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Faculty of Medicine

Norwegian University of Science and Technology

Student thesis

The pharmacodynamic effects of thiazides from a computational physiological perspective

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January 4, 2017

Acknowledgements

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Abstract

Background. Primary hypertension is the leading risk factor for mortality in low-, middle- and high-income countries and affects more than one billion people world-wide. Multi-scale and multi-organ computational models seeking to explain the emergence and maintenance of primary hypertension at the individual level are now emerging, but in order to be used in personalized hypertension therapy these models have to be able to describe the dynamic effects of drug administration.

Objective. To provide an overview of the thiazides' hemodynamic effects in humans, with a particular focus on compiling and assessing information instrumental for constructing physiological computational models capable of describing the effects of this drug class in individuals.

Method. We did a systematic literature search in the PubMed data-base on hemodynamic effects of thiazides and loop- diuretics in monotherapy. The loop diuretics were included for comparison. After the systematic literature search, we extracted the quantitative data and did a meta-analysis of the hemodynamic and hormonal effects of thiazides and loop diuretics. Our meta-analysis findings were then compared with those of high impact papers in the field. Based on the data from the meta-analysis we sought to describe the causal mechanisms underlying the thiazides' effects on the vascular system in order to ease the mathematization of our findings.

Result. The thiazides have an immediate diuretic effect, followed by a decline in plasma volume. The body partially compensates for this fluid loss by activating the renin angiotensin aldosterone system (RAAS). A new steady state is established. However, after four to six weeks plasma volume and cardiac output paradoxically return towards pretreatment levels while the total peripheral resistance (TPR) decreases. Blood pressure remains lowered. The cause of this shift is unclear. The two main hypotheses seeking to explain this shift are named the direct vasodilation hypothesis and the reverse autoregulation hypothesis. In addition to providing the basic scaffold for modelling the effects of thiazides, we suggest how these two competing hypotheses might be tested in a computational physiology setting.

Conclusion. The meta-analysis shows that there is a dramatic variability in how individuals respond to thiazides. This variability appears to be poorly understood, in particular how the thiazides effect the TPR. Considering the therapeutic prominence of this drug class, this is somewhat surprising, and it suggests that we have a long way to go before we are in position to individualize the administration of thiazides and other anti-hypertensive drugs. And due to the complexities involved, it is hard to see how such individualization can be achieved without extensive use of computational models capable of accounting for the physiological variability of humans.

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Abbreviations

ATII angiotensin II

CO cardiac output

DBP diastolic blood pressure

ECV extra cellular fluid volume

eGFR estimated glomerular filtration rate

ESH/ESC European Society of Hypertension / European Society of Cardiology

FBF forearm blood flow

HUT head-up tilt

MAP mean arterial pressure

PCWP pulmonary capillary wedge pressure

PRA plasma renin activity

PRC plasma renin concentration

RAAS renin angiotensin aldosterone system

RAP right atrial pressure

RCT randomized controlled trial

SBP systolic blood pressure

TEA tetraethylammonium

TPR total peripheral resistance

WHO World Health Organisation

1 Introduction

1.1 High blood pressure as a global medical challenge

Hypertension is the leading risk factor for mortality in the world according to the 2009 Global Health Risk report from the World Health Organisation (WHO) [1]. The report relies on data from 2004. The aetiology of hypertension is poorly understood. "Secondary hypertension" is the term for high blood pressure caused by another medical condition and accounts for only 5-6% of the cases [2,3]. In the remaining cases the physicians find no obvious cause and the patient is given the diagnosis "primary" or "essential hypertension". The treatment of secondary hypertension is aimed at treating the underlying medical condition, whereas with primary hypertension the cause itself is unknown. Physicians are therefore left with manipulating blood pressure regulating systems instead of eliminating the cause, whatever that may be. According to uptodate.com, a well trusted clinical decision support website, *"the primary factors determining the blood pressure are the sympathetic nervous system, the RAAS, and the plasma volume (largely mediated by the kidneys)"* [1]. We can manipulate these systems with several kinds of drugs. One class of such drugs are diuretics.

1.2 The role of diuretics in treatment of hypertension

The diuretics play an important role in the treatment of hypertension. The general idea is that diuretics decrease plasma volume, and thus also blood pressure, by increasing the diuresis. The diuretics are divided into different groups based on their mechanism of action: osmotic agents, carbonic anhydrase inhibitors, loop diuretics, thiazides and potassium sparing agents. The 2013 European Society of Hypertension / European Society of Cardiology (ESH/ESC) Guidelines for management of arterial hypertension state that *"Diuretics have remained the cornerstone of antihypertensive treatment since at least the first Joint National Committee (JNC) in 1977(..)"*.(p.32) [4] In the choice of drug treatment the guidelines emphasize that reducing blood pressure is *the* important factor in reducing risk for cardiovascular events. They state that *"(...) the main benefits of antihypertensive treatment are due to lowering of BP per se and are largely independent of the drugs employed."*(p.32) [4]. Thus the guidelines conclude that diuretics, specifically thiazides, chlorthalidone and indapamide (thiazide-like diuretics), are all suitable for treatment of hypertension in the same manner as other antihypertensive drugs in both initial and maintenance therapy. Spironolactone, a potassium sparing agent, is suggested as a third or fourth

line drug. Loop diuretics are not mentioned in ordinary treatment, but are recommended to replace thiazides if serum creatinine is above 1.5 mg/dL or estimated glomerular filtration rate (eGFR) is under 30 mL/min/1.73 m^2.

The thiazides are a group of drugs defined by their action in the distal convoluted tubule in the nephron [5]. As mentioned in the ESH/ESC guidelines, thiazide and thiazide-like diuretics are the recommended diuretics in antihypertensive treatment. Since only hydrochlorothiazide and bendroflumethiazide are approved by the Norwegian Medicines Agency, only these will be subject to this review.

1.3 Computational physiology and hypertension

Computational physiology can be seen as part of the systems biology approach that has become increasingly popular the last two decades. Denis Noble, current President of the International Union of Physiological Sciences states that: *"Systems biology is where we are moving to(...). It is about putting together rather than taking apart, integration rather than reduction"* [6] (p. xi). The gain in popularity for making use of systems approaches in biomedical research is to a large degree due to the realization that the complexity and interwovenness of physiological systems prevent deeper understanding unless one makes extensive use of nonlinear system dynamics tools to guide and interpret experimental research. The subject matter of physiological research is indeed to understand the mechanisms and principles underlying biological systems behavior, and computational physiology has a long track record of applying systems dynamics models to understand biological function. The utility of computational models in physiology arises from their capacity to connect a comprehensive amount of empirical data into a functional whole, by enforcing explicit formulations of various hypotheses, by explicating the prediction space of hypotheses, by initiating and canalizing experimental or empirical work by pointing out key questions and the type of data needed, and by functioning as highly efficient interfaces between a range of disciplines.

Primary hypertension is a field in medicine where we have acquired huge amounts of information for more than 70 years, but the fact that there is still no consensus on what causes primary hypertension suggests that its etiology is highly complex. There have indeed been some attempts to make use of computational physiology to elucidate this etiology [7, 8], but these efforts are still dwarfed by experimental work guided by mostly simple conceptual schemes. It is reason to believe that substantial progress will be made in the hypertension field when one starts to establish strong theoretical-

experimental research programs where the experimental work is tightly linked to the construction and validation of computational models describing the development and maintenance of hypertension across a range of individual physiological profiles. At the Norwegian University of Science and Technology (NTNU) the NTNU Hypertension project tries, in collaboration with several international groups, to establish such a theoretical-experimental research program. One major goal of this program is to extend validated individualized models capturing the etiology of hypertension with the capacity to predict the effects of antihypertensive drugs. This capacity will make it possible to make use of the models as therapeutic tools. As a preparation for this work, one needs a compilation of the large amounts of information that exist on the effects of the most common drugs. Considering the clinical prominence of thiazides, a compilation of what is known about the effects of this drug class is arguably the most natural starting point.

The objective of this paper is thus to provide an overview of the thiazides' hemodynamic effects in humans with a particular focus on compiling and assessing information instrumental for constructing physiological computational models capable of describing the effects of this drug class in individuals.

2 Method

2.1 Data selection

In order to investigate the hemodynamic effects of thiazides we performed a literature search in the PubMed database. After trying out different synonyms and search words we ended up with sixteen words which we combined with the different thiazides and loop diuretics approved by the Norwegian Medicines Agency. The thiazides were hydrochlorothiazide and bendroflumethiazide, while the loop diuretics were furosemide and bumetanide. The search words were 'hemodynamics', 'blood volume', 'vasoconstriction', 'vasodilation', 'mechanism', 'plasma volume', 'blood pressure', 'vascular', 'baroreflex', 'pharmacodynamics', 'stroke volume', 'diuresis', 'urine output', 'plasma renin activity', 'pulse pressure', 'cardiac output'. Each drug was combined with each of these search words giving a total of 64 searches and 2405 hits.

Out of these 2405 hits only 233 were included in our database based on the following criteria. We only included studies on humans. The study had to be written in English and contain quantitative information on parameters we found to be suited for a computer model, such as hemodynamic and hormonal parameters. The subjects had to be 18 years of age or older and have no other medical condition than uncomplicated essential hypertension. The drug used had to be among those outlined above and ideally given as monotherapy. The only combined therapy studies we included involved combination of thiazides with potassium supplements. We excluded case-reports from our material.

We organized the selected publications in a Mendeley database. Based on the abstracts we categorized and tagged the articles after search word, study design, drugs given, parameters and population. In agreement with the project group we subjectively categorized the studies in three different groups based on how relevant each study seemed to be for the mathematical model. After this period of categorizing we went through the articles one more time. Based on the abstracts and results we revised the tags and wrote a little note about the most important findings in each study. During this process 14 more studies were excluded from the database according to the exclusion criteria above. After the systematic literature search and exclusion process, the database included 214 studies. However, some studies were found to be relevant despite not being found through a systematic literature search. Such studies were stored in a special folder and served to broaden our perspective on the matter. For this group, containing 39 articles, the inclusion criteria were not as stringent. These were for example trials done with types of thiazides not registered in

Norway or studies picked from the reference list from the 2013 ESH/ESC Guidelines for management of arterial hypertension [4]. Three [9–11] of these 39 studies were included in our meta-analysis.

The next step was to summarize our findings and give a qualitative presentation of them for our supervisors and the staff working with the mathematical model. Based on their feedback we decided to perform a meta-analysis for each parameter based on the studies in our Mendeley database. For loop diuretics we evaluated all the identified articles. Regarding the thiazides, we started with the ones we had initially categorized as most relevant. Subsequently we looked through all studies categorized on specific parameters except from those that only investigated blood pressure, plasma renin activity (PRA), and body weight. This is because we already had investigated a sufficient number of studies on these parameters. In this manner we could focus our time on studies that described parameters we needed more data on. Within the available time frame, we succeeded in evaluating 112 of the 214 selected articles. Out of 117 articles on thiazides, 67 were not evaluated. From the titles and abstract of the 67 papers we did not evaluate, we have reason to believe that these did not contain information that would change the main conclusions of our meta-analysis.

2.2 Data pooling

Most of the studies in our Mendeley database contain quantitative information on predefined parameters of interest. The majority of these studies also have information on the variance of their data, i.e. standard error of the mean or standard deviation. We performed a meta-analysis of each parameter in order to better describe the true effect size of the drug intervention. Note that data from both healthy and hypertensive subjects were pooled together.

We extracted quantitative data from each parameter in each study and wrote the data into a .txt-file. The data we extracted were: 1) time after drug administration, 2) value of parameter, 3) standard deviation or standard error, 4) number of observations (i.e. number of subjects). These .txt-files were categorized in directories based on the parameter they described. The data pooling itself was done using a self-written Python 2.7.12 script (see A B). Python is a widely used programming language. The script is based on the algorithm described here: http://www.burtonsys.com/climate/composite_standard_deviations.html.

2.3 Specific challenges regarding data pooling

Some studies used different units describing the same parameter. An example of this is that most studies on PRA use the unit ng angiotensin I produced per ml per hr. One study, however, used the unit ng angiotensin I produced per ml per 3hr. Both units reflect the concentration of renin, but are they comparable? The amount of angiotensin I produced as a function of time is not linear as it is critically dependent on the remaining angiotensinogen in the blood sample. We were therefore unable to convert the units, at least without some kind of model for the enzyme kinetics. We chose to include the study with the 3hr time unit in our metaanalysis without correcting for the difference in units. We assume that the difference in results obtained due to the different units after normalization is negligible.

Another major problem in our data pooling is that different studies used different measuring positions, age groups as well as patients with very different initial parameter values. For example, some studies looked at the blood pressure effect for patients with extreme hypertension, while other studies looked at patients with borderline hypertension. If we pool these studies we would get a quite large pooled standard deviation even at the time of drug administration. One way to deal with this would be to stratify the studies based on all these different initial settings. A relatively low number of studies per parameter kept us from doing this in fear of having too few studies for each meta-analysis. We chose to work around this problem by converting the data from each study from absolute values to relative values. Each study was defined as having parameter value = 1 at time = 0. The rest of the values would be relative to the initial value. This relative-effect-procedure was performed prior to the data-pooling.

