Departamento de Medicina

METABOLISMO ENERGÉTICO POSTPRANDIAL Y SU RELACIÓN CON EL SÍNDROME METABÓLICO EN PACIENTES CON ENFERMEDAD CORONARIA

POSTPRANDIAL LIPID METABOLISM AND ITS RELATIONSHIP WITH METABOLIC SYNDROME IN PATIENTS WITH CORONARY DISEASE

TESIS DOCTORAL
JUAN FRANCISCO ALCALÁ DÍAZ
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TITULO: *Metabolismo energético postprandial y su relación con el síndrome metabólico en pacientes con enfermedad coronaria*

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TÍTULO DE LA TESIS: METABOLISMO ENERGÉTICO POSTPRANDIAL Y SU RELACIÓN CON EL SÍNDROME METABÓLICO EN PACIENTES CON ENFERMEDAD CORONARIA.

DOCTORANDO/A: JUAN FRANCISCO ALCALÁ DÍAZ

INFORME RAZONADO DEL/DE LOS DIRECTOR/ES DE LA TESIS

(se hará mención a la evolución y desarrollo de la tesis, así como a trabajos y publicaciones derivados de la misma).

D. JOSÉ LÓPEZ MIRANDA, CATEDRÁTICO DE MEDICINA DEL DEPARTAMENTO DE MEDICINA DE LA UNIVERSIDAD DE CÓRDOBA Y D. JAVIER DELGADO LISTA, PROFESOR DEL DEPARTAMENTO DE MEDICINA DE LA UNIVERSIDAD DE CÓRDOBA,

HACEN CONSTAR:

Que el trabajo titulado "METABOLISMO ENERGÉTICO POSTPRANDIAL Y SU RELACIÓN CON EL SÍNDROME METABÓLICO EN PACIENTES CON ENFERMEDAD CORONARIA" ha sido realizado por D. JUAN FRANCISCO ALCALÁ DÍAZ, bajo nuestra dirección, en el Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), dentro del grupo GC9: NUtrigenómica. Síndrome Metabólico.

Este trabajo ha conseguido un nivel científico de suficiente relevancia como para derivar en la publicación de tres artículos en revistas internacionales, incluidas dentro de Q1 de su categoría (Atherosclerosis. 2013 Jan;226(1):258-62; PLoS One. 2014 May 6;9(5):e96297; J. Eur J Clin Invest. 2014 Nov;44(11):1053-64), con unos índices de impacto de 3.971, 3.23 y 2.734 respectivamente en el momento de su publicación.

A nuestro juicio, reúne los méritos suficientes para ser defendido ante el tribunal correspondiente y poder optar al grado de Doctor.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 28 de NOVIEMBRE de 2016

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ABSTRACT

INTRODUCTION

Postprandial lipemia (PPL) influences the development of atherosclerosis. However, there are still significant gaps to fully understanding of postprandial metabolism and its regulating factors.

OBJECTIVES

Main objective: To determine whether metabolic syndrome (MetS) traits influence the PPL of coronary patients, and whether this influence depends on the number of MetS criteria.

Secondary objectives: 1) To investigate whether the number of criteria of metabolic syndrome may predict the degree of postprandial response in patients with normal fasting triglycerides (TGs); 2) To determine the exact contribution of the presence of MetS to age-associated enlarged PPL; 3) To explore the phenotypic flexibility of high risk patients, measured with an oral fat tolerance test (OFTT), according to different cardio-metabolic abnormalities and body mass index (BMI).

METHODS

We developed two independent studies: A first one where we compared the PPL response to a rich fatty meal of 88 healthy young men (<30 years old) and 97 older participants (77 MetS patients aged > 40; and 20 healthy people > 65) (all ApoE3/E3), at fasting state and at 2nd and 4th postprandial hours; and a second one where 1002 coronary artery disease patients from the CORDIOPREV study were submitted at the beginning of the study to an OFTT with 0.7 g fat/kg body weight and serial blood test analyzing lipid fractions were drawn at 0, 1, 2, 3 and 4 hours during the postprandial state. Patients were classified according to the presence of MetS and the number of its traits. We also explored in that cohort the dynamic response according to six body size phenotypes: (i) normal weight, metabolically healthy; (ii) normal weight, metabolically abnormal; (iii) overweight, metabolically healthy; (iv) overweight, metabolically abnormal; (v) obese, metabolically healthy; and (vi) obese, metabolically abnormal.

