Diabetes-induced lipid panel particularities in hypertensive patients: A pilot NMR spectroscopy study

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Abstract

Cardiovascular diseases are the leading cause of death globally, constantly increasing morbidity and mortality each year, despite significant advances in diagnosis and treatment. Among the multiple cardiovascular risk factors, hypertension, diabetes mellitus type II and dyslipidemia are the most frequent and show the best correlations with the risk of further developing a major cardiovascular event. We set out to assess the influence of diabetes on the lipid panel in hypertensive patients as a sub-analysis of a larger study that we have previously developed regarding the need for fasting prior to blood sampling in a cohort of subjects admitted in a tertiary cardiovascular emergency unit with acute coronary syndromes. It was observed that the fasten triglycerides levels, the VLDL and IDL particle number, apolipoprotein A1, A2, B100 levels, as well as the ratio of apo B100/apo A1 are all potentially useful markers that could be successfully used to differentiate between the strictly hypertensive patients and the ones that associate type II diabetes mellitus. The study also confirmed once more our previous reports that the fasting status upon sampling has little influence on the lipid panel final value.

Keywords: hypertension, diabetes, cardiovascular diseases, NMR spectroscopy, lipidomics, metabolomics.

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Introduction

Cardiovascular disease (CVD) currently represents a continuously increasing global health issue and the underlying cause of more than one-third of all deaths globally. CVD is the most common cause of death in European Society of Cardiology (ESC) member countries, with ischemic heart disease (IHD) accounting for 45% of these deaths in females and 39% in males [1].

There are many already well-known cardiovascular risk factors (CVRF) that have proven to be associated with the further development of CVD, such as diabetes mellitus, high blood pressure, cigarette smoking, obesity, lack of physical activity and lipid abnormalities. Among these, high BP is associated with the strongest evidence for causation and has a high prevalence of exposure [2].

Hypertension (HTN) is defined, according to the latest ESC good clinical practice guidelines, as an office SBP value of at least ≥140 mmHg and/or a diastolic BP (DBP) value of at least ≥90 mmHg. The same classification is used for other populational categories (younger, middle-aged and older people). In contrast, BP centiles are used in children and teenagers, in whom data from interventional trials are not available.

Despite significant advances in diagnosis and treatment over the past 30 years, the disability-adjusted life years (DALY) attributable to hypertension have increased by 40% since 1990. Both office and out-of-office BP levels have an independent and continuous relationship with the incidence of several CV events, such as ischaemic or hemorrhagic stroke, myocardial infarction, sudden death, heart failure, end-stage renal disease and peripheral artery disease. The continuous relationship between BP and risk of events has been shown at all ages and in all ethnic groups and extends from high BP levels to relatively low values, close to the ones considered to be in a normal range [3].

On the other side, dyslipidemia is another important established risk factor for cardiovascular events and it is defined as increased levels of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or a decreased serum high-density lipoprotein cholesterol (HDL-C) concentration [4].

Elevated serum lipids lead to vessel wall responses, including endothelial dysfunction, smooth muscle cell proliferation, lipid accumulation, foam cell formation, and, eventually, necrosis and plaque development [5]. The current practice regarding standardized biochemical blood analysis is to sample it in a fasting status. However, there are recent reports questioning the need for fasting before sampling, at least for a selected set of biochemical parameters. There are few studies to cover this area of interest, and most of the already published have evaluated just the main lipoprotein parameters and only through standardized biochemical methods [6].

We have previously demonstrated that there are no significant changes before and after food intake regarding the lipid profile, with the exception of triglycerides [7]. We now set out to assess the influence of diabetes on the lipid panel in hypertensive patients. This is a sub-analysis of a larger study that we have previously developed regarding the need for fasting prior to blood sampling in a cohort of subjects admitted to a tertiary cardiovascular emergency unit with acute coronary syndromes.

Material and methods

We have selected a group of 30 hypertensive patients (15 strictly hypertensive and 15 who were also diagnosed with diabetes mellitus type II, aged 38 to 78 years old, out of which 13 were males and 14 were smokers) who also associated lipid profile abnormalities, in order to create the study group. Some of them were already on statin treatment prior to their admittance, while others were newly introduced to this treatment. Only 16 of them were on some form of antihypertensive treatment at the time of their enrollment.

