

# Increased mean aliphatic lipid chain length in left ventricular hypertrophy secondary to arterial hypertension

## A cross-sectional study

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### Abstract

About 77.9 million (1 in 4) American adults have high blood pressure. High blood pressure is the primary cause of left ventricular hypertrophy (LVH), which represents a strong predictor of future heart failure and cardiovascular mortality. Previous studies have shown an altered metabolic profile in hypertensive patients with LVH. The goal of this study was to identify blood metabolomic LVH biomarkers by <sup>1</sup>H NMR to provide novel diagnostic tools for rapid LVH detection in populations of hypertensive individuals. This cross-sectional study included 48 hypertensive patients with LVH matched with 48 hypertensive patients with normal LV size, and 24 healthy controls. Two-dimensional targeted M-mode echocardiography was performed to measure left ventricular mass index. Partial least squares discriminant analysis was used for the multivariate analysis of the <sup>1</sup>H NMR spectral data. From the <sup>1</sup>H NMR-based metabolomic profiling, signals coming from methylene (–CH<sub>2</sub>–) and methyl (–CH<sub>3</sub>) moieties of aliphatic chains from plasma lipids were identified as discriminant variables. The –CH<sub>2</sub>–/–CH<sub>3</sub> ratio, an indicator of the mean length of the aliphatic lipid chains, was significantly higher ( $P < 0.001$ ) in the LVH group than in the hypertensive group without LVH and controls. Receiver operating characteristic curve showed that a cutoff of 2.34 provided a 52.08% sensitivity and 85.42% specificity for discriminating LVH (AUC = 0.703,  $P$ -value < 0.001). We propose the –CH<sub>2</sub>–/–CH<sub>3</sub> ratio from plasma aliphatic lipid chains as a biomarker for the diagnosis of left ventricular remodeling in hypertension.

**Abbreviations:** <sup>1</sup>H NMR = proton nuclear magnetic resonance, AUC = area under the curve, BMI = body mass index, –CH<sub>2</sub>– = methylene, –CH<sub>3</sub> = methyl, DBP = diastolic blood pressure, E/A = early/late ventricular filling velocities ratio, E/Ea = early diastolic transmitral velocity to early mitral annular diastolic velocity ratio, LA = left atrium, LV = left ventricular, LVEF = left ventricular ejection fraction, LVH = left ventricular hypertrophy, LVIDd = left ventricular internal diameter at end-diastole, LVM = left ventricular mass, LVMI = left ventricular mass index, PET = positron electron tomography, PLS-DA = partial least square discriminant analysis, PWTd = left ventricular posterior wall thickness at end-diastole, ROC = receiver operating characteristic, SBP = systolic blood pressure, SWTd = septum wall thickness at end-diastole.

**Keywords:** biomarker, hypertension, left ventricular hypertrophy, left ventricular remodeling, methylene/methyl ratio

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## 1. Introduction

Hypertension is the principal etiology of pathologic left ventricular hypertrophy (LVH).<sup>[1]</sup> Pressure-dependent hemodynamic overload induces left ventricular remodeling as an adaptive response to minimize wall stress. Over time, LVH becomes maladaptive and emerges as a strong and independent risk factor for cardiovascular morbidity (e.g., heart failure, coronary artery disease, cerebrovascular accidents, ventricular arrhythmia) and mortality.<sup>[2–6]</sup> Indeed, studies indicate that in 20%<sup>[7]</sup> to 36%<sup>[8]</sup> of chronic hypertensive patients, the myocardium undergoes profound structural remodeling, characterized by enhanced cardiomyocyte growth, increased rate of cardiomyocyte apoptosis, accumulation of fibrosis, and microcirculatory changes. This structural remodeling disrupts myocardial excitation–contraction coupling and eventually leads to heart failure.<sup>[9,10]</sup> The increase in the cardiomyocyte stretching is the main factor that induces the cardiac hypertrophic growth. However, a host of nonhemodynamic factors (neuroendocrine stimulation, renin angiotensin aldosterone system, endothelin-1) substantially contribute to modulating the hypertrophic response.<sup>[11]</sup> Notable relevance in the regulation of LVH has been found in the defective vasodilatation due to desensitization of  $\beta$  adrenergic receptors by an increase of G-protein coupled receptor kinases.<sup>[12–15]</sup>

During hypertensive LVH, metabolic changes have been observed in the cardiac muscle, which switches its preference from fatty acids to glucose for ATP generation. Rodent models of spontaneous hypertension,<sup>[16]</sup> and humans with essential hypertension,<sup>[17,18]</sup> display a decrease in myocardial fatty acid uptake and an increased reliance on glucose and lactate for energy provision. In the acute setting, this shift is considered beneficial to the contractile function because enhanced glucose metabolism allows the heart to cope with the increased workload by producing more ATP per molecule of oxygen consumed.<sup>[19]</sup> However, chronic hypertension induces the reactivation of the fetal gene program and renders persistent metabolic remodeling.<sup>[20]</sup> Lastly, in advanced pathological hypertrophy, myocardial glucose metabolism is also decreased due to the development of insulin resistance, leaving the heart unable to generate sufficient ATP to sustain the high workload.<sup>[21]</sup>

Because LVH commonly precedes cardiac failure in patients with hypertension, the early detection of left ventricular (LV) remodeling could potentially optimize health care for hypertensive patients at risk of developing heart failure. Echocardiography and electrocardiography are presently the most readily available tools for identifying LVH, though they may prove to be time consuming for physicians and costly for patients. Several studies have proposed the use of positron electron tomography (PET) scans to detect the early metabolic alterations in myocardial tissue during LVH.<sup>[22,23]</sup> However, PET scanning cannot be used for routine LVH detection because of high running costs, the fact that it exposes the patient to ionizing radiation and to radioactive substances, and because the equipment is only available in a minority of medical centers. Circulating biomarkers have also been investigated in hypertensive heart disease (i.e., LVH secondary to hypertension): the quantification of cardiotrophin-1,<sup>[24]</sup> annexin A5,<sup>[25]</sup> and propeptide of procollagen type I<sup>[26]</sup> has been proposed for screening of the disease, but none of these biomarkers are currently used in clinical practice due to their low sensitivity or lack of specificity.