2.4 Graphical visualization

Each pooled data point is represented by a red circle. The area of the circle represents the number of subjects for each data point relative to the number of subjects at t=0. The number of subjects at t = 0 is written in a small box in each figure. Each circle is accompanied with an error bar representing the pooled standard error of the mean.

In addition to the red data points we also performed a "locally weighted scatterplot smoothing" (LOWESS) to better visualize a trend in our data. This regression is represented by a green line. Usually a LOWESS is performed on a scatter plot, whereas we only had data on sample means and their variance. We therefore regenerated a possible sample from each sample mean prior to the LOWESS regression.

Using a regenerated sample does not cause bias for this purpose as the LOWESS regression only relies on the average y-values for an interval of x-values. This regression line must be assessed with some caution though, as it shows no estimate of the data variance. For some data sets the data was too sparse to justify using LOWESS and thus only the red circles and their error bars are shown. The LOWESS function we used can be found here: http://statsmodels.sourceforge.net/devel/generated/statsmodels.nonparametric.smoothers_lowess.lowess.html

The studies used for each graph can found in the list of figures.

2.5 Blood pressure calculations

We calculated the mean arterial pressure (MAP) and pulse pressure based on the systolic blood pressure (SBP)- and diastolic blood pressure (DBP)-data. MAP was calculated using equation 1. Pulse pressure was calculated as the difference between SBP and DBP. In order to calculate the correct data variance for MAP and pulse pressure one would need access to the SBP- and DBP-data for each individual. As we only had access to the entire sample mean and its variance we were unable to calculate the correct data variance for MAP and pulse pressure. We therefore chose to make the graphs based on a worst-case scenario, i.e. the max theoretical variance. We calculated the highest possible variance by defining the correlation between SBP and DBP as minus one, which is probably very unlikely biologically. We therefore probably grossly overestimate the uncertainty associated with the MAP and pulse pressure-graphs. The formulas used were:

$$\text{MAP} = \frac{1}{3}(2\text{DBP} + 1\text{SBP}) \tag{1}$$

$$\text{Var}(aX - bY) = a^2\text{Var}(X) + b^2\text{Var}(Y) - 2ab\text{Cov}(X, Y) \tag{2}$$

$$\text{Corr}(X, Y) = \frac{\text{Cov}(X, Y)}{\sigma_x \sigma_y} \tag{3}$$

Where X and Y are two variables, σ is the standard deviation, $\text{Cov}(X, Y)$ is the covariance and $\text{Corr}(X, Y)$ is the correlation.

3 Results

In the following section we first go through each individual parameter and summarize some of the most important articles and reviews. Secondly, we present the results of our meta-analyses and compare these to the high impact papers. For each parameter we start with thiazides and end with loop diuretics. Note that studies on loop diuretics often have a shorter period of observation as this drug class is generally used for treating acute or semiacute conditions. These studies are therefore only comparable with thiazides for the equally short time-span. Note that the time units for our graphs are days for thiazides and hours for loop diuretics.

3.1 Plasma volume

There is a broad agreement that thiazides initially decrease plasma volume. Then after approximately 4 to 6 weeks the plasma volume increases and approaches pre-treatment level [5, 9, 12–14]. Figure 1 is copied from Shah et al. [12] and is a good example of the changes seen on plasma volume in many of the studies we reviewed. Notably, plasma volume was only measured in eight subjects in this study. There is some dispute whether plasma volume goes all the way up to the pre-treatment level or not. Some of the studies show that the volume goes all the way up [9, 15]. Others show that it stabilizes under pre-treatment levels [5, 10, 16]. However, Tarazi et al. [17] found that discontinuation of long-term thiazide therapy, ranging from 6 months to 2 years, gave a rapid volume increase. This indicates that the treated individuals are in a physiologically hypovolemic state throughout long-term therapy.

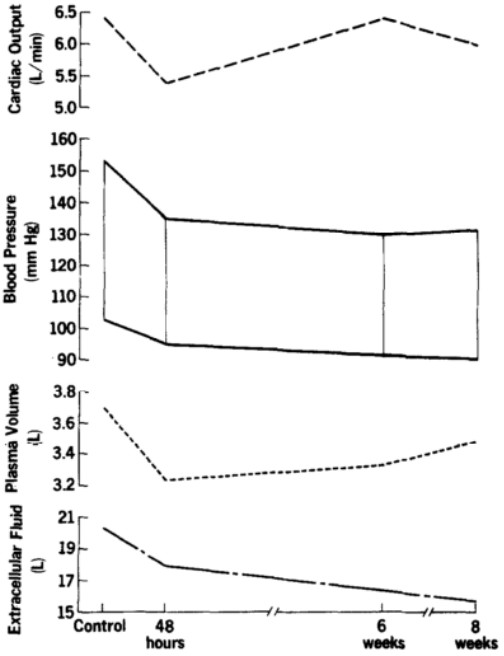

Fig. 1. Mean changes in cardiac output, blood pressure, plasma volume, and extracellular fluid volume (thiocyanate space) before as well as 48 hours, 6 weeks, and 8 weeks after treatment with hydrochlorothiazide.

Figure 1: Shah et al. [12]

When comparing the findings from these studies with our meta-analysis we find similarities, but also some differences (Fig. 2). Our LOWESS regression line of pooled relative change of plasma volume shows a rapid decrease the first days. After approximately 14 days the plasma volume stabilizes and possibly increases towards

pre-treatment levels. After 130 days the regression line decreases a second time. However, note that this decrease is based on one small study with only 15 patients. We would like to stress that the regression line does not give an estimate of the uncertainty, and that the reader must assess the uncertainty him- or herself. We assess the apparent secondary decrease in plasma volume as too uncertain. In fact, a central part of the theories regarding the long-term effects of thiazides is to try to explain the apparent long-term increase towards pre-treatment values. Later in this paper we will therefore assert that there is no secondary decrease in plasma volume. Note that our regression line before day 130 seems to follow the trend equal to that of Shah et al. (Fig. 1). Our meta-analysis suggests a sustained decrease of plasma volume compared to pre-treatment. However, interstudy and interindividual variability is so large that this average shift may not always occur.

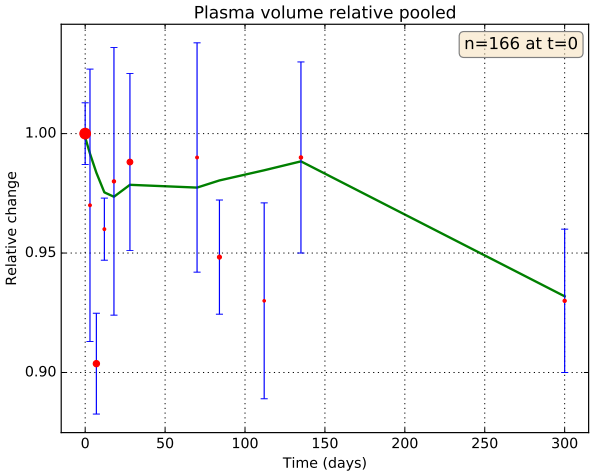

Figure 2: Plasma volume, thiazides

The data are even more sparse when it comes to the loop diuretics. One study with 8 patients shows a decline in plasma volume of 8% (SEM=1.3%) two hours after intravenous administration of 0.5mg/kg furosemide [21]. Another study found insignificant changes in plasma volume and extra cellular fluid volume (ECV) after one month of 80mg/day furosemide administered orally [22]. Interestingly, these data support to some degree the claim of some authors that plasma volume paradoxically normalizes itself after a couple of weeks of diuretic treatment.

3.2 Extracellular fluid volume

The ECV decreases rapidly the first days after initiation of thiazide therapy according to Shah and van Brummelen [12, 18]. As seen in Shah's data (Fig. 1) ECV stabilizes at a new level significantly below pre-treatment levels. Contrary to plasma volume the ECV does not seem to increase markedly during long-term therapy. Tarazi [17] measured a non-significant increase in ECV after discontinuation of thiazide therapy, but this was only based on five subjects. These studies were the only three we found

on ECV related to thiazides. Only van Brummelen's study contained information on data variance. Hence, we were not able to do a meta-analysis of the ECV. Figure 3 shows the changes in bromide space volume (proxy for ECV) observed by van Brummelen et al. [18] after 1 week and four months of hydrochlorothiazide treatment.

Based on van Brummelen, Shah and Tarazi, it seems as though ECV stabilizes on a proportionally lower level than the plasma volume after long-term therapy. This is surprising because it would imply that the equilibrium between plasma volume and ECV changes during long-term treatment. However, all these studies are based on small numbers of individuals, with a total of 22 subjects. The results are therefore uncertain, and we were not in position to decide whether the apparent difference in ECV and plasma volume trends are statistically significant or not.

The only data on ECV concerning loop diuretics was one study with 8 patients which showed insignificant changes in ECV after one month of 80 mg/day oral furosemide [22].

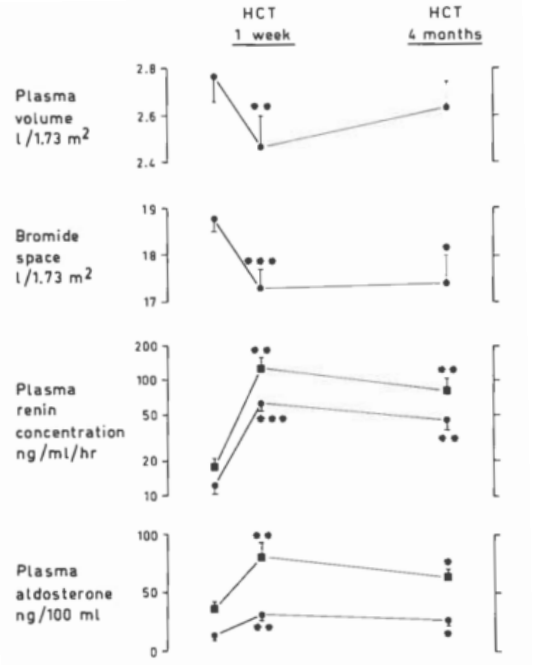

Fig. 1. Plasma volume, bromide space, renin and aldosterone before treatment and after 1 week and 4 months of hydrochlorothiazide treatment (mean ± S.E.M.). Significance of differences compared with pretreatment values $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 3: van Brummelen et al. [18]

3.3 Cardiac output

According to a review from 2009 [5], the cardiac output (CO) decreases the first 2-4 weeks before it returns to pre-treatment levels after months with thiazide therapy. Most of the studies we found agree with the initial decrease. The following return to pre-treatment levels is more disputed. Shah [12] and van Brummelen [18] found that CO returns all the way to pre-treatment levels. However, Lund-Johansen [10] and Dahlöf

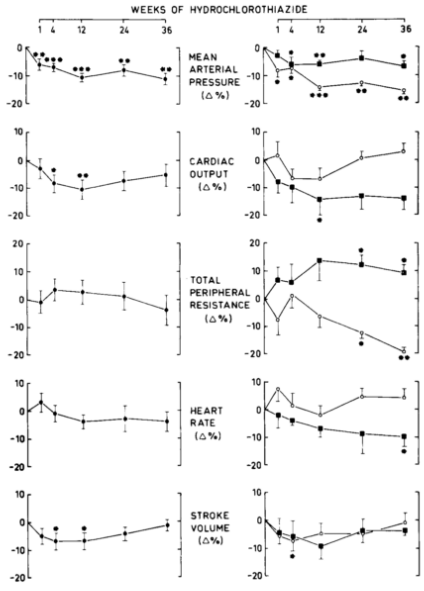

Fig. 1. Percentile changes in hemodynamic variables during treatment of essential hypertension with hydrochlorothiazide. Mean values ± SE are shown. *Left panel:* All patients; *right panel:* responders *(circles)* and nonresponders *(squares)*. The number of patients is given in Table II. Significance of deviation from placebo values:* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Figure 4: van Brummelen et al. [19]

[23] found that it increased, but not entirely to pre-treatment levels. Van Brummelen et al. [19] made an interesting finding when they differentiated between blood pressure responders and non-responders (Fig. 4). The subjects who had a blood pressure fall less than 10 mmHg were defined as non-responders. The study showed that during long-term therapy, the CO among the responders returned to pre-treatment level. On the other hand, the non-responders stabilized under the pre-treatment level. This difference in CO-response was not significant, however.

The LOWESS regression line based on our meta-analysis (Fig. 5) shows an initial decrease in CO that lasts several weeks into the thiazide treatment period. It shows a trend to increase towards pre-treatment levels after several weeks, but does not seem to continue all the way to pre-treatment level. Figure 6 shows a 20% reduction in CO the first two hours after intravenous administration of loop diuretics.