The control group included 17 otherwise healthy individuals (ages between 25 and 42 years, 5 males, 5 smokers), without any known comorbidities and without any long-term treatment at the moment of sampling. A blood sample of 6 mL was collected before and two hours after meals in standardized red vacutainers without any anticoagulant, used for biochemistry analysis and centrifuged after 30 min at room temperature at 3500 rpm for 15 min for serum separation. The separated serum was then aliquoted in 1 mL cryovials and stored at -80°C until the NMR analysis was performed. The cryovials were kept at -80°C until up to 60 minutes before the NMR analysis. We then developed a mixture with 400 microL of plasma and 400 microL of 5 mM sodium 3-(trimethylsilyl)-[2,2,3,3-d4]-1-propionate (TSP) in Na2HPO4/NaN3/D2O buffer (Bruker Biospin, Ettlingen, Germany), which was homogenized at room temperature with a plasma rotator (VWR). A volume of 600 microL of the homogenized sample was then transferred into a 5 mm NMR tube (Wilmad 507) previously checked as described before [8] and further loaded into the NMR sample changer. Five different types of NMR experiments have been recorded at 300.0 K with pulse sequences and parameters, as follows: ERETIC for quantification, JRESOLVED 2D for signal assignment, CPMG for lipid suppression, 1D DIFFUSION for the suppression of low molecular metabolites and 1H GRADIENT for quality control as previously described [7]. The full set of assessed metabolites and lipoprotein parameters has also been previously detailed [7].

Results and discussion

As we describe below, there are several NMR spectroscopy-revealed markers that might be able to differentiate between otherwise healthy hypertensive patients and those with both hypertension and diabetes mellitus. On the other hand, the present study supports our previous report [7], indicating that except for triglycerides, the fasting state has

no significant influence on the lipid profile. In order to document this statement, we present in all subsequent graphs both the fasting (blue) and postprandial (red) states. In terms of "classical" markers, the most significant ones for differentiating the three groups, *i.e.* controls (cases 1–17), hypertensives (HTN) (cases 18–32) and hypertensives with DM type II (cases 33–47), as revealed by NMR spectroscopy, are the following: creatinine (below 0.1 mmol/L in controls and above 0.1 mmol/L in HTN and HTN-DM type II, Figure 1), fasting state glucose (below 6 mmol/L in controls and HTN, and above 6 mmol/L in HTN-DM type II, Figure 2), triglycerides (below 70 mg/dL in controls and above 90 mg/dL in HTN and HTN-DM type II, Figure 3), HDL cholesterol (above 55 mg/dL in most of controls and well below 55 mg/dL in most of the HTN and HTN-DM type II patients, Figure 4), apolipoprotein A1 (above 140 mg/dL in most of the controls and below 140 mg/dL in most of the HTN and HTN-DM type II patients, Figure 5), apolipoprotein A2 (well above 30 mg/dL in most of the controls and well below 30 mg/dL in most of HTN and HTN-DM type II, Figure 6), ratio apolipoprotein B100/apolipoprotein A1 (below 0.6 mg/dL in controls and above 0.6 mg/dL in more than half of the HTN and HTN-DM type II patients, Figure 7).

In terms of differentiating between the HTN and HTN-DM type II groups, in addition to the before mentioned fasten glucose levels (Figure 2), the fasten triglycerides levels could be helpful, with levels below 100 mg/dL for HTN and above 100 mg/dL for HTN-DM type II (Figure 3). Furthermore, analyzing the apolipoprotein A1 and apolipoprotein A2 levels could help differentiate between the two. The lowest levels seem to be noted in the HTN group, followed by the HTN-DM type II group, while the control group showed the highest serum levels overall.

It is interesting to mention that both total cholesterol and LDL cholesterol levels are comparable in all three groups. This can be explained by the efficiency of the statin treatment for both HTN and HTN-DM type II patients (Figures 8, 9). However,

the protective level of HDL fraction was not restored either in the HTN group or in the HTN-DM type II one as a result of the statin treatment, as it was detailed above in Figure 3.

While there is not a notable difference between the levels of apolipoprotein B100 in the studied groups (Figure 10), the ratio of apo B100/apo A1 can still distinguish between controls and the two HTN groups (Figure 7), although this ratio is less powerful than apolipoprotein A1 itself (Figure 5). Similarly, the LDL/HDL cholesterol ratio is a less efficient marker in distinguishing between groups (with most of the controls below 2 mg/dL and half of the HTN and HTN-DM type II above 2 mg/dL, Figure 11) than the HDL cholesterol levels alone (Figure 4).

The emerging markers for CVD include lipoprotein subfractions and particle numbers instead of concentrations. As NMR spectroscopy is a powerful tool that enables the assessment of these modern markers, we present below the most significant findings from our study.

A very suitable marker for distinguishing between controls and HTN patients is the VLDL particle number (well below 80 nmol/L for controls and well above 120 nmol/L for both HTN and HTN-DM type II, Figure 12). The IDL particle number is also extremely helpful in differentiating between the groups (with values in general below 70 nmol/L for controls and above 70 nmol/L for HTN and HTN-DM type II, Figure 13).

It is remarkable that, equally to the LDL concentration levels described above (Figure 9), the LDL particle number is corrected by treatment to similar values in all three studied groups (Figure 14).

What is also worth mentioning is that the particle number of all LDL subfractions, including the small-dense ones that were shown to be highly correlated with the risk of further developing CVD (L5-6PN as shown in recent studies), is maintained by statins within the same range for both the control and the HTN group, with very few exceptions (Figures 15–17) [9, 10].

