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LIPOPROTEIN INDEX BY NUCLEAR MAGNETIC RESONANCE AND INSULIN SENSITIVITY

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ABSTRACT

Introduction: dyslipidemia is an alteration in both quantity and quality of lipids and lipoproteins that can trigger cardiovascular events. Although LDL cholesterol is the main lipid risk factor, 50% of myocardial infarctions occur in patients with normal LDL. Most of these patients have atherogenic dyslipidemia which is characterized by increased triglycerides, normal LDLc transported by an increased number of small LDL particles and low HDLc. Insulin resistance is the main trigger for atherogenic dyslipidemia and is currently evaluated using the HOMA index. Given that lipid profile is routinely examined, which is not the case for insulin sensitivity, and that insulin resistance causes qualitative and quantitative changes in lipoprotein profile, we hypothesized that monitoring lipid profile by MRI would allow early detection of signs of insulin resistance.

Objective: to establish a lipoprotein index to assess insulin resistance based on lipid values measured by NMR, applicable to patients of different BMI and both sexes.

Methods: The study was conducted as a population-based cohort study (Di@bet.es) in 2008-2010, using cluster sampling of the Spanish population. The initial sample is 5072 subjects (> 18 years, 51.9% women) randomly selected by the National Health System. From a blood sample, the lipoprotein profile was determined using the 1H-NMR-based Liposcale® Test which was used to measure the number and concentration of lipoproteins of different sizes.

Results: Following linear regression models, 9 of the lipoprotein parameters measured by NMR, were those that best estimated the HOMA index (R^2: 0.17; p<0.001) (total VLDL particles, large VLDL particles, small VLDL particles, VLDL diameter, large LDL particles, medium LDL particles, total HDL particles, large HDL particles and HDL diameter).
These parameters were used to create the LipoINRE index, which significantly correlates with the HOMA index (r=0.43; p<0.001). The LipoINRE index correlates with BMI (r=0.37; p<0.001), even adjusting for age (r=0.31; p<0.001). The LipoINRE index correlated with HOMA index and BMI respectively in both men and women (men r=0.40, p<0.001 and r=0.31, p<0.001, women r=043, p<0.001 and r=0.37, p<0.001). Finally, the correlation

between LipoINRE index and BMI increases significantly as BMI increases ($r=0.207$, $p<0.001$ in thin; $r=0.31$, $p<0.001$ in overweight; and $r=0.37$, $p<0.001$ in obese).

Conclusion: the lipoprotein profile measured by NMR provides qualitative and quantitative information on the lipid profile. This not only allows us to assess cardiovascular risk but also lipoprotein changes related to insulin sensitivity assessed by the HOMA index.

RESUMEN

Introducción: la dislipemia es una alteración tanto en cantidad como en calidad de los lípidos y las lipoproteínas que puede desencadenar eventos cardiovasculares. Aunque el colesterol LDL es el principal factor de riesgo lipídico, un 50% de los infartos de miocardio se dan en pacientes con LDL normal. La mayoría de estos individuos presentan una dislipemia aterógena caracterizada por un aumento de triglicéridos, LDLc normal con aumento de partículas LDL pequeñas y nivel bajo de HDLc. La resistencia a la insulina es el principal detonante de la dislipemia aterógena y suele valorarse a través del índice HOMA. Dado que el perfil lipídico se examina de forma rutinaria, cosa que no sucede con la sensibilidad a la insulina, y que la resistencia a la insulina provoca cambios cualitativos y cuantitativos en el perfil lipoproteico, hipotetizamos que un seguimiento del perfil lipídico mediante RMN permitiría detectar prematuramente signos de resistencia a la insulina.

Objetivo: establecer un índice lipoproteico que permita valorar la resistencia a la insulina a partir de valores lipídicos medidos por RMN, aplicable a pacientes de diferentes IMC y ambos sexos.

Métodos: El estudio se ha realizado mediante un estudio de cohortes de base poblacional Di@bet.es. La muestra inicial es de 5072 sujetos (> 18 años, 51,9% mujeres) seleccionados aleatoriamente por el Sistema Nacional de Salud. A partir de una muestra de sangre se determinó el perfil lipídico mediante el Liposcale® Test que midió el número y la concentración de lipoproteínas de distintos tamaños.

