Article

Assessment of selected clock proteins (CLOCK, CRY1) and their relationship with biochemical, anthropometric and lifestyle parameters in hypertensive patients

Aniceta Ada Mikulska [1], Teresa Grzelak [2,*], Marta Pelczyńska [3], Paweł Bogdański [3] and Krystyna Czyżewska [4]

[1] Chair and Department of Physical Pharmacy and Pharmacokinetics, Poznan University of Medical Sciences, 6 Święcickiego Street, 60-781 Poznań, Poland; amikulska@ump.edu.pl
[2] Chair and Department of Physiology, Poznan University of Medical Sciences, 6 Święcickiego Street, 60-781 Poznań, Poland; tgrzelak@ump.edu.pl
[3] Chair and Department of Treatment of Obesity, Metabolic Disorders and Clinical Dietetics, Poznan University of Medical Sciences, 84 Szamarzewskiego Street, 60-569 Poznań, Poland; pbogdanski@ump.edu.pl (P.B.); mpelczynska@ump.edu.pl (M.P.)
[4] Department of Nursing, Stanislaw Staszic State University of Applied Sciences in Pila, 10 Podchorążych Street, 64-920 Piła; czyzew@ump.edu.pl
* Correspondence: tgrzelak@ump.edu.pl; Tel.: +48-(0)6-1854-6435

Abstract: Circadian rhythms misalignment is associated with hypertension. The aim of the study was to evaluate the concentration of selected clock proteins- cryptochrome 1 (CRY1) and circadian locomotor output cycles kaput (CLOCK) and to determine their relationships with biochemical and anthropometric parameters and lifestyle elements (diet, physical activity, quality of sleep) in hypertensive patients. In 25 female with hypertension (HT) and 49 non-hypertensive women (NHT), the CRY1 and CLOCK concentrations, total antioxidant status (TAS), lipid profile and glycemia were conducted. Blood pressure and anthropometric measurements, nutritional, exercise and sleep analyses were performed. In HT the CRY1 level was 40.2% lower than in the NHT. No differences were noted in CLOCK concentration between groups. The study showed no relationship between CRY1 or CLOCK concentrations and glucose or lipids profile, amount of physical activity and sleep quality, although CRY1 was associated with some anthropometric parameters. There was a negative correlation between the CLOCK concentration and the amount of saturated fatty acids consumed (24h) in HT. That indicates the possible influence of the diet on the CLOCK level. In a detailed prognosis of the risk of hypertension, it is worth considering the measurement of the level of CRY1 in populations with abnormal anthropometric indices.

Keywords: circadian clock; cryptochrome 1; circadian locomotor output cycles kaput; hypertension; total antioxidant status; diet; chrononutrition

1. Introduction

The circadian clock is as an endogenous mechanism that generates and synchronizes the course of many physiological processes over time. It acts as a primary regulator of adapting internal mechanisms to external environmental factors. The circadian clock generates endogenous 24-hour rhythms. It controls diurnal variations in behavioral and physiological functions such as sleep-wake regulation, blood pressure (BP), lipid synthesis and metabolism and hormone secretion [1–5]. Circadian rhythms are controlled by the circadian clock that consists of a central clock and a peripheral clocks. The central clock is located in the suprachiasmatic nucleus (SCN) within the hypothalamus and synchronized by light/dark signals. SCN receives ganglionic input from retinal photosensitive cells. Peripheral oscillators are found in almost all tissues, including liver, intestine

and adipose tissue [3,5–8]. Peripheral clocks predominate in local physiological cycles, including heart rate, glucose and lipid homeostasis, secretion of some hormones and digestive juices, normalization of body temperature and functioning of the digestive system. These oscillators are synchronized in response to diet and light cues and communicating through hormonal and neuronal signals. Desynchronization of the circadian system, such as shift work, is associated with increased risk for many adverse health effects, including hypertension [8–10]. For example, studies have shown that SCN ablation resulted in the disappearance of daily rhythmic blood pressure variations [11].

The molecular mechanism regulating circadian rhythms is based on transcription-translation feedback loop (TTFL), which produce oscillations in the expression of circadian clock genes and protein activity, controlling quantitative changes in their subordinate genes CCGs (clock-controlled genes), which ultimately regulate the course of physiological processes in most body cells [5,12,13]. At the feedback center are two active transcription factors - CLOCK (circadian locomotor output cycles kaput) and BMAL1 (Brain-Muscle-Arnt-like 1), which form a heterodimeric complex and bind to E-box sequences mediating transcription of Period (Per1, Per2, Per3) and Cryptochrome (Cry1, Cry2) genes, which leads to a gradual accumulation of protein products of these genes throughout the day. The negative feedback loop consists of the periods and cryptochromes proteins that inhibit CLOCK and BMAL1-mediated transcription [5,6,12,14–16]. In addition to the primary loop, there are many interactions between biological clock genes, their proteins, and key biochemical pathways regulating intracellular metabolism [15,17]. The proteins encoded by clock genes perform important functions in various metabolic processes, regulating biological functions as part of circadian monitoring [18].

Recent publications have present the role of the circadian clock genes in the regulation of processes in the heart, kidney, blood vessels and the metabolic organs, which are essential for blood pressure regulation. Convincing evidence from many animal studies shows a relevant role for the circadian clock in the regulation of blood pressure [19,20].

Circadian clock proteins play an essential role in regulating and coordinating metabolism, because most of the clock deficient animal models present metabolic abnormalities. Cryptochromes appear relevant to the pathogenesis of metabolic syndrome (MetS) because they participate in glucocorticoid regulation of gluconeogenesis and steroidogenesis. CRY proteins inhibit fasting-induced gluconeogenesis in the liver by influencing glucocorticoid signalling: while CRY1 and CRY2 directly interact with the glucocorticoid receptor [21]. Additionally, circadian disruption may lead to changes in the secretion of hormones such as aldosterone, renin, angiotensin II and glucocorticoids, which is partly responsible for the daily regulation rhythm of blood pressure and inflammation [8,22].

Blood pressure exhibits a daily rhythm that has clinical value. In healthy people, blood pressure drops during nocturnal sleep compared to diurnal wakefulness. The nocturnal reduce in blood pressure is called “dipping”. Lowering blood pressure at night from 10% to 20% of daily blood pressure levels is indicative of the dipping response [20,23–27]. „Non-dippers” are individuals who have nocturnal blood pressure that decrease less than 10% compared to diurnal blood pressure [20,25–27]. Studies showed that „non-dippers” have increased risk of metabolic disorders, cardiovascular diseases (CVD), the progression of chronic kidney disease, mortality and complications for individuals already diagnosed with hypertension [20,24,27–29].

Variation in BP during a day depends on internal and exogenous factors. Internal factors include autonomic nervous activity and humoral factors (cortisol, renin, vasoactive intestinal peptide, aldosterone). External factors contain physical activity, diet, emotional state, and sleep/awake states [22,30]. Peripheral clock regulators of blood pressure are kidneys, brain, nervous system, and of course - vasculature and heart [23].

Studies have concluded an association between sleep deprivation, excessive exposure to artificial light at night, shift work schedule or altered mealtime with metabolic imbalances that can alter the circadian system and lead to dysregulation of metabolic homeostasis [7,8,31–34]. Several studies have reported that the desynchronization of the circadian pattern of BP is associated with increased risk for cardiovascular disease, but the mechanism of this dysregulation is still unknown [23,31,35,36]. Moreover, circadian misalignment increases 24-hour blood pressure and decreases parasympathetic activity, and this chronic condition leads to the development of cardiovascular

diseases [31,36]. A cross-sectional study showed that disruption of the BP circadian rhythm is also associated with metabolic syndrome in male population [37]. Other studies confirmed that the circadian misalignment in healthy adults and shift workers could cause a significant increase in blood pressure and inflammatory markers and lead to CVD [31,36], and maintaining normal blood pressure is associated with decreased risk of cardiovascular events and death [38].