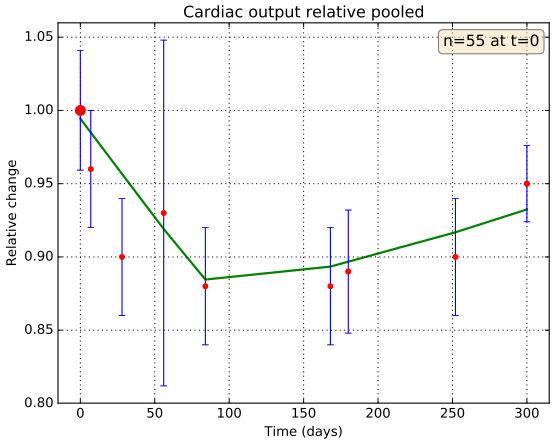

Figure 5: Cardiac output, thiazides

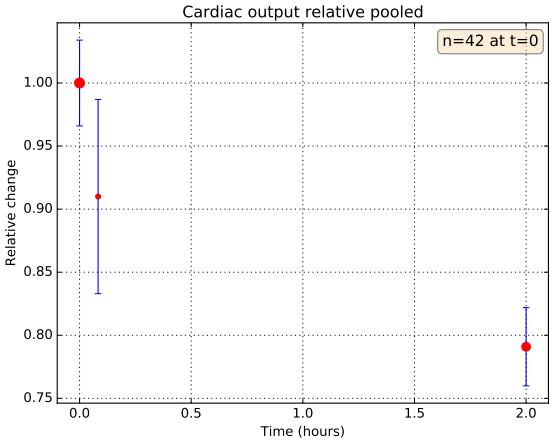

Figure 6: Cardiac output, loop diuretics

In a study by Iwasaki et al. [27] (Fig. 7) they describe changes in CO, pulmonary capillary wedge pressure (PCWP), right atrial pressure (RAP) and stroke volume after administration of furosemide. PCWP is used as an estimate of the left atrial pressure. Interestingly the PCWP and RAP decreases by a dramatic 40%, whereas CO and stroke volume only decreases 10% and 20% respectively.

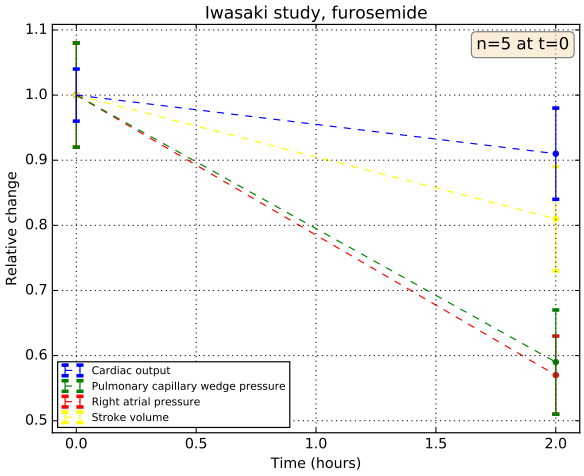

Figure 7: Iwasaki et al.

3.4 Plasma renin activity or concentration

To measure plasma renin levels one may use PRA or plasma renin concentration (PRC). PRA measures the amount of angiotensin 1 produced per time unit (usually 1 hour) while PRC measures the concentration of renin in the plasma. The two different methods correlate well [39]. We have included studies using both measurements in our meta-analysis. For the rest of this article we will refer to either of the two terms as "plasma renin" for the sake of clarity.

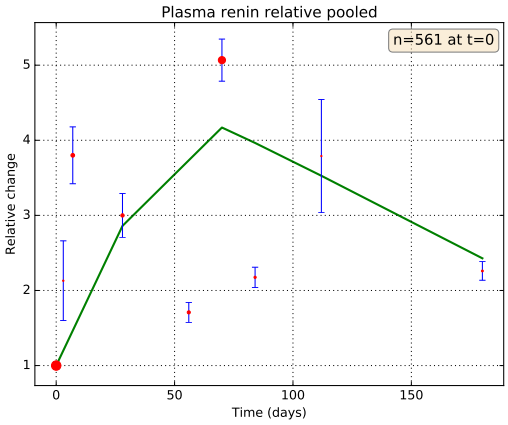

Figure 8: Plasma renin, thiazides

According to Ernst [5] and several others [13, 18, 29, 30, 40, 41] the plasma renin level increases the first days to weeks during thiazide therapy. After several months the plasma renin continues to stay high, but stabilizes at a level slightly below the one observed shortly after therapy initiation. Tarazi [17] showed that after discontinuation of thiazide therapy the plasma renin decreases towards pre-treatment level. The trend of our LOWESS regression line (Fig. 8) corresponds well with apparent current consensus regarding plasma renin changes during thiazide therapy. The plasma renin increases rapidly. It peaks at level about 4 times the initial plasma concentration and continues to stay elevated throughout the treatment period.

The increase in plasma renin and activation of the RAAS is thought to attenuate the antihypertensive effect of thiazides. Thus when thiazides are combined with RAAS-inhibitors the antihypertensive efficacy is markedly increased [49–51]. Some patients fail to lower the blood pressure adequately with thiazide therapy. One could suggest that this is due to an effective activation of the RAAS system. Some small studies do not support this theory [13, 30]. However, a big study with 343 patients receiving hydrochlorothiazide found a significantly

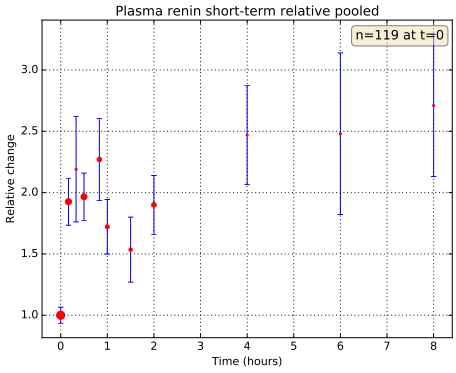

Figure 9: Plasma renin, short-term, loop diuretics

larger increase of plasma renin in the patients that either needed maximum doses (200mg/day) or did not respond at all, compared to the patients responding to the lowest doses (25 and 50 mg/day) [32].

Van Brummelen et al. [18] investigated which parameters correlated with the plasma renin level during short and long-term therapy. Their results showed that during the first week of therapy the supine plasma renin correlated directly with heart rate and inversely with plasma volume and ECV. However, after 4 months of therapy, this correlation was absent and the supine plasma renin correlated with serum concentration of sodium instead. The authors therefore suggest that the initial elevation in plasma renin is due to reduced fluid volumes and increased neuronal activity in the sympatetic nervous system. They further suggest serum sodium to be a more important factor for the sustained elevation in plasma renin during long-term therapy. This is in conflict with the findings of Tarazi et al. [17] which showed a substantial reduction in plasma renin after discontinuation of long-term thiazide therapy (6-24 months). In this study plasma renin correlated with the increase in plasma volume seen after thiazide discontinuation, but not with the serum sodium concentration. Based on these two studies it is difficult to conclude which factor is the most important for the plasma renin level during long-term treatment. Both of the studies are small. Van Brummelen et al. [18] studied 9 subjects and Tarazi et al. [17] only 8, where one of the 8 subjects got chlorothiazide instead of hydrochlorothiazide. Another important factor is that van Brummelen et al. [18] standardized the sodium and potassium intake the last 10 days before treatment to 50 mmol sodium and 90 mmol potassium/day. Tarazi et al. [17] did no such standardization. Since both potassium and sodium have an effect on the renin release, this is something that should be taken into consideration. None of the studies had placebo control groups.

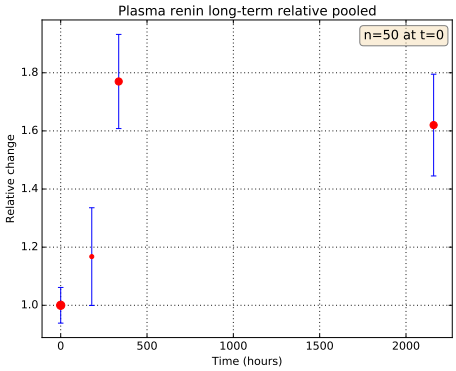

Figure 10: Plasma renin, long-term, loop diuretics

Plasma renin increases twofold within half an hour after intravenous administration of loop diuretics (Fig. 9). If the subjects are given oral tablets for 2-3 months (Fig. 10) the plasma renin levels are still about two times higher than the initial value. Thus, both thiazides and loop diuretics appear to give a persistent and strong increase in plasma renin.

3.5 Angiotensin II

No studies in our database describe changes in angiotensin II (ATII) after thiazide treatment. However, two studies measured ATII after administration of furosemide and bumetanide (Fig. 11). They show that ATII increases about 50% within half an hour after giving the drug.

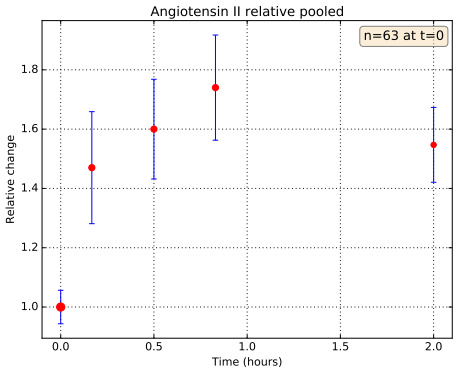

Figure 11: Angiotensin II, loop diuretics

3.6 Heart rate

There are some small variations between the different studies concerning thiazides' effect on heart rate. Some show a slight increase in heart rate the first days followed by a normalization after weeks [18, 54]. Other studies found the heart rate to be unchanged [12]. When we pool the data from all the studies on heart rate in our database we find that the heart rate deviates minimally from pre-treatment level (Fig. 12). This suggests that the initial decrease in CO described above is caused by reduced stroke volume. The heart rate seems to play a less important role in that matter. One could expect that the heart would increase its rate to compensate for the volume loss. However, this does apparently not happen. Heart rate seems to be stable up to 8 hours after loop diuretic administration (Fig. 13).

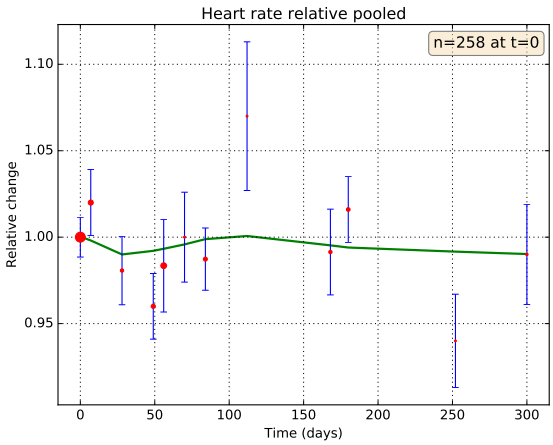

Figure 12: Heart rate, thiazides

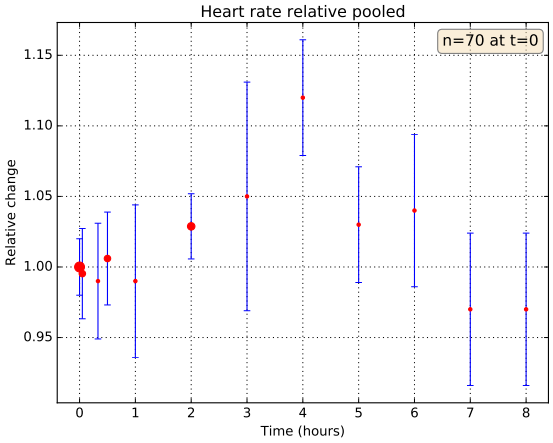

Figure 13: Heart rate, loop diuretics

3.7 Total peripheral resistance

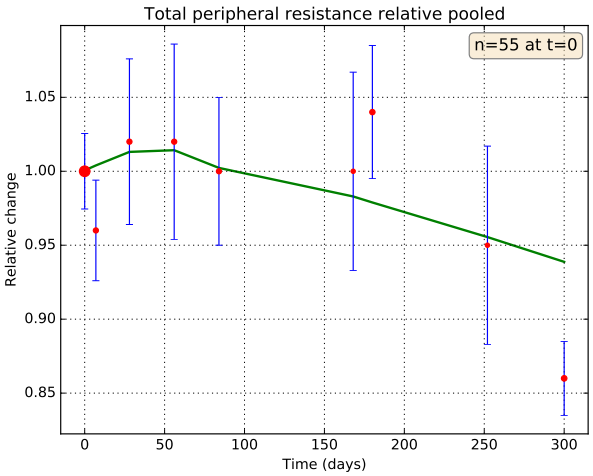

Figure 14: Total peripheral resistance, thiazides

TPR is calculated by dividing the difference in arterial and venous pressure by CO. Thus, TPR is measured indirectly. Shah et al. [12] observed a 16% increase in TPR the first 48h of thiazide therapy, with a p-value of 0,09 compared to pre-treatment. After six weeks the TPR decreased to a mean below pre-treatment, although not significantly. Van Brummelen et al. [19] observed neither an initial increase nor any long-term decrease after 24 and 36 weeks when they lumped responders and non-responders together. When differentiating between the two groups they found that the responders had a significant decrease in TPR after 24 and 36 weeks compared to placebo. The non-responders had on the other hand a significant increase in TPR after 24 and 36 weeks compared to placebo. See Figure 4 for details. Even though these two studies include few subjects, they are both well designed, and both suggest a long term-term reduction in TPR. Our pooled graph (Fig. 14) is based on four studies, including that of van Brummelen et al. [19]. It suggests a small increase of 2-3% the first 60 days, before it decreases below pre-treatment levels during long-term therapy. This two-phase pattern is, as the error bars show, uncertain. We were unable to integrate the study of Shah et al. [12] in our meta-analysis as it lacked data on SEM or SD. If we were able able to integrate their data in our meta-analysis, it would have been more in favor of the two-phase pattern, as Shah observed an increase of 16% whereas our meta-analysis only has a peak of about 2-3%. Although we believe the evidence for this two-phase pattern is relatively sparse it seems to be the consensus, as many authors have published papers trying to explain this pattern [61]. We will discuss these efforts in detail later.