Resultados: Siguiendo modelos de regresión lineal, 9 de los parámetros lipoproteicos medidos por RMN se identificaron como aquellos que mejor estiman el índice HOMA (R^2: 0,17; $p<0,001$) (partículas VLDL totales, partículas VLDL grandes, partículas VLDL pequeñas, diámetro VLDL, partículas LDL grandes, partículas LDL medianas, partículas HDL totales, partículas HDL grandes y diámetro HDL).

Estos parámetros se utilizaron para crear el “índice LipoINRE”, que se correlaciona con el índice HOMA ($r=0,43$; $p<0,001$). El índice LipoINRE se correlaciona con el IMC ($r=0,37$; $p<0,001$), incluso ajustando por la edad ($r=0,31$; $p<0,001$). El índice LipoINRE también se correlacionó con el índice HOMA y el IMC respectivamente tanto en hombres como en mujeres (hombres $r=0,40$, $p<0,001$ y $r=0,31$, $p<0,001$, mujeres $r=043$, $p<0,001$ y $r=0,37$, $p<0,001$). Por último, la correlación entre el índice LipoINRE y el IMC aumenta de forma significativa a medida que aumenta el IMC ($r=0,207$, $p<0,001$ en delgados; $r=0,31$, $p<0,001$ en sobrepeso; y $r=0,37$, $p<0,001$ en obesos).

Conclusión: el perfil lipoproteico medido por RMN aporta información cualitativa y cuantitativa del perfil lipídico. Ello no solo permite valorar el riesgo cardiovascular sino también cambios lipoproteicos relacionados con la sensibilidad a la insulina valorada mediante el índice HOMA.

RESUM

Introducció: la dislipèmia és una alteració tant en quantitat com en qualitat dels lípids i les lipoproteïnes que pot desencadenar esdeveniments cardiovasculars. Encara que el colesterol LDL és el principal factor de risc lipídic, un 50% dels infarts de miocardi es donen en pacients amb LDL normal. La majoria d'aquests individus presenten una dislipèmia aterògena caracteritzada per un augment de triglicèrids, LDLc normal amb augment de partícules LDL petites i nivell baix de HDLc. La resistència a la insulina és el principal detonant de la dislipèmia aterògena i es sol valorar a través de l'índex HOMA. Atès que el perfil lipídic s'examina de manera rutinària, cosa que no succeeix amb la sensibilitat a la insulina, i que la resistència a la insulina provoca canvis qualitatius i quantitatius en el perfil lipoproteic, hipotetitzem que un seguiment del perfil lipídic mitjançant RMN permetrà detectar prematurament signes de resistència a la insulina.

Objectiu: establir un índex lipoproteic que permeti valorar la resistència a la insulina a partir de valors lipídics mesurats per RMN, aplicable a pacients de diferents IMC i tots dos sexes.

Mètodes: L'estudi s'ha realitzat mitjançant un estudi de cohorts de base poblacional Di@bet.es. La mostra inicial és de 5072 subjectes (> 18 anys, 51,9% dones) seleccionats aleatòriament pel Sistema Nacional de Salut. A partir d'una mostra de sang es va mesurar el perfil lipídic mitjançant el Liposcale® Test que mesura el número i concentració de lipoproteïnes de diferents mides.

Resultats: Seguint models de regressió lineal, 9 dels paràmetres lipoproteics mesurats per RMN van ser identificar com aquells que millor estimen l'índex HOMA (R^2: 0,17; p<0,001) (partícules VLDL totals, partícules VLDL grans, partícules VLDL petites, diàmetre VLDL, partícules LDL grans, partícules LDL mitjanes, partícules HDL totals, partícules HDL grans i diàmetre HDL).