We aimed to identify biomarkers of LV remodeling in the plasma of hypertensive patients using untargeted metabolomics.

Metabolomics, a high-throughput technology widely used in clinical and epidemiological research, has successfully been employed to screen for cardiovascular biomarkers.<sup>[27]</sup> Myocardial metabolism is the first responder to changes in cardiac homeostasis and therefore it may identify metabolites (small molecules of atomic mass <1.5 kDa) as biomarkers of cellular stress, even before structural or functional changes can be observed by clinical imaging techniques. For example, pattern-recognition techniques applied to proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra of human serum correctly diagnose the presence and severity of coronary artery disease.<sup>[28]</sup>

In this study, our purpose was to identify metabolomic biomarkers in the plasma of hypertensive patients that could indicate the presence of LV remodeling.

## 2. Methods

### 2.1. Subjects

The study population was enrolled in the cardiology department of Rangueil Hospital, Toulouse, France to constitute the Identification Of Blood Markers For Asymptomatic Ventricular Dysfunction (IBLOMAVED) cohort. During the period 2007 to 2010, among 600 screened patients, 221 presented with hypertension. According to the inclusion and exclusion criteria of the study, and in order to match the cardiovascular risk factors in the 2 hypertensive groups, the final number of patients included was 120. Patients were sorted into 3 groups for the present analysis: 24 healthy controls, 48 patients with hypertension and normal LV size, and 48 patients with hypertension and LV hypertrophy. The 2 comparative groups of hypertensive patients were matched for age, obesity, body mass index (BMI), diabetes, and dyslipidemia. Inclusion criteria were age over 18 years, signed informed consent, fasting blood sampling, and presence of appropriate echocardiographic data. Exclusion criteria included presence of acute or chronic heart failure, any form of cardiomyopathy (e.g., familial obstructive hypertrophic cardiomyopathy, dilated cardiomyopathy, or toxic cardiomyopathy), myocardial necrosis, heart rhythm problems, active smoking, or smoking cessation in the past 3 years, hemoglobinopathies, septicemia, hepatitis, or kidney failure requiring dialysis. All subjects underwent a complete cardiac evaluation, including medical history, physical examination, blood pressure measurement, and echocardiography. This research protocol was registered in a clinical database (ClinicalTrials.gov NCT01024049) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The protocol was approved by the institution's human research (COSSEC) and regional ethics committee (Comité de Protection des Personnes (CPP) # DC2008-452). Written informed consent was obtained from all participants.

### 2.2. Blood pressure measurement

All hypertensive subjects underwent a 24-hour ambulatory blood pressure measurement recorded with validated ambulatory recorders (TM 2420 and TM 2421, A & D Engineering, CA, San Jose).<sup>[29]</sup> A trained nurse fitted a cuff on the left arm of the patients and monitors were programmed to record blood pressure every 30 minutes. Patients completed a diary card and pressed the event marker on the monitor to identify sleep-wake cycle. Patients with a 24-hour average blood pressure  $\geq 140/85$  mm Hg were defined as hypertensive.

### 2.3. Echocardiography

All subjects underwent complete M-mode, 2-dimensional, and Doppler echocardiography to determine left ventricular ejection fraction (LVEF), left ventricular mass (LVM), and valve function. The LVEF was calculated according to the modified Simpson rule. LV dimensions were acquired in systole and diastole by measurement of the septal wall thickness (SWT), posterior wall thickness (PWT), and LV internal diameter (LVID) from the parasternal long-axis view. We calculated LV mass as  $0.8 \times \{1.04 [(LVIDd + PWTd + SWTd)^3 - (LVIDd)^3] + 0.6\text{g}\}$ .<sup>[30]</sup> LV mass was indexed to the body surface area calculated as  $0.0235 \times \text{height (cm)}^{0.42246} \times \text{weight (kg)}^{0.51456}$  to derive the LV mass index (LVMI,  $\text{g/m}^2$ ).<sup>[31]</sup> LVH was defined according to the previously established criterion (LVMI > 125  $\text{g/m}^2$  for men and LVMI > 110  $\text{g/m}^2$  for women).<sup>[32]</sup> Anteroposterior left atrium (LA) diameter was obtained using a parasternal long-axis view and LA area using a 4-chamber apical window in a 2-dimensional echocardiography.

### 2.4. Blood sampling

Venous blood samples were collected after overnight fasting in Becton Dickinson Vacutainer CPT tubes with sodium heparin. Plasma was immediately separated by centrifugation (3000 RCF/4°C/10 min) and aliquots were stored at  $-80^\circ\text{C}$ . Total plasma cholesterol was measured by the CHOD-PAP method with kit A11A01634 (HORIBA ABX Diagnostic, Montpellier, France).

