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**HYPOVITAMINOSIS D AND ORGAN DAMAGE IN PATIENTS WITH
ARTERIAL HYPERTENSION: A MULTICENTER DOUBLE BLIND RANDOMISED CONTROLLED TRIAL OF
CHOLECALCIFEROL SUPPLEMENTATION (HYPODD).**

Study design, clinical procedures and treatment protocol.

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Abstract

At this time, good quality randomized clinical trials assessing the effects of vitamin D supplementation on cardiometabolic outcomes are lacking in the international literature. To fill this gap, the Working Group on Vitamin D and Cardiorenal Disorders established jointly by the Italian Society of Hypertension (SIIA) and the Forum in Bone & Mineral Research conceived the HYPODD study (HYPOVitaminosis D and organ Damage). HYPODD is a no-profit multicenter 12-month parallel-group double-blind placebo controlled randomized trial aiming to assess the effects of cholecalciferol supplementation on blood pressure control, antihypertensive drugs consumption and progression of target organ damage in patients with essential hypertension and 25-hydroxyvitamin D serum level lower than 20 ng/ml (vitamin D deficiency). HYPODD is coordinated by the European Society Excellence Center of Hypertension of Federico II University, Naples, and involves 12 academic institutions in Italy (Ancona, Milan, Padua, Perugia, Rome, Siena, Trieste, Turin, Udine, Varese, and Verona). It has been registered at the Agenzia Italiana del Farmaco – Osservatorio sulla Sperimentazione Clinica del Farmaco (AIFA- OsSC) and EUDRACT sites (n° 2012-003514-14) and has been approved by the Ethical Committees of all the Centers involved in the study. The patients' recruitment is currently underway.

Background and HYPODD definition.

For many decades the vitamin D-vitamin D receptor (VDR) biological system has been considered the “key regulator” of calcium-phosphate homeostasis and bone metabolism (1). In the last 20 years, several clinical and experimental observations have significantly modified our knowledge regarding the properties and the activities of this biological system (2). The new evidence can be summarized in three points: i) at least 36 different cell types express the VDR and are responsive to stimulation by 1,25-dihydroxyvitamin D; ii) a paracrine production of 1,25-dihydroxyvitamin D takes place in over 10 extra-renal organs, iii) the 1,25-dihydroxyvitaminD-VDR endocrine-paracrine system directly or indirectly controls the expression of about 3% of the whole human genome and regulates complex processes as cell differentiation and growth (3). Based on these acquisitions, a variety of human diseases has been related to vitamin D insufficiency or deficiency, respectively defined as 25-hydroxyvitamin D circulating levels lower than 30 or lower than 20 ng/ml (4). In particular, experimental and observational studies support the contention that vitamin D deficiency contributes to the pathogenesis of most common cardio-metabolic disorders such as hypertension, type 2 diabetes and their complications, among which coronary artery disease and congestive heart failure(5-8). The epidemiological and clinical relevance of these observations is emphasized by the notion that worldwide almost 1billion people of any age have 25-hydroxyvitamin D circulating levels lower than 20 ng/ml (9). However, despite the large number of observational studies available and their relative consistency, a causal effect of vitaminD deficiency in cardiometabolic disorders is not definitely established. For this reason, correction of vitamin D deficiency is not officially recommended nowadays for cardiovascular disease prevention (10, 11). To this end, good quality randomized clinical trials that assess the effects of vitamin D supplementation on cardiometabolic outcomes are needed (12).

The HYPOvitaminosis D and organ Damage(HYPODD) evaluation trial described in this report aims to contribute to fill this gap. HYPODD is a no-profit, placebo controlled trial aiming to evaluate the effects of cholecalciferol supplementation on blood pressure (BP) control, antihypertensive drug consumption and

progression of target organ damage in patients with essential hypertension and 25-hydroxyvitamin D serum level lower than 20 ng/ml (i.e. vitamin D deficiency).

Objective and study design

The main objective of HYPODD is to assess the efficacy of cholecalciferol supplementation in improving BP control and slow down target organ damage progression in patients with idiopathic hypertension and vitamin D deficiency, as indicated by a serum level of 25-hydroxyvitamin D lower than 20 ng/ml at enrollment.