No studies in our database describe changes in TPR after loop diuretic treatment.

3.8 Adrenaline and noradrenaline

We found no studies on the short-term change in plasma adrenaline or noradrenaline levels during thiazide therapy. The earliest measurements were done after four weeks

[33]. Our data on 85 patients from five studies seem to show divergent responses, although most of the patients have an increase of about 20% after about 8 weeks (Fig. 15, 16). One interesting study measured 7 supine and after 5 and 20 minutes head-up tilt (HUT). We can clearly see that the individuals' posture during measurement affects the levels of noradrenaline and adrenaline far more than the drug therapy itself (Fig. 17).

Noradrenaline clearly increases after intravenous administration of loop diuretics (Fig. 18).

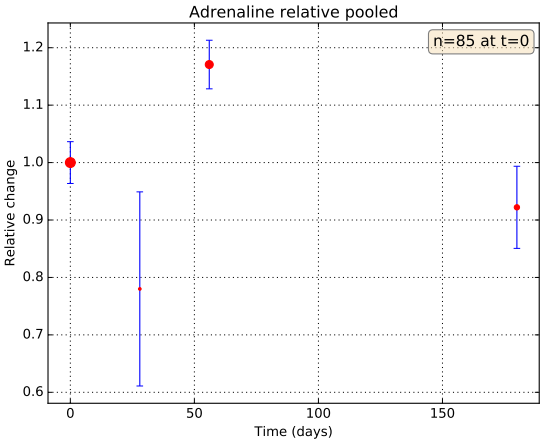

Figure 15: Adrenaline, thiazides

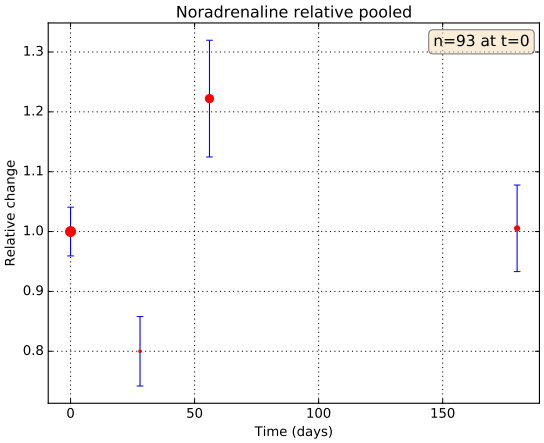

Figure 16: Noradrenaline, thiazides

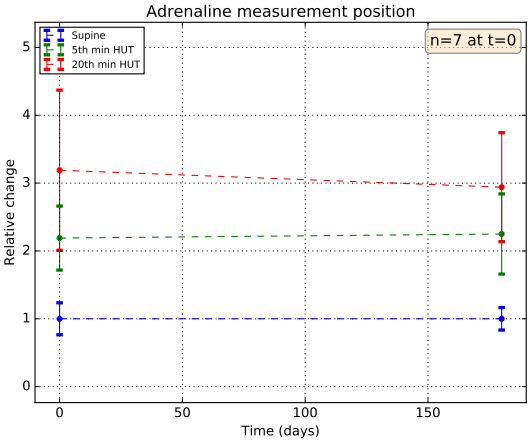

Figure 17: Adrenaline measurement position, thiazides

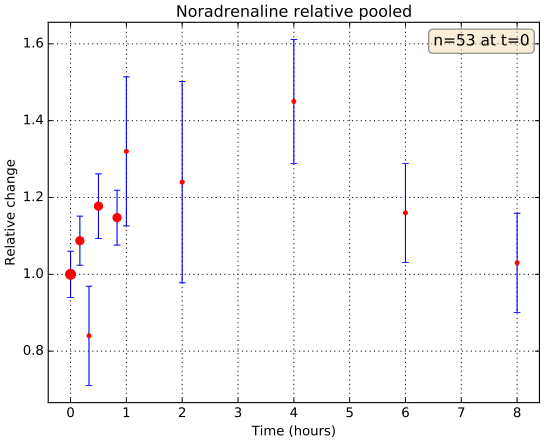

Figure 18: Noradrenaline, loop diuretics

3.9 Antidiuretic hormone

Okada [63] measured ADH before treatment and after 180 days with thiazide therapy. The measurements were done in supine and in 60 degree HUT position after five and 20 minutes. The only change was found in HUT position after five minutes, from ADH levels of 0.54±0.11 pg/ml before treatment to 0.70±0.53 pq/ml at 6 months. This was the only study we found on changes in ADH after thiazide therapy.

No studies in our database describes change in ADH with data variance after administration of loop diuretics.

3.10 Body weight

Several studies observed a stable decrease in body weight during thiazide therapy [9, 12, 13, 18]. Our regression line shows the same trend (Fig. 19). This reflects that the diuresis increases and the ECV and plasma volume go down. It may suggest that the body is in a hypovolemic state throughout treatment. Based on our meta-analysis we can not conclude whether the weight loss continues throughout treatment or if it stabilizes on a new steady level.

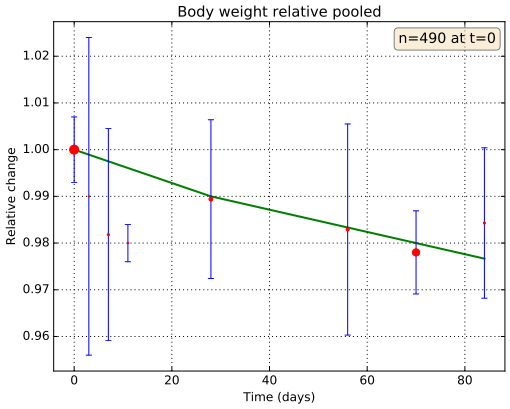

Figure 19: Body weight, thiazides

When it comes to loop diuretics body weight seems to decrease within hours and may or may not persist (Fig. 20, 21).

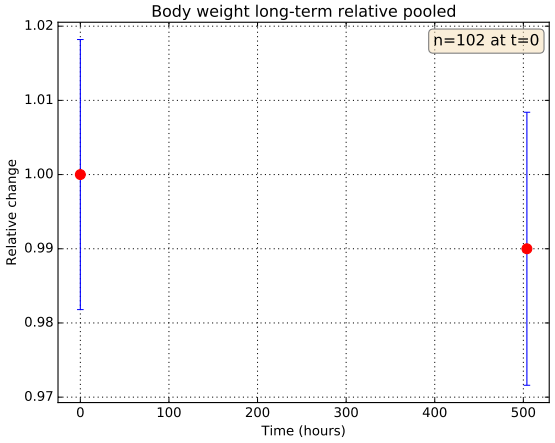

Figure 20: Body weight, long-term, loop diuretics

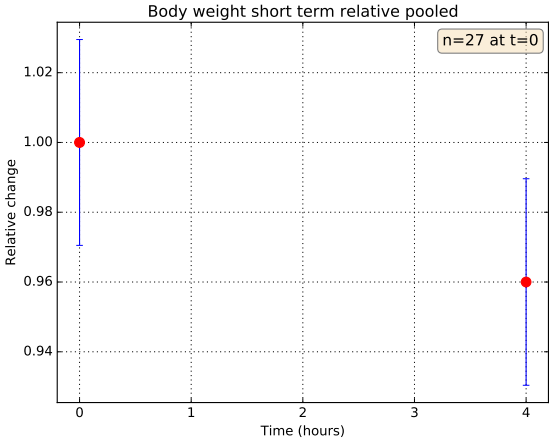

Figure 21: Body weight, short-term, loop diuretics

3.11 Aldosterone

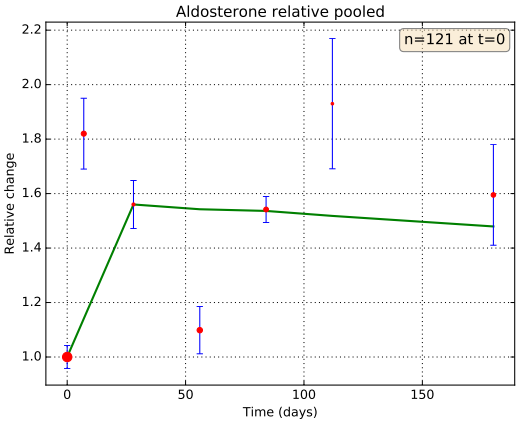

Figure 22: Aldosterone, thiazides

The plasma level of aldosterone appears to increase during thiazide therapy. Van Brummelen [18] measured an increase from 5.7±0.9 ng/100ml (±SEM), to 8.5±0.6 after one week. After 12 weeks of therapy the plasma aldosterone level was still high: 8.7±0.6. Our pooled graph (Fig. 22) has more deviations and uncertainty than that of van Brummelen (Fig. 3). It is although consistent with an increase in plasma aldosterone level which is maintained throughout therapy. This corresponds well with the changes in plasma renin levels seen above and is consistent with a considerable activation of the RAAS after administration of thiazides.

No studies in our database describe changes in aldosterone after administration of loop diuretics.

3.12 Blood pressure

The antihypertensive effect of thiazides appears to be significant and stable over time. In a meta-analysis from 2010 [66] the average SBP response before 4 weeks was -16 mmHg. For subjects receiving hydrochlorothiazide over a period ranging from 12 to 52 weeks the average SBP response was -19 mmHg from baseline. This suggests that the blood pressure declines rapidly the first 4 weeks, before it starts to stabilize. These data are from studies using doses between 12,5 - 25mg/day. A six-year follow-up study on bendroflumethiazides [67] shows that the reduced blood pressure is maintained throughout the whole period of treatment.

A Cochrane meta-analysis from 2014 [68] found that thiazides lower SBP more than DBP, giving a decrease in the pulse pressure by 4 to 6 mmHg, as would be expected from a decrease in stroke volume (Cf. 3.6 Heart rate). The same meta-analysis concluded that hydrochlorothiazide has a dose-related antihypertensive effect, while the other thiazides have a maximal effect on the lowest doses. There were no significant differences between the antihypertensive effects of the thiazides when they were given at a maximal dose.

Duarte's (2010) review [61] on the mechanisms underlying thiazides' antihyper-

tensive effects suggests that the stable blood pressure reduction throughout treatment is due to two different mechanisms, one operating at a short time-scale and the other operating at a much longer time-scale. This suggestion was based on two studies where they infused dextran, a volume expander, intravenously during thiazide therapy. In the first study [69] they infused dextran after 2 weeks to 3 months of thiazide therapy. In the other study [70] they infused dextran after 2 weeks to 9 months. In the first study, with a mean treatment duration on 1.4 months, the dextran infusion restored the blood pressure to pre-treatment level. In the second study, with a mean treatment duration on 2.5 months, the blood pressure was not restored. Duarte [61] uses this to claim that the mechanisms underlying the short term effect is dependent on the plasma volume, while the long term effect is less dependent on plasma volume. This corresponds well with the long term increase in plasma volume and CO and the reduction in TPR described above. However, we would like to emphasise that the treatment periods in the two dextran studies overlap. Besides, only one of the studies standardized salt intake and in the first study the patients continued their other anti-hypertensive medications throughout the study. Based on this we are concerned whether these studies and their data are reliable to make such a claim which Duarte [61] does. However, based on the observations made on plasma volume CO and TPR, we agree that there may be a difference in the mechanisms underlying the short- and long-term effects of thiazides. We will discuss this in detail later (3.13. Interpretation of empirical data).

There is a marked interindividual and interracial variation in the blood pressure response to thiazides [3, 32, 71, 72]. One study [3] compared the blood pressure response in two separate treatment periods with the same individuals. They found that the mean reduction in SBP and DBP correlated significantly the first and second treatment period. However, the individual response varied between the two treatment periods. This indicates that it is challenging to predict how an individual will respond to thiazide therapy.

In a randomized controlled trial (RCT) [71], 1292 subjects received placebo or one of six different antihypertensive drugs. 188 men were treated with hydrochlorothiazide (HCTZ). After one year of therapy, 46% of the men in the HCTZ-group, responded to therapy and reached the target DBP. White hypertensive men under 60 years of age had a response rate of 32%. Those over 60 had a response rate of 52%. Black hypertensives over 60 years had a response rate of 58%. Those under 60 had a response rate of 40%. This indicates that the black and elderly respond better to thiazides than the young and white.