Most of the mechanisms underlying the role of CRY1 and CLOCK in a human organism have not been explained. To the best of our knowledge, yet there are no published results on the associations between circadian clock proteins serum concentrations and metabolic disorders. The aim of the study was to evaluate the serum concentrations of CRY1 and CLOCK and to determine their associations with biochemical and anthropometric parameters and individual lifestyle elements (diet, physical activity, quality of sleep) in hypertensive patients.

2. Materials and Methods

2.1. Study Population

We analyzed 74 Caucasian women. 25 women (age range 24-61 years) were included in the hypertensive patient group (HT, study group). Patients were recruited at the Department of Internal Medicine, Metabolic Disorders and Hypertension (Poznan University of Medical Sciences). Hypertension was diagnosed when morning blood pressure twice time exceeded at least 140/90 mmHg or when patients were already diagnosed as having this disease and were taking antihypertensive medication. Additionally, 49 non-hypertensive female subjects (age range 25-63 years) were recruited as controls (NHT). Exclusion criteria in the study group included secondary obesity or secondary hypertension, a positive history of cancer over the last 5 years, chronic heart or liver failure, autoimmune diseases, hepatic, renal, adrenal, or thyroid disorders, pregnancy and breastfeeding. The control group comprised of healthy females of a similar age as members of the study group. Female who had poor health (mental or somatic), according to physical examination and laboratory analyses, following an interview or were pregnant or breastfeeding, were excluded from the control group. All participants had a similar circadian rhythm (shift workers were not included in the study).

The Local Bioethics Committee has given its permission to conduct the research project (regulation no. 729/17 and 326/18). Participation in the analysis was voluntary. Each participant provided written informed consent after having been informed about the project's purpose and course. The study was carried out in accordance with the Declaration of Helsinki [39].

2.2. Study Design

Blood pressure measurement

Blood pressure was measured manually three times in the morning with the patient sitting using a sphygmomanometer and an appropriate-sized cuff after 10 minutes of rest while patients were seated, according to standard guidelines of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) [24]. In accordance with this ESC/ESH guidelines hypertension is defined as office systolic blood pressure (SBP) values at least 140 mmHg and/or diastolic blood pressure (DBP) values at least 90 mmHg (Table 1) [24]. Most of the other definition is also based on these criteria [40].

Table 1. Classification and definitions of office blood pressure in adults

Category	Systolic [mmHg]		Diastolic [mmHg]
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89

Grade 1 h hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

Base on: [24,40]

Anthropometric parameters

The anthropometric measurements were taken after overnight fasting for a minimum of 12 h (body weight and height analyses with accuracy to 0.1 kg (certified weigh) and to 0.1 cm (stadiometer) in light underwear, with no shoes). Waist circumference was measured at the midway between the costal arch and the upper iliac crest and hip circumference at the level of the greater trochanters. Obtained data were further used to calculate a body mass index (BMI), calculated as weight divided by height squared (kg/m^2) and WHR (Waist-Hip Ratio) calculated as waist measurement divided by hip measurement. In addition, body composition measurements were assessed using bioelectrical impedance analysis, carried out with a TANITA BC-418 device (Tanita Corp., Tokyo, Japan). It was used to estimate total body adipose tissue, lean body mass and water content.

Diet content analysis

The study populations also completed nutritional interviews (prospective and retrospective studies). The study used the current recording method to evaluate the nutrition of women, which consists of completing the 3-day dietary diary (1st day, 14th day and 28th day of the study). Then the data was incorporated into a dietetics software (DIETA 5.0, recommended by the National Centre of Nutritional Education, Warsaw, Poland), which allows to calculate calorific value of the diet, the amount of micronutrients and macronutrients, including carbohydrates, fats, fatty acids, proteins of animal or plant origin, minerals, vitamins and fibre.

Physical activity and quality of sleep

Physical activity (number of steps taken, distance travelled) and the quality of sleep for 28 days (sleep and wakefulness over a 24-hour period) were analyzed using a specialized device with motion sensors (Beurer Bluetooth® Activity Tracker AS80 with Health Manager Application, Germany).

Biochemical parameters

Blood samples (5 ml) were collected from each study participant after overnight fasting and an all-night rest between 7:00 and 8:00 in the morning. The blood was allowed to clot (at room temperature, 30 min) prior to centrifugation at 350 × g. Most of the obtained serum samples were subjected to biochemical analyses immediately after collection, whereas another part of the samples, required to assay proteins and other molecules, was appropriately secured and frozen at −80ºC for further biochemical analyses. Biochemical tests included measurements of glycemia and lipid profile. Fasting blood glucose (FBG) and lipid profile - total cholesterol (TC), high density lipoprotein (HDL), and triglyceride (TG) were determined using enzymatic methods with standardized commercial tests (Cobas c, Roche Diagnostic, Mannheim, Germany). Low density lipoprotein (LDL) concentration (mmol/L) was calculated as LDL=TC−(HDL+TG/2.2), because triglyceridemia was lower than 4.52 mmol/L [41].

CLOCK and CRY1 measurement

The analysis of CLOCK and CRY1 protein concentration in serum was carried out closely in accordance with the guidelines of the manufacturer, using commercial immunoenzymatic tests (Cloud-Clone Corp., USA). Microtiter plates with the fixed primary antibody (specific for the

determined molecules) were incubated with serum (containing the antigen, namely the measured protein) and later with a secondary antibody marked with peroxidase. Subsequently, a reaction was provoked with a substrate for chromogen and the colour was monitored on an ELISA MR−96 microplate reader manufactured by Clindiag Systems B.V.B.A. (Pollare, Belgium). Protein levels were calculated on the basis of calibration curves which were determined using a 4-parameter-algorithm (SigmaPlot 11.00 software) prepared each time for a specific set of assays. Intra-assay and inter-assay coefficients of variation (CVs) were respectively for CRY1 7.1% and 9.2%, for CLOCK 6.2% and 10.0%.

TAS measurement

The serum parameter of extracellular protection against pro-oxidant activity - TAS (Total Antioxidant Status) was determined by Randox Total Antioxidant Status kits (Crumlin, UK). TAS analysis was based on the incubation of 2.2-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) with peroxidase (metmyoglobin) and hydrogen peroxide, which produced a radical cation ABTS+ with characteristic colour (changes in absorbance were determined at 600 nm wavelength).

2.3. Statistical Analysis

Statistical analysis of the results was conducted using Statistica 13 software with Medical Set (StatSoft, Tulsa, OK, USA). The results are presented as mean ± standard deviations (SDs). The results were analyzed statistically, using elements of descriptive statistics and statistical procedures, such as correlation analysis (with Pearson test for normal and Spearman test for non-normal distributions). Comparisons between groups were performed using t-Student test or Mann-Whitney U test (for respectively normal or non-normal data distributions). Stepwise multiple regression analyses were conducted to determine the predictor for protein levels in blood. The receiver operator characteristics (ROC) curve with area under the curve (AUC) were used to compare the usefulness of different parameters to describe of risk of a small level of CRY1 and CLOCK levels in blood. The normality of the data distribution was assessed using the Shapiro-Wilk test. The level of statistical significance was taken as $p < 0.05$.