Aquests paràmetres es van utilitzar per a crear l'índex LipoINRE, que es correlaciona amb l'índex HOMA (r=0,43; p<0,001). L'índex LipoINRE es correlaciona amb l'IMC (r=0,37;

p<0,001), fins i tot ajustant per l'edat (r=0,31; p<0,001). L'índex LipoINRE es va correlacionar amb l'índex HOMA i l'IMC respectivament tant en homes com en dones (homes r=0,40, p<0,001 i r=0,31, p<0,001, dones r=043, p<0,001 i r=0,37, p<0,001). Finalment, la correlació entre l'índex LipoINRE i l'IMC augmenta de manera significativa a mesura que augmenta l'IMC (r=0,207, p<0,001 en prims; r=0,31, p<0,001 en sobrepès; i r=0,37, p<0,001 en obesos).

Conclusió: el perfil lipoproteic mesurat per RMN aporta informació qualitativa i quantitativa del perfil lipídic. Això no sols permet valorar el risc cardiovascular sinó també canvis lipoproteics relacionats amb la sensibilitat a la insulina valorada mitjançant l'índex HOMA.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in Europe. Primary and secondary prevention are essential to reduce the consequences of CVD. One of the main CVD risk factors is dyslipidemia, which consists of a quantitative or qualitative alteration of lipids and lipoproteins **(1)**. Dyslipidemia can evolve into an atherosclerotic disease that develops a cholesterol deposit in the vascular endothelium, producing stenosis of medium and large caliber arteries, which impedes blood flow, developing ischemic cardiovascular events **(2)**.

LDL cholesterol lowering therapy has been shown to reduce the rate of cardiovascular events in patients with or without significant risk **(3)**. However, 50% of patients with atherosclerosis and ischemic events have normal lipid concentrations in routine biochemistry blood tests. This leads us to hypothesize that there might be other lipid characteristics that are not assessed in the routine analysis and that might also be of importance in the pathogenesis of the disease **(4)**.

There is a large group of subjects in the general population at high cardiovascular risk who have normal lipid levels: type 2 diabetics (T2D). They frequently present the so-called atherogenic dyslipidemia, a group with a high prevalence of lipid abnormalities and atherosclerosis. Atherogenic dyslipidemia is characterized by high levels of TG, increased small LDL particles and low HDLc, and is an important risk factor for myocardial infarction **(5)**.

Triglycerides are the driving force of atherogenic dyslipidemia and are a direct consequence of insulin resistance **(6)**. An increased fatty acid flux from adipose tissue triggered by insulin resistance results in hepatic overproduction of large TG-rich very low-density lipoproteins. In circulation and through the action of cholesteryl ester transfer protein (CETP) and hepatic lipase (HL), large VLDL results in cholesterol-depleted HDL and smaller LDL particles. Insulin resistance is a very prevalent health problem worldwide, especially in developed countries, as it is one of the 10 leading causes of mortality in the world, having increased by 70% since 2000 **(7)**.

Therefore, atherogenic dyslipidemia is not only a quantitative alteration of lipoproteins in the blood, but also qualitative, with small particles being the most harmful to the vascular endothelium. However, this characteristic is not measurable by means of a conventional blood test, but requires a specific technique, namely nuclear magnetic resonance **(8)**.

Thus, the onset of insulin sensitivity not only leads to atherogenic dyslipidemia, it represents an early sign of T2D incidence in children and adolescents, where prevalence continues to increase. On the other hand, it is estimated that the percentage of undiagnosed individuals is greater than 50%, so further investigation of the disease and its pathophysiology is necessary in order to achieve an earlier diagnosis and try to predict its evolution.

One of the tools for assessing insulin resistance and predicting the risk of diabetes and metabolic syndrome is the HOMA Index, calculated mainly from analytical values of glucose and insulin and useful for assessing the function of pancreatic beta cells, since under normal conditions there is a balance between hepatic production of glucose and pancreatic secretion of insulin **(9)**.

While lipoprotein profile is normally assessed as part of routine health check, insulin sensitivity is not.

Currently, the routine study of lipid levels is very systematized globally, unlike insulin levels, which are not usually requested in a conventional analysis. Since insulin resistance promotes certain qualitative changes in the lipoprotein profile, the aim of this study is to detect early signs of insulin resistance through a combination of changes in lipid and lipoprotein parameters determined by NMR.