The study was designed as a multicenter 12-month parallel-group double-blind placebo controlled randomized trial. It has been conceived by the Working Group on Vitamin D and Cardiorenal Disorders established jointly by the Italian Society of Hypertension (SIIA) and the Forum in Bone & Mineral Research coordinated by the Federico II University of Naples ESH Excellence Center. HYPODD involves academic institutions in Ancona, Milan, Padua, Perugia, Rome (La Sapienza University), Siena, Trieste, Turin, Udine, Varese, and Verona. An ancillary genetic study has been further scheduled to evaluate the possible influence of VDR allelic variants on the main study outcomes, to be performed in collaboration with the “Adriano Buzzati-Traverso” Institute of the Italian National Research Council (CNR) in Naples.

Inclusion and exclusion criteria

To be eligible for participation in the study, patients must fulfill at enrollment the following criteria: a) have well-controlled idiopathic hypertension [i.e. systolic BP (SBP) lower than 140 mmHg and diastolic BP (DBP) lower than 90 mmHg at two separate clinic visits (13)]; b) follows stable anti-hypertensive drug therapy since for at least 4 weeks; and c) have serum levels of 25-hydroxyvitamin D levels lower than 20 ng/ml. Exclusion criteria are a) age lower than 35 and higher than 75 years, b) body mass index lower than 20 Kg/m² or higher than 35 Kg/m²; c) secondary hypertension (13); d) personal or family history of rickets and/or osteomalacia; e) serum calcium and/or intact PTH higher than the laboratory reference range; primary or secondary hyperparathyroidism (14); f) glucose fasting levels higher than 126 mg/dl; g) glomerular filtration rate < 60 ml/min/1.73 m²; g) renal tubular acidosis (urinary pH >7, low potassium

serum levels and positive urinary net charge) (15); h) serum TSH level <0.5 mUI/ml or >4.5 mUI/ml (16); i) altered nutritional status, i.e. weight loss higher than 10% of usual weight during the last six months, weight loss greater than 5% of usual weight during the last 30 days, personal history positive for chronic diarrhea, caloric intake (estimated using a food frequency questionnaire) inappropriate for age and gender (17); l) personal history positive for sarcoidosis, type 1 and type 2 diabetes mellitus, nephrolithiasis, atrial fibrillation, atrial flutter, atrial or ventricular extrasystoles (Lown class ≥ 2); m) ECG rhythm different from sinus rhythm; n) drugs history positive for antiarrhythmics drugs including digitalis and/or digoxin, anticonvulsant drugs, glucocorticoids drugs, warfarin, magnesium, antacids with aluminium hydroxide, cholestyramine or colestipol, rifampicin, orlistat, bisphosphonate, and/or 25 hydroxy vitamin D.

Recruitment

Study Centers. This multicenter RCT will be conducted at the Hypertension Centers of Ancona, Milan, Naples (Federico II University, coordinator center), Padua, Perugia, Siena, Trieste, Turin, Udine, Varese, and Verona.

Patients. Each Centre will receive from the coordinator center a copy of the study protocol including the list of the inclusion and exclusion criteria above exposed and will enroll a similar number of male and female participants. Altogether, the HYPODD study population will be made of 240 participants. The majority of centers will recruit 16 patients, whereas the Naples, Perugia and Padua centers will recruit 40, 32 and 10 patients respectively. The patients attending the above mentioned Centers and who are willing to participate will receive information about the aims, content, privacy issues and risks related to participation in the study. After signing a written informed consent to participate, they will undergo a clinical examination. A letter will then be sent to their primary care physicians with a detailed description of the study. Each enrolled patient will be univocally identified with a sequential alphanumeric code, according to the enrollment order. This alphanumeric code is formed by three parts: the first part (two letters) identifies the recruitment center, the second part (three numbers) identifies the patient according to order of inclusion in the study; the third part (one letter and two numbers) identifies the clinical examination number.