Freis et al. [32] differentiated between four patterns of blood pressure response to hydrochlorothiazides. This was based on a 10 week titration trial with doses from 50 to 200 mg hydrochlorothiazides/day. 52 % of the responders reached the DBP goal at 90 mmHg with a dose of 50 mg/day and an average weight loss on 1.58 kg (SD=1.91). The patients with such a response pattern were categorized as group 1. Group 2, comprising 29% of the responders, needed twice as high thiazide dose as the first group to reach the blood pressure goal. This was achieved with approximately the same amount of weight loss (1.63 kg, SD=1.72). Group 3 reached the blood pressure goal with a dose on 200 mg hydrochlorothiazide/day and twice the weight loss of the two first groups (3.14 kg, SD=2.50). This group comprised 19% of the responders. The fourth group were the non-responders. These failed to reach the blood pressure goal at the highest dose (200mg/day) and comprised 35% of the 305 patients in the study. Their average weight loss was 1.66 kg (SD=2.18), which was significantly lower than the third group. These observations show that there is a great diversity between individuals in their blood pressure responsiveness to thiazides. The mechanism behind this diversity is not solely based on different responsiveness to the thiazides' diuretic effect itself. It is also dependent on how our bodies respond to the volume loss, here measured by weight loss. It looks like some are more resistant towards the diuretic effect and hence lose less plasma volume (group 2), while others are more resistant to the volume loss itself and therefore need a larger decrease in plasma volume to reach the blood pressure goal (group 3). We suggest that the differences in the diuretic effect is due to reduced bioavailiabilty of thiazides or to reduced sensitivity for thiazides in the NaCl-symporter in the distal convoluted tubules. For the third group we suggest that they have effective compensating mechanisms such as the RAAS and sympathetic nervous system. This is supported by the changes seen in PRA in the same study. The two first groups had significantly smaller changes in PRA compared to the third group and the non-responders. Interestingly, the black subjects had a larger blood pressure fall an higher responsive rate than the white. The amount of body weight loss was approximately the same between black and white subjects.

As shown in Figure 4, van Brummelen et al. [19] separated responders and non-responders by classifying the responders as those with more than 10% decline in MAP after 36 weeks compared to baseline levels. This study showed that the two groups had a similar decrease in blood pressure the first 4 weeks. However, after these 4 initial weeks the responders continued their blood pressure decline to below 10% of baseline levels, while the non-responders stabilized on the same level as after 4 weeks. This suggests that the differences between responders and non-responders first appear

after 4 weeks.

Our pooled data on blood pressure are in line with the consensus view that thiazides lower both SBP and DBP, as well as the MAP (Fig. 23, 24, 25). Our data is also in agreement with the Cochrane meta-analysis [68] that pulse pressure is lowered (Fig. 26). We would emphasize that the data are sparse after 100 days, and one should not pay too much attention to the regression line after this point. The error bars for MAP and pulse pressure represent a theoretical max (cf. 'Blood pressure calculations' in Method).

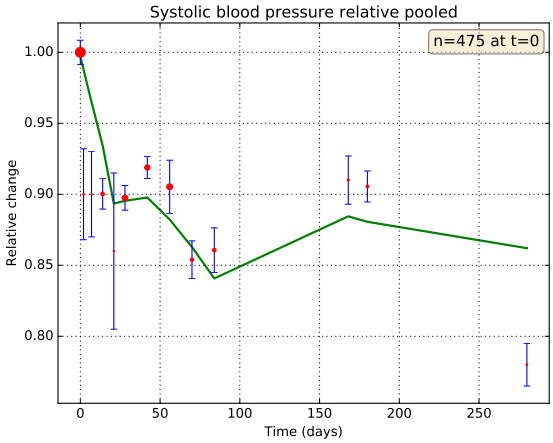

Figure 23: Systolic blood pressure, thiazides

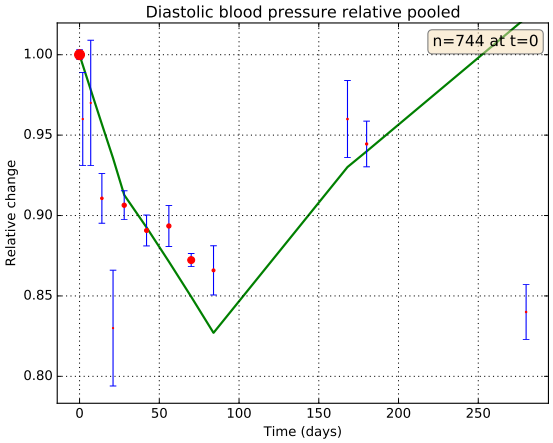

Figure 24: Diastolic blood pressure, thiazides

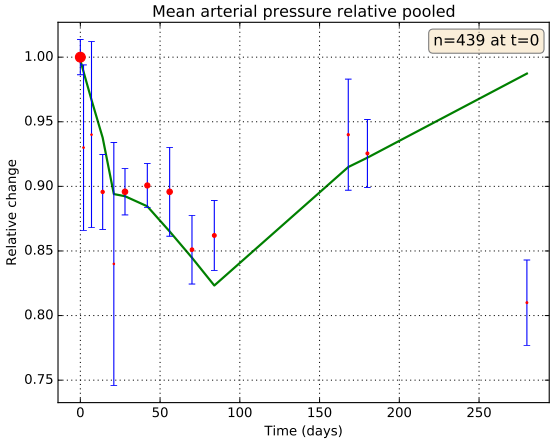

Figure 25: Mean arterial pressure, thiazides

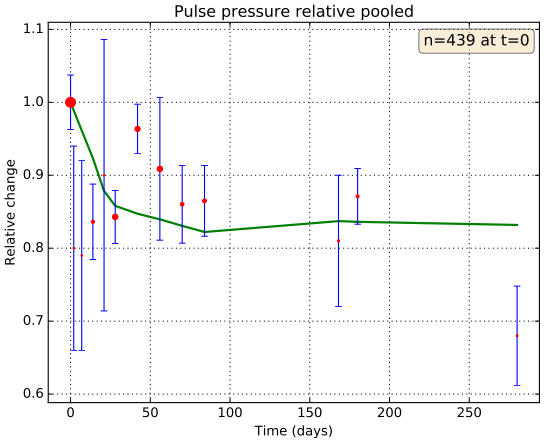

Figure 26: Pulse pressure, thiazides

The picture is the same for loop diuretics for the same time-span as the thiazides (Fig. 27, 28, 29, 30). The short-term effect is however a little bit surprising. DBP does not seem to decline at all the first hours, whereas SBP seems to decline quite notably (Fig. 31, 32). Pulse pressure is therefore reduced markedly in the first hours (Fig. 33). MAP is virtually unaffected (Fig. 34).

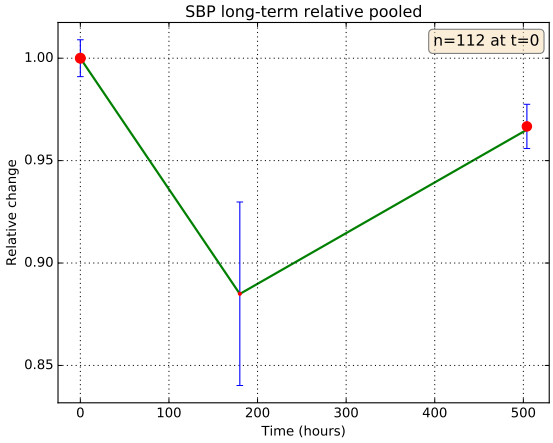

Figure 27: Systolic blood pressure, long-term, loop diuretics

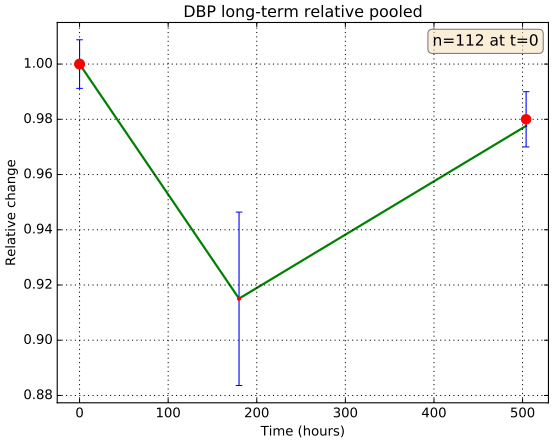

Figure 28: Diastolic blood pressure, long-term, loop diuretics

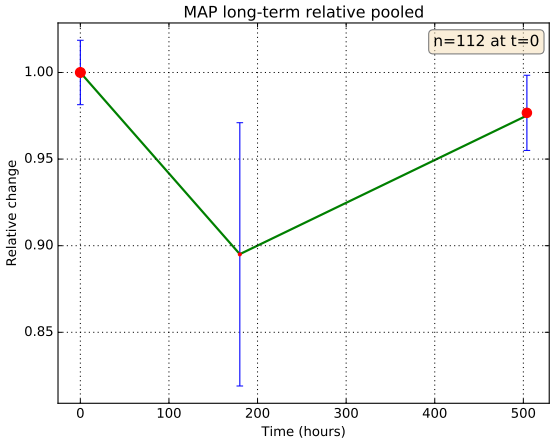

Figure 29: Mean arterial pressure, long-term, loop diuretics

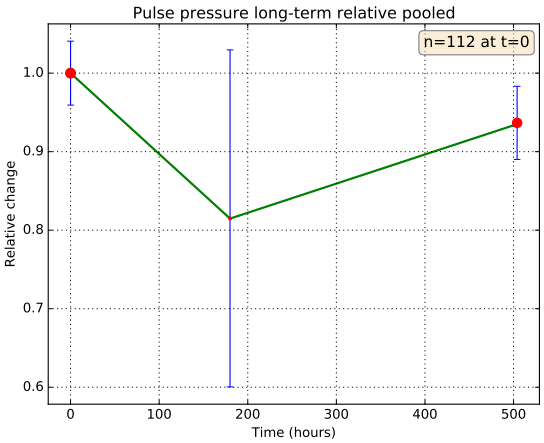

Figure 30: Pulse pressure, long-term, loop diuretics

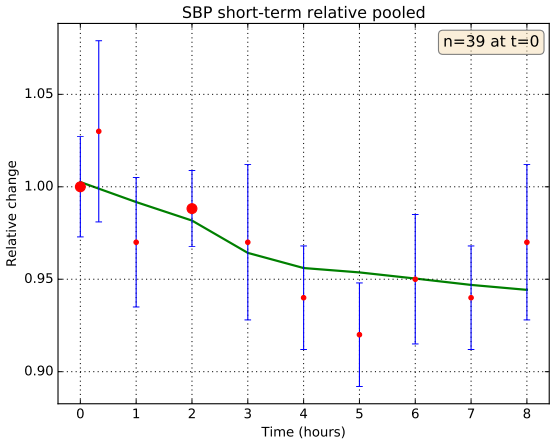

Figure 31: Systolic blood pressure, short-term, loop diuretics

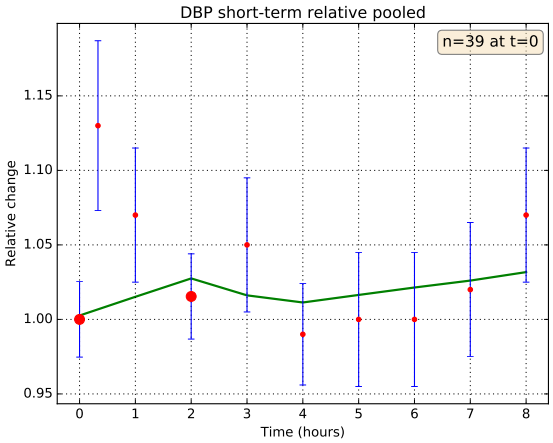

Figure 32: Diastolic blood pressure, short-term, loop diuretics

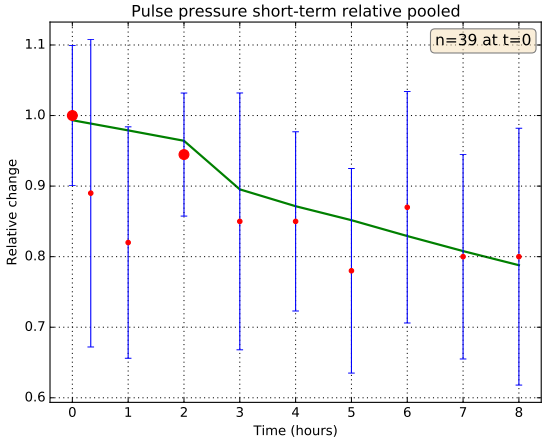

Figure 33: Pulse pressure, short-term, loop diuretics

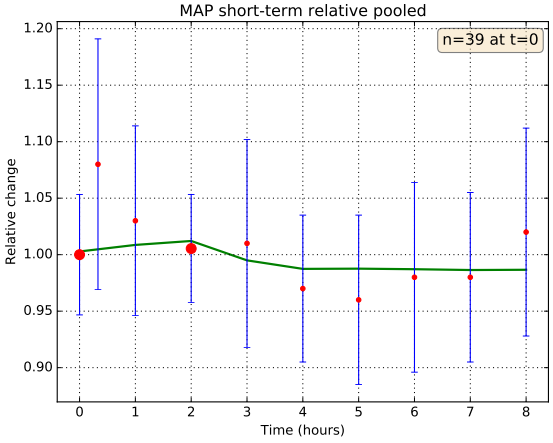

Figure 34: Mean arterial pressure, short-term, loop diuretics

3.13 Interpretation of empirical data

Above, we have focused on the thiazides' effects on separate measurable variables of the human body. We have looked on each system or parameter isolated. However, in the human body, few changes come isolated. Most often, one alteration has an effect on multiple other sites too. Causality is as always difficult to prove, but in the following we will try to flesh out the primary and secondary effects of thiazides in terms of clear experimental patterns that need to be accounted for. This is of course

a bit speculative, but quoting Novalis, hypotheses are like nets, only he who casts will catch. We will also present some hypotheses trying to explain the physiological changes observed during thiazide therapy. The table below sums up the change in each parameter. A central question is: How do these parameters act on each other and in what order?