MATERIALS AND METHODS

Study design, setting and population. Samples and data were based on the population-based, cohort study Di@bet.es epidemiological trial.

The initial cross-sectional study of the Di@bet.es was undertaken in 2008–2010 from a random cluster sampling of the Spanish population **(10)**. The Di@bet.es study sample consisted of 5072 subjects more than 18 years old, randomly selected from National Health System registries distributed into 100 clusters. Subjects with severe disease such as cancer or hepatitis were excluded by protocol. Detailed information on the methodology of the Di@bet.es cohort study has been previously described **(11)**.

The research was carried out in accordance with the Declaration of Helsinki (WHO 2011) of the Word Medical Association. Written informed consent was obtained from all the participants. The study was approved by the Ethics and Clinical Investigation Committee of the Hospital Regional Universitario de Málaga (Malaga, Spain) in addition to other regional ethics and clinical investigation committees all over Spain.

Data collection and laboratory measurements. Participants were invited to attend an examination visit at their health center with a nurse specially trained for this project. Information was collected using an interviewer administered structured questionnaire, followed by a physical examination and blood sampling. Anthropometric and sociodemographic variables were collected.
Insulin resistance was estimated by the homeostasis model assessment (HOMA), and the HOMA 75th percentile of our population excluding subjects with T2D was calculated as the insulin resistance risk category (HOMA-IR).

Lipoprotein analysis. Before 1H-NMR analysis, 200 µl of serum were diluted with 50 µl deuterated water and 300 µl of 50 mM phosphate buffer solution (PBS) at pH 7.4. 1H-NMR spectra were recorded at 306 K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600.20 MHz.

Lipoprotein analysis was made by using Liposcale® Test, a novel advanced lipoprotein test based on 2D diffusion-ordered 1H-NMR spectroscopy. The methyl signal was deconvoluted by using 9 lorentzian functions to determine the lipid concentration of the large, medium and small subclasses of the main lipoprotein classes (VLDL, LDL and HDL), and their size associated diffusion coefficients. Then, we combined the lipid concentration with their associated particle volume in order to quantify the number of particles required to transport the measured lipid concentration of each lipoprotein subclass. Finally, weighted average VLDL, LDL and HDL particle sizes were calculated from various subclass concentrations by summing the known diameter of each subclass multiplied by its relative percentage of subclass particle number. The variation coefficients for particle number were between 2% and 4%, and for the particle sizes were lower than 0.3%.

Statistical analysis: statistical software SPSS, version 23 was use to analysed all data. Continuous variables normally distributed are presented as the mean and standard deviation (SD). Categorical variables are presented as the percentage and the number of individuals. Bivariate correlations were estimated using Pearson correlation coefficient for normal variables and Spearman correlation coefficient for non-normally distributed variables. p-value of <0.05 was considered statistically significant.
The HOMA variable was defined as LipoINRE formed by the words: lipoprotein (LIPO), insulin (-IN-) and resistance (-RE). To estimate the HOMA variable, stepwise multiple linear regression models were adjusted with the addition of Liposcale variables and known confounders. The R-squared (R^2) statistic was used to select the best model fitted and to provide an estimate of the percentage of the HOMA variable that was explained by the linear model.

RESULTS

The Di@bet.es cohort is composed by a total of 5072 participants. Since HOMA index was not normally distributed among the entire cohort because of some participants with extremely high HOMA index, we selected those with HOMA values ≤5, thus we

have finally studied a total of 3985 subjects. The baseline characteristics are summarized in **Table 1.**

Table 1.