### 2.5. NMR analysis

NMR analysis was performed as previously reported.<sup>[33]</sup> Briefly, 1 plasma aliquot was used for diluted plasma  $^1\text{H}$  NMR analysis (250  $\mu\text{L}$  was diluted into 755  $\mu\text{L}$  final mixture of 500  $\mu\text{L}$  0.9% saline in  $\text{D}_2\text{O}$  and 5  $\mu\text{L}$  100 mM sodium 3-(trimethylsilyl) propionate-2,2,3,3-d<sub>4</sub> (TSP)) immediately before the NMR spectra acquisition. A second plasma aliquot was submitted to an extraction process to isolate the hydrophilic and lipophilic plasma metabolites into 2 separate fractions that were analyzed serially on the following day. Simultaneous extraction of lipophilic and polar metabolites was performed with ice-cold methanol, chloroform, and water (2:2:1.3, v/v/v). The aqueous fraction of the extract was reconstituted in 600  $\mu\text{L}$  of  $\text{D}_2\text{O}$  phosphate-buffered solution with 10  $\mu\text{L}$  of a 10 mM 3-(trimethylsilyl)-1-propanesulfonate sodium salt (TMPS) before NMR analysis. The organic fraction of the extract was reconstituted in 1 mL  $\text{CDCl}_3$  with 10  $\mu\text{L}$  TCB (100 mM) and maintained under nitrogen atmosphere at  $-80^\circ\text{C}$  until NMR analysis.  $^1\text{H}$  NMR spectra were recorded at 300K on a Bruker Avance DRX 600 spectrometer operating at 600.13 MHz and equipped with a 5 mm triple axis inverse (TXI) gradient cryoprobe. Four plasma spectra were acquired for each patient: spectrum of diluted plasma sample acquired with presaturation of the water signal and using 1-pulse sequence, namely the Zg-spectrum; spectrum of diluted plasma acquired with presaturation of the water and carr-purcell-meiboom-gill spin-echo sequence, namely the cpmg-spectrum; spectrum of aqueous fraction of the plasma extract, namely the Aq-spectrum; spectrum of the organic fraction of the plasma extract, namely the Org-spectrum. The Zg-spectrum and CPMG spin-echo sequence (cpmg-spectrum) had an echo loop time (2np) of 320 milliseconds. A total of 64 transients were sampled with a spectral width of 12 ppm, 32K data point on time domain (2.3 seconds acquisition time) and 2.5 seconds additional relaxation delay. Spectra of aqueous and organic fractions were serially acquired using an automatic sampling changer (B-ACS

60). Spectra of aqueous fractions (Aq. spectrum) were obtained with similar parameters to the one-pulse spectrum of the diluted plasma whereas spectra of the organic fraction (Org. spectrum) were acquired with an additional delay of 4 seconds and without solvent suppression.  $^1\text{H}$  NMR spectra were processed using the TOPSPIN (version 2.1, Bruker BioSpin SA, France, Wissembourg) and AMIX (Bruker Analytik, Rheinstetten, Germany) software packages. Typical processing parameters were 65K zero-filling and an exponential apodizing function (0.3 Hz) applied before Fourier transformation. Phase and base-line corrections of spectra were performed by operator and referenced with AMIX software to methyl resonance of TMPS, lactate or TCB for diluted plasma, aqueous fraction, and organic fraction, respectively.  $^1\text{H}$  NMR spectra were processed using the TOPSPIN (version 2.1, Bruker BioSpin SA) and AMIX (Bruker Analytik) software packages. To perform data reduction and pattern recognition, each of the 4 NMR spectra obtained per patient were bucketed to obtain spectral data sets using the AMIX (Bruker Analytik) software package. The generated variables were identified with the central chemical shift value of the bins as suffix and Zg, cpmg, Aq, or Org as prefixes. This raw data matrix was exported into the SIMCA-P+ (version 12.0, Umetrics, Umea, Sweden) software to be separately orthogonalized with an orthogonal signal correction (OSC) filtering function prior fused in a normalized matrix of 672 rows (X-block) and 126 lines. To maximize separation between the groups, partial least squares discriminant analysis (PLS-DA) was performed by using LV mass index as Y (Y-block). The statistical results obtained by PLS-DA methods are able to detect which variables in the X-block are relevant to determine the dependent variables (Y-block) by means of the variable influence on projection parameter (IP) values. The IP values reflect, in fact, the importance of terms in the model both with respect to Y, that is, its correlation with all the responses, and with respect to X.

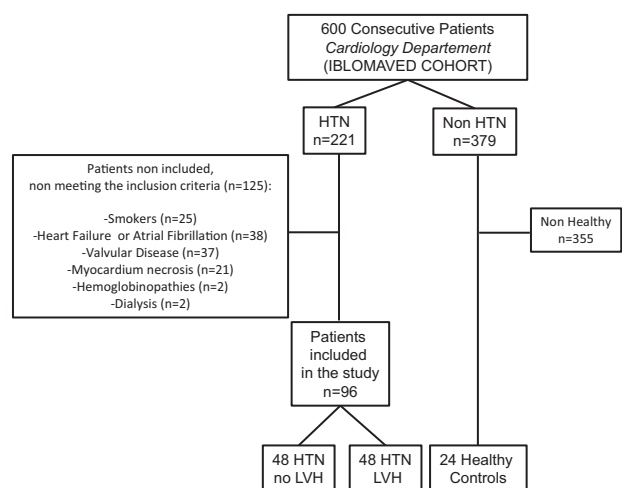
### 2.6. Statistical analysis

Continuous variables are presented as mean  $\pm$  SD and categorical as numbers and percentages. Continuous variables were compared with the use of 1-way ANOVA followed by Tukey multiple comparison test when  $P$ -value was  $<0.05$ , or Kruskal–Wallis rank sum test followed by Dunn multiple comparisons test (for a  $P$ -value  $<0.05$ ), when normality or an equal variance test failed. Categorical variables were compared with the use of the Pearson Chi-square (sigma stat). Statistical analyses were performed using Graph Pad Prism software 6.0 (www.graphpad.com). Receiver operating characteristic (ROC) curve analysis was performed using MedCalc 15.8 software (www.medcalc.be).