Randomization. The ABIAGEN drug company has produced an active drug and an identical placebo with equal organoleptic properties and has proceeded to their packaging in boxes using an alphanumeric code including two letters and two progressive numbers identifying the study center and the patient, respectively. The company has also taken care of the randomization procedure allocating the patients to the active drug or the placebo arm by a computer generated schedule. The investigators are blinded to the patient allocation as the randomization list is preserved by the ABIAGEN Industries.

Intervention. At the start of the intervention, each patient will receive a box containing a drug or placebo supply sufficient for the entire study. Those in the intervention group will be instructed to take 50,000 UI of cholecalciferol every week for 8 weeks and subsequently 50,000 UI of the same substance every month for ten months, whereas patients in the control arm will take the placebo preparation at the same time points. Both drugs and placebo will be taken orally (4).

Biochemical and clinical evaluation. Before initiation of the treatment, the patients will undergo a clinical session including the collection of demographic data (age, sex, living environment, education, physical activity, migration status and/or cultural background), information on life style habits and clinical findings (personal and family history, anthropometric measurements, occurrence of selected symptoms or signs of clinical disorders, ongoing drug treatments) and BP measurements according to standardized procedure by SIIA criteria (18). The study protocol also includes the assessment of the biochemical serum, plasma and urinary parameters reported in table 1 and the collection of biological samples (whole blood, plasma, serum and urine) that will be stored in aliquots which will constitute the HYPODD biobank. The biological specimens will be unequivocally identified using a progressive alphanumeric code defining a) the recruiting center; b) the participant's code; c) the examination date and d) the type of biological material stored according to criteria suggested by the Italian Society of Human Genetics[SIGU, Società Italiana di Genetica Umana (19)]. In addition to the above, at T=0 the patients will undergo a 1) standard 12-lead electrocardiogram, recorded and analysed according to Meek and colleagues (20, 21) and by Edhouse and colleagues (22); 2) a two- and three-dimensional transthoracic echocardiogram with acquisition of parameters of left and right ventricular function and geometry, including tissue-doppler procedures,

according to the criteria of the American Society of Echocardiography(23); 3) the assessment of central hemodynamic parameters [central systolic blood pressure (SBPc), central pulse pressure (PPc), augmentation pressure (AP), augmentation index (Aix), augmentation index adjusted for heart rate (Aix75)] and arterial stiffness [carotid-femoral pulse wave velocity (PWV)]. These parameters will be assessed by a high-fidelity applanation tonometer and analysed by the SphygmoCor device (24), 4) a carotid Doppler Ultrasound Scan with evaluation of the arterial intimal medial thickness (25); and 5) a 24 hour ambulatory BP monitoring (ABPM) performed and analysed according to the ESH/ESC criteria (13). The instrumental tests previously listed in points 1, 2, 3, 4 and 5 will be recorded and then examined in a centralized way. After this initial clinical assessment, the participants will receive a) a schedule for BP home monitoring, b) a normocaloric (2100 Kcal), normal calcium (1000 mg/day), reduced sodium diet (1500 mg/day); c) appropriate suggestions to promote an increased cutaneous biosynthesis of 25-hydroxy vitamin D, according to the International Osteoporosis Foundation (IOF)(20).

Monitoring. A monthly clinical evaluation of the enrolled participants is planned (figure 1). At each clinic visit BP is measured and the patient's pharmacological treatment is checked and modified, if necessary, according to the flow-chart shown in figure 2. The antihypertensive treatment is reduced if, at two consecutive monthly clinical evaluations, SBP is lower than 130 mmHg and DBP lower than 85 mmHg. In patients taking a single drug, the drug dosage will be halved until the minimum dosage reported in table 2. If this minimum dosage is reached and a further dosage reduction is necessary, the physician stops the drug administration. In patients assuming two or more antihypertensive drugs, the physician first reduces the dosage of one of the two drugs, according to the subsequent stepwise schedule: 1st) Diuretic; 2nd) Beta-blocker; 3rd) Calcium antagonist; 4th) Renin-Angiotensin-Aldosterone (RAAS) inhibitor. Conversely, the pharmacological treatment is increased if a) SBP is higher than 160 mmHg and/or DBP is higher than 95 mmHg; b) SBP is higher than 140 mmHg and/or the DBP is higher than 90 mmHg and the mean of home diary BP measurements is higher than 130 mmHg for SBP and higher than 85 mmHg for DBP; c) at two consecutive monthly clinic visits, SBP is higher than 140 mmHg and/or DBP is higher than 90 mmHg. In patients taking a single drug, the drug dosage is increased until the maximum dosage reported in table 1