3. Results

A total of 74 women aged 41.64 ± 9.41 years were enrolled into the study. They were divided into two groups that are comparable in age. The mean BMI of the entire study population was 26.97 ± 5.79 kg/m2 and was significantly higher in the HT, at 30.56 ± 6.78 kg/m2, than in NHT (25.14 ± 4.22 kg/m2, $p < 0.0003$, Table 2). According to the study assumption, the mean SBP and DBP in the study group was significantly higher than in the control group (Table 2). The mean systolic and diastolic blood pressure of all participants were 124.14 ± 20.12 and 77.44 ± 13.32 mmHg, respectively, which were higher in the hypertensive patients ($p < 0.0000001$). The study also showed the presence of the metabolic syndrome in a statistically higher percentage of people in HT ($p < 0.0000001$).

We found that HT had statistically higher body weight (17% higher; $p < 0.006$), BMI (22% higher; $p < 0.0003$) and total body fat mass (23% higher; $p < 0.00009$) as compared with NHT (Table 2). In addition, the study group had significantly higher levels of FBG (12% higher; $p < 0.002$) and TG (60% higher; $p < 0.0009$). The concentrations of TC and LDL were similar in HT (5.04 ± 0,8 mmol/L and 2.98 ± 0.82 mmol/L) and NHT (4.9 ± 1.0 mmol/L and 3.0 ± 0.9 mmol/L). In addition, the concentration of HDL was slightly lower in the study group in comparison to healthy group (1.59 ± 0.42 mmol/L vs. 1.73 ± 0.44 mmol/L), but the difference was not statistically significant ($p > 0.05$) (Table 2).

As shown in Table 2, statistically significant differences between the groups occurred in CRY1 concentration. The blood concentration of CRY1 in HT was 40.2% lower ($p < 0.008$) than in NHT (Table 2). However, no differences ($p < 0.05$) were noted in serum CLOCK concentration and TAS status between hypertensive patients and control subjects.

Interestingly, the research carried out showed that HT declared statistically significantly lower (by 15.8%) average daily caloric intake than NHT (1421.50 ± 311.51 kcal/day vs. 1646.46 ± 390.24 kcal/day, $p < 0.01$). Moreover, a similar relationship was seen for the level of the total amount of

carbohydrates consumed daily. People with hypertension consumed less (by 19.1%) of them than people without this disease (182.11 ± 43.99 kcal/day vs. 216.84 ± 60.20 g/day, $p < 0.007$). Total daily protein and fat intake were also lower, but the differences were not statistically significant (61.78 ± 15.17 vs. 67.90 ± 15.28 g/day, $p = 0.11$ and 56.63 ± 14.85 vs. 64.47 ± 19.58 g/day, $p < 0.13$).

The study showed that HT is characterized by statistically significant less physical activity during the 24h than NHT. The average number of steps performed during the day in the study group was 7007.43 ± 1877.56 steps (about 5.13 ± 1.70 kilometres) and in the control subjects 8597.20 ± 3195.00 steps (about 5.89 ± 2.17 kilometres) ($p < 0.009$). Moreover, the study showed no statistically significant difference in the amount of sleep during the day between the studied populations (485.59 ± 99.48 vs. 446.48 ± 92.68 minutes/day, $p > 0.05$, respectively).

Table 2. Anthropometric and biochemical characteristics of hypertensive individuals (HT) and non-hypertensive subjects (NHT)

Parameter [unit]	**HT (n=25)**	**NHT (n=49)**	**P value**
Age [years]	44.60±11.09	40.12±8.13	NS*
SBP [mmHg]	143.72±21.42	114.15±9.21	p < 0.0000001**
DBP [mmHg]	90.28±13.49	70.89±7.01	p < 0.0000001**
Body weight [kg]	83.39±20.08	71.12±12.36	p < 0.006**
BMI [kg/m^2]	30.56±6.78	25.14±4.22	p < 0.0003**
WHR	0.85±0.06	0.81±0.16	p < 0.0002**
Total body fat mass [%]	38.23±6.66	31.19±6.93	p < 0.00009*
FBG [mmol/L]	5.63±1.16	5.04±0.43	p < 0.002**
TC [mmol/L]	5.04±0.80	4.90±1.00	NS*
TG [mmol/L]	1.48±0.89	0.93±0.47	p < 0.0009**
HDL [mmol/L]	1.59±0.42	1.73±0.44	NS*
LDL [mmol/L]	2.98±0.82	2.97±0.94	NS*
CLOCK [ng/mL]	1.87±0.73	1.95±0.65	NS**
CRY1 [ng/mL]	0.64±0.37	1.07±0.93	p < 0.008*
TAS [mmol/L]	1.35±0.30	1.33±0.23	NS*

Parameters are shown as means (±standard deviations); n – number of individuals studied; p – level of statistical significance for HT vs. NHT groups according to t-Student test or Mann-Whitney U test** (for respectively normal or non-normal data distributions); NS – difference not statistically significant; SBP – systolic blood pressure; DBP – diastolic blood pressure; BMI – body mass index; WHR – Waist-Hip Ratio; FBG – fasting blood glucose; TC – total cholesterol; TG – triglycerides; HDL – high density lipoprotein; LDL – low density lipoprotein; CLOCK – circadian locomotor output cycles kaput; CRY1 – cryptochrome 1; TAS – total antioxidant status.*

In the entire study population, no relationship was observed between the concentration of CRY1 or CLOCK and biochemical, anthropometric and lifestyle parameters. The hypertensive patients showed no relationship between the concentrations of CRY1 or CLOCK and biochemical parameters. Of the anthropometric parameters the concentration of CRY1 was significantly negatively correlated with body weight: ($R = -0.40$, $p < 0.049$), BMI ($R = -0.48$, $p < 0.015$) and WHR ($R = -0.43$, $p < 0.034$) (Table 3). In addition, a positive correlation was found between CRY1 level and age in this group ($R = 0.42$, $p < 0.038$). A negative correlation existed between CLOCK concentration and the amount of saturated fatty acids consumed during the day ($R = -0.40$, $p < 0.049$) in HT. We have not shown any association between CRY1 or CLOCK concentrations and the amount of physical activity or sleep quality in study group. Furthermore, a positive correlation was noted between values of CLOCK and TAS in hypertensive patients ($R = 0.57$, $p < 0.003$), as well as between concentrations of CRY1 and TAS ($R = 0.54$, $p < 0.006$). Similar positive correlations were observed between CLOCK and CRY1 concentration ($R = 0.79$, $p < 0.000003$) in HT.

Table 3. Indices of correlation and levels of statistical significance in cases of analyses the relationship between CRY1, CLOCK and selected anthropometric parameters in HT

Parameter [unit]	**Body weight** [kg]	**BMI** [kg/m^2]	**TAS** [mmol/L]
CRY1 [ng/mL]	R = -0.40** p < 0.049**	R = -0.48** p < 0.015**	R = 0.54* p < 0.006*
CLOCK [ng/mL]	NS**	NS**	R = 0.57** p < 0.003**

Parameters are shown as R – coefficient of Pearson or Spearman** (for respectively normal or non-normal data distributions) and p – level of statistical significance, NS – statistically non-significant difference; HT– hypertensive individuals.*

The study also showed that CLOCK concentration in HT can help to distinguish the risk of 1. quarter (Q25) of CRY1 level with fair accuracy, as indicated by AUC (Cut-off value <1.611 ng/mL, sensitivity 95%, specificity 87%, AUC 0.951+/-0.024, 95%CI: 0.904-0.998, p=0.0001, Figure 1). There was no such relationship in the control group and other parameters.