Several other subfractions could also facilitate the differentiation between the control group
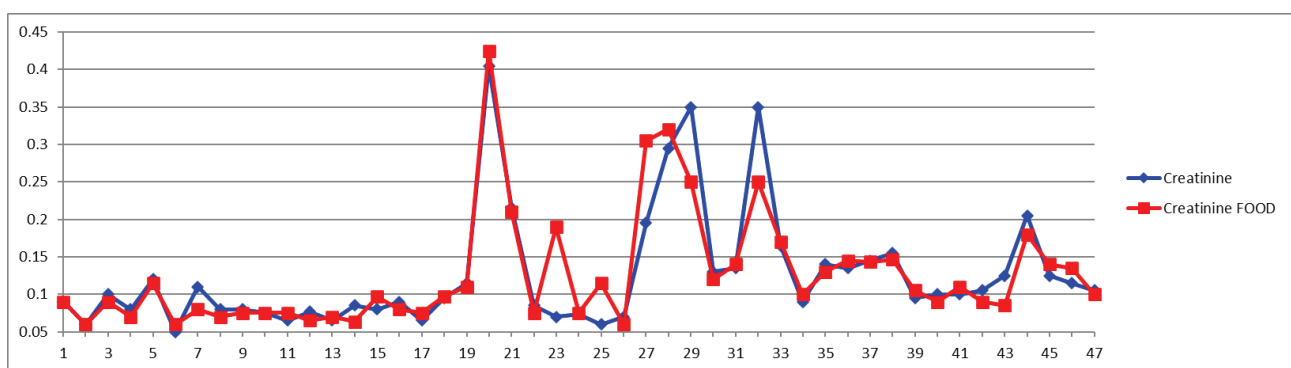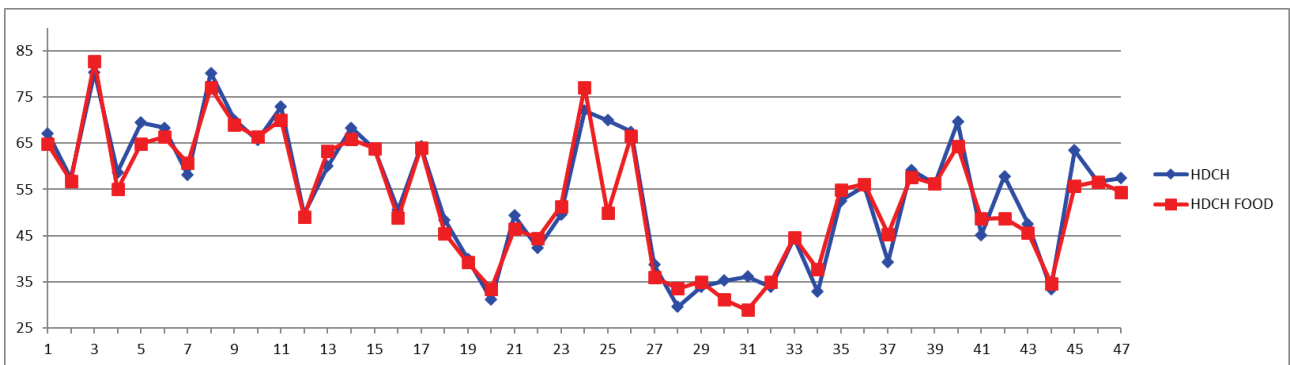