RESULTS

In the first study, we didn't find differences between the healthy young men and the healthy elderly. MetS patients displayed a higher postprandial TG area below the curve than the other two cohorts $p < 0.001$. In the CORDIOPREV study, PPL response was directly related to the presence of MetS. We found a positive association between the number of MetS criteria and the response of postprandial plasma TGs ($p= 0.001$), area under the curve (AUC) of TGs ($p= 0.001$) and incremental AUC of TGs ($p= 0.001$). However, the influence of them on postprandial TGs remained statistically significant only in those patients without basal hypertriglyceridemia. Only fasting TGs, fasting glucose and waist circumference appeared as significant independent contributors ($p < 0.05$). Metabolically healthy patients displayed lower PPL compared with those metabolically abnormal, independently whether or not they were obese ($p < 0.001$ and $p < 0.01$, respectively).

CONCLUSIONS

MetS may account for the differences in PPL that have been attributed to age. Fasting TGs are the major contributors to the postprandial TGs levels. MetS influences the PPL in patients with coronary heart disease, particularly in non-hypertriglyceridemic patients. Finally, our findings showed that certain types of the metabolic phenotypes of obesity are more favorable modulating their response to a fat load test. To identify these phenotypes may be the best strategy for personalized treatment of obesity.

RESUMEN

INTRODUCCIÓN

La lipemia postprandial (LPP) influye en el desarrollo de arteriosclerosis. Sin embargo, existen importantes áreas de incertidumbre para comprender completamente el metabolismo energético postprandial y sus factores reguladores.

OBJETIVOS

Objetivo principal: Determinar si los rasgos de síndrome metabólico (SMet) influyen en la LPP en pacientes coronarios, y si esta influencia depende del número de criterios presentes de SMet.

Objetivos secundarios: 1) investigar si el número de criterios de SMet puede predecir el grado de respuesta postprandial en pacientes con triglicéridos (TGs) plasmáticos en ayunas normales; 2) determinar la contribución exacta de la presencia de SMet en la respuesta anormal de LPP asociada a la edad; y 3) explorar la flexibilidad fenotípica de pacientes de alto riesgo, medida a través de un test de sobrecarga oral de grasa (TSOG), de acuerdo a diferentes anormalidades cardiometabólicas y al índice de masa corporal (IMC).

MÉTODOS

Se han desarrollado dos estudios independientes: un primer estudio comparando la respuesta en la LPP a una comida rica en grasa en 88 hombres jóvenes sanos (<30 años) y 97 participantes mayores (77 pacientes con SMet >40 años, y 20 sanos >65 años) (todos ApoE3/E3), en ayunas y a las 2 y 4 horas tras la sobrecarga; y un segundo estudio donde 1002 pacientes con enfermedad coronaria pertenecientes al estudio CORDIOPREV completaron al inicio del mismo un TSOG con 0.7 g de grasa/kg, con extracciones sanguíneas a las 0, 1, 2, 3 y 4 horas durante el estado postprandial. Los pacientes fueron clasificados según la presencia de SMet y el número de sus criterios. También se exploró en esta cohorte de pacientes coronarios su respuesta dinámica de acuerdo a seis fenotipos diferentes corporales: (i) normopeso, metabólicamente sano; (ii) normopeso, metabólicamente enfermo; (iii) sobrepeso, metabólicamente sano; (iv) sobrepeso, metabólicamente enfermo; (v) obeso, metabólicamente sano; y (vi) obeso, metabólicamente enfermo.