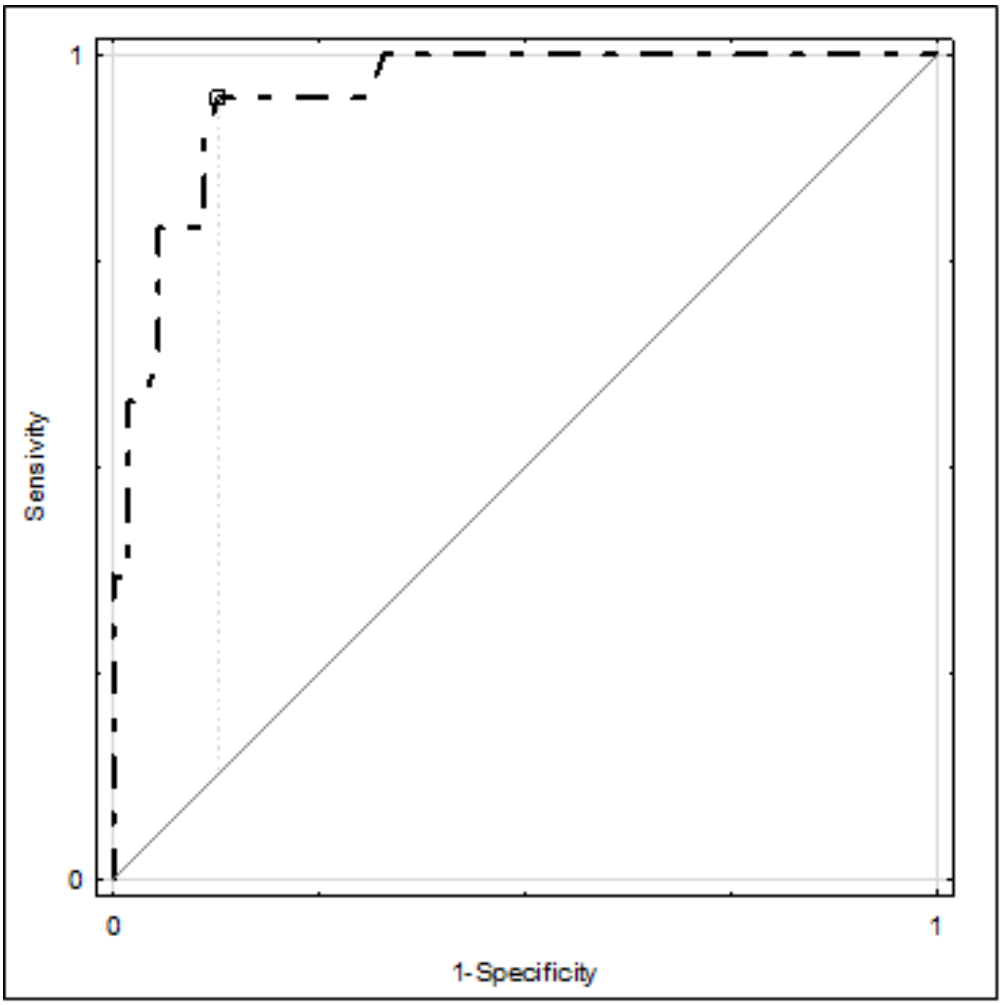

Figure 1. Receiver Operating Characteristic (ROC) curves for CLOCK level for the differentiation between 1. quarter (Q25) of CRY1 level and rest group (sum of 2., 3. and 4. quarter of CRY1 level subgroups) in HT – hypertensive individuals.

Moreover, tree variables: SBP, TAS level and CLOCK level can account for almost 22% of variation of CRY1 levels in the entire examined population (HT+NHT) ($p < 0.0007$, R^2_{adj} (adjusted R-squared) = 21.6%) (Table 4). Analogical relationships did not receive in the case of variation of CLOCK levels.

Table 4. Comparison of the stepwise multiple regression model explaining variations of CRY1 levels before and after adding the third variable (CLOCK level) the basic model, which includes SBP and TAS status in the entire examined population (HT+NHT, n = 74).

Statistical parameters	A model including 2 variables: SBP and TAS level	An extended model with a new variable (CLOCK level)
R^2_{adj}	0.030	0.216
F	1.097	6.415
P value	0.339	<0.0007

TAS – total antioxidant status; SBP – systolic blood pressure; n – number of females; R^2_{adj} – adjusted R-squared; p – level of statistical significance in multiple regression; HT– hypertensive individuals and NHT – non-hypertensive subjects.

In NHT in turn, the concentration of CRY1 correlated positively only with body weight and BMI ($R = 0.30$, $p < 0.0354$ and $R = 0.35$, $p < 0.0135$, respectively).

4. Discussion

Recent years have shown that circadian mechanisms play an essential role in the regulation of blood pressure. Most of the results regarding the meaning of the circadian clock in BP variability were conducted on rodents, not on humans [19,20,23,31,36]. Cry1 gene has been shown to be the primary circadian repressor [42]. Cry1/2 null mice characteristic salt-sensitive hypertension due to the dramatically high synthesis of the mineralocorticoid aldosterone by the adrenal gland [43]. Moreover, a later study demonstrated that Cry1/2 null mice have increased kidney damage on normal and high salt diets [44]. Furthermore, CRY1 interacts with several nuclear receptors and modulate transcriptional activity, that suggests a widespread mechanism of circadian nuclear receptor in the regulation of carbohydrate metabolism [45,46]. A recent study has shown that CLOCK Δ19 mutant mice with partially restored liver clock function under the high-fat diet normalized body weight, energy expenditure and rescued 24-h food intake rhythms similar to wild-type mice [47]. CLOCK-deficient mice exhibit also reduced arterial blood pressure and altered urinary sodium excretion compared to wild-type mice [48] and have decreased kidney microsomes and urinary levels of 20-hydroxyeicosatetraenoic acid (regulator of BP and blood flow) [49]. Furthermore, CLOCK Δ19 knockout mice have a blunted daily difference in mean blood pressure and a higher prevalence of cardiac hypertrophy and fibrosis compared to wild-type [50]. These studies suggest the role of CLOCK in renal and cardiovascular function. Moreover, Anea et al. demonstrated that mutation in the CLOCK gene induced endothelial dysfunction that was associated with the attenuation of Akt signalling and a subsequent the decrease in nitric oxide production that may be significant in the progression of vascular diseases [51]. Data on the concentration of circadian clock protein are scarce [52]. In our study, CRY1 levels in the hypertensive group were significantly lower than in subjects with normal blood pressure. Such a relationship has not been demonstrated for CLOCK concentration. Kovanen et al. indicated that several Cry1 SNPs were associated with metabolic syndrome, arterial hypertension and elevated blood pressure in human [53]. Only one more study (beside of our research) has evaluated the concentration of CRY1 in the human serum (in two patient groups with obstructive sleep apnea (OSA): with and without primary aldosteronism, PA) and differed significantly from our results (where women with primary aldosteronism or other forms of secondary hypertension were rejected). Tedjasukmana et al. received compared results of CRY1 level in both analyzed groups. Their research has been conducted on small population: 13 males (M) and 3 females (F) with OSA+PA and 24 persons (with OSA, without PA; control group: 13 M/11 F) [52].

In our study, CRY1 level was positively correlated with TAS only in the hypertension group (which characteristics high value in such anthropometric parameters as BMI, WHR and body mass). Some analogues this relationship may be found in interesting conclusion received in study of Tedjasukmana et al. that this protein concentration in blood decreased in severe hypoxia in OSA patients [52]. Because the destabilization of CRY1 is done by phosphorylation catalyzed by kinase activated by AMP (AMPK) and on the other hand, the activity of this kinase is regulated by the availability of glucose or the inflow of Ca2+ ions into the cell [54,55], so our study group with hypertension and high FGB levels at the same time - may be characterized by low CRY1 concentration in blood. Moreover, CRY1 status (similarly to blood pressure status [56]) in men and women is connected with different mechanisms, which are a little knowledge, so further investigation is warranted.