Figure 1. Creatinine concentrations (mmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
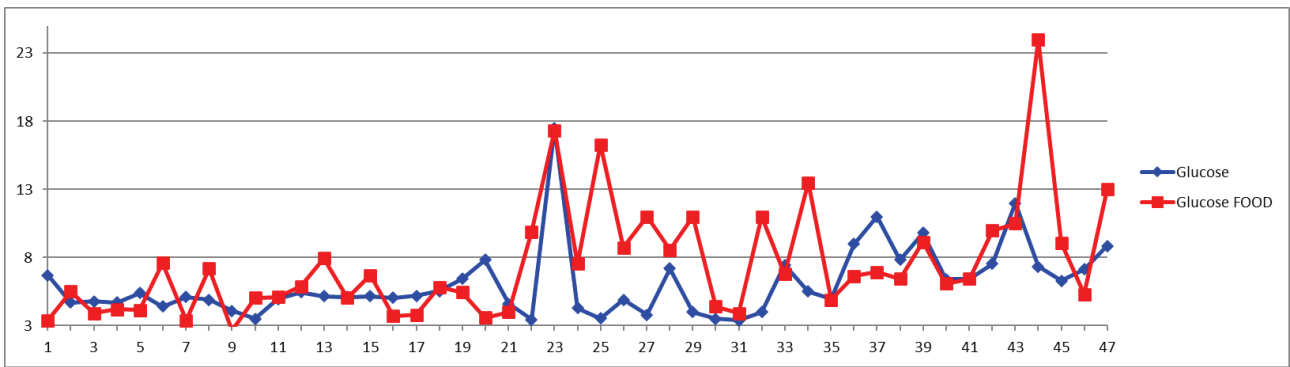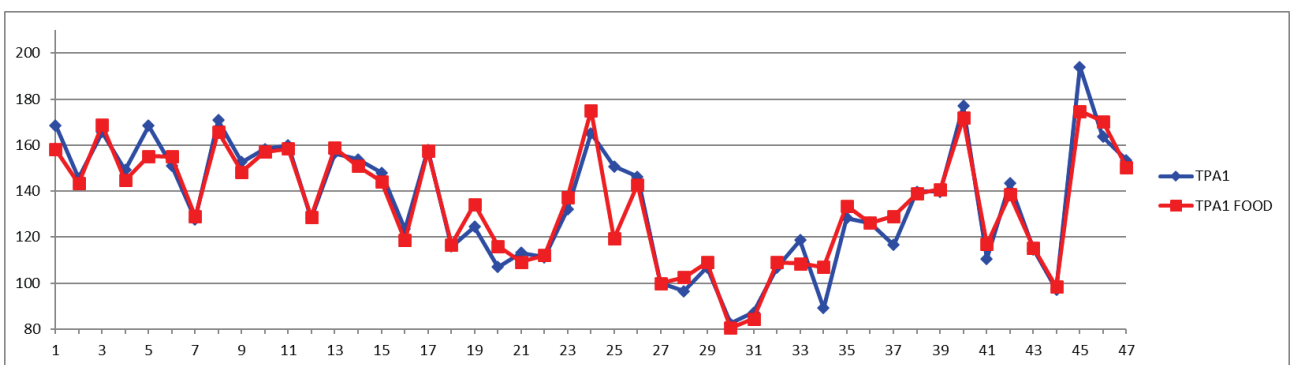
Figure 2. Glucose concentrations (mmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
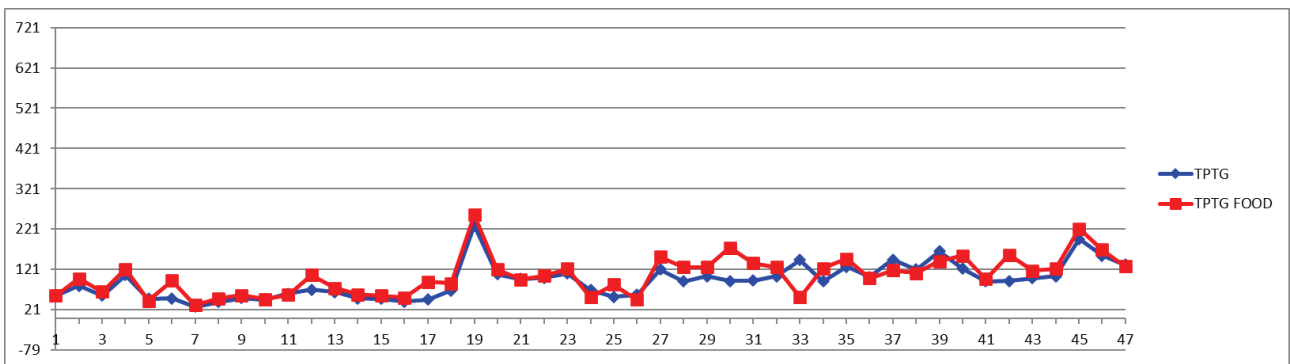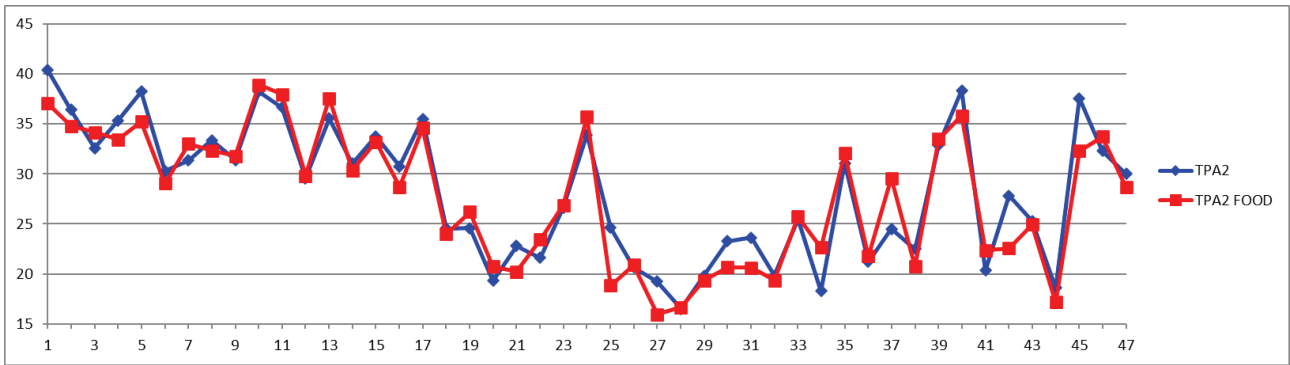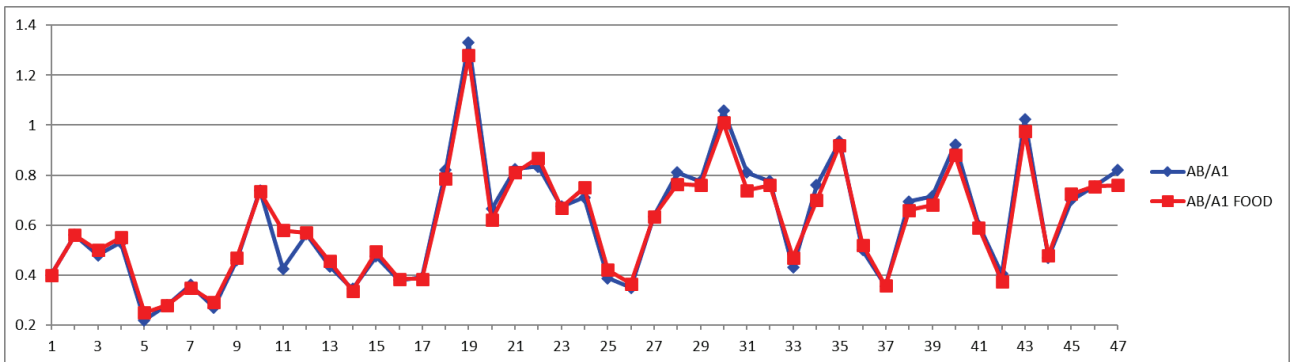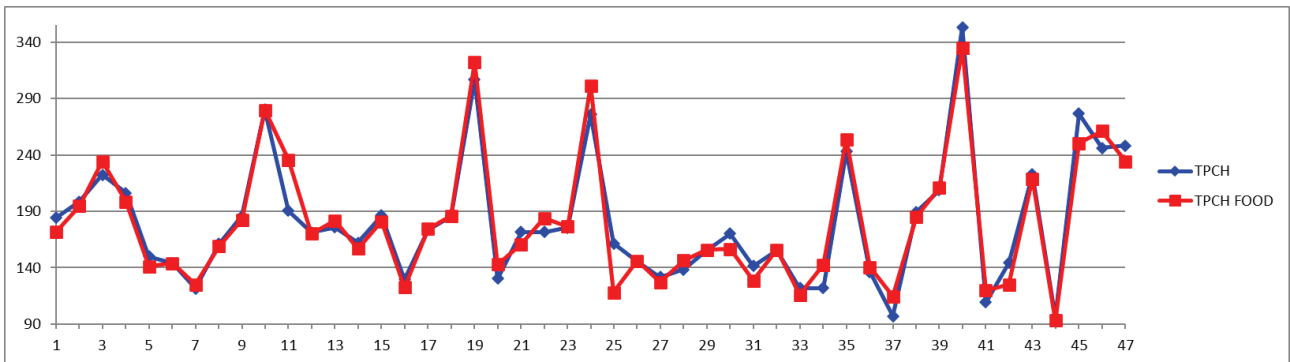