	N=3985
Anthropometric data	
Age (years)	49.98 (17.01)
Gender (% men)	42.1
Body mass index (kg/m^2)	27.65 (4.87)
Routine lipid parameters	
Total cholesterol (mmol/L)	5.05 (1.03)
LDL cholesterol (mmol/L)	2.70 (0.77)
HDL cholesterol (mmol/L)	1.35 (0.33)
Triglycerides (mmol/L)	1.30 (0.81)
NMR lipid profile	
VLDL particles (nmol/L)	50.56 (35.62)
large VLDL particles (nmol/L)	1.32 (0.84)
medium VLDL particles (nmol/L)	4.56 (4.28)
small VLDL particles (nmol/L)	44.67 (32.04)
LDL particles (nmol/L)	1388.7 (275.67)

large LDL particles (nmol/L)	190.15 (36.75)
medium LDL particles (nmol/L)	405.48 (136.58)
small LDL particles (nmol/L)	793.1 (164.41)
HDL particles (micromol/L)	28.66 (5.02)
large HDL particles (micromol/L)	0.27 (0.04)
medium HDL particles (micromol/L)	9.35 (1.62)
small HDL particles (micromol/L)	19.03 (4.07)
VLDL diameter (nm)	41.97 (0.40)
LDL diameter (nm)	20.99 (0.34)
HDL diameter (nm)	8.26 (0.09)
Insulin resistance	
Glucose (mmol/L)	4.59 (1.20)
Insulin	1.01 (0.08)
HOMA index	1.89 (0.98)

Data is presented as the mean and standard deviation (SD).

With regard to the participants who took part in the study, a series of characteristics should be highlighted. The mean age is 49.98 years, 57.9% of the participants are women and 42.1% are men. The mean BMI is 27.65 kg/m², in the overweight range. At the level of lipid parameters measured by routine analysis, the mean in our participants is in the normal range.

As for lipids values measured by NMR, total LDL, large, medium and small LDL particles are altered, unlike VLDL and HDL which are within normal values.

At the level of glucose metabolism, mean glucose values are in normal ranges. On the other hand, the mean insulin ranges are decreased. The mean values of the HOMA index of insulin resistance are within the normal range.

ESTIMATION of HOMA INDEX based on lipoprotein concentration and diameter

Lineal regression models were performed to identify those lipoprotein parameters determined by NMR that best estimate the HOMA index. The model including the following 9 lipid parameters (total VLDL particles, large VLDL particles, small VLDL particles, VLDL diameter, large LDL particles, medium LDL particles, total HDL particles, large HDL particles and HDL diameter) showed a significant association with HOMA index (R^2: 0,17; p<0,001).

The beta coefficients of the nine variables included in the model are depicted in **Table 2**. Total VLDL, large VLDL, medium LDL and large HDL particles are associated with higher HOMA index, while small VLDL, large LDL, total HDL, and size of VLDL and HDL particles, are associated with lower HOMA index. And large HDL particles having the greatest influence on the HOMA index of insulin resistance.

Table 2.

	b	**95% CI**	**p**
VLDL particles (nmol/L)	0.016	0.004 / 0.028	0.007
Large VLDL particles (nmol/L)	0.284	0.180 / 0.389	< 0.001
Small VLDL particles (nmol/L)	-0.019	-0.032 / -0.006	0.005
VLDL diameter (nm)	-0.376	-0.485 / -0.266	< 0.001

Large LDL particles (nmol/L)	-0.007	-0.009 / -0.005	< 0.001
Medium LDL particles (nmol/L)	0.002	0.001 / 0.002	< 0.001
HDL particles (micromol/L)	-0.065	-0.075 / -0.056	< 0.001
Large HDL particles (micromol/L)	5.546	4.145 / 6.948	< 0.001
HDL diameter (nm)	-1.754	-2.209 / -1.299	< 0.001

Adjusted b linear regression estimates with 95% confidence intervals of the association of NMR lipid parameters with HOMA index.