RESULTADOS

En nuestro primer estudio, no encontramos diferencias entre los varones sanos jóvenes y los mayores sanos. Los pacientes con SMet mostraron mayor magnitud en la respuesta de TGs postprandiales que los otros dos grupos ($p < 0.001$). En el estudio CORDIOPREV, la magnitud en la respuesta de la LPP se relacionó directamente con la presencia de SMet. Encontramos una asociación positiva entre el número de criterios de SMet y la respuesta de TGs plasmáticos postprandiales ($p= 0.0001$), ABC de TGs ($p= 0.0001$) y el incremento del area bajo la curva (AUC) de TGs plasmáticos ($p= 0.001$). La influencia de estos criterios sobre los TGs postprandiales sólo se mantuvo significativa en aquellos pacientes sin hipertrigliceridemia basal. Tan sólo la cifras de TGs y glucosa en ayunas, así como el perímetro de cintura se mantuvieron como predictores independientes significativos de LPP ($p < 0.05$). Los pacientes metabólicamente sanos mostraron menor LPP comparados con aquellos metabólicamente enfermos, independientemente de si eran obesos o no ($p< 0.001$).

CONCLUSIONES

La presencia de SMet puede modular las diferencias en LPP que han sido atribuidas a la edad. Las cifras de TG en ayunas son el factor que más influye en el grado de respuesta de TGs plasmáticos postprandiales. El SMet influye en la magnitud de la LPP de pacientes coronarios, especialmente en aquellos sin hipertrigliceridemia basal. Finalmente, nuestros hallazgos muestran que ciertos fenotipos metabólicos de obesidad son más favorables modulando su respuesta a un TSOG. Identificar estos fenotipos podría constituir la mejor estrategia para un tratamiento personalizado de la obesidad.

INDEX

Chapter 1

INTRODUCTION

I. Chapter 1: INTRODUCTION

Cardiovascular disease (CVD) is one of the major causes of death and young age disability in developed countries, accounting for more than 30% of all deaths(1). Although there are entities, such as coronary vasospasm or sudden death that have a special pathophysiology, the underlying pathological mechanism of CVD in most cases is atherosclerosis. This slow and almost irreversible closeness of the vessel lumen develops insidiously over the years and is closely related to lifestyle risk factors (modifiable) and risk markers (unmodifiable). To date, the most recognized CVD risk factors, also known as classic risk factors, are smoking(2), hypertension(3), hyperlipidemia(4), obesity(5) and diabetes(6). The relation of these factors with CVD has been stated as causal.

Although classic risk factors have been identified to play an important role in the development of atherosclerosis, they cannot account for the entire risk for incident coronary events. Furthermore, the strict control of these factors in clinical trials leads to a modest decline in CVD rate. This factor is known as residual risk, and may account up to 33%(7, 8). Thus, emerging, “novel”, or nontraditional cardiovascular risk factors have been proposed, in an effort to improve risk assessment for CVD. They include postprandial hypertriglyceridemia(9), C-reactive protein (CRP)(10), carotid intima-media thickness (CIMT)(11), homocysteine(12) or lipoprotein (a) (Lp(a))(13) among others(14).

At population level, there are different entities that have been linked to a higher risk for CVD. Among these, elevated age and disturbed metabolism/obesity are a current focus of research. Two factors may be behind this interest: First, the global increase in life expectancy, and the importance towards achieving a healthy aging. Second, the increased prevalence of obesity worldwide, and the related Metabolic Syndrome (MetS).

The so called MetS describes a clustering of risk factors for cardiovascular disease and type 2 diabetes mellitus (T2DM) including dyslipidemia, glucose intolerance, hypertension and central obesity. As stated above, this entity is increasing its prevalence to epidemic proportions worldwide and the health care costs and burden are enormous. As an example, MetS in Europe in adults includes over 30%(15), and in Spain 31% in general population and 50% among cardiovascular patients(16, 17). Although the importance of MetS includes a two-fold risk of cardiovascular disease or five-fold risk for T2DM(15, 18), the exact underlying mechanisms of the complex pathways of the MetS are still to be fully known.