## 3. Results

### 3.1. Study population

The flowchart for patient inclusion is shown in Fig. 1. Patient's characteristics are shown in Table 1. Hypertensive patients were older, had a higher BMI, and were more likely to have diabetes and dyslipidemia. The sex distribution, renal function, and LV ejection fractions were similar across all cohorts. Control individuals had lower rates of diabetes, dyslipidemia, or obesity than the hypertensive cohort. However, within the hypertensive cohort the rates of these pathologies were similar. The hypertensive population had a systolic blood pressure (SBP) of  $149 \pm 20$  mm Hg versus  $125 \pm 13$  mm Hg for the control group.



**Figure 1.** Flow diagram of the study. The Identification Of Blood Markers For Asymptomatic Ventricular Dysfunction (IBLOMAVED) cohort was used for this study. HTN=arterial hypertension, LVH=left ventricular hypertrophy.

The hypertensive population with LVH received a higher total number of antihypertensive treatments in comparison to hypertensive patients with normal LV size, and, although not statistically significant, had 1 mm Hg of SBP and 4 mm Hg of diastolic blood pressure (DBP) lower than hypertensive patients

with normal LV size. Patients with LV hypertrophy had greater LV mass and a LV thickness/radius ratio (h/r) of  $52 \pm 8$ , both significantly greater ( $P < 0.001$ ) versus hypertensive patients without LV remodeling and compared to healthy controls. Lastly, hypertensive patients with and without LVH had higher deceleration time, early diastolic transmitral velocity to early mitral annular diastolic velocity ratio (E/Ea ratio), and left atrial area, but lower early/late ventricular filling velocities ratio (E/A ratio) compared to control. E/A ratio and LA area were not different between the 2 hypertensive groups, while LA diameter index tended to increase in the LVH group ( $P = 0.0567$ ).

### 3.2. $^1\text{H}$ NMR metabolomic profiling

Data from the  $^1\text{H}$  NMR spectra of plasma were analyzed using partial least squares discriminant analysis (PLS-DA). A PLS-DA score plot of spectral data revealed sample clustering according to the clinical status (Fig. 2A). In the loading plot (Fig. 2B), discriminant variables clearly separate the hypertensive patients with LVH, hypertensive patients with normal LV size, and healthy subjects into unique cohorts. The 10 most discriminating variables in the PLS-DA corresponded to specific signals (framed in red in Fig. 2B) from plasma lipids. Comparing the distribution of these variables with the distribution of individuals, we found that variables called orgOSC: 1.2 and 1.39 had a greater mean value in the group of hypertensive patients with LVH, while orgOSC: 0.91 and 0.97 were higher in the group of hypertensive patients with normal LV size and control individuals. Signals

**Table 1**

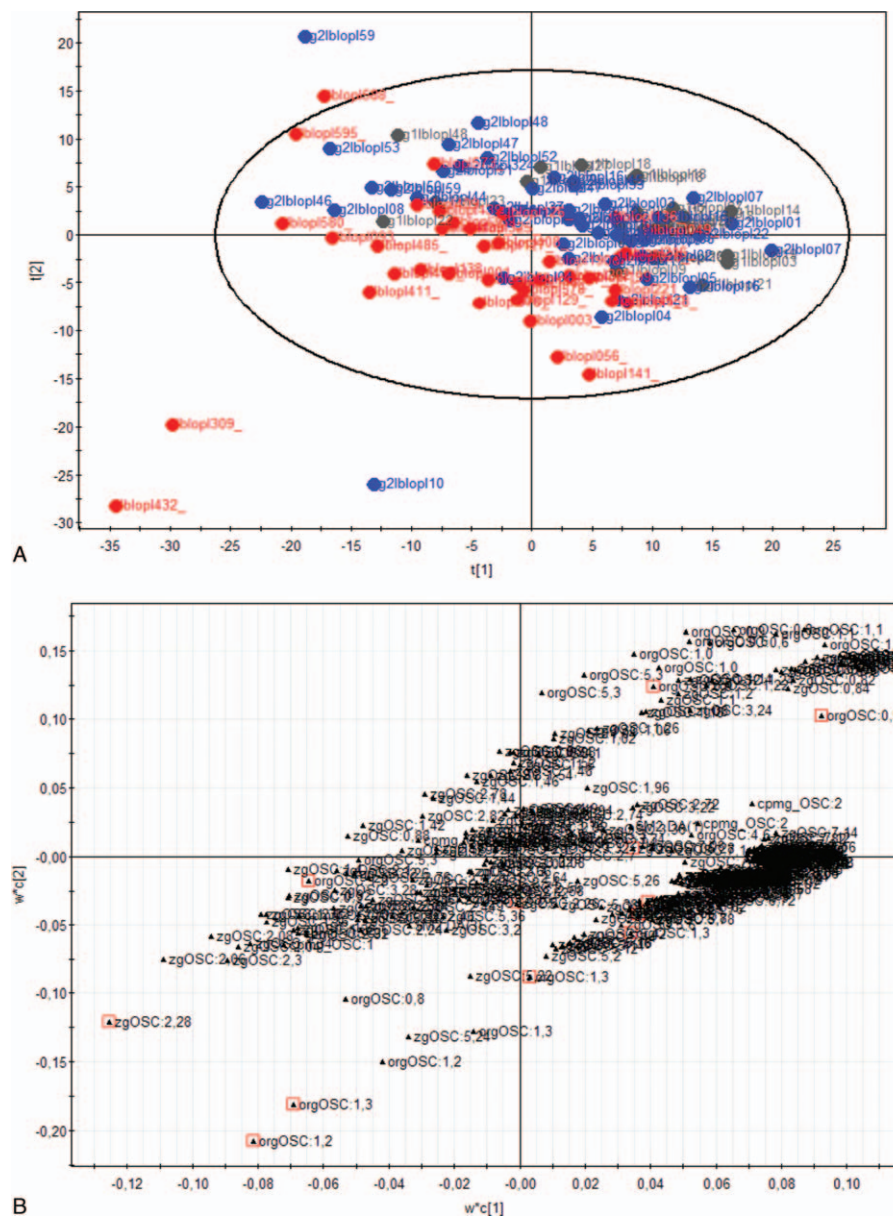
**Clinical characteristics, echocardiographic parameters, and hemodynamic levels.**