Parameter	**Response to thiazides**
Plasma volume	2-3% reduction initially, long-term return towards pre-treatment levels.
Extra cellular fluid volume	Initial decrease. No signs of return towards pre-treatment levels. Uncertain.
Cardiac output	10% reduction initially, long-term increase towards pre-treatment levels.
PRA/PRC	Peaks about 4 x above pretreatment levels. May or may not stabilize. Large interstudy variation.
Heart rate	Minimal change, possibly unaffected.
Total peripheral resistance	A possible initial increase, followed by a long-term decrease to or below pretreatment levels. Uncertain.
Adrenaline and Noradrenaline	No clear trend.
Antidiuretic hormone	A possible long-term increase, very uncertain.
Body weight	A clear trend of a stable decrease of 1-3%.
Aldosterone	Initial increase that remains about 50% above pretreatment level. Large interstudy variation.
Systolic blood pressure	Initial decrease that stabilizes 10-15% below pretreatment level.
Diastolic blood pressure	Initial decrease that stabilizes 10-15% below pretreatment level.
Pulse pressure	Initial decrease that stabilizes 10-15% below pretreatment level.

Table 1: Overview of thiazides' effects, according to our study.

3.13.1 The initial effects

Some of the most marked effects of thiazides are the initial decreases in plasma volume, ECV and CO. We find it likely that these changes come as a consequence of a period of increased diuresis. This is also reflected in the body weight reduction. The reduction in plasma volume and ECV lowers venous return to the heart. The decreased filling pressure of the heart leads to reduced stroke volume and thus a reduction in CO. As a consequence of reduction in CO the blood pressure declines. Simultaneously the plasma renin and aldosterone levels increase. This could be explained by the compensating mechanisms for hypovolemia and/or hypotension, such as the baroreflex in the aortic arch and the carotid sinuses, as well as the renal perfusion systems. All these mechanisms contribute to increased sympathetic activity and activate the RAAS. The data from the studies we found show that the blood pressure response is maintained in most individuals despite the evident counter regulatory response. However, some patients fail to decrease their blood pressure further. This may be because they compensate too well with systems such as the RAAS-system. Up to this point the increased diuresis and reduction in plasma volume can explain most of the alterations of the other parameters. One exception is that the data on noradrenaline and adrenaline do not show a clear increase that would be expected by increased sympathetic activity. This apparent mismatch may be explained by the notion that noradrenaline and adrenaline perhaps only react to rapid changes in an individual's hemodynamics (Fig. 17). This claim is supported by Guyton and Hall Textbook of Medical Physiology [77] which states that baroreceptors (which are thought to be closely related with the release of adrenaline and noradrenaline): *"(...) tend to reset in 1 to 2 days to the pressure level to which they are exposed."*(p.222). We were, however, unable to find a reference in Guyton and Hall's textbook for their claim.

In the table on the next page we try to highlight the causal pathway of the short-term alterations observed with thiazide therapy. We have tried to make it as clear and straight forward as possible. This is meant as a summary of the clear experimental pattern that the computer model should be able to predict.

Step	Alteration	Effect	Effect on BP
1.	↑ diuresis	↓ plasma volume, ↓ ECV	- BP
2.	↓ plasma volume, ↓ ECV	↓ CO, - HR	↓ BP
3.	↓ CO, - HR	↑ renin, ↑ aldosterone	↓ BP
4.	↑ renin, ↑ aldosterone	↑ TPR, - HR	- BP

Table 2: Short term effects of thiazides, as observed in our material. The symbols ↑, ↓ and - represent an increase, decrease and no change from the previous step respectively.

3.13.2 The long-term effects

The long-term effects are defined as those seen after four weeks and later. After the initial decline the plasma volume gradually increases towards pre-treatment levels. The same happens with the CO. Despite this, the blood pressure remains low. This means there must be a simultaneous reduction in TPR and/or a reduction in venous pressure. This reduction has to step in after the initial phase and last for the whole treatment period. Note also that the ECV seems to fail to regain as much as plasma volume after the initial decline. This implies that the normal equilibrium of hydrostatic and osmotic forces in the capillary bed has been altered during long-term therapy. However, the data we have on ECV only stem from a few studies [12,17,18]. It would be interesting to see more studies being done on the relationship between ECV and plasma volume.

As mentioned above in the TPR-section the alleged long-term reduction in TPR is associated with some uncertainty, at least in our meta-analysis. However, based on the increase in CO and maintenance of low blood pressure, we believe it is likely that the TPR decreases during long-term therapy.

What could be the mechanism for the long-term reduction in TPR towards, or even below, pre-treatment levels? Several explanations have been proposed to account for this reduction is mediated. In a comprehensive review on the thiazides antihypertensive effects Duarte [61] categorize these explanations into two major groups:

a) The direct vasodilation theories.
b) The indirect vasodilation theories.

The main idea of the direct vasodilation theories is that the thiazide molecules have a more or less direct effect on the systems regulating tension of the vascular wall. On the other hand, the indirect vasodilation theories claim that the vasodilation is a

consequence of a long cascade of events that all originate from the increased diuresis. This is a highly disputed topic. In the following we will try to give an overview of the different theories, we will compare them with our pooled data and evaluate which theory that is most likely in our opinion, outlining possible causal mechanisms in tables.

Direct vasodilation theories. Puscas [78] showed that thiazides have an inhibitory effect on the enzyme carbonic anhydrase 1 in vascular smooth muscle cells. These cells are lining the arterial walls and are able to constrict and dilate the arterioles. Carbonic anhydrase 1 is important for the pH regulation inside the vascular smooth muscle cells. In the membranes of these cells there are some important potassium channels. They are called conductance calcium activated channels (KCa). These channels are pH dependent. The theory claims that thiazides through inhibition of the calcium anhydrase alter the intracellular pH in smooth vascular muscle cells. This could lead to an activation of the KCa channels which again gives vasodilation [61,78]. The theory is based on two facts: that thiazides inhibit carbonic anhydrase 1 and that KCa-channels are pH dependent. Although these facts are interesting we believe they are insufficient to say that this is in fact the vasodilatory mechanism of thiazides. Besides, as mentioned by Duarte [61], bendroflumethiazide have a weaker inhibitory effect on carbonic anhydrase, but a strong long-term antihypertensive effect. How is this possible if the vasodilation is solely based on inhibition of carbonic anhydrase 1? It would be interesting to see if bendroflumethiazide has a similar effect profile to that of hydrochlorothiazide. However, as only about 5% of the studies in our database are based on bendroflumethiazide and 95% are based on hydrochlorothiazide, this asymmetry prevented a sound comparison of the two drugs.

Pickkers et al. [79] offer another theory on the vasodilation. They showed that intravascular administration of hydrochlorothiazide led to a 55% increase in forearm blood flow (FBF). This effect was attenuated by tetraethylammonium (TEA) reducing the increase in FBF to only 13%. TEA is a potassium channel blocker that inhibits KCa. Hence, they claim that vasodilation is *"(...) mediated by opening of vascular calcium-activated potassium channels, resulting in hyperpolarization and reduction in intracellular calcium in the smooth muscle cell"*. They also claim that this effect is independent of the kidneys. This is based on data from two subjects with Gitelmans syndrome, a genetic disorder with lack of thiazide sensitive Na-Cl cotransporters in the kidneys. These subjects had the same vasodilatory response as the other subjects. One major problem with this study was that the plasma levels of hydrochlorothiazide

were far above the therapeutic levels. Pickkers et al. argue that these findings might be relevant anyway since, according to him, the thiazides are accumulated in smooth muscles cells over time [79]. Duarte [61] argues against this theory. His point is that normotensive subjects had a similar grade of vasodilatory response as the hypertensives. This contrasts the antihypertensive effect which normally is lower in normotensives compared to hypertensives. It is, however, interesting that they found an effect on the subjects with Gitelmans syndrome. If this is something that is seen consistently one can argue that the kidneys are of less importance for the vasodilatory effect.

Indirect vasodilation theories. The most important of the indirect vasodilation theories is the reverse autoregulation theory. According to Shah [12] this theory was first suggested by Tobian [80] around 1974. This theory says that there is no need for a direct vasodilatory effect of thiazides. The reduction in plasma volume is allegedly sufficient to explain the long-term reduction in TPR. Following Shah [12], this conclusion is based on observations from experimental induction of hypertension through provision of high doses of salt to subjects with reduced renal mass [81], through provision of high doses of licorice [82] and through experimental induction of renovascular hypertension in rats [83]. In these studies an increase in plasma volume led to an increase in CO and blood pressure the first days while TPR was not elevated. After several days with induced hypervolemia, TPR gradually increased while CO decreased towards normal levels. The hypertension persisted. This means that what started as a hypervolemic hypertension characterized by increased CO and normal TPR, shifted into a normovolemic hypertension characterized by normal CO, and increased TPR. Tobian [80] suggested that the opposite hemodynamic changes are observed with thiazide therapy, which is virtually the inverse of giving salt and dextran. When thiazides increase urinary output and lowers plasma volume, the CO decreases while the TPR remains normal. Then after weeks we see the same shift as above, but now in the opposite direction. The body's initial response to thiazides, a relative hypotension characterized of decreased CO and normal TPR, is thus reversely autoregulated back to a state of relative hypotension characterized by normal CO and reduced TPR.

The molecular basis for the reversed autoregulation theory is poorly understood. However, Blaustein [84] suggested the hormone oubain as a possible molecular candidate. Ouabain is a crystal glycoside originally used as dart poison by African tribes [85]. It has an effect similar to that of the heart medicine digitalis [51]. Some

evidence indicate that the body produce an endogenous form of ouabain as a response to long-term increase in plasma volume [84,86,87]. The function of ouabain is that it blocks the Na-K-ATPase [51]. The Na-K-ATPase is an enzyme located in the cellular wall which pumps sodium out and potassium into the cell [84]. Inhibition of the Na-K-ATPase gives a higher intracellular concentration of sodium. This influences the current through another membrane molecule: the Na/Ca-exchanger. With increased intracellular sodium, more sodium exits through this exchanger. Consequently, more calcium comes into the cell leading to constriction of the smooth muscular cell and hence, increased TPR. Uptodate.com suggests that the opposite thing is happening during thiazide therapy: *"By inducing volume depletion, diuretics would diminish the secretion of this hormone, leading sequentially to a rise in Na-K-ATPase activity, a fall in cell sodium concentration (since more sodium is pumped out of the cell), and increased calcium efflux from the cells. The ensuing decline in the cell calcium concentration then leads to vasodilation and a fall in systemic vascular resistance. This theory, however, has yet to be confirmed."* [51]

As described above, when it comes to the initial effects of thiazides, our meta-analysis support that these effects are mediated through reduced plasma volume. Concerning the long-term effects, the picture is blurrier. Based on our meta-analysis it is hard to conclude which theory is the correct one. However, one important finding is that the plasma volume and CO seem to show a trend in not returning entirely to pre-treatment levels. These effects are relatively uncertain, but if we assume that the trend is correct, and remember that the renin levels are increased, it indicates that the body is in a hypovolemic state throughout the treatment period. This is also supported by Tarazi's [17] observations of increased plasma volume after discontinuation of thiazide-therapy. All of this is in favor of the indirect vasodilation theories. According to the reverse autoregulation theory, hypovolemia is all that is necessary to give a long-term hypotensive effect. However, if hypovolemia were the only explanation for thiazides' hypotensive effect, one would expect loop diuretics, with its superior diuretic effect [88], to have a stronger hypotensive effect than thiazides. Instead loop diuretics seem to have a weaker [88] and shorter [89] long-term hypotensive effect than the thiazides. When it comes to the direct vasodilation theories our analyses on hemodynamic parameters are not suited to say much about the molecular mechanisms that these theories promote. What we can say is that it is possible that thiazides have a direct effect on the vascular wall. We find it difficult to explain a direct mechanism that initially increase the TPR, and then suddenly after weeks, decreases it. However, one cannot ignore the findings of Pickkers et al. [79]

that showed direct vasodilatory effects of high dose thiazides. We believe that neither of these theories are sufficient to explain the hemodynamic changes alone. We find it most likely that the reverse autoregulation theory, possibly mediated through ouabain, works in concert with one or several direct vasodilatory mechanisms.