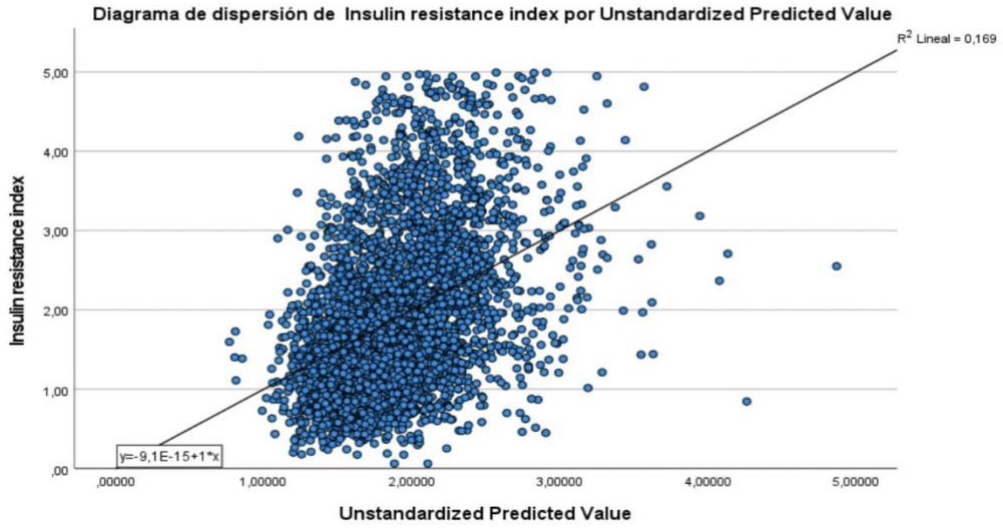
CORRELATION BETWEEN THE SURROGATE INDEX, HOMA INDEX and BMI

Those 9 lipid parameters identified, were used to create a new surrogate index based on lipid parameters equivalent to HOMA index called LipoINRE.

The LipoINRE was obtained after summing up the beta coefficient multiplied to the value of each 9 lipid parameter identified previously.

LipoINRE correlated significantly and positively with HOMA index (r=0,43; p<0,001) **(Figure 1).**

Figure 1.



Correlation between HOMA index (insulin resistance index) and LipoINRE (unstandardized predicted value).

We then tested the correlation between LipoINRE with those confounders clearly influencing lipid parameters. LipoINRE positively and significantly correlated with BMI (r=0,37; p<0,001) and it remained significant after adjusting for age (r=0,31; p<0,001). **Table 3**

Table 3.		HOMA index	LipoINRE index
LipoINRE index	r	**0.43**	1.000
	p	**< 0.001**	.
BMI	r	0.51	**0.37**
	p	< 0.001	**<0.001**

Correlation between LipoINRE and HOMA index and BMI.

We then stratified the correlation of LipoINRE with HOMA index and BMI according to gender and obesity. LipoINRE positively and significantly correlated with HOMA index and BMI both in men (r=0.40; p<0.001 and r=0.31; p<0.001, respectively) and in women (r=0.43; p<0.001 and r=0.37; p<0.001, respectively), and it remained significantly when adjusted for age. **Table 4**

Table 4.			HOMA index	IMC
Men	**LipoINRE index**	r	**0.40**	**0.31**
		p	**<0.001**	**<0.001**
Women	**LipoINRE index**	r	**0.43**	**0.37**
		p	**<0.001**	**<0.001**

Correlation between LipoINRE and HOMA index and BMI according to gender.

The correlation between LipoINRE and HOMA index was positive and significant and it was stronger as the BMI increased (r=0.207, p<0.001 in thin subjects; r=0.31, p<0.001 in overweight subjects; and r=0.37, p<0.001 in obese subjects), and it remained significantly when adjusted for age. **Table 5**

Table 5.			LipoINRE Index	HOMA index
Thin	LipoINRE index	Correlation	1.000	**0.207**
		p	.	**< 0.001**
Overweigth	LipoINRE index	r	1.000	**0.31**
		p	.	**< 0.001**
Obese	LipoINRE index	r	1.000	**0.37**
		p	.	**< 0.001**

Correlation between LipoINRE and HOMA index and BMI according to BMI classification.

DISCUSSION

Insulin resistance triggers lipid and lipoprotein alterations characteristic of atherogenic dyslipidemia in subjects with type 2 diabetes mellitus conferring high cardiovascular risk. Some of these changes are only detectable using advanced methodologies such as NMR **(12)**.

The HOMA index is the most widely used marker to assess the status of insulin sensitivity and is based on glucose and insulin levels. Our aim was to describe a new metabolomic index of lipoprotein insulin resistance based on the lipid and lipoprotein parameters obtained with NMR.