Increased body mass index has been also associated with excess mortality risk(5). Obesity is a chronic disease which has an outstanding impact on public health due to its increasing prevalence and to the high impact on cardiometabolic diseases. However, recent evidence suggests that not all obese subjects display a clustering of metabolic and cardiovascular risk factors, and, likewise, not all lean subjects present a healthy metabolic and disease-free profile. Thus, recently more attention has been paid to the different metabolic phenotypes of obesity, suggesting that individuals in the same body mass index (BMI) category can have substantial heterogeneity on their metabolic control(19). These metabolic faults become more easily evident in the situations in which there is an increased stress of the metabolic pathways, such as the postprandial state. This fact may support the

idea that obesity is a multi-systemic disease with loss of flexibility in one or more metabolic processes involved(20). It has been shown that the persons with a given BMI with a poorer metabolism control have a higher risk for developing T2DM and CVD(21).

Linking the two above metabolic diseases, one of the current lines of study on the pathophysiology of CVD is postprandial lipemia (PPL). PPL is the period of time after a fatty meal (>40 mg fat) in which dietary lipids are digested and metabolized until they return their levels in blood to baseline. Additionally to the increase of lipids, PPL is a situation characterized by the generation of an atherogenic environment in the bloodstream. This fact is derived by the conjunction of the direct atherogenic properties of some lipid particles, especially those carried in the triglyceride-rich lipoproteins (TRLs), and by the activation of the inflammatory and hemostatic system(22). A disregulation of this capacity of the body to deal effectively with the increase of postprandial lipids has been identified as loss of “phenotypic flexibility”(23), and highlights the fact that, although the metabolic machinery of a subject may work properly, there may be a problem to overcome to a metabolic overload, and, during that hours, biochemical, inflammatory and haemostatic factors may increase CV risk. This altered PPL is not only circumscribed to metabolic Syndrome or to certain altered metabolic phenotypes, but may be an isolated phaenomenon, which makes even harder to identify subjects at a high cardiovascular risk, because probably they would never conduct a posptprandial lipid determination, and, hence, they would remain undiagnosed. A fact that speaks about the importance of PPL in human metabolism is that due to the several meals ingested over the day, and considering that PPL lasts 8-12 hours, humans of modernized countries spend most of their lives in postprandial situation.

In line with the above, recent studies have even suggested that the evaluation of the postprandial lipemic response may be important to identify disturbances in lipid metabolism and correlates better with CVD risk than measurements taken in the fasting state. Large population studies (e.g. Women´s Health Study and the Copenhagen City Heart Study) have assessed the association between non-fasting triglycerides (TGs) and the risk of cardiovascular disease (CVD) events, and they state that postprandial TG levels are excellent markers of risk for coronary artery disease, peripheral vascular disease and cerebrovascular disease. In this regard, it has been shown that non-fasting TG (5 mmol/L vs. <1 mmol/L, 438.6 mg/dL vs. 87.7 mg/dL) confer an increased risk of myocardial infarction, ischemic stroke and risk of early death in women and men in the general population(24-28).

Many factors have been described influencing postprandial metabolism(29). Dietary background has been identified as the main extrinsic factor influencing postprandial lipemia(30, 31), while genetics, gender and age have been identified as intrinsic modifiers(30, 32, 33). The influence of gender or genetic factors is relatively easy to study, due that the factor studied is well characterized. However, the study of the cause of the increased PPL associated to age is much more complex. Aging is a process associated to other CVD risk factors, and age is often associated to related conditions, such as the appearance of metabolic syndrome. The exact contribution of the presence of metabolic syndrome to age-associated enlarged postprandial lipemia has not been explored deeply in the literature.

The purpose of this thesis is to revise the knowledge and expose new findings regarding the regulation of PPL metabolism. We will focus on age associated PPL changes, and study

the consequences of human metabolism disruption. To study this, we will explore two situations. First, the well stablished model of MetS. Other, the so called body size cardiometabolic phenotypes, a relatively novel classification of metabolic status based on the addition of inflammatory and insulin resistance status to the classic MetS factors. With this, we aim to further characterize the pathophysiology of postprandial lipemia and identify subpopulations at high CVD risk.