Parameter	Group 1, control (n=24)	Group 2, HTN normal LV size (n=48)	Group 3, HTN LVH (n=48)	$P_1$ Group 1 vs 2	$P_2$ Group 1 vs 3	$P_3$ Group 2 vs 3
Age, y	53 ± 10	62 ± 10	65 ± 10	0.0014	<0.0001	0.3092
Female, % (n)	42 (10)	50 (24)	22 (11)	0.9999	0.3689	0.0191
BMI, kg/m <sup>2</sup>	24 ± 3	28 ± 5	28 ± 4	0.0008	0.0008	0.9999
SBP, mmHg	125 ± 13	149 ± 21	148 ± 20	<0.0001	<0.0001	0.9650
DBP, mm Hg	79 ± 9	88 ± 12	84 ± 12	0.0061	0.1936	0.2065
Diabetes, n	0	10 (21%)	14 (29%)	0.0873	0.0095	0.5497
Dyslipidemia, n	0	28 (58%)	24 (50%)	<0.0001	<0.0001	0.6365
Obesity, n	0	13 (27%)	14 (29%)	0.024	0.0136	0.966
Cockcroft, mL/min	77 ± 8	78 ± 9	78 ± 7	0.8729	0.8729	>0.9999
LV ejection fraction, %	69 ± 7	69 ± 7	68 ± 9	>0.9999	0.8673	0.8079
LV mass indexed, g/m <sup>2</sup>	74 ± 18	81 ± 18	115 ± 33	0.5072	<0.0001	<0.0001
h/r	36 ± 5	37 ± 4	52 ± 8	0.7887	<0.0001	<0.0001
E/A ratio	1.3 ± 0.4	0.9 ± 0.2	0.8 ± 0.3	<0.0001	<0.0001	0.2119
Deceleration time, ms	180 ± 39	222 ± 53	246 ± 77	0.0203	0.0001	0.1418
E/Ea ratio	7.1 ± 3	8.2 ± 2	8.8 ± 2	0.1239	0.008	0.3887
LA area, cm <sup>2</sup>	16.2 ± 3	19.2 ± 4	19.3 ± 6	0.0346	0.0278	0.9942
LA diameter/BSA, mm/m <sup>2</sup>	17.2 ± 1.2	18.3 ± 2.5	19.5 ± 3	0.1955	0.0012	0.0567
Antihypertensive medication, % (n)						
ACE I	0 (0)	27 (13)	42 (20)	0.0032	<0.0001	0.197
ARBs	0 (0)	31 (15)	48 (23)	0.0014	<0.0001	0.1436
Diuretics	0 (0)	40 (19)	50 (24)	0.001	<0.0001	0.4118
BBs	0 (0)	40 (19)	50 (24)	0.001	<0.0001	0.4118
AAs	0 (0)	4 (2)	0 (0)	0.5493	>0.999	0.4974
CCBs	0 (0)	40 (19)	46 (22)	0.0001	<0.0001	0.681
CAAs	0 (0)	10 (5)	17 (8)	0.1619	0.0457	0.5523
Total antihypertensive medications	0 (0)	92	121	<0.0001	<0.0001	0.0202

Average ± SD.

AAs=aldosterone antagonists, ACE I=angiotensin-converting-enzyme inhibitors, ARBs=angiotensin II receptor blockers, BBs=beta-blockers, BMI=body mass index, BSA=body surface area, CAAs=centrally acting antihypertensive drugs, CCBs=calcium channel blockers, DBP=diastolic blood pressure, E/A=early/late ventricular filling velocities ratio, E/Ea=early diastolic transmitral velocity to early mitral annular diastolic velocity ratio, h/r=thickness/radius ratio, HTN=arterial hypertension, LA=left atrium, LV=left ventricular, LVH=left ventricular hypertrophy, SBP=systolic blood pressure.





