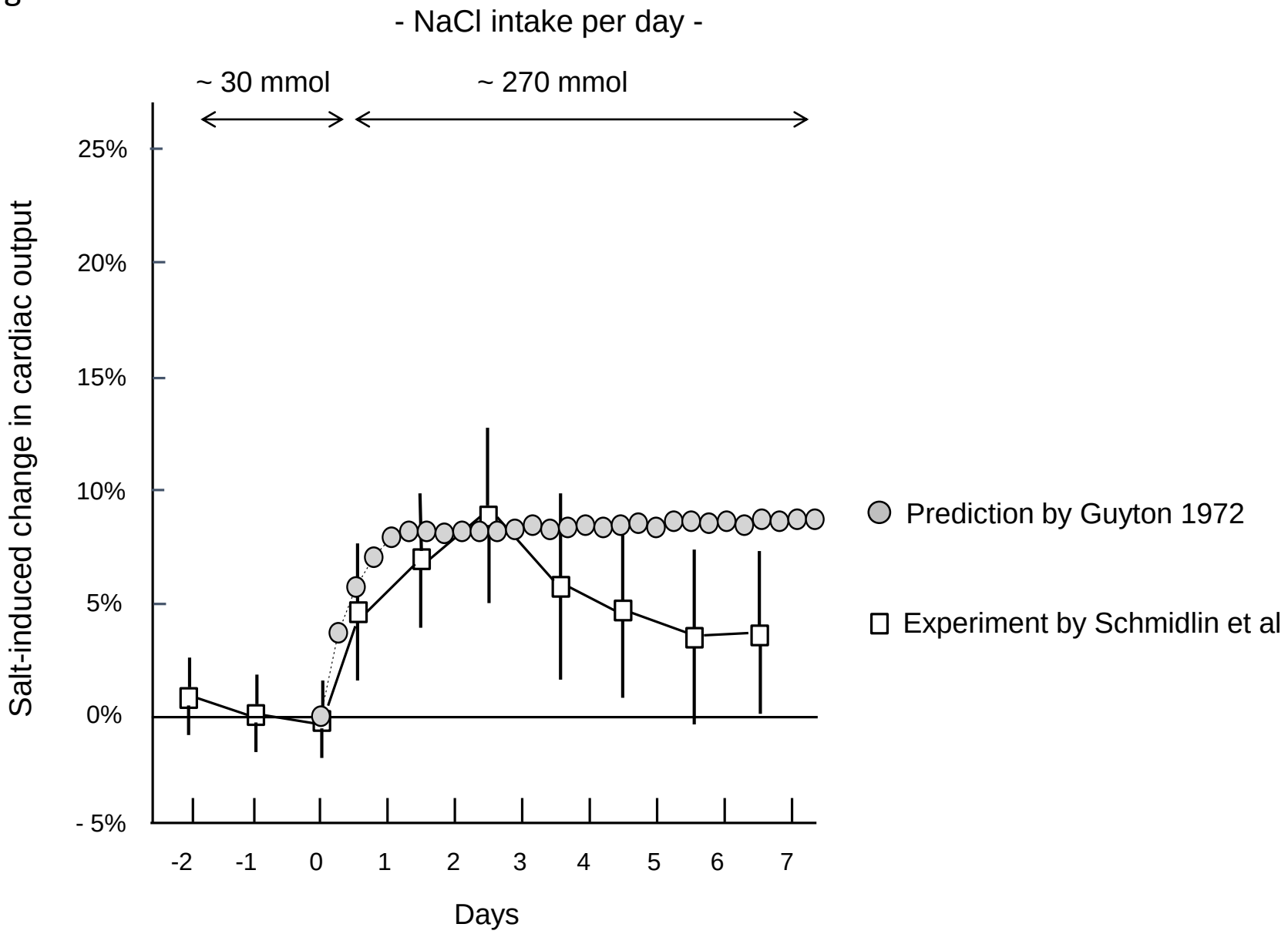
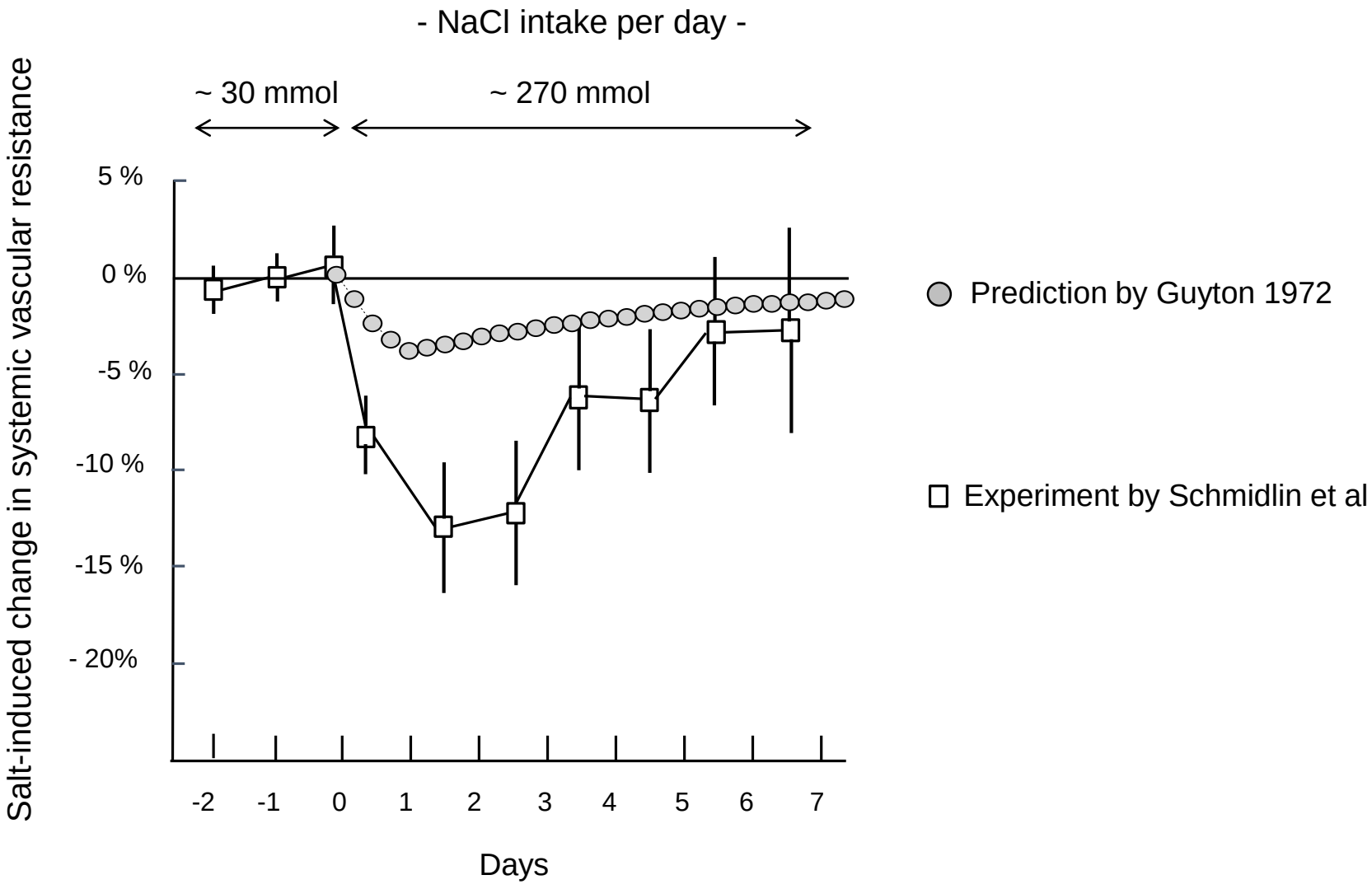

- NaCl intake per day -
~ 30 mmol
~ 270 mmol
Salt-induced change in cardiac output
25%
20%
15%
10%
5%
0%
- 5%
-2
-1
0
1
2
3
4
5
6
7
Days
Prediction by Guyton 1972
Experiment by Schmidlin et al

Figure S11


- NaCl intake per day -
~ 30 mmol
~ 270 mmol
Salt-induced change in systemic vascular resistance
5 %
0 %
-5 %
-10 %
-15 %
- 20%
-2
-1
0
1
2
3
4
5
6
7
Days
Prediction by Guyton 1972
Experiment by Schmidlin et al