or, alternatively, the physician may add another antihypertensive drug according to the subsequent schedule: 1st choice RAAS inhibitor; 2nd) Calcium antagonist; 3rd) Beta-blocker; 4th) Diuretic. In patients taking combination therapy, the physician increases the dosage of one of the drugs being currently used to reach the maximum dosage reported in table 1 or, alternatively, another antihypertensive drug can be added according to the previously reported schedule.

At days 120, 240 and 360 (i.e. T=120, T=240 and T=360), biochemical serum and urinary parameters are measured and biological samples (whole blood, plasma, serum and urine) are collected and stored in aliquots in the HYPODD biobank. At the end of the study (T= 360), in addition to biochemical analyses, clinical examinations aimed at the assessment of target organ disease are performed as at the enrollment (T= 0). A flow chart of the study is given in Figure 1.

Study endpoints and outcome measures

The first primary endpoint of the study is the assessment of the effect of cholecalciferol supplementation on the consumption of antihypertensive drugs during the study needed to maintain optimal BP control, according to the ESH/ESC criteria (13). The consumption of antihypertensive drugs at the end of the study (T=360) minus the one at patients' enrollment (T=0) is the outcome measure for this endpoint.

A further primary endpoint is the evaluation of the effect of cholecalciferol supplementation on the progression of organ damage. This is a composite endpoint and its outcome measures are the echocardiographic -estimated left ventricular mass, the aPWV, the albuminuria levels and the atrial natriuretic peptides plasma levels.

Sample size calculation. The sample size of the HYPODD study was estimated based on an expected reduction in antihypertensive drug consumption of 20% or more in the vitamin D intervention group compared with the placebo group at T=360 (power: 80%; alpha error: 5%). In addition, the HYPODD study is expected to have a power of 80% (alpha error: 5%) to identify a statistically significant reduction of left ventricular mass equal to or greater than $7 \text{ g/m}^{2.7}$ and/or a reduction of PWV equal to or greater than 0.9 m/s, and/or a reduction of albuminuria levels equal to or greater than 45 mg/24h, and/or a reduction of

atrial natriuretic peptide plasma levels equal to or greater than 600 fmol/ml between the two study cohorts. A dropout rate of up to 15% was taken into account in the sample size calculation.

Statistics. The statistical analyses will be performed using the Statistical Package for the Social Sciences (SPSS) version 12. The Student's T test, with Bonferroni correction when required, and contingency χ^2 -table will be used for univariate analysis of interval and nominal variables, respectively. Linear regression analysis and Pearson's correlation will be performed to detect possible associations between the variables under study. Non-parametric tests will be used for nominal variables. Logistic regression models will be generated, based on the results of univariate analyses, to assess the relative impact of interrelated factors on the outcome variables. The genetic data obtained from the ancillary study will be analyzed using the FINETTI program at <http://ihg.ghs.de/cgi-bin/hw/hwa1.pl> and the Estimated Haplotype Linkage Program version 5.1.

Ethics and legal aspects

HYPODD is a no-profit study. The study costs are partially covered by a SIIA research grant (to LDE). The ABIOTEN Industries support the study by providing the drug and placebo preparations at no cost; they are also in charge of the participants' random allocation to one or the other treatment arm and the only ones aware of the participants allocation. According to the Declaration of Helsinki (27) and to the criteria of the Convention of Oviedo (28), each patient enrolled for this study is required to sign an informed consent for collection and storage of clinical data and biological materials and for their use for diagnostic and research purposes, without any financial interest. The signed informed consent includes the explicit possibility of withdrawing the given consent at any time. All biological samples are identified using an unambiguous and progressive alphanumeric code and stored according to the SIGU criteria (19). The criteria for identification, storage and eventual destruction of the biological samples are clearly declared on the informed consent model. The dosage at which cholecalciferol is administered during the study is the one suggested by Holick for the correction of vitamin D deficiency and supported by the Endocrine Society (4, 29). The antihypertensive treatment protocol conforms to the ESH/ESC guidelines for the clinical management of hypertension (13). Based on the above considerations, participation to the study is at no

added risk for the participants as compared to that inherent to the currently recommended treatment of high BP and vitamin D deficiency.