**Figure 2.** PLS-DA analysis of  $^1H$  NMR spectral data. (A) PLS-DA  $t_1/t_2$  score plot derived from  $^1H$  NMR metabolic profiling. Gray represents control, blue hypertensive patients without LV hypertrophy (LVH), and red hypertensive with LV hypertrophy (alphanumeric codes represent individuals). The ellipse defines the Hotelling  $t^2$  confidence region (95%). (B) PLS-DA weight plot ( $w^*c_1$  vs  $w^*c_2$ ). Metabolites are indicated with an alphanumeric code. The 10 most discriminating metabolites were framed in red and correspond to specific signals from plasma lipids. Metabolites in the lower left contribute considerably to the definition of the group of hypertensive patients with LVH, while those in the upper right contribute to define the group of hypertensive patients with normal LV size or healthy control.

coming from these variables were identified based on their chemical shift and corresponded to protons located in the methylene ( $-CH_2-$ ) group (1.2–1.39) and in the methyl ( $-CH_3$ ) group (0.91–0.97) of aliphatic chains from plasma lipids in the organic fraction of plasma. In more detail, signals in the organic fraction came from a peak of the  $^1H$  NMR spectra at 1.3 ppm (part per million) for the hypertensive patients with LVH, while the signals for hypertensive patients with no LVH and healthy controls came from a peak at 0.9 ppm.

Chemical groups derived from the aliphatic chains of plasma lipids are shown in Fig. 3. The methylene/methyl ratio was found to be significantly higher in hypertensive patients with LVH when compared to hypertensive patients with normal LV size or to control individuals (Fig. 4). The methylene/methyl ratio was

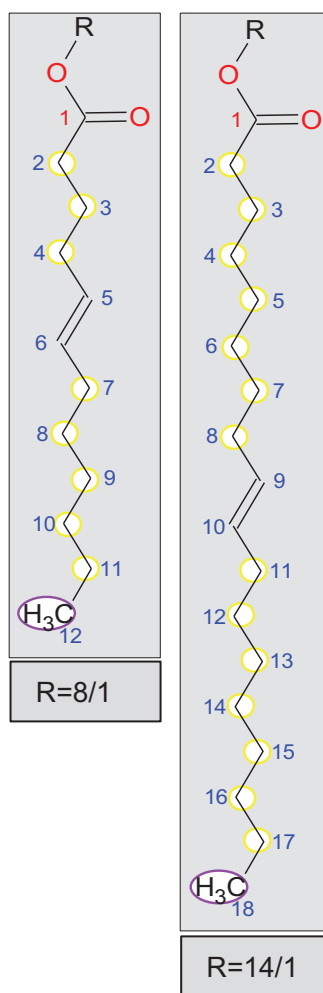
similar between hypertensive patients with normal LV size and control individuals.

### 3.3. Cholesterol level

There was no difference between the 3 groups for cholesterol plasma levels (Fig. 5).

### 3.4. Methylene/methyl ratio as predictor of left ventricular hypertrophy

The diagnostic performance of the methylene/methyl ratio with respect to LV mass index for LVH was evaluated using ROC curve analysis (Fig. 6). The methylene/methyl ratio showed an



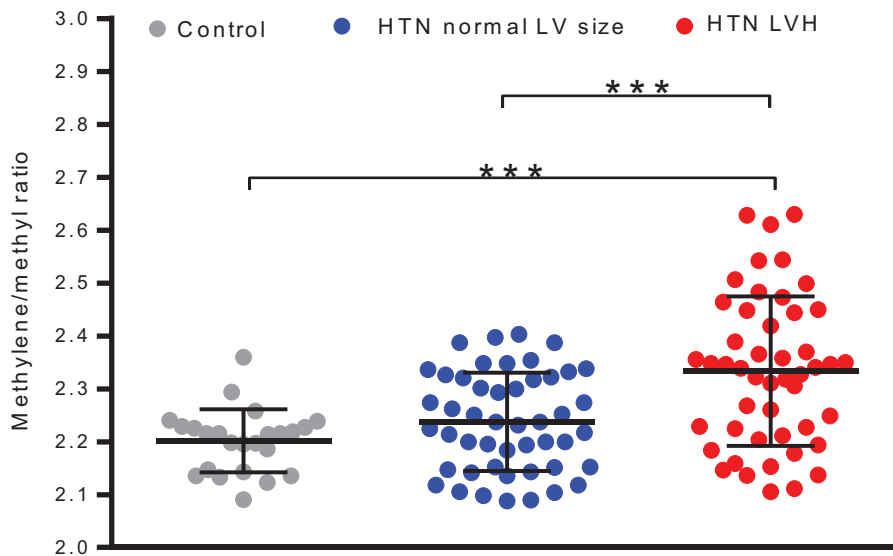
**Figure 3.** Methylene ( $-CH_2-$ ) and methyl ( $-CH_3$ ) moieties of aliphatic chains from plasma lipids ratio (R). Formulae illustrate  $-CH_2-$ / $-CH_3$  ratios according to the fatty acid chain length. Yellow points represent methylene ( $-CH_2-$ ).

area under the curves (AUCs) of 0.703 (95% CI: 0.601–0.792;  $P < 0.001$ ). The optimum cut-off point determined by the Youden index was at 2.337, with 52.08% sensitivity and 85.42% specificity. The positive and negative predictive values were 78.13% and 64.06%, respectively.