Chapter 2

REVIEW OF LITERATURE

II. Chapter 2: REVIEW OF LITERATURE

A. TRIGLYCERIDE-RICH LIPOPROTEINS AND POSTPRANDIAL LIPEMIA

As stated above, the postprandial state is the period from food intake to post-absorptive state. It is a dynamic condition, with a continuous fluctuation in the degree of lipemia and glycemia over the day, in which there is a rapid continuous remodeling of the lipid levels, and a host of other metabolic adaptations compared to the relatively stable conditions in the fasting state. The duration of the postprandial period depends on the composition of the diet, but typically it reaches its peak between the third and fourth hour, and lasts between 8 and 12h after a fat meal(34).

There is an increasing awareness on the importance of postprandial events in the development and exacerbation of atherosclerosis. Several epidemiological studies have demonstrated that the presence of postprandial hypertriglyceridemia is an independent risk factor for cardiovascular disease. Although this fact will be described in Section 1.4. in detail, in Table 1 we show the epidemiologic studies reporting strong links between remnant cholesterol and atherosclerosis.

Study/First Author [ref.]	Population	Follow-up	Main outcomes	Remarks
Norwegian counties study[178]	43,641 men and 42, 600 women free of CVD	Prospective, 27 years	HRs (95%CI) per 1 mmol/L increase in non-fasting TGs for all causes, CVD, IHD, and stroke mortality: Women: 1.16 (1.13–1.20), 1.20 (1.14–1.27), 1.26 (1.19–1.34) and 1.09 (0.96–1.23) Men: 1.03 (1.01–1.04), 1.03 (1.00–1.05), 1.03 (1.00–1.06) and 0.99 (0.92–1.07).	Adjustment for major cardiovascular risk factors attenuated the effect
Copenhagen City Heart study [177,176,175]	Random population sample of 6391 men and 7581 women	Prospective, 33 years	HRs (95%CI) for total mortality by non-fasting TGs: (TG < 1 mmol/L: HR 1) TG 1.0–1.99 mmol/L: 1.1 (95%CI: 1.0–1.2) in women and 1.1 (95%CI: 1.1–1.2) in men TG 2.0–2.99 mmol/L: 1.3 (95%CI: 1.2–1.4) in women and 1.2 (95%CI: 1.1–1.4) in men TG 3.0–3.99 mmol/L: 1.4 (95%CI: 1.2–1.7) in women and 1.3 (95%CI: 1.1–1.4) in men TG 4.0–4.99 mmol/L: 1.4 (95%CI: 1.1–1.9) in women and 1.4 (95%CI: 1.2–1.6) in men TG > 5 mmol/L: 2.0 (95%CI: 1.5–2.7) in women and 1.5 (95%CI: 1.2–1.7) in men HRs (95%CI) for ischemic stroke by non-fasting TGs: (TG < 1 mmol/L: HR 1) TG 1.0–1.99 mmol/L: 1.2 (95%CI: 0.9–1.7) in women and 1.2 (95%CI: 0.8–1.7) in men TG > 5 mmol/L: 3.9 (95%CI: 1.3–11.1) in women and 2.3 (95%CI: 1.2–4.3) in men	The best predictor for MI in women was non-fasting TG and in men non-fasting cholesterol The remnant cholesterol increased stepwise as a function of non-fasting TG and cholesterol in cross-sectional analysis of 53,629 subjects
The Women's Health study[24]	26,509 initially healthy US women of which 6391 had non-fasting samples	Prospective, 11 years	HR for CVD event by non-fasting TG: 2nd tertile: 1.44 (95% CI 0.90–2.29) 3rd tertile: 1.98 (95% CI 1.21–3.25)	TG measured 2 to 4 h postprandially had the strongest association with CVD events (fully adjusted HR [95% CI] for highest vs. lowest tertiles of levels, 4.48 [1.98–10.15] [P < 0.001 for trend])
The Framing ham study[174]	1567 women offspring of the original Framingham cohort: 83 with and 1484 without CVD	Cross-sectional	RLP-chol + 15.6%; P b 0.0001 and RLP-TG +27.0%; P < 0.0002 in women with prevalent CVD	Adjusted RLP-chol was significantly associated with prevalent CVD in women in logistic regression analysis
Kugiyama et al.[173]	147 consecutive patients with CAD	Prospective follow-up until coronary event or 36 months	OR for developing coronary event: 2nd tertile of remnant levels 2.43 (95%CI: 1.1–5.8) 3rd tertile of remnant levels 6.38 (95%CI:2.3–17.6)	Remnant levels were independent predictors of future coronary event in multivariate model

CI, confidence interval; CHD, coronary heart disease; CVD, cardiovascular disease; HR, hazard ratio; MI, myocardial infarction; OR, odds ratio; RLP, remnant-like particles; TG, triglycerides.