Of the three parameters analyzed in the multivariable model - SBP level, TAS status and CLOCK concentration - the latest parameter had the greatest importance in shaping the CRY1 level and proved to be an independent predictor in the entire examined population. Partly these data were also confirmed by the results from ROC curve for the hypertensive group: low blood concentrations (first quartile, 25%) for CRY1 occurred mainly in subjects with CLOCK concentrations in blood below 1.611 ng/mL (with high values of sensitivity and specificity). This data may suggest that there is a direct

relationship between the concentrations of these proteins in the blood, as there is an interaction at their transcriptional level (i.e. CLOCK-BMAL1 complex activates the expression of the cryptochromes gene families during the day) [13,16].

Hypertension is a modifiable risk factor for cardiovascular disease. It often occurs with other cardiovascular risk factors such as central obesity, dyslipidaemia and glucose intolerance [24,40]. The mean BMI in both studied populations was above 24.9 kg/m2, which indicates overweight, however, BMI values were significantly higher in women with hypertension compared to control group. Moreover, HT had higher body weight compared with NHT. Our findings are supported by previous studies that have identified a direct relationship between BMI and BP that is continuous and almost linear [38]. Some other studies also indicate that BMI showed a significant association with hypertension [57–59].

In our study, HT was characterized also by a more frequent occurrence of metabolic syndrome compared to NHT. These results are consistent with findings of other researchers, as abnormal blood pressure values are a component of the metabolic syndrome [60,61]. In the Korean National Health and Nutrition Examination Survey (KNHANES) study MetS prevalence in hypertension patients was reaching almost 60%. Moreover, the presence of metabolic syndrome in the hypertensive population was associated with increased organ damage [61].

The current findings revealed that patients with hypertension had higher values of FBG and TG compared to those without, but TC, LDL and HDL levels did not differ between the two populations. Different results were obtained in the cross-sectional study with 4202 participants where all lipid profile variables (despite HDL level) and fasting blood glucose level were significantly higher in hypertensive individuals compared to the control group with normal BP [57]. Another study, conducted among 234 participants, showed that serum levels of TC, TG and LDL were statistically higher, while HDL level was lower in patients with hypertension compared to normotensive subjects [58]. Because in our study, HT was treated with lipid-lowering medications in most cases, so there are no statistical differences in lipaemia status between study and control groups.

The present study documented an equivalent value of the concentration of TAS in HT and NHT. The results of other authors' researches are very diverse. Kharroubi et al. documented increased TAS levels in obese compared to non-obese subjects (2.12 vs. 1.85 mmol/L Trolox). Moreover, this study showed that people with SBP above 140 mmHg had higher TAS levels compared to those with normal SBP (2.15 vs. 1.97 mmol/L Trolox), as in the case with abnormal DBP and the normal DBP values (2.22 and 1.96 mmol/L Trolox, respectively) [62]. Different results were obtained in the study by Chaudharya et al. They found that TAS levels in hypertensive subjects were significantly reduced (1.74 mmol/L Trolox Equivalent) compared to healthy subject ≥60 and <60 years old (2.03 and 2.29, mmol/L Trolox Equivalent, respectively) [63]. Antioxidants status are connected with different factors (i.e. age, anthropometric parameters, antioxidative vitamins and other substance in diet, physical activity) [62,64,65]. Because large obesity and intensive physical activity promote oxidative stress [62–65] and our control group was thinner and more active than HT, so levels of TAS in both groups maybe comparable. In the development and treatment of hypertension, the most important modifiable risk factors are diet and physical activity [66,67].

Our study documented statistically significant lower activity during the day in HT than NHT. Several studies showed that decreased level of daily physical activity is associated with a higher risk of hypertension and cardiovascular diseases. Moreover, studies demonstrated beneficial effects of exercise on blood pressure causing reductions in systolic and diastolic blood pressure about 5-7 mmHg [67–69].

Surprisingly, in our study HT declared lower intake of calories during the day than NHT, but the difference between energy consumption (15.8% lower than control group) and physical activity (22.7% lower value than NHT in case of the number of steps performed during the 24-period) indicates a positive energy balance. Studies indicate that restriction caloric intake during the day can lower SBP, DBP and mean blood pressure compared to a standard diet [70]. This caloric reduction may be the critical change in the lifestyle of the studied patients and could have a measurable impact on further blood pressure values. Moreover, the results from a meta-analysis of 17 randomized controlled trials suggest that healthy dietary patterns, for example, DASH diet (Dietary Approaches

to Stop Hypertension), or a Mediterranean diet can significantly reduce SBP and DBP (by 4.26 mm Hg and 2.38 mm Hg, respectively). These diets are based on vegetables, fruits, whole grains, legumes, nuts, seeds, fish, and avoid meat and sweets [71]. Therefore, the diet of the study population should be based on these principles. Moreover, our study has shown a negative correlation between CLOCK concentration and the amount of saturated fatty acids consumed during the day in HT. Animal studies revealed that the high-fat diet alters the function of the circadian clock. [72–74]. For example, the consumption of high-fat diet in CLOCK mutant mice disturbed the circadian rhythm and caused weight gain [72].

In both studied populations of individuals with HT as well as without, the average length of sleep was around 7.5-8 hours per day. A recent meta-analysis has found an association between short sleep duration and hypertension risk. Both excessively longer and shorter sleep periods can be risk factors for this disease, and these relationships are stronger in women than in men. The lowest risk of an increase in blood pressure occurs with 7 hours of sleep a day [32]. These results are confirmed by a later analysis carried out by Grandner et al. with 700000 adults [75]. Moreover, in a new cross-sectional study from 2019 involving 19407 participants, an association was found between short sleep times (<7 hours a day) and an increased risk of hypertension [76].

Currently, no data are available on the correlation between CLOCK or CRY1 and anthropometric and biochemical parameters or lifestyle elements to compare our results. Our study is the first analysis of these associations in human. The study showed a relationship between the concentration of CRY1 and anthropometric parameters (body weight, BMI and WHR) in our entire study population (negatively and positively, depending on the group considered). There was also a negative correlation between the CLOCK concentration and the amount of saturated fatty acids consumed during the day in HT. Moreover, in hypertensive patients, increased CLOCK or CRY1 values were associated with high TAS level. On the other hand, we have not shown any association between CLOCK level in blood and anthropometric parameters and between CRY1 or CLOCK concentrations and the amount of physical activity or sleep quality.

4.1 Limitations and strength of the study

This study has some limitations. The first limitation is represented by the small size of the study and control groups. It is worth considering expanding biochemical analyses to larger research groups. Moreover, the study contained only female Caucasians, so the results of this study should not be generalized to the general population. Besides, more studies should be done, including more diverse populations (different races, male patients, etc.) to look into the role of circadian clock proteins, especially CRY1 and CLOCK, in the regulation of blood pressure. Another limitation is the lack of 24-hour blood pressure monitoring. We decided to analyse the blood pressure in the morning because research has shown that the morning surge of BP is correlated with a higher incidence of stroke, cardiovascular events or sudden cardiac death than other times of the day and independently of 24-hour average BP [70].

The key strengths of the study included the in-depth comprehensive analysis of biochemical, anthropometric and lifestyle parameters in hypertensive patients. Another strength of this study is the exclusion of patients with secondary obesity or secondary hypertension and obtaining homogeneity of the research group. Furthermore, this is the first study to identify a serum level of CRY1 and CLOCK in hypertensive individuals as well as its association with anthropometric, biochemical and lifestyle parameters.