Study strengths and limitations.

Major strengths of the study are its multicenter randomized controlled trial design and the double blindness of both the participants and the trial investigators to the patients' treatment allocation.

During the entire study, BP is monitored and maintained within the limits recommended by the ESH/ESC guidelines for hypertension management (13) in both study cohorts (active drug and placebo). The effects of BP optimal control on the biochemical and clinical indices of organ damage are thus equally distributed in both study cohorts. Thus, any difference in the antihypertensive drug consumption and/or in the clinical expression of organ damage between the two study cohorts will be reasonably attributable to the correction of vitamin D deficiency in the active drug study cohort.

The major study limitation is the absence of "hard" cardiovascular endpoints (i.e. cardiovascular morbidity and mortality) due to the lack of the necessary budget.

Perspectives

Depending on HYPODD study results, substantial innovations could be proposed for the diagnostic process and the treatment protocol of high BP. The study could help clarify important aspects of the extraskeletal biological activities of the vitamin D system, providing novel evidence in this respect that could be the basis for the design and the implementation of larger trials with harder endpoints. The ancillary genetic study aiming to the assessment of the possible influence of VDR allelic variants on the main study outcomes may provide further important information on the pathophysiological bases of the cardiovascular and metabolic effects of the vitamin D system. In general, the biological bank created in connection with HYPODD implementation may inspire further studies in this area.

Trial status.

The HYPODD study has been registered at the Agenzia Italiana del Farmaco – Osservatorio sulla Sperimentazione Clinica del Farmaco (AIFA- OsSC) and EUDRACT sites (n° 2012-003514-14) and has been approved by the Carlo Romano Ethical Committee of the Federico II University (prot. 41/12). The study

protocol has been submitted to all the Ethical Committees of the participating Centers and received their approval. The patients recruitment is currently underway at all Centers.

References

1. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol*. 2005;289(1):F8-28.
2. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *Am J Clin Nutr*. 2008;88:491Se9S
3. Rendina D, De Filippo G, Strazzullo P. Should vitamin D status be assessed in patients with congestive heart failure? *Nutr Metab Cardiovasc Dis*. 2010;20(9):627-32.
4. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266-81.
5. Rendina D, De Filippo G, Muscariello R, et al. Vitamin D and cardiometabolic disorders. *High Blood Pres Cardiovasc Prev*. 2014;21(4):251-6.
6. Pilz S, Tomaschitz A, Ritz E, et al. Vitamin D status and arterial hypertension: a systematic review. *Nat Rev Cardiol*. 2009;6(10):621-30.
7. Parker J, Hashmi O, Dutton D, et al. Levels of vitamin D and cardiometabolic disorders: systematic review and meta-analysis. *Maturitas*. 2010;65(3):225-36.
8. Brandenburg VM, Vervloet MG, Marx N. The role of vitamin D in cardiovascular disease: from present evidence to future perspectives. *Atherosclerosis*. 2012;225(2):253-63.
9. Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*. 2006;81:353-73
10. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911-30.
11. Rosen CJ, Abrams SA, Aloia JF, et al. IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab*. 2012;97(4):1146-52.
12. Kupferschmidt K. Uncertain verdict as vitamin D goes on trial. *Science*. 2012;337(6101):1476-8.
13. Mancia G, De Backer G, Dominiczak A, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European

- Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2007;25(6):1105-87
14. Bilezikian JP, Silverberg SJ. Clinical practice. Asymptomatic primary hyperparathyroidism. *N Engl J Med*. 2004;350:1746–51.
 15. Smulders MY, Frissen PHJ, Slaats EH, et al. Renal tubular acidosis. Pathophysiology and diagnosis. *Arch Intern Med*. 1996;156:1629-36.
 16. Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. *Endocr Rev*. 2008;29:76-131
 17. <http://www.medicochirurgica.it/documenti/Linee%20guida%20SINPE%20per%20la%20Nutrizione%20Artificiale%20Ospedaliera%202002.pdf>
 18. <http://siia.it/wp-content/uploads/2011/06/linee-guida-sulla-misurazione-convenzionale-e-auto-243.pdf>
 19. http://www.sigu.net/index.php?option=com_docman&task=cat_view&gid=46&Itemid=74
 20. Meek S, Morris F. ABC of clinical electrocardiography. Introduction. I-Leads, rate, rhythm, and cardiac axis. *BMJ*. 2002;324(7334):415-8.
 21. Meek S, Morris F. Introduction. II--basic terminology. *BMJ*. 2002;324(7335):470-3.
 22. Edhouse J, Thakur RK, Khalil JM. ABC of clinical electrocardiography. Conditions affecting the left side of the heart. *BMJ*. 2002;324(7348):1264-7.
 23. Ehler D, Carney DK, Dempsey AL, et al. Guidelines for cardiac sonographer education: recommendations of the American Society of Echocardiography Sonographer Training and Education Committee. *J Am Soc Echocardiogr*. 2001;14(1):77-84
 24. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. 2006;27(21):2588-605.
 25. Grant EG, Benson CB, Moneta GL, et al. Carotid artery stenosis: gray-scale and Doppler US diagnosis--Society of Radiologists in Ultrasound Consensus Conference. *Radiology*. 2003;229(2):340-6.

26. <http://www.iofbonehealth.org/>
27. <http://www.wma.net/en/30publications/10policies/b3/>
28. <http://conventions.coe.int/Treaty/en/Treaties/Html/164.htm>
29. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96(7):1911-30.

Figure Legends

Figure 1.

HYPODD study. flow chart. T: time expressed in days. BP: blood pressure. ECG: electrocardiogram. US: ultrasonography. ABPM: 24h-ambulatory blood pressure measurement.

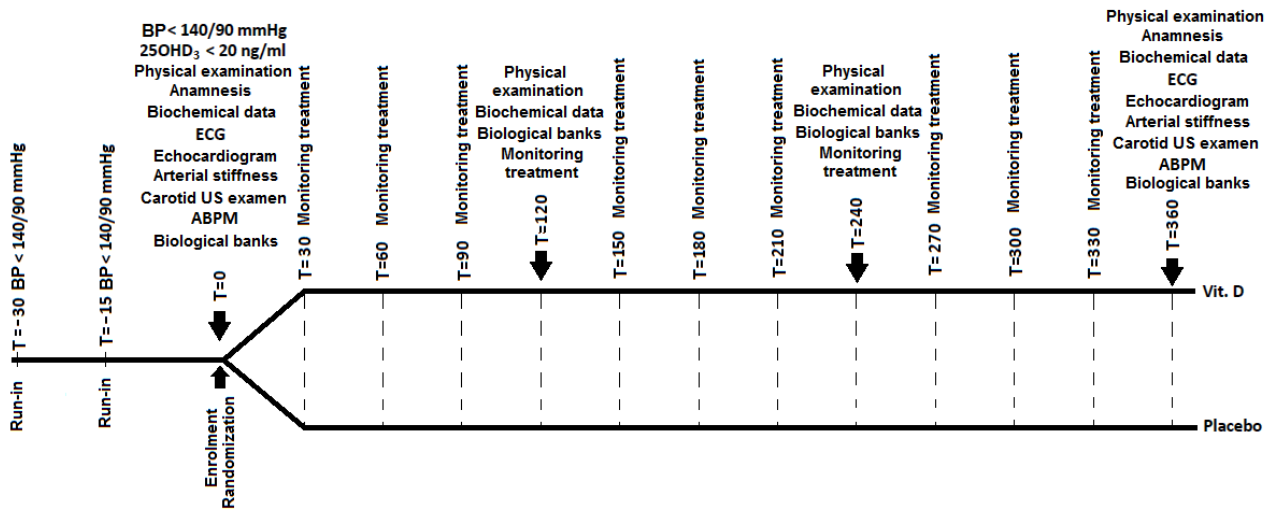


Figure 2.