Table 1. Epidemiologic studies reporting non-fasting triglycerides (TG) or remnant lipoproteins and the risk for cardiovascular morbidity and mortality. Adapted from Borén J et al, Clinica Chimica Acta (2014)(9).

The underlying metabolic abnormalities that may explain the increased risk of CVD associated to an enlarged PPL are principally initiated by overproduction and/or decreased catabolism of TRLs. The effects of this unbalance, such as enhanced inflammation, endothelial dysfunction and higher concentrations of atherogenic particles, are summarized in Figure 2.

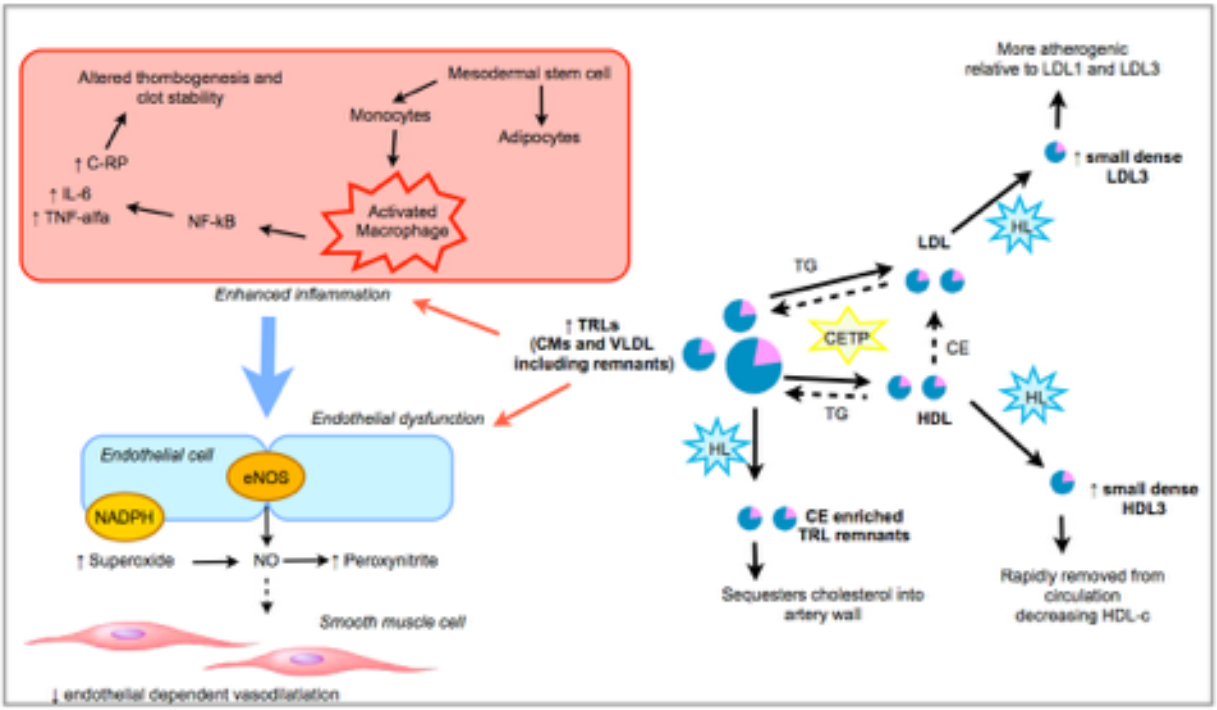

Figure 2. Impact of delayed clearance of triglyceride rich lipoprotein on high density lipoprotein and low density lipoprotein metabolism, inflammation and vascular function. Adapted from Jackson et al, Atherosclerosis (2012)(35). Abbreviations: CE, cholesteryl ester; CETP, Cholesteryl ester transfer protein; CMs, chylomicrons; C-RP, C-reactive protein; eNOS, Endothelial nitric oxide synthase; HDL3, High-density lipoprotein 3; HDL-c, High-density lipoprotein cholesterol; HL, Hepatic lipase; IL-6, Interleukin 6; LDL3, Low-density lipoprotein 3; NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, Nitric oxide; TG, Triglycerides; TNF-alfa, Tumor necrosis factor alpha; TRLs, Triglycerides rich lipoproteins; VLDL, Very-low-density lipoprotein.