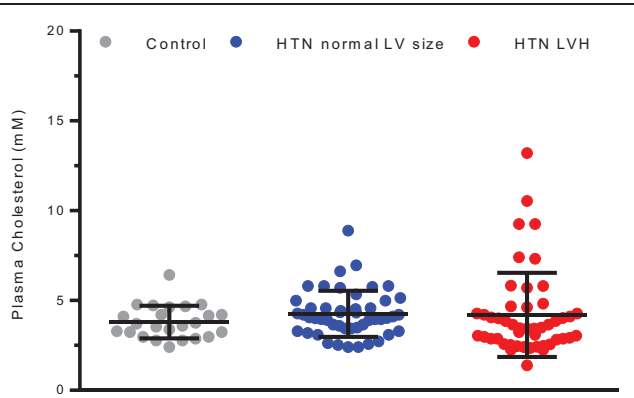
**4. Discussion**

Prolonged hypertension may result in LV structural remodeling, alteration of cardiac function, and chronic heart failure.<sup>[2]</sup> Early detection and treatment of LV hypertrophy in hypertensive patients is important in order to prevent the progression toward advanced cardiac disease. Cardiac hypertrophy biomarkers could be a quick, sensitive and cost-effective tool to complement the use of echocardiography in differentiating hypertensive patients with LV remodeling from those with normal LV size. Such a biomarker may be able to help in the screening of hypertensive patients because a simple blood test could rule-out the presence of LV structural remodeling. This situation would optimize the use of echocardiography for confirmation of LV structural remodeling and help clinicians adapt therapeutic strategies to improve a patient’s life expectancy.

We looked for plasma biomarkers of left ventricular remodeling in hypertensive patients using plasma metabolomics. Plasma offers the advantage of representing an “average” of tissue biochemical information and is commonly available in clinical practice. Discriminant variables were identified as signals from the methylene and methyl moieties of plasma lipid aliphatic chains. The methylene/methyl ratio, an indicator of the mean aliphatic lipid chain length, was increased in the plasma of hypertensive patients with left ventricular remodeling. Long-chain fatty acids are the preferred substrate for energy provision in the normal heart. The higher concentration of long-chain fatty acids in the plasma of hypertensive patients with LVH could reflect an impairment of their utilization at the myocardial level. Indeed, patients with very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, the enzyme responsible for the catabolism of fatty acids with an acyl chain length between 14 and 18



**Figure 4.** Methylene/methyl ratio in aliphatic lipid chains from plasma lipids of control, hypertensive patients with normal LV size (HTN normal LV size) and hypertensive patients with LV hypertrophy (HTN LVH). The methylene/methyl ratio is higher in the HTN LVH group compared to HTN normal LV size group and control. \*\*\* $P < 0.001$ .



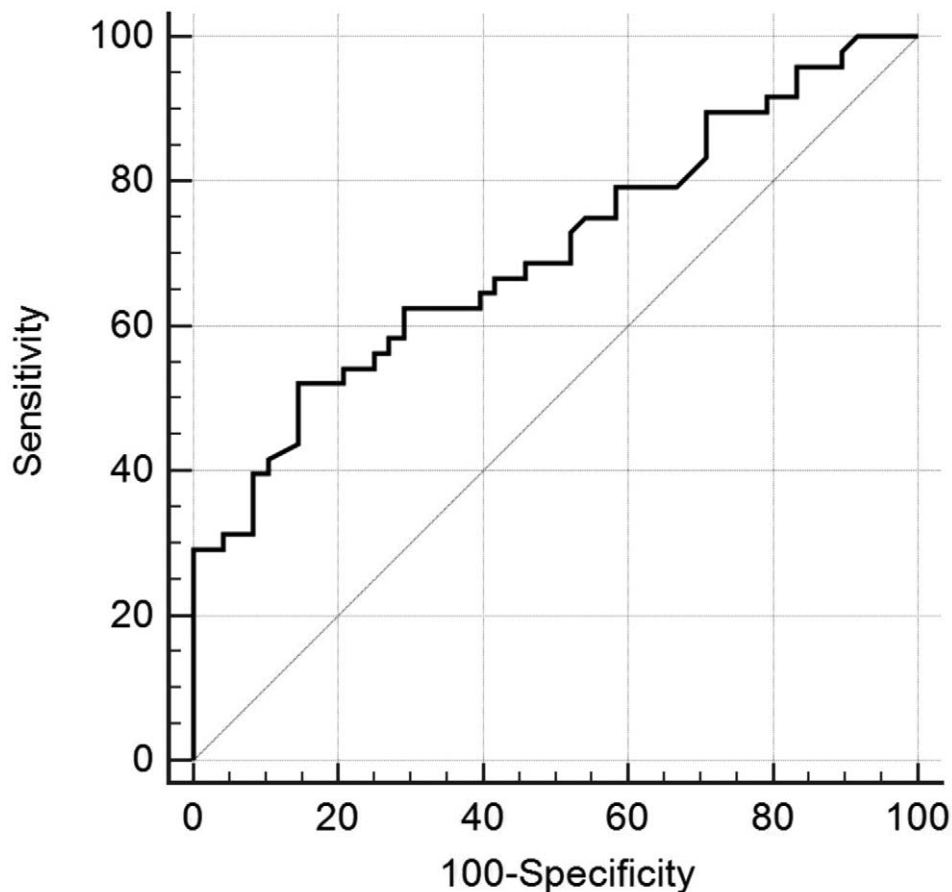
**Figure 5.** Plasma cholesterol concentration in control, hypertensive patients with normal LV size (HTN normal LV) and hypertensive patients with LV hypertrophy (HTN LVH). No difference was found among groups.

carbons, exhibit cardiac hypertrophy.<sup>[34]</sup> Moreover, blood profiles from animals and patients with VLCAD deficiency are characterized by large accumulations of long chain acyl carnitines.<sup>[35,36]</sup>