Figure 3. Triglycerides (TPTG) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

Figure 4. HDL cholesterol (HDCH) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

Figure 5. Apolipoprotein A1 (TPA1) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
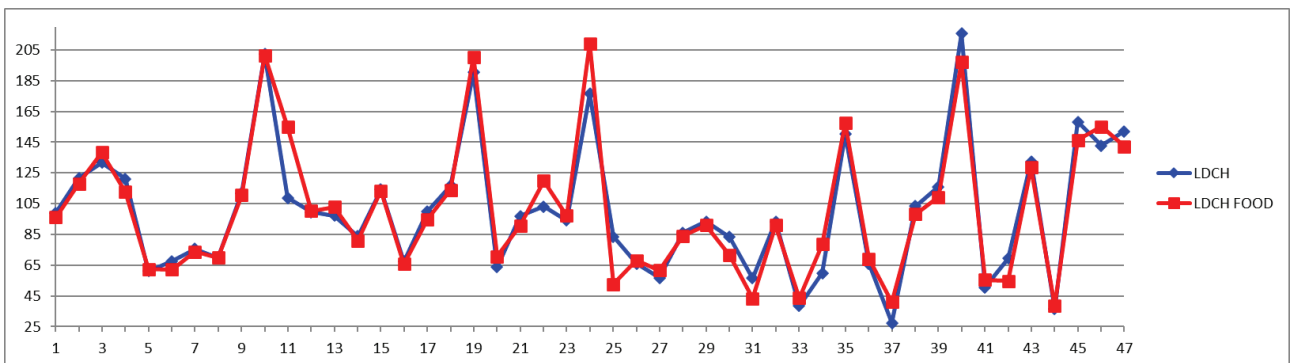
Figure 6. Apolipoprotein A2 (TPA2) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
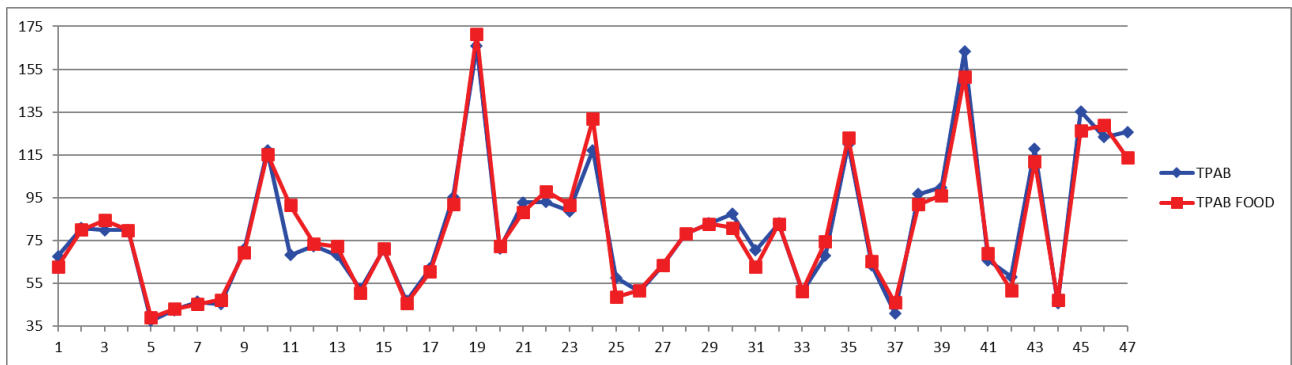