The majority of the studies on loop diuretics only measure parameters up to 24 hours after drug administration. This makes it difficult to contrast the effects of thiazides and loop diuretics. The studies done on a timescale comparable with the thiazides generally show the same effects as the thiazides. This may be considered in favor of the indirect vasodilation theory. However, we don't even know whether the apparent normalization of plasma volume and reduction of TPR for thiazides is true for loop diuretics as well.

The following table is an effort in trying to break down the effects of the alterations seen during long term thiazide therapy. Note that all these alterations are categorized in one step. However, the end point is a stabilisation of the blood pressure on the same level or slightly below the initial phase level.

Step	**Alteration**	**Effect**	**Effect on BP**
5.	↑ plasma volume ↑ CO - ECV - HR	- renin - aldosterone ↓ TPR	-BP

Table 3: Long term effects of thiazides, hypothesis 1.

An alternative chain of events is that the reduction in TPR is the driving factor for the increase in CO and plasma volume. This is summarized in the table below. The weakness with this chain of events is the increase in plasma volume. Is that a logical consequence of reduced TPR? We suggest that the sustained elevation in renin and aldosterone levels could be the reason for the increase in plasma volume.

Step	**Alteration**	**Effect**	**Effect on BP**
5.	↓ TPR	↑ CO, - renin, - aldosterone	- BP
6.	↑ CO, -renin, - aldosteron	↑ plasma volume, -HR, -ECV	- BP

Table 4: Long term effects of thiazides, hypothesis 2.

Another thing that would be interesting to test in a computational physiology model is how renin affects the blood pressure response. How large must the increase in renin be to suppress the antihypertensive effect? As observed by Freis et al [32] the patients that did not respond to the lowest doses had a greater increase in plasma renin than the patients that responded. Besides, as shown by van Brummelen et al. [19], the non-responders failed to decrease their TPR. It would be interesting to see if a model would be able to predict these inter-individual differences.

4 Discussion

In this study of the pharmacodynamic effects of thiazides from a computational physiological perspective, the most striking observation is arguably the huge variability in the data. Almost all the graphs of the different parameters show large variability, both between different studies, as well as between individuals in the same study. Some of this variability might be explained by different study models, dosage regimes and quality of the studies, but based on the large variability within each study, we believe that a considerable part of the variability is a consequence of real, inter-individual variation. In other words, despite being one of the worlds most frequently used anti-hypertensive drugs, we know remarkably little about how thiazides affect the human physiology in general and the cardiovascular subsystem in particular.

The therapeutic discourse in Western medicine is currently undergoing a significant shift from the "one size fits all" approach of conventional, group-based Evidence-Based Medicine (EBM) to a "personalized medicine" or "precision medicine" approach, aiming to serve the unique features of each particular individual. Instead of just giving one anti-hypertensive drug and hope for reduced blood pressure, we hope it will soon be possible to measure for instance heart rate, renin and aldosterone and pick a drug that with a high degree of certainty would help this patient. Computational physiological models are seen as key to realizing this vision. However, as we have already noted, this milestone is still far away. We find it surprising that so little is actually known about the mechanisms of such a renowned an important used diuretic, even on a conventional group level.

4.1 Methodological limitations

As outlined in the method section, our analysis does not cover all data retrieved by our literature search. We were unable to evaluate all studies in our Mendeley database that contained data variance. This was simply due to lack of time. Most of the remaining studies described the blood pressure effect of thiazides. It is generally accepted that thiazides lower blood pressure on a group level. We therefore believe the remaining studies would not affect the total picture of this study had they in fact been evaluated.

4.2 Limitations in the available data

The fact that the majority of the studies in our database only measure a few parameters each makes it difficult to deduce any relationships between the parameters. Whenever you come across an interesting pattern, you have to ask yourself whether this is a real pattern or whether the apparent pattern is a result of different methodology, patient groups, drug doses etc.? The ideal situation for modelers would be to have a few large RCTs which measured all the relevant parameters at equal time points. This would allow them, through multivariate analysis, to identify clear patterns that are easily confounded when analyzing a pile of small studies addressing a subsample of parameters. These patterns are likely to provide an unprecedented information source for computational physiology modeling.

Another problem is that all 4 studies in our meta-analysis that describe TPR calculate it on the basis of only CO and arterial blood pressure. Recall that it is the *difference* in arterial *and* venous blood pressure that equals TPR multiplied with CO. If a study uses the arterial blood pressure as an estimate of the difference between arterial and venous blood pressure it would give an incorrect TPR-value should venous blood pressure change during therapy. However, a large relative change in venous blood pressure may not affect TPR that much as the venous pressure is quite low originally. We therefore chose to ignore that the studies calculated TPR incorrectly.

4.3 Suggestions for further work

Further pharmacodynamic work with computational physiological models should aim at predicting the inter-individual variability of drug treatment effects. Before individualized, precision medicine can be realized, the models should be able to track down even small alterations, since these might together have a big contribution to the final effect in the complex, human physiology.

The intention of this review was to provide some of the necessary information needed for the development of a physiologic computational model. We found the process of doing a rigorous literature search in the classical way was very time demanding and challenging. In other words, if future computational models of drug pharmacodynamics are expected to proceed in such a manner, progress might be relatively slow. The ideal situation for a modeler, we think, would be to have access to some sort of database where modelling-relevant information from studies are stored in a way that is easily accessible. Envision for example "PubMeds cousin" where all the metadata in a study such as drug type, dose, patient attributes, measurement method etc. are

stored in addition to the drug effect. This would enable the programmer to easily identify and utilize information from the studies he or she wishes in a computational physiology project without having to wait for research collaborators to extract the relevant information from conventional publications. It is hard to see how the manual curation of such a database would we funded, though. But knowledge management and machine learning tools now emerging are in the not too distant future likely to be capable of performing such a task to a large degree.

Even though our meta-analysis show that the overall short-term effects of thiazides are well in line with established cardiovascular physiology, it showed that mechanisms underlying the large inter-individual variability deserves much more attention. Moreover, the analysis showed that the long-term effects, characterized by normalization of CO, decreased TPR and maintenance of elevated plasma renin levels, are still poorly understood. A more consolidated understanding of the mechanisms underlying the long-term effects of thiazides might be in position to throw some new explanatory light on the etiology of primary hypertension as such. And also in this case attention should be given to explain the large inter-individual variability. A computational physiology approach is likely to be instrumental for improving the current situation.