Several investigations in spontaneously hypertensive rats<sup>[16,37,38]</sup> and in humans with essential hypertension<sup>[22]</sup> have proposed a direct link between decreased myocardial fatty acid transport and a prohypertrophic cardiac response. A recent study

found that the heart of hypertensive humans with LV remodeling expresses a truncated form of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ).<sup>[18]</sup> PPAR- $\alpha$  is a key transcriptional regulator of the expression of fatty acid carriers and  $\beta$ -oxidation genes. Increased expression of the truncated PPAR $\alpha$  is associated with decreased expression of the PPAR $\alpha$  target genes involved in fatty acid transport and oxidation.<sup>[18]</sup> Moreover, decreased PPAR $\alpha$  expression and/or activity in the heart could be linked to the development of local inflammation and fibrosis.<sup>[39]</sup> Supporting the concept that defects in fatty acid transport and oxidation play an important role in ventricular hypertrophy is the fact that mutations in the fatty acyl translocase CD36, a plasma membrane transporter involved in the uptake of long chain fatty acids by cardiomyocytes, have been linked to cases of inherited hypertrophic cardiomyopathy.<sup>[40]</sup> Further, the inhibition of myocardial fatty acid metabolism has been reported as an independent predictor of the cardiac hypertrophic response in hypertensive patients evaluated by PET scan.<sup>[22]</sup>

The use of the ROC curve analysis with the methylene/methyl ratio data allowed us to describe the performance of the biomarker. Despite a modest sensitivity (52%) this biomarker displayed high specificity (>85%) in detecting LVH, supporting the diagnostic value of this biomarker. It has been previously reported that cholesterol, insulin resistance, and adiposity are significantly correlated with left ventricular mass in hypertensive patients.<sup>[41,42]</sup> In our study, there was no significant difference in insulin levels, obesity, or dyslipidemia between the hypertensive



**Figure 6.** Receiver operating characteristic (ROC) curve analysis of methylene/methyl ratio used to identify LVH in hypertensive population. The AUC (area under the curve) is 0.703.

groups. Moreover we did not find any difference in cholesterol levels between hypertensive patients with or without left ventricular remodeling. This is in agreement with a study conducted in Japanese patients where total cholesterol was not found associated to LVH in essential hypertension.<sup>[42]</sup> In our study, hypertensive patients with LV remodeling maintained a normal LV ejection fraction. This finding agrees with previous reports of increased ventricular mass index and relative wall thickness with preserved ejection fraction.<sup>[43]</sup> In fact, LVH secondary to hypertension is a physiological process of compensation to minimize wall stress, allowing preservation of LVEF.<sup>[2]</sup> However, concentric LVH commonly precedes dilated cardiac failure in patients with hypertension.<sup>[2]</sup>

An abnormal diastolic relaxation in the absence of systolic impairment is often seen in patients with hypertension, and a reduction in compliance is observed when LVH occurs.<sup>[44]</sup> Attention has recently been concentrated on echocardiographic measurement of left atrial size, as it frequently correlates with LVH and is predictor of cardiovascular events.<sup>[45]</sup> In our study, there were no differences in standard parameters used for diastolic and left atrial function evaluation in the 2 groups of hypertensive patients. However, left atrial diameter index tended to increase in our hypertensive patients with LVH compared to hypertensive patients with no LV remodeling. This evidence suggests a diastolic dysfunction in hypertensive patients with LV remodeling and points out the limitations<sup>[45]</sup> of current techniques such as Doppler and 2D echocardiography in the evaluation of diastolic<sup>[46]</sup> and left atrial function.<sup>[47]</sup> New tools such as 2D speckle tracking echocardiography<sup>[48]</sup> should be useful for early detection of cardiac malfunction due to arterial hypertension.<sup>[48–50]</sup>

A recent study has reported that exercise-induced arterial hypertension leads to myocardial hypertrophy in a similar way to that found in pathological conditions with arterial hypertension.<sup>[51]</sup> As some biomarkers, both cardiac troponin I and NTproBNP have been found to be increased in runners,<sup>[52]</sup> it would be interesting to measure the level of the methylene/methyl ratio in these athletes. In addition, low-intensity exercise was shown to unmask hypertension in patients with apparent normal clinic blood pressure but elevated blood pressure outside the office environment.<sup>[53]</sup> We used a 24-hour ambulatory blood pressure measurement and did not evaluate patients this way in our study. However, after low-intensity exercise testing of patients, methylene/methyl ratio monitoring could provide useful information about possible LV remodeling.

The absence of other physiological differences between the 2 hypertensive groups in our study reinforces the possibility that the methylene/methyl ratio may be used as a diagnostic and screening tool for hypertensive left ventricular remodeling. Moreover, methylene/methyl ratio was not different between controls and hypertensive patients without LVH. Although evidence is currently lacking, we believe that methylene/methyl ratio is only sensitive to the LV remodeling and thus not different in normal participants and hypertensive patients that did not show any clinical manifestation toward LV remodeling during the screening.

Our study proposes plasma methylene/methyl ratio as a metabolic biomarker for the detection of LV remodeling in hypertensive patients. However, as this result stems from a small single center cohort, further validation through a large international multicenter study is needed. Secondly, the timing of the methylene/methyl ratio modification, that is, whether an increase in the methylene/methyl ratio can be detected in

hypertensive patients at risk to develop LVH or whether it is only a post-LVH consequence, remains to be established. Thirdly, as it is known that genetic variants and polymorphisms are implied in hypertensive related cardiovascular complications,<sup>[54–57]</sup> genetic variants of key fatty acids metabolic enzymes regulating the hypertrophic phenotype need to be evaluated. Lastly, potential confounders to increases in the methylene/methyl ratio need to be determined and the precise molecular mechanisms leading to the alteration of fatty acid species in the bloodstream remain to be investigated.

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