Figure 7. Apo-B100/Apo-A1 (AB/A1) ratio in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
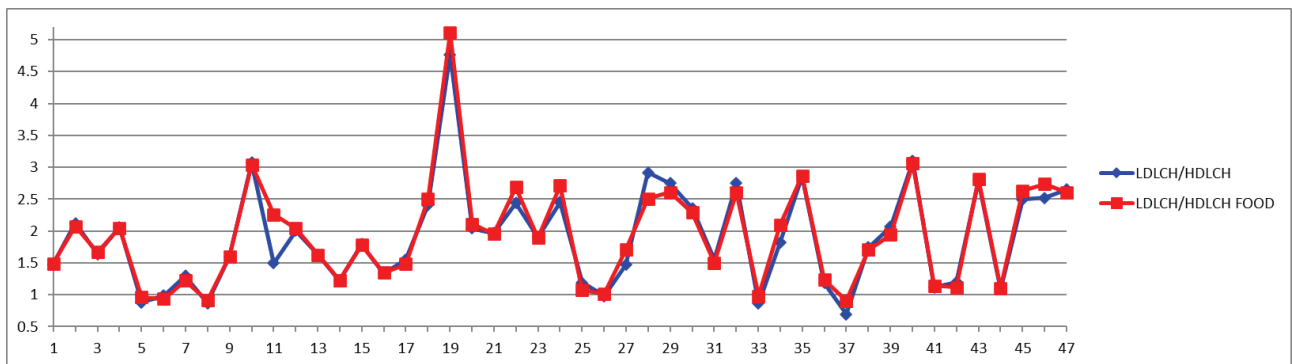

Figure 8. Total cholesterol (TPCH) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
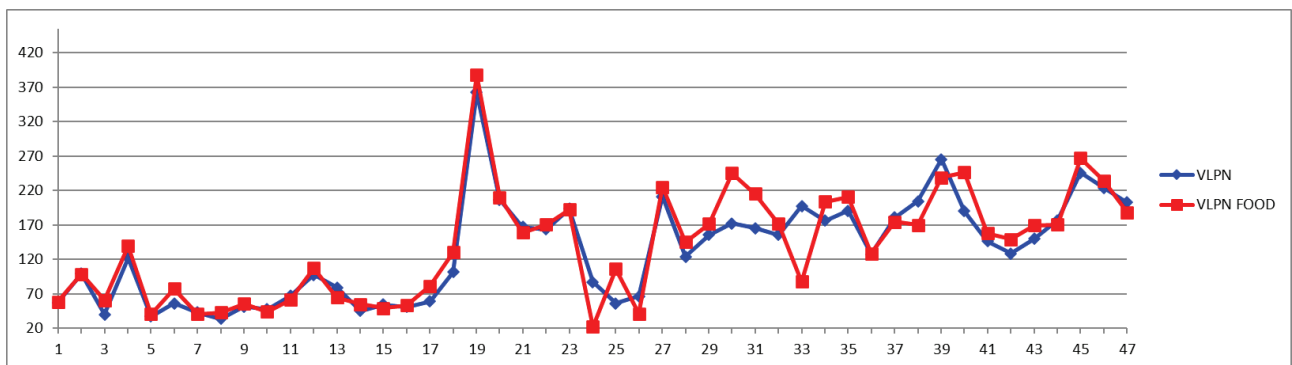