HYPODD study: decision-making. ABP: ambulatory blood pressure measurement. HBP: home blood pressure measurement. A: In patients taking a single drug, the drug dosage will be halved until the minimum dosage reported in table 1. If this minimum dosage is reached and an ulterior dosage reduction is necessary, the physician will interrupt the drug administration. In patients assuming two or more antihypertensive drugs, the reduction of anti-hypertensive drugs should occur according to this diagram: 1st) Diuretics; 2nd) Beta-blocker; 3rd) Calcium antagonist; 4th) Renin-Angiotensin-Aldosterone (RAAS) inhibitor. B: In patients taking a single drug, the drug dosage will be increased until the maximum dosage reported in table 1 or, in alternative, the physician could added another antihypertensive drug according to this diagram: 1st) RAAS inhibitor; 2nd) Calcium antagonist; 3rd) Beta-blocker; 4th) Diuretic

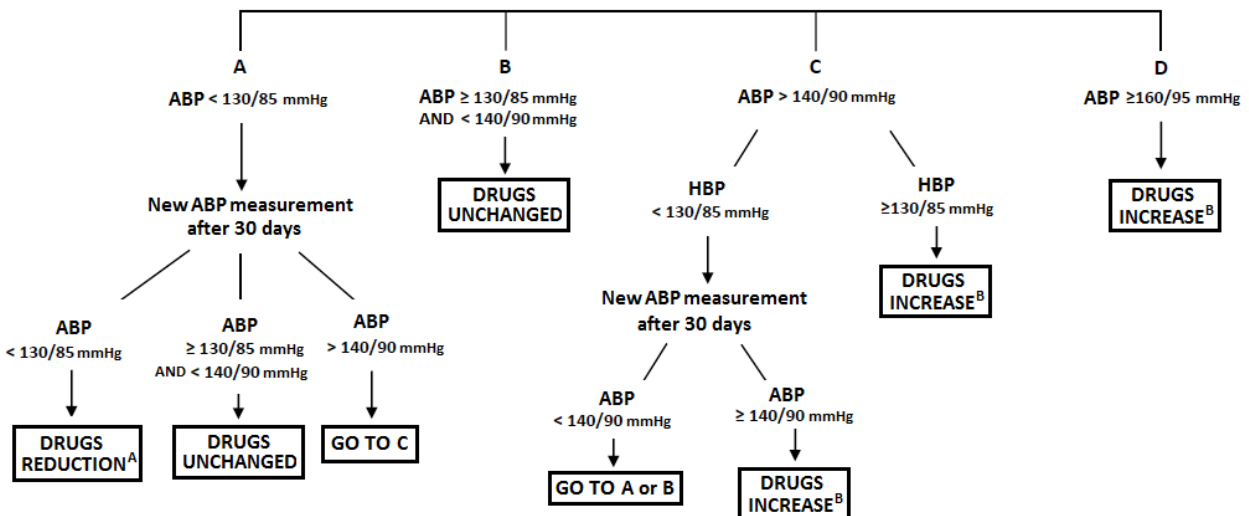


Table 1

Biochemical parameters assessed in the HYPODD study cohorts

Serum and plasma parameters		Complete Blood Count
Glucose	Sodium	Urinary parameters
Insulin	Potassium	pH
Total Cholesterol	C-reactive Protein	Cortisol*
HDL- Cholesterol	TSH*	Proteinuria*
LDL- Cholesterol	25 hydroxy vitamin D*	Albuminuria*
Triglycerides	PTH*	Total Calcium*
Creatinine	Renin*	Magnesium*
Total Calcium	Aldosterone*	Phosphate*
Albumin	Pro atrial natriuretic peptides*	Citrate*
Magnesium		Sodium*
Phosphate		Potassium*
Chloride		Urate*

HDL High Density Lipoprotein; LDL Low Density Lipoprotein. *parameters measured on biological specimens stored at the HYPODD biobank.