5. Conclusions

Our results confirm the hypothesis that hypertensive patients show altered serum level of CRY1, but not in the case of CLOCK concentration. In this study, patients with HT showed reduced serum CRY1 level concomitantly to higher body weight, total body fat mass, BMI, FBG and TG compared to healthy peers. The present study demonstrated the relationship between the concentration of CRY1 and some anthropometric parameters (body weight, BMI and WHR). There was a negative

correlation between the CLOCK concentration and the amount of saturated fatty acids consumed during the day in HT, which indicates the possible influence of the diet on the concentration of CLOCK in this population. Furthermore, in hypertensive patients, increased CLOCK or CRY1 values were associated with high TAS level, which can exhibit their protective effects in these patients. CLOCK concentration in blood had the greatest importance in shaping the CRY1 level and proved to be an independent predictor of CRY1 level in the entire examined population. In conclusion, the serum level of CRY1 could be considered in detailed diagnostics of hypertension (primarily in populations with abnormal anthropometric indices), to help broaden knowledge about arterial blood pressure disorders.

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References

1. Dibner, C.; Schibler, U.; Albrecht, U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* **2010**, *72*, 517–549, doi:10.1146/annurev-physiol-021909-135821.
2. Aoyama, S.; Shibata, S. The Role of Circadian Rhythms in Muscular and Osseous Physiology and Their Regulation by Nutrition and Exercise. *Front. Neurosci.* **2017**, *11*, doi:10.3389/fnins.2017.00063.
3. Richards, J.; Gumz, M.L. Advances in understanding the peripheral circadian clocks. FASEB J. **2012**, *26*, 3602–3613, doi:10.1096/fj.12-203554.
4. Marcheva, B.; Ramsey, K.M.; Peek, C.B.; Affinati, A.; Maury, E.; Bass, J. Circadian clocks and metabolism. *Handb. Exp. Pharmacol.* **2013**, 127–155, doi:10.1007/978-3-642-25950-0_6.
5. Partch, C.L.; Green, C.B.; Takahashi, J.S. Molecular architecture of the mammalian circadian clock. *Trends Cell Biol.* **2014**, *24*, 90–99, doi:10.1016/j.tcb.2013.07.002.
6. Albrecht, U.; Eichele, G. The mammalian circadian clock. *Curr. Opin. Genet. Dev.* **2003**, *13*, 271–277, doi:10.1016/s0959-437x(03)00055-8.
7. Yamanaka, Y.; Hashimoto, S.; Masubuchi, S.; Natsubori, A.; Nishide, S.; Honma, S.; Honma, K. Differential regulation of circadian melatonin rhythm and sleep-wake cycle by bright lights and nonphotic time cues in humans. *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* **2014**, *307*, R546–R557, doi:10.1152/ajpregu.00087.2014.
8. Xie, Y.; Tang, Q.; Chen, G.; Xie, M.; Yu, S.; Zhao, J.; Chen, L. New Insights Into the Circadian Rhythm and Its Related Diseases. *Front. Physiol.* **2019**, *10*, doi:10.3389/fphys.2019.00682.
9. Buijs, F.N.; León-Mercado, L.; Guzmán-Ruiz, M.; Guerrero-Vargas, N.N.; Romo-Nava, F.; Buijs, R.M. The Circadian System: A Regulatory Feedback Network of Periphery and Brain. *Physiol. (Bethesda)* **2016**, *31*, 170–181, doi:10.1152/physiol.00037.2015.
10. Hermida, R.C.; Ayala, D.E.; Smolensky, M.H.; Mojón, A.; Fernández, J.R.; Crespo, J.J.; Moyá, A.; Ríos, M.T.; Portaluppi, F. Chronotherapy improves blood pressure control and reduces vascular risk in CKD. *Nat. Rev. Nephrol.* **2013**, *9*, 358–368, doi:10.1038/nrneph.2013.79.
11. Witte, K.; Schnecko, A.; Buijs, R.M.; Vliet, J. van der; Scalbert, E.; Delagrange, P.; Guardiola-Lemaitre, B.; Lemmer, B. Effects of Scn Lesions on Orcadian Blood Pressure Rhythm in Normotensive and Transgenic Hypertensive Rats. *Chronobiol. Int.* **1998**, *15*, 135–145, doi:10.3109/07420529808998678.
12. Dierickx, P.; Van Laake, L.W.; Geijsen, N. Circadian clocks: from stem cells to tissue homeostasis and regeneration. *EMBO Rep.* **2018**, *19*, 18–28, doi:10.15252/embr.201745130.
13. Asher, G.; Sassone-Corsi, P. Time for Food: The Intimate Interplay between Nutrition, Metabolism, and the Circadian Clock. *Cell* **2015**, *161*, 84–92, doi:10.1016/j.cell.2015.03.015.

14. Froy, O. Circadian rhythms, nutrition and implications for longevity in urban environments. *Proc. Nutr. Soc.* **2018**, *77*, 216–222, doi:10.1017/S0029665117003962.
15. Johnston, J.D. Physiological links between circadian rhythms, metabolism and nutrition. *Exp. Physiol.* **2014**, *99*, 1133–1137, doi:10.1113/expphysiol.2014.078295.
16. Bollinger, T.; Schibler, U. Circadian rhythms – from genes to physiology and disease. *Swiss Med. Wkly.* **2014**, *144*, doi:10.4414/smw.2014.13984.
17. Bass, J. Circadian topology of metabolism. *Nature* **2012**, *491*, 348–356, doi:10.1038/nature11704.
18. Zhang, R.; Lahens, N.F.; Ballance, H.I.; Hughes, M.E.; Hogenesch, J.B. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 16219–16224, doi:10.1073/pnas.1408886111.
19. Richards, J.; Diaz, A.N.; Gumz, M.L. Clock genes in hypertension: novel insights from rodent models. *Blood Press. Monit.* **2014**, *19*, 249–254, doi:10.1097/MBP.0000000000000060.
20. Rhoads, M.K.; Balagee, V.; Thomas, S.J. Circadian Regulation of Blood Pressure: of Mice and Men. *Curr. Hypertens. Rep.* **2020**, *22*, 40, doi:10.1007/s11906-020-01043-3.
21. Lamia, K.A.; Papp, S.J.; Yu, R.T.; Barish, G.D.; Uhlenhaut, N.H.; Jonker, J.W.; Downes, M.; Evans, R.M. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* **2011**, *480*, 552–556, doi:10.1038/nature10700.
22. Bhatnagar, A. Environmental Determinants of Cardiovascular Disease. *Circ. Res.* **2017**, *121*, 162–180, doi:10.1161/CIRCRESAHA.117.306458.
23. Douma, L.G.; Gumz, M.L. Circadian clock-mediated regulation of blood pressure. *Free Radic. Biol. Med.* **2018**, *119*, 108–114, doi:10.1016/j.freeradbiomed.2017.11.024.
24. Williams, B.; Mancia, G.; Spiering, W.; Agabiti Rosei, E.; Azizi, M.; Burnier, M.; Clement, D.L.; Coca, A.; de Simone, G.; Dominiczak, A.; et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. *J. Hypertens.* **2018**, *36*, 1953–2041, doi:10.1097/HJH.0000000000001940.
25. Timio, M.; Venanzi, S.; Lolli, S.; Lippi, G.; Verdura, C.; Monarca, C.; Guerrini, E. "Non-dipper" hypertensive patients and progressive renal insufficiency: a 3-year longitudinal study. *Clin. Nephrol.* **1995**, *43*, 382–387.
26. Krzych, Ł.J.; Bochenek, A. Blood pressure variability: Epidemiological and clinical issues. *Cardiol. J.* **2013**, *20*, 112–120, doi:10.5603/CJ.2013.0022.
27. Mahabala, C.; Kamath, P.; Bhaskaran, U.; Pai, N.D.; Pai, A.U. Antihypertensive therapy: nocturnal dippers and nondippers. Do we treat them differently? *Vasc. Health Risk Manag.* **2013**, *9*, 125–133, doi:10.2147/VHRM.S33515.
28. de la Sierra, A.; Gorostidi, M.; Banegas, J.R.; Segura, J.; de la Cruz, J.J.; Ruilope, L.M. Nocturnal hypertension or nondipping: Which is better associated with the cardiovascular risk profile? *Am. J. Hypertens.* **2014**, *27*, 680–687, doi:10.1093/ajh/hpt175.
29. Aksit, E.; Gursul, E.; Aydin, F.; Samsa, M.; Ozcelik, F. Non-dipper hypertension is associated with slow coronary flow among hypertensives with normal coronary angiogram. *Cardiovasc. J. Afr.* **2017**, *28*, 14–18, doi:10.5830/CVJA-2016-045.
30. Takeda, N.; Maemura, K. Circadian clock and cardiovascular disease. *J. Cardiol.* **2011**, *57*, 249–256, doi:10.1016/j.jjcc.2011.02.006.
31. Morris, C.J.; Purvis, T.E.; Hu, K.; Scheer, F.A.J.L. Circadian misalignment increases cardiovascular disease risk factors in humans. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E1402-1411, doi:10.1073/pnas.1516953113.
32. Wang, Y.; Mei, H.; Jiang, Y.-R.; Sun, W.-Q.; Song, Y.-J.; Liu, S.-J.; Jiang, F. Relationship between duration of sleep and hypertension in adults: A meta-analysis. *J. Clin. Sleep Med.* **2015**, *11*, 1047–1056, doi:10.5664/jcsm.5024.
33. Wehrens, S.M.T.; Christou, S.; Isherwood, C.; Middleton, B.; Gibbs, M.A.; Archer, S.N.; Skene, D.J.; Johnston, J.D. Meal timing regulates the human circadian system. *Curr. Biol.* **2017**, *27*, 1768-1775.e3, doi:10.1016/j.cub.2017.04.059.
34. Serin, Y.; Acar Tek, N. Effect of Circadian Rhythm on Metabolic Processes and the Regulation of Energy Balance. *Ann. Nutr. Metab.* **2019**, *74*, 322–330, doi:10.1159/000500071.
35. Chen, L.; Yang, G. Recent advances in circadian rhythms in cardiovascular system. *Front. Pharmacol.* **2015**, *6*, doi:10.3389/fphar.2015.00071.