Throughout the study we developed a "LipoINRE index" based on a weighted score of the 9 lipid profile variables obtained by the use of NMR technology that significantly estimate the HOMA index. Some of these variables estimate elevations of the HOMA index (total VLDL, large VLDL, medium LDL and large HDL particles) while some estimate decreases of HOMA (small VLDL, large LDL, total HDL particles, VLDL size and HDL size). Most of these associations are in accordance with the disturbances in lipid metabolism linked to insulin resistance **(13)**.

In further detail, under insulin resistance conditions, the adipose tissue is unable to properly control lipid storage and induces an increased fatty acid efflux, which promotes increased synthesis and secretion of TG-rich lipoproteins (VLDL) by the liver **(14)**. These VLDLs are larger, contain more TG and possess a greater proportion of proteins such as apoC-III compared with apoC-II; therefore, the enzyme lipoprotein lipase is not normally active and less hydrolysis of TG occurs **(15)**. The derived LDL particles are poorly recognized by both the LDLR and the LRP (LDLR-related protein), which are responsible for the elimination of these particles from the circulation **(16)**.

This increases the time that these lipoproteins remain in the circulation and allows for the formation of smaller LDL and remnant particles because of the action of enzymes such as CETP (cholesteryl ester transfer protein) and HL (hepatic lipase). These small dense LDL particles are highly susceptible to oxidation and are more atherogenic, as they are associated with at least a 3-fold increase in CVD risk **(17)**.

CETP and HL also promote changes that cause large cholesterol-rich HDL particles to become TG-rich and cholesterol poor **(18)**; the hydrolysis of TG induces rapid changes that yield much smaller HDL particles, which are subject to renal excretion due to their smaller sizes.

The "LipoINRE index" positively correlated with the HOMA index. These correlations were independent of age, and were stronger among women and among overweight and obese subjects.

Since insulin resistance is an early step of the dyslipoproteinemia and metabolic complications associated with T2D promoting CVD, identifying markers for early detection in groups of interest is of critical importance **(19)**.

In this sense, the fact that the “LipoINRE index” seems to be more sensitive to insulin resistance in women is very interesting since men are traditionally more a matter of concern because they have higher risk of atherosclerosis and CVD. This is in accordance to previous studies suggesting that HOMA thresholds should be adapted to women **(20)**.

Finally, the “LipoINRE index” correlates more strongly in individuals with a higher BMI, and this relation is supported by the well-known association between dysfunctional adipose tissue and insulin resistance **(21)**.

We believe that the limitations of the study are reflected by the following: the use of nuclear magnetic resonance for the study of lipid parameters is currently not fully implemented. It is currently only used for research purposes. In addition, there is currently insufficient scientific evidence to make this method of lipid and cardiovascular risk assessment cost-effective.

The main strength of this study, we believe, is the fact that few studies currently offer such significant empirical data in relation to obtaining the risk of insulin resistance and type 2 diabetes mellitus from lipid data measured by nuclear magnetic resonance.

We believe that these results can provide a starting point to promote research on the "LipoINRE index" and its possible application to clinical practice by means of NMR lipid measurements alone.

On the other hand, it should be noted that research on this subject, at the international level, is still very scarce, so this work makes a substantial contribution to the scientific literature currently available.

In addition, this study provides results with great statistical power, since the results have been obtained from a final sample of 3985 patients.

CONCLUSIONS

In summary, nowadays, lipid results from routine blood test, are not sufficiently detailed to fully characterize the risk associated to insulin resistance, type 2 diabetes mellitus, arteriosclerosis and cardiovascular disease, since it has been shown that a lipoprotein study by nuclear magnetic resonance can reveal a lipid profile at risk **(8)**.

Therefore, this new surrogate for insulin resistance is a complementary test that could be introduced at hospital level for early diagnosis and better primary prevention, especially in women, the obese and the elderly. However, it is also statistically significant in males, people with normal or low BMI and young people.

For this reason, we believe that this study contains findings of great importance in a hitherto unknown area, representing a possible statistically significant starting point for future research and to be able, in the future, to obtain sufficient scientific evidence on its usefulness and possible applicability at the clinical level.

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