Figure 9. LDL cholesterol (LDCH) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
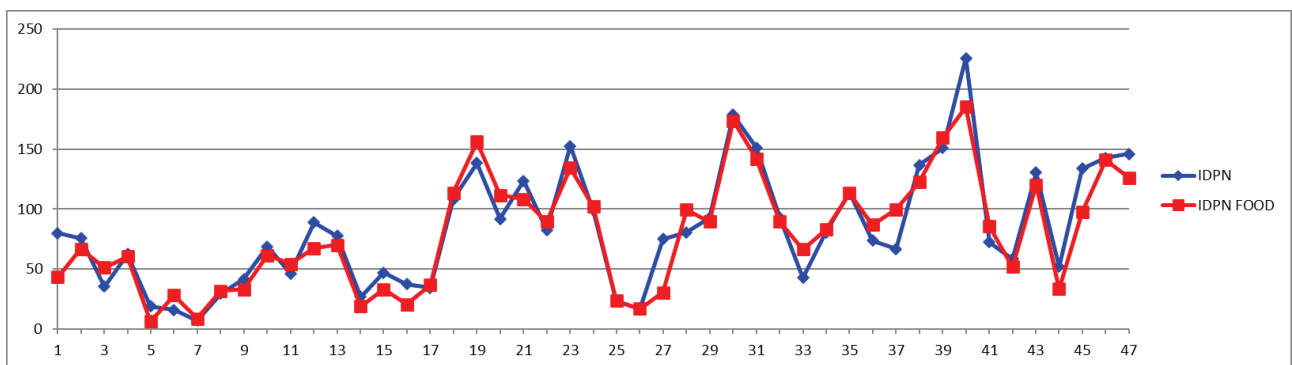
Figure 10. Apolipoprotein B100 (TPAB) concentrations (mg/dL) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
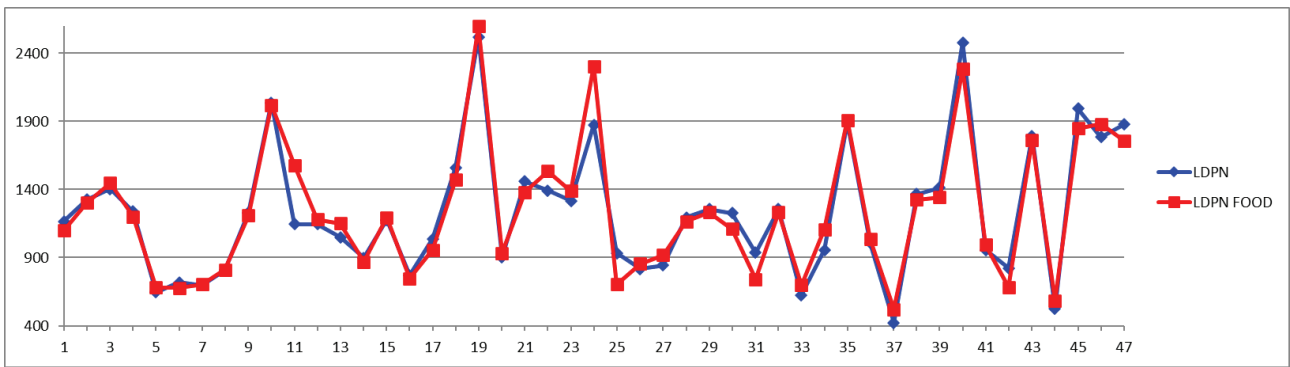

Figure 11. LDL/HDL cholesterol ratio in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
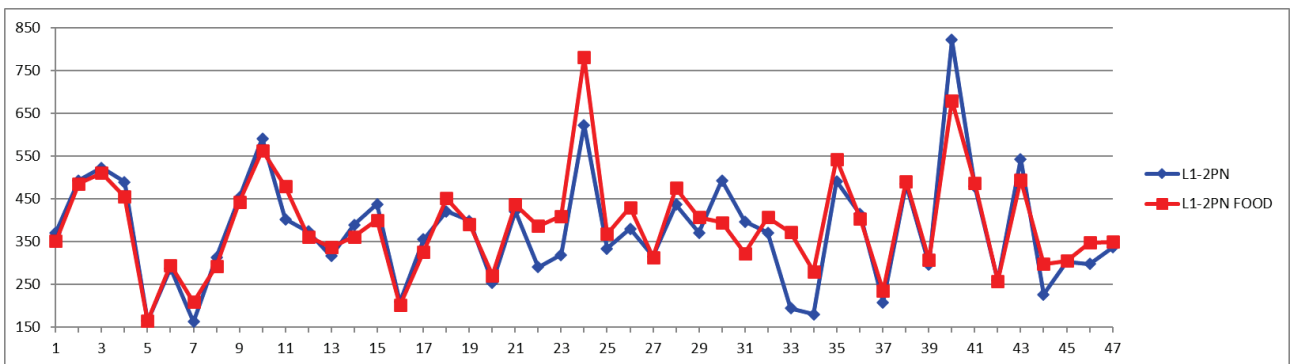