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List of Figures

A Python script 1

functions.py Thu Dec 15 17:33:05 2016 1

```
#!/usr/bin/env python
# -*- coding: utf-8 -*-
'''
15 december 2016
Author: Hakon Thomas Kiaer
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This script defines some handy functions.
These functions are imported to the script 'metaanalysator.py' where they are executed.
'''

####Comments####
#Each .txt-file represents a set of data
#The data in the .txt-file is represented as follows:
        #time   value   SEM     n       SD*
        #0      5       3       9
        #3      7       4       9
#Or like this, depending on whether the study contains SEM or SD:
        #time   value   SEM     n       SD
        #0      5       0       9       3
        #3      7       0       9       4
#Note that SD is left empty (we didn't write 0) if the study doesnt contain SD.
        #This is exploited downstream in the script to identify which
        #studies have SEM and which have SD.
#One study may have several datasets
        #The .txt-files would then be named e.g.
        #'lib_study_1_25mg.txt' and 'lib_study_1_50mg.txt'
#Datapooling
        #The algorithm used is described here:
        #http://www.burtonsys.com/climate/composite_standard_deviations.html

# * SEM = Standard error of the mean
#   n   = number of subjects
#   SD  = Standard deviation

from matplotlib import pyplot as plt
import numpy as np
import sys
import os
import shutil
import statsmodels.api as sm
lowess = sm.nonparametric.lowess

#Defining functions:#
def amountofstudies():
        #Returns the number of different studies and the number of datasets
        #in the "Original filenames"-dir.
        list_s = []     #Each study number x will be appended to this list.
        dest   = os.getcwd()
        src    = ("%s/Original filenames" % dest)
        src_files = os.listdir(src)
        for file_name in src_files:
                if file_name.startswith('lib_study'):
                        a = file_name[10:12]    #Finding the study number.
                        if a[1] == "_":
                                a = a[0]
                        list_s.append(a)

        set_list_s = set(list_s)
        amount_of_studies  = len(set_list_s)
        amount_of_data_sets = len(list_s)
        return (amount_of_studies, amount_of_data_sets)

def copying():
        #Copies the files in 'Original filenames'-dir to the
        #dir the script is running from (the parent dir).
        dest  = os.getcwd()
        src   = ("%s/Original filenames" % dest)
```

```
        src_files = os.listdir(src)
        for file_name in src_files:
                full_file_name = os.path.join(src, file_name)
                if file_name.startswith('lib'):
                        shutil.copy(full_file_name, dest)

def renaming():
        #Renames study 'lib_xxx.txt' to 'Study_1-2-3-4.txt'
        for f in os.listdir('.'):
                if f.startswith('lib'):
                        i=1
                        while os.path.exists('Study_%s.txt' % i):
                                i=i+1
                        os.rename(f, 'Study_%s.txt' % i)

def convertinguncertainty(amount_of_data_sets):
        #Computes standard deviation from standard error of the mean or vice versa
        k = int(amount_of_data_sets+1)
        for i in range(1, k):
                data= np.loadtxt('Study_%s.txt' % i)
                a   = data[0, :]
                c   = len(data) #Equals amount of rows in .txt-file.
                if len(a)==5:   #If true, the script assumes that SD is known.
                        fh  = open('Study_%s_convertedtoSEM.txt' % i, 'w')
                        fh.writelines('#x       y       SEM     n       SD      D_s\n')
                        for m in range(0, c):
                                a   = data[m, :]
                                x   = a[0]
                                y   = a[1]
                                SD  = a[4]
                                n   = a[3]
                                SEM = SD/(n**0.5)       #Calculates SEM from SD and n.
                                D_s = 1                 #D_s = datasets.
                                Var = SD**2

                                SEM = round(SEM, 3)
                                SD  = round(SD, 3)
                                Var = round(Var, 3)

                                fh.writelines('\n')
                                fh.writelines('%s       ' % x)
                                fh.writelines('%s       ' % y)
                                fh.writelines('%s       ' % SEM)
                                fh.writelines('%s       ' % n)
                                fh.writelines('%s       ' % SD)
                                fh.writelines('%s       ' % D_s)
                                fh.writelines('%s       ' % Var)

                        fh.close()
                        d = ('Study_%s_convertedtoSEM.txt' % i)
                        b = ('Study_%s.txt' % i)
                        os.rename(d,b)

                elif len(a)==4: #If true, the script assumes that SEM is known.
                        fh  = open('Study_%s_convertedtoSEM.txt' % i, 'w')
                        fh.writelines('#x       y       SEM     n       SD      D_s\n')
                        c   = len(data) #Equals amount of rows in .txt-file.
                        for m in range(0, c):
                                a   = data[m, :]
                                x   = a[0]
                                y   = a[1]
                                n   = a[3]
                                SEM = a[2]
                                SD  = SEM*(n**0.5)
                                D_s = 1
                                Var = SD**2

                                SEM = round(SEM, 3)
```

```
                                SD  = round(SD, 3)
                                Var = round(Var, 3)

                                fh.writelines('\n')
                                fh.writelines('%s       ' % x)
                                fh.writelines('%s       ' % y)
                                fh.writelines('%s       ' % SEM)
                                fh.writelines('%s       ' % n)
                                fh.writelines('%s       ' % SD)
                                fh.writelines('%s       ' % D_s)
                                fh.writelines('%s       ' % Var)

                        fh.close()
                        d = ('Study_%s_convertedtoSEM.txt' % i)
                        b = ('Study_%s.txt' % i)
                        os.rename(d,b)  #Overwriting the original file.

def pooling(a,b,fh):
#Pools pairs of .txt-files using algorithm described in Comments-section.

        Lxa = a[:, 0]   #Lxa contains the x-column in study A.
        Lxb = b[:, 0]   #Lxb contains the x-column in study B.

        Lxa_max_item = max(Lxa) #Biggest x-value in study A.
        Lxb_max_item = max(Lxb) #Biggest x-value in study B.
        Lxa = [i for i in Lxa if i <= Lxb_max_item]     #Redifining Lxa
        Lxb = [i for i in Lxb if i <= Lxa_max_item]     #Redifining Lxb

        #Contains the x-values that just were removed from Lxa and LXB.
        Lxa_remainder = [i for i in a[:, 0] if i > Lxb_max_item]
        Lxb_remainder = [i for i in b[:, 0] if i > Lxa_max_item]

        Fx = set(Lxa) & set(Lxb)        #Fx contains common values in Lxa and Lxb
        LFx = len(Fx)
        c = len(Lxa)+len(Lxb)-LFx
        #c is the number of iterations that are needed
        #in the pooling-procedure below.

        f = 0   #These are used to correct the iteration if study A
        h = 0   #has an x-value that study B does not, and vice versa.

        for i in range(0, c):
                d    = a[i+f, :]                #Contains row number i in A
                e    = b[i+h, :]                #Contains row number i in B
                xa   = d[0]                     #X-value for the current row in A
                xb   = e[0]                     #X-value for the current row in B
                na   = d[3]                     #Amount of subjects for in A
                nb   = e[3]                     #Amount of subjects for in B
                N    = d[3]+e[3]                #Sum of subjects for both rows.
                ya   = d[1]                     #Y-value for the current row in A
                yb   = e[1]                     #Y-value for the current row in B
                SEMa = d[2]                     #SEM for the current row in A
                SEMb = e[2]                     #SEM for the current row in B
                y    = (d[1]*d[3]+e[1]*e[3])/N  #Mean Y-value
                SDa  = d[4]                     #SD for current row in A
                SDb  = e[4]                     #SD for current row in B
                Vara = SDa**2                   #Variance for current row in A
                Varb = SDb**2                   #Variance for current row in B
                ESSa = Vara*(d[3]-1)            #Error sum of squares for current row in A
                ESSb = Varb*(e[3]-1)            #Error sum of squares for current for in B
                ESS  = ESSa+ESSb                #Error sum of squares for A and B
                MaGM = ya-y                     #GM=Grand Mean
                MbGM = yb-y                     #GM=Grand Mean
                GSSa = (MaGM**2)*d[3]           #Group sum of squares for current row in A
                GSSb = (MbGM**2)*e[3]           #Group sum of squares for current row in A
                TGSS = GSSa+GSSb                #Total group sum of squares
                GV   = (TGSS+ESS)/(N-1)         #Grand variance
                GSD  = GV**0.5                  #Grand standard deviation
```

```
                GSEM = GSD/(N**0.5)             #Grand standard error of the mean
                As_a = d[5]                     #Amount of datasets in current row in A
                As_b = e[5]                     #Amount of datasets in current row in B
                As   = d[5]+e[5]                #Sum of datasets for A and B.

                GSEMf= round(GSEM, 4)           #Rounding to 4 decimals
                GSDf = round(GSD, 4)
                yf   = round(y, 4)
                SDaf = round(SDa, 4)
                SDbf = round(SDb, 4)
                SEMa = round(SEMa, 4)
                SEMb = round(SEMb, 4)
                SDa  = round(SDa, 4)
                SDb  = round(SDb, 4)

                if xa==xb:      #If x-values are equal, pooled data is written
                        fh.writelines('%s       ' % xa)
                        fh.writelines('%s       ' % yf)
                        fh.writelines('%s       ' % GSEMf)
                        fh.writelines('%s       ' % N)
                        fh.writelines('%s       ' % GSDf)
                        fh.writelines('%s       \n' % As)

                elif xa<=xb:    #If xa<xb, the values are just copied from current row in A
                        fh.writelines('%s       ' % xa)
                        fh.writelines('%s       ' % ya)
                        fh.writelines('%s       ' % SEMa)
                        fh.writelines('%s       ' % na)
                        fh.writelines('%s       ' % SDa)
                        fh.writelines('%s       \n' % As_a)

                        h = h-1 #Since the x-value in B was bigger than the x-value in A,
                                #the iteration process for study B is halted
                                #for one loop so that study A may catch up.

                else:           #If xa>xb, the values are just copied from current row in B
                        fh.writelines('%s       ' % xb)
                        fh.writelines('%s       ' % yb)
                        fh.writelines('%s       ' % SEMb)
                        fh.writelines('%s       ' % nb)
                        fh.writelines('%s       ' % SDbf)
                        fh.writelines('%s       \n' % As_b)

                        f = f-1 #Since the x-value in A was bigger than the x-value in B,
                                #the iteration process for study A is halted
                                #for one loop so that study B may catch up.


        if Lxa_remainder:       #If study A had the biggest x-value, this will be 'true'.
                for i in range(0, len(Lxa_remainder)):  #Copying remaining part of A
                        d    = a[i+len(Lxa), :] #Row 'i' for remaining part of study A
                        x    = d[0]             #X-value in current row
                        n    = d[3]             #Amount of subjects in the current row in A
                        y    = d[1]             #Y-value in current row in A
                        SEM  = d[2]             #SEM for current row in A
                        SD   = d[4]             #Standard deviation for current row in A
                        As_a = d[5]             #Amount of data sets in current row

                        SEM  = round(SEM, 4)
                        SD   = round(SD, 4)
                        y    = round(y, 4)

                        fh.writelines('%s       ' % x)
                        fh.writelines('%s       ' % y)
                        fh.writelines('%s       ' % SEM)
                        fh.writelines('%s       ' % n)
                        fh.writelines('%s       ' % SD)
                        fh.writelines('%s       \n' % As_a)
```

```
        if Lxb_remainder:       #If study B had the biggest x-value, this will be 'true'.
                for i in range(0, len(Lxb_remainder)):  #Copying remaining part of B.
                        d    = b[i+len(Lxb), :]
                        x    = d[0]
                        n    = d[3]
                        y    = d[1]
                        SEM  = d[2]
                        SD   = d[4]
                        As_b = e[5]

                        SEM  = round(SEM, 4)
                        SD   = round(SD, 4)
                        y    = round(y, 4)

                        fh.writelines('%s       ' % x)
                        fh.writelines('%s       ' % y)
                        fh.writelines('%s       ' % SEM)
                        fh.writelines('%s       ' % n)
                        fh.writelines('%s       ' % SD)
                        fh.writelines('%s       \n' % As_b)

        fh.close()


def metaanalysator(amount_of_data_sets, s):
        #This function runs the function 'pooling' the necessary number of times.
        r = int(amount_of_data_sets)
        fh = open("Study_pooled_1.txt", "w")

        fh.write('#x    y       GSEM    n       GSD     D_s\n')
        fh.write('\n')

        a = np.loadtxt('Study_1.txt')
        b = np.loadtxt('Study_2.txt')

        pooling(a,b,fh)

        v = r-1         #r is a global variable representing Study_1-r.txt.
        for i in range(1, v):
                a = np.loadtxt('Study_pooled_%s.txt' % i)
                q = i+2
                b = np.loadtxt('Study_%s.txt' % q)

                u=i+1
                fh = open("Study_pooled_%s.txt" % u, "w")

                fh.write('#x    y       GSEM    n       GSD     D_s\n')
                fh.write('\n')

                pooling(a,b,fh)

        if r > 2:
                for i in range(1, v):
                        os.remove("Study_pooled_%s.txt" % i)

        u = r - 1
        os.rename("Study_pooled_%s.txt" % u, "%s.txt" % s) #s is a global user-input.

def findxylim(s):
        #Returns an appropriate x- and y-lim for the pyplot.
        x_max =-np.inf
        y_min = np.inf
        y_max =-np.inf

        data = np.loadtxt('%s.txt' % s)
```

```
        x    = data[:, 0]
        y    = data[:, 1]
        GSEM = data[:, 2]

        x_max = np.max(x) + np.max(x)/20
        x_min = np.min(x) - np.max(x)/20
        y_min = np.min(y-GSEM)-(np.max(y+GSEM) - np.min(y-GSEM))/20
        y_max = np.max(y+GSEM)+(np.max(y+GSEM) - np.min(y-GSEM))/20


        return (x_min, x_max, y_min, y_max)


def relativeeffect(amount_of_data_sets):
        #Normalises the data to show relative effect.
        k = int(amount_of_data_sets+1)
        for m in range(1, k):
                fh   = open('Study_%s_relativetemp.txt' % m, 'w')
                fh.writelines('#x       y       SEM     n       SD      A_d     Var\n')
                fh.writelines('\n')
                data = np.loadtxt('Study_%s.txt' % m)
                c = len(data[:, 0])
                for i in range(0, c):
                        a    = data[i, :]
                        b    = data[0, :]
                        y0   = b[1]
                        y    = a[1]/y0
                        x    = a[0]
                        n    = a[3]
                        SEM  = a[2]/y0
                        SD   = a[4]/y0
                        D_s  = a[5]
                        Var  = SD/(y0**2)

                        SEM  = round(SEM, 3)
                        SD   = round(SD, 3)
                        y    = round(y, 2)
                        fh.writelines('%s       ' % x)
                        fh.writelines('%s       ' % y)
                        fh.writelines('%s       ' % SEM)
                        fh.writelines('%s       ' % n)
                        fh.writelines('%s       ' % SD)
                        fh.writelines('%s       ' % D_s)
                        fh.writelines('%s       \n' % Var)

                fh.close()
                d = ('Study_%s.txt' % m)
                e = ('Study_%s_relativetemp.txt' % m)
                os.rename(e, d)


def delete(amount_of_data_sets):
        #deletes datasets
        k = int(amount_of_data_sets+1)
        for i in range(1, k):
                os.remove("Study_%s.txt" % i)


def sampleregen(amount_of_data_sets):
        #Regenerates a sample satisfying the sample mean and variance.
        k = int(amount_of_data_sets+1)
        fh = open('scatterpoints.txt', 'w')
        fh.writelines('#x=x-value, y=y-value\n#x        y\n')

        for u in range(1,k):
                data = np.loadtxt('Study_%s.txt' % u)
                len_d= len(data)
```

```
                for p in range(0,len_d):
                        row_p = data[p,:]
                        x     = row_p[0]
                        y_m   = row_p[1]        #y_m denotes y mean.
                        n     = row_p[3]        #n denotes amount of subjects
                        v     = row_p[6]        #v denotes variance

                        #Calculates 2 y-points satisfying the mean and
                        #variance input, given that the rest
                        #of the y-points are equal to the mean.
                        #In retrospect the variance didn't
                        #have to be satisfied in order to use LOWESS...
                        y1 = float(y_m + (((n-1)*v)**0.5)/(2**0.5))
                        y2 = float(y_m - (((n-1)*v)**0.5)/(2**0.5))

                        fh.writelines('%d       %f\n' %(x,y1))
                        fh.writelines('%d       %f\n' %(x,y2))

                        n = int(n)
                        for i in range(2,n):
                                fh.writelines('%d       %f\n' %(x,y_m))
        fh.close()

def lowessplot(figname, s, z_, time):
        #Creates a lowess regression line in addition
        #to the scatter plot with error bars.
        data  = np.loadtxt('scatterpoints.txt')
        x     = data[:,0]
        y     = data[:,1]

        z = lowess(y, x, frac=7./10)
        z_x = z[:,0]
        z_y = z[:,1]

        data1   = np.loadtxt("%s.txt" % s)
        x1      = data1[:,0]
        y1      = data1[:,1]
        GSEM    = data1[:,2]
        N       = data1[:,3]

        (x_min, x_max, y_min, y_max) = findxylim(s)

        fig, ax = plt.subplots(1)
        ax.errorbar(x1,y1, yerr=GSEM, fmt='none', zorder=3)
        sct_s = [80*(N[i]/N[0]) for i in range(0,int(len(N)))]
        ax.scatter(x1,y1, s=sct_s, c='r', edgecolors='r', zorder=4)

        #This is the textbox giving number of subjects at t=0
        props = dict(boxstyle='round', facecolor='wheat', alpha=0.5)
        textstr = 'n=%d at t=0' % int(N[0])
        ax.text(0.98, 0.97, textstr,
                            transform=ax.transAxes,
                            fontsize=14,
                            verticalalignment='top',
                            ha='right',
                            bbox=props)

        #Some plot adjustments
        ax.plot(z_x,z_y, lw='2', zorder=2)
        ax.grid(zorder=1)
        plt.ylabel(z_)
        plt.xlabel(time)
        plt.title(s)
        plt.xlim(x_min, x_max)
        plt.ylim(y_min, y_max)
        plt.savefig(figname)

        os.remove('scatterpoints.txt')
```

B Python script 2

```
metaanalysator.py       Thu Dec 15 17:51:38 2016       1
#!/usr/bin/env python
# -*- coding: utf-8 -*-
'''
15 december 2016
Author: Hakon Thomas Kiaer
E-mail: haakontk@ntnu.stud.no
'''


import functions as fct

time = ("Time (days)")
s_i = raw_input('Write parameter name:')
z = raw_input('Write parameter (unit):')

#absolute effects
a_s, d_s = fct.amountofstudies()
fct.copying()
fct.renaming()
fct.convertinguncertainty(d_s)
fct.sampleregen(d_s)
s = ("%s absolute pooled" % s_i)
fct.metaanalysator(d_s, s)
figname = "%s absolute lowess.pdf" % s_i
fct.lowessplot(figname, s, z, time)

#Relative effects (only these are shown in
#the thiazide review)
fct.relativeeffect(d_s)
fct.sampleregen(d_s)
s = ("%s relative pooled" % s_i)
z = ("Relative change")
fct.metaanalysator(d_s, s)
figname = "%s relative lowess.pdf" % s_i
fct.lowessplot(figname, s, z, time)
fct.delete(d_s)
```