1. METABOLISM OF POSTPRANDIAL LIPEMIA

After a meal ingestion, TG are hydrolyzed in the human intestine by lipases to produce fatty acids (FAs) and monoacylglycerol, which are then absorbed into the enterocytes. Into the enterocyte they can be: 1) repacked into chylomicron (CM) lipoprotein particles for distribution to the body tissues, 2) stored within the enterocyte in a lipid droplet or TG storage pool, 3) used for synthesis of cholesteryl esters or phospholipids, and 4) oxidized(9). Although the incorporation of dietary fatty acids into TG or other lipids within the enterocyte may depend on chain length and structure, the majority of dietary fatty acids are processed as TG-rich CM and secreted into lymph(36). Once in the circulation, chylomicrons rapidly undergo hydrolysis to produce cholesterol-dense lipoprotein remnants which are taken up by the liver(37). Eventually, these fatty acids stored in the liver may be reassembled again and returned to the blood as very low-density lipoproteins (VLDL)(38).

The elevation of postprandial triglycerides observed in plasma is due to raised concentrations of chylomicrons, VLDL, and their respective remnants, collectively known as TRLs(29). These TRLs are composed of a core of neutral lipids, mainly TG but also some cholesterol esters, surrounded by a monolayer of phospholipids, proteins and free cholesterol. Each TRL particle has one molecule of apolipoprotein B (ApoB), which is the ligand for the low-density lipoprotein (LDL) receptor(39). ApoB exists in two forms, ApoB-100 and ApoB-48, and both forms are coded by the same gene. ApoB-48 is formed in the intestine through editing of ApoB-100 mRNA by apolipoprotein B mRNA-editing enzyme 1 (apobec-1), which function relies on introducing a stop codon into ApoB mRNA(40). Therefore, the resulting molecule corresponds to the amino-terminal 48% of apoB-100.

Thus, apoB48 is present on CMs and CM remnants, and apoB-100 on VLDL, intermediate-density lipoprotein (IDL) and LDL.

The exogenous CMs and endogenously produced VLDL share the same metabolic pathway. The intravascular lipolysis of these TRLs by lipoprotein lipase (LPL) results in the formation of smaller remnant particles that are TG depleted and enriched in cholesteryl esters(41). TGs are removed from the lipoproteins by LPL allowing the delivery of free FAs to be used by peripheral tissues, such as muscle and adipose tissue. As the TGs are extracted and density increases, CMs become CM remnants, and large TG-rich VLDL1 particles become smaller VLDL2 and subsequently IDL. IDL can be further hydrolyzed by hepatic lipase (HL) to LDL, which is catabolized mainly by hepatic uptake of LDL through LDL receptors. Since the TRLs contain a substantial amount of cholesteryl esters, the smaller remnant particles formed by TG hydrolysis are enriched in cholesteryl esters(9).

Due to limited LPL availability, competition at the level of this enzyme may induce accumulation of TRLs, specially when fasting high TG plasma levels are present. On the other hand, hepatic removal of remnant lipoproteins and direct chylomicron uptake are mechanisms that also determine triglyceride plasma levels. Some studies in T2DM patients have reported that, in this disease, the hepatic uptake of VLDL, IDL and LDL is decreased, resulting in increased plasma residence time of these lipoproteins. Thus, individuals with insulin resistance exhibit an impaired lipid tolerance with a severely delayed postprandial lipemia due to suppressed removal of TRL remnants(42-44).