36. Morris, C.J.; Purvis, T.E.; Mistretta, J.; Hu, K.; Scheer, F.A.J.L. Circadian Misalignment Increases C-Reactive Protein and Blood Pressure in Chronic Shift Workers. *J. Biol. Rhythms* **2017**, *32*, 154–164, doi:10.1177/0748730417697537.
37. Yan, B.; Yan, H.; Sun, L.; Yan, X.; Peng, L.; Wang, Y.; Wang, G. Novel Association Between the Reverse-Dipper Pattern of Ambulatory Blood Pressure Monitoring and Metabolic Syndrome in Men But Not in Women. *Medicine (Baltimore)* **2015**, *94*, doi:10.1097/MD.0000000000002115.
38. Whelton Paul K.; Carey Robert M.; Aronow Wilbert S.; Casey Donald E.; Collins Karen J.; Dennison Himmelfarb Cheryl; DePalma Sondra M.; Gidding Samuel; Jamerson Kenneth A.; Jones Daniel W.; et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* **2018**, *71*, e13–e115, doi:10.1161/HYP.0000000000000065.
39. Association, W.M. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA* **2013**, *310*, 2191–2194, doi:10.1001/jama.2013.281053.
40. Tykarski, A.; Filipiak, K.J.; Januszewicz, A.; Litwin, M.; Narkiewicz, K.; Prejbisz, A.; Ostalska-Nowicka, D.; Widecka, K.; Kostka-Jeziorny, K. 2019 Guidelines for the Management of Hypertension — Part 1–7. *Arter. Hypertens.* **2019**, *23*, 41–87, doi:10.5603/AH.a2019.0008.
41. Knopfholz, J.; Disserol, C.C.D.; Pierin, A.J.; Schirr, F.L.; Streisky, L.; Takito, L.L.; Massucheto Ledesma, P.; Faria-Neto, J.R.; Olandoski, M.; da Cunha, C.L.P.; et al. Validation of the friedewald formula in patients with metabolic syndrome. *Cholesterol* **2014**, *2014*, 261878, doi:10.1155/2014/261878.
42. Chen, R.; Schirmer, A.; Lee, Y.; Lee, H.; Kumar, V.; Yoo, S.-H.; Takahashi, J.S.; Lee, C. Rhythmic PER abundance defines a critical nodal point for negative feedback within the circadian clock mechanism. *Mol. Cell* **2009**, *36*, 417–430, doi:10.1016/j.molcel.2009.10.012.
43. Doi, M.; Takahashi, Y.; Komatsu, R.; Yamazaki, F.; Yamada, H.; Haraguchi, S.; Emoto, N.; Okuno, Y.; Tsujimoto, G.; Kanematsu, A.; et al. Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. *Nat. Med.* **2010**, *16*, 67–74, doi:10.1038/nm.2061.
44. Nugrahaningsih, D.A.A.; Emoto, N.; Vignon-Zellweger, N.; Purnomo, E.; Yagi, K.; Nakayama, K.; Doi, M.; Okamura, H.; Hirata, K. Chronic hyperaldosteronism in Cryptochrome-null mice induces high-salt- and blood pressure-independent kidney damage in mice. *Hypertens. Res.* **2014**, *37*, 202–209, doi:10.1038/hr.2013.143.
45. Jordan, S.D.; Kriebs, A.; Vaughan, M.; Duglan, D.; Fan, W.; Henriksson, E.; Huber, A.-L.; Papp, S.J.; Nguyen, M.; Afetian, M.; et al. CRY1/2 Selectively Repress PPARδ and Limit Exercise Capacity. *Cell Metab.* **2017**, *26*, 243-255.e6, doi:10.1016/j.cmet.2017.06.002.
46. Kriebs, A.; Jordan, S.D.; Soto, E.; Henriksson, E.; Sandate, C.R.; Vaughan, M.E.; Chan, A.B.; Duglan, D.; Papp, S.J.; Huber, A.-L.; et al. Circadian repressors CRY1 and CRY2 broadly interact with nuclear receptors and modulate transcriptional activity. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 8776–8781, doi:10.1073/pnas.1704955114.
47. Meyer-Kovac, J.; Kolbe, I.; Ehrhardt, L.; Leliavski, A.; Husse, J.; Salinas, G.; Lingner, T.; Tsang, A.H.; Barclay, J.L.; Oster, H. Hepatic gene therapy rescues high-fat diet responses in circadian Clock mutant mice. *Mol. Metab.* **2017**, *6*, 512–523, doi:10.1016/j.molmet.2017.03.008.
48. Zuber, A.M.; Centeno, G.; Pradervand, S.; Nikolaeva, S.; Maquelin, L.; Cardinaux, L.; Bonny, O.; Firsov, D. Molecular clock is involved in predictive circadian adjustment of renal function. *Proc. Natl. Acad. Sci.* **2009**, *106*, 16523–16528, doi:10.1073/pnas.0904890106.
49. Nikolaeva, S.; Pradervand, S.; Centeno, G.; Zavadova, V.; Tokonami, N.; Maillard, M.; Bonny, O.; Firsov, D. The circadian clock modulates renal sodium handling. *J. Am. Soc. Nephrol. JASN* **2012**, *23*, 1019–1026, doi:10.1681/ASN.2011080842.
50. Alibhai, F.J.; LaMarre, J.; Reitz, C.J.; Tsimakouridze, E.V.; Kroetsch, J.T.; Bolz, S.-S.; Shulman, A.; Steinberg, S.; Burris, T.P.; Oudit, G.Y.; et al. Disrupting the key circadian regulator CLOCK leads to age-dependent cardiovascular disease. *J. Mol. Cell. Cardiol.* **2017**, *105*, 24–37, doi:10.1016/j.yjmcc.2017.01.008.
51. Anea, C.B.; Zhang, M.; Stepp, D.W.; Simkins, G.B.; Reed, G.; Fulton, D.J.; Rudic, R.D. Vascular disease in mice with a dysfunctional circadian clock. *Circulation* **2009**, *119*, 1510–1517, doi:10.1161/CIRCULATIONAHA.108.827477.
52. Tedjasukmana, R.; Purba, J.S.; Wanandi, S.I.; Suyatna, F.D. Neuroglobin correlates with cryptochrome-1 in obstructive sleep apnea with primary aldosteronism. *PLOS ONE* **2018**, *13*, e0204390, doi:10.1371/journal.pone.0204390.