Figure 12. VLDL particle numbers (VLPN, nmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

Figure 13. IDL particle numbers (IDPN, nmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).
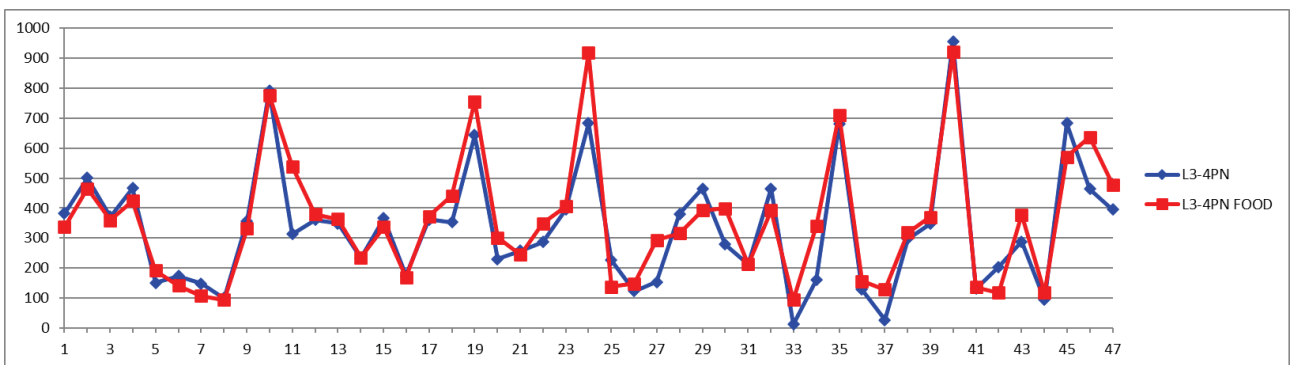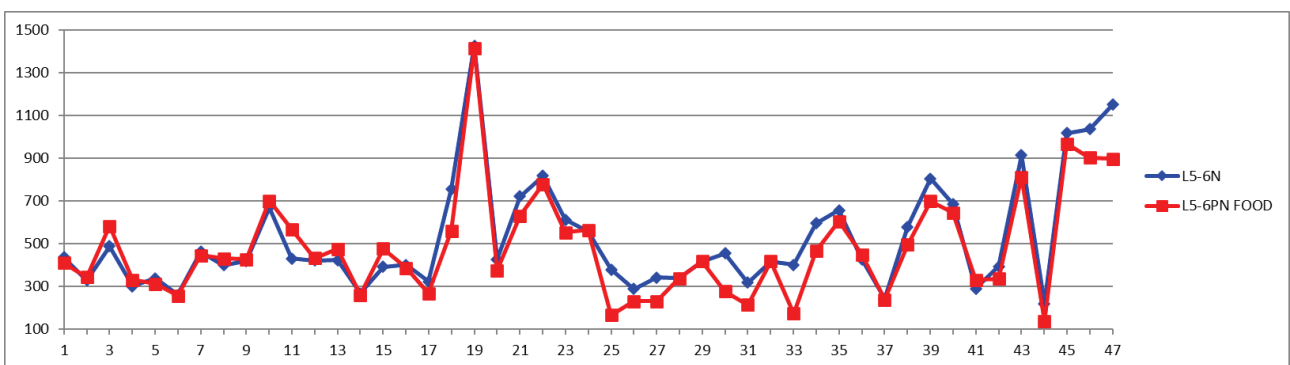
Figure 14. LDL particle numbers (LDPN, nmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

Figure 15. Large LDL subfractions particle numbers (L1-2PN, nmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

Figure 16. Medium LDL subfractions particle numbers (L3-4PN, nmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

Figure 17. Small-dense LDL subfractions particle numbers (L5-6PN, nmol/L) in fasting (blue) and postprandial (red) status for control cases (1–17), HTN (18–32), and HTN-DM type II cases (33–47).

subjects and the hypertensive ones, even when they are under statin treatment.

Conclusions

We have evaluated the lipid panel differences induced by diabetes mellitus type II in hypertensive patients, and we have compared the results with an otherwise healthy control group. We have identified the NMR spectroscopy markers that could be able to spot subtle differences in the lipid profiles of the two study groups (two hypertensive groups, one consisting of strictly hypertensive patients, the other one including hypertensive patients with already known type II diabetes mellitus), even when LDL levels have been lowered to normal levels due to statin treatment.

We have therefore observed that the fastened triglycerides levels, the VLDL and IDL particle number, apolipoprotein A1, A2, and B100 levels, as well as the ratio of apo B100/apo A1, are all potentially useful markers that could be successfully used to differentiate between the strictly hypertensive patients and the ones that associate type II diabetes mellitus.

The study also confirmed once more our previous reports that the fasting status upon sampling has little influence on the lipid panel final values.

Conflict of interest

The authors declare no conflict of interest.

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