In summary, the fasting TG levels, the rate of TRLs synthesis, the hydrolysis of TGs mediated by LPL and the hepatic uptake of chylomicron remnants are the cornerstones of

the TRLs metabolism(9). Thus, the inter-individual PPL response can be explained by modification of those elements under genetic or environmental circumstances (Figure 3).

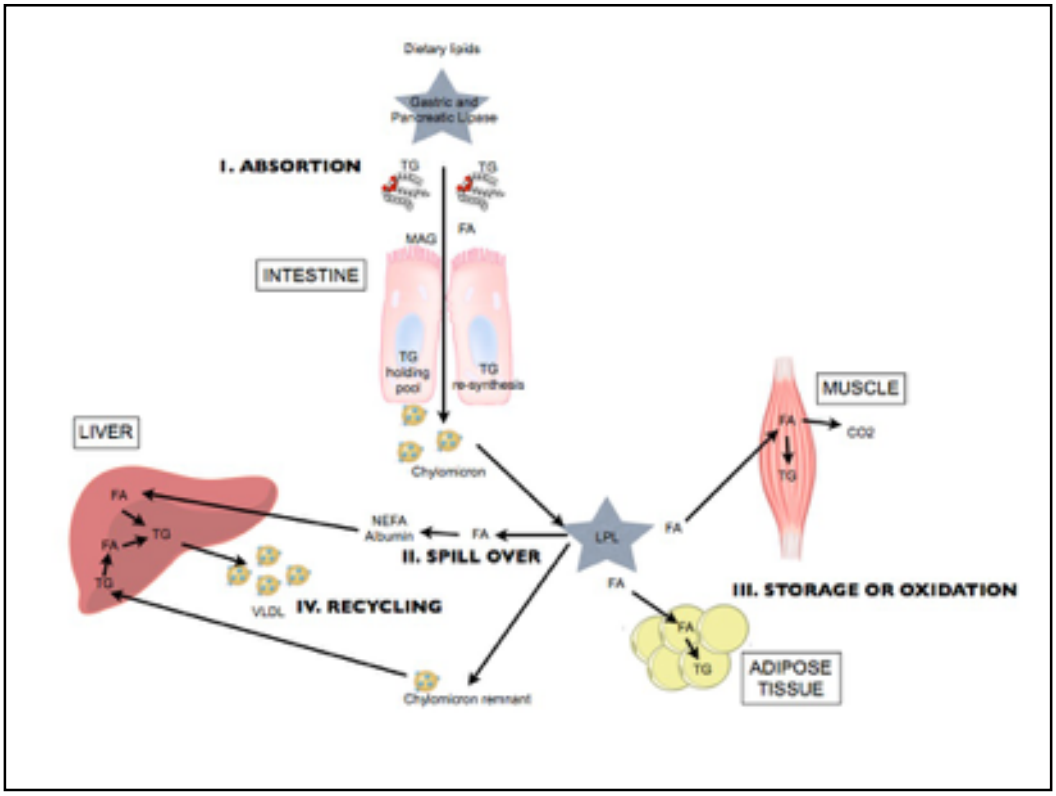

Figure 3. Metabolism of postprandial triglycerides. Adapted from Lambert et al, Biochimica et Biophysica Acta (2012)(36). Abbreviations: CO2, Carbon dioxide; FA, fatty acids; LPL, lipoprotein lipase; MAG, Monoacylglycerol; NEFA, non-esterified fatty acid; TG, Triglyceride; VLDL, Very-low-density lipoprotein.

2. FACTORS AFFECTING THE POSTPRANDIAL LIPEMIA

Several studies have linked the extent of PPL to the incidence of coronary heart disease (CHD), and it has been proposed that it is modulated by dietary patterns, food composition, conditions associated with lifestyle (physical activity, smoking and alcohol consumption), physiological factors (age, gender, genetic background and postmenopausal status) and cardiometabolic conditions such as fasting TGs levels(45-49), T2DM, insulin resistance and obesity (50-52) (FIGURE 4). Of all of these, fasting TG seems to be the strongest predictor, but, in turn, it may be influenced by the rest of the factors. This highlights the fact that, although we will expose the influence of these factors separately, on a pedagogic basis, all the cited factors are intimately related among them.