53. Kovanen, L.; Donner, K.; Kaunisto, M.; Partonen, T. CRY1 , CRY2 and PRKCDBP genetic variants in metabolic syndrome. *Hypertens. Res.* **2015**, *38*, 186–192, doi:10.1038/hr.2014.157.
54. Lamia, K.A.; Sachdeva, U.M.; DiTacchio, L.; Williams, E.C.; Alvarez, J.G.; Egan, D.F.; Vasquez, D.S.; Juguilon, H.; Panda, S.; Shaw, R.J.; et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* **2009**, *326*, 437–440, doi:10.1126/science.1172156.
55. Hardie, D.G. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 774–785, doi:10.1038/nrm2249.
56. Faulkner, J.L.; Belin de Chantemèle, E.J. Sex Differences in Mechanisms of Hypertension Associated With Obesity. *Hypertens.* **2018**, *71*, 15–21, doi:10.1161/HYPERTENSIONAHA.117.09980.
57. Nwankwo, M.; Okamkpa, J.C.; Danborno, B. Association between high blood pressure with risk of type 2 diabetes, metabolic syndrome and its predictors: A cross-sectional study. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2019**, *13*, 1549–1554, doi:10.1016/j.dsx.2018.11.065.
58. Choudhury, K.N.; Mainuddin, A.K.M.; Wahiduzzaman, M.; Islam, S.M.S. Serum lipid profile and its association with hypertension in Bangladesh. *Vasc. Health Risk Manag.* **2014**, *10*, 327–332, doi:10.2147/VHRM.S61019.
59. Aronow, W.S. Association of obesity with hypertension. *Ann. Transl. Med.* **2017**, *5*, doi:10.21037/atm.2017.06.69.
60. Mulè, G.; Calcaterra, I.; Nardi, E.; Cerasola, G.; Cottone, S. Metabolic syndrome in hypertensive patients: An unholy alliance. *World J. Cardiol.* **2014**, *6*, 890–907, doi:10.4330/wjc.v6.i9.890.
61. Lee, S.-R.; Cha, M.-J.; Kang, D.-Y.; Oh, K.-C.; Shin, D.-H.; Lee, H.-Y. Increased prevalence of metabolic syndrome among hypertensive population: Ten years' trend of the Korean National Health and Nutrition Examination Survey. *Int. J. Cardiol.* **2013**, *166*, 633–639, doi:10.1016/j.ijcard.2011.11.095.
62. Kharroubi, A.T.; Darwish, H.M.; Akkawi, M.A.; Ashareef, A.A.; Almasri, Z.A.; Bader, K.A.; Khammash, U.M. Total Antioxidant Status in Type 2 Diabetic Patients in Palestine. *J. Diabetes Res.* **2015**, *2015*, 461271, doi:10.1155/2015/461271.
63. Chaudhary, P.; Pandey, A.; Azad, C.S.; Tia, N.; Singh, M.; Gambhir, I.S. Association of oxidative stress and endothelial dysfunction in hypertension. *Anal. Biochem.* **2020**, *590*, 113535, doi:10.1016/j.ab.2019.113535.
64. Yavari, A.; Javadi, M.; Mirmiran, P.; Bahadoran, Z. Exercise-Induced Oxidative Stress and Dietary Antioxidants. *Asian J. Sports Med.* **2015**, *6*, doi:10.5812/asjsm.24898.
65. Villaverde, P.; Lajous, M.; MacDonald, C.-J.; Fagherazzi, G.; Bonnet, F.; Boutron-Ruault, M.-C. High dietary total antioxidant capacity is associated with a reduced risk of hypertension in French women. *Nutr. J.* **2019**, *18*, 31, doi:10.1186/s12937-019-0456-0.
66. Ozemek, C.; Laddu, D.R.; Arena, R.; Lavie, C.J. The role of diet for prevention and management of hypertension. *Curr. Opin. Cardiol.* **2018**, *33*, 388–393, doi:10.1097/HCO.0000000000000532.
67. Pescatello, L.S.; Buchner, D.M.; Jakicic, J.M.; Powell, K.E.; Kraus, W.E.; Bloodgood, B.; Campbell, W.W.; Dietz, S.; Dipietro, L.; George, S.M.; et al. Physical Activity to Prevent and Treat Hypertension: A Systematic Review. *Med. Sci. Sports Exerc.* **2019**, *51*, 1314–1323, doi:10.1249/MSS.0000000000001943.
68. Diaz, K.M.; Shimbo, D. Physical Activity and the Prevention of Hypertension. *Curr. Hypertens. Rep.* **2013**, *15*, 659–668, doi:10.1007/s11906-013-0386-8.
69. Rossi, A.; Dikareva, A.; Bacon, S.L.; Daskalopoulou, S.S. The impact of physical activity on mortality in patients with high blood pressure: a systematic review. *J. Hypertens.* **2012**, *30*, 1277–1288, doi:10.1097/HJH.0b013e3283544669.
70. Nicoll, R.; Henein, M.Y. Caloric Restriction and Its Effect on Blood Pressure, Heart Rate Variability and Arterial Stiffness and Dilatation: A Review of the Evidence. *Int. J. Mol. Sci.* **2018**, *19*, doi:10.3390/ijms19030751.
71. Ndanuko, R.N.; Tapsell, L.C.; Charlton, K.E.; Neale, E.P.; Batterham, M.J. Dietary Patterns and Blood Pressure in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Adv. Nutr. (Bethesda)* **2016**, *7*, 76–89, doi:10.3945/an.115.009753.
72. Turek, F.W.; Joshu, C.; Kohsaka, A.; Lin, E.; Ivanova, G.; McDearmon, E.; Laposky, A.; Losee-Olson, S.; Easton, A.; Jensen, D.R.; et al. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* **2005**, *308*, 1043–1045, doi:10.1126/science.1108750.
73. Kohsaka, A.; Laposky, A.D.; Ramsey, K.M.; Estrada, C.; Joshu, C.; Kobayashi, Y.; Turek, F.W.; Bass, J. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab.* **2007**, *6*, 414–421, doi:10.1016/j.cmet.2007.09.006.

74. Pendergast, J.S.; Branecky, K.L.; Yang, W.; Ellacott, K.L.J.; Niswender, K.D.; Yamazaki, S. High-fat diet acutely affects circadian organisation and eating behavior. *Eur. J. Neurosci.* **2013**, *37*, 1350–1356, doi:10.1111/ejn.12133.
75. Grandner, M.; Mullington, J.M.; Hashmi, S.D.; Redeker, N.S.; Watson, N.F.; Morgenthaler, T.I. Sleep Duration and Hypertension: Analysis of > 700,000 Adults by Age and Sex. *J. Clin. Sleep Med.* **2018**, *14*, 1031–1039, doi:10.5664/jcsm.7176.
76. Li, M.; Yan, S.; Jiang, S.; Ma, X.; Gao, T.; Li, B. Relationship between sleep duration and hypertension in northeast China: a cross-sectional study. *BMJ Open* **2019**, *9*, e023916, doi:10.1136/bmjopen-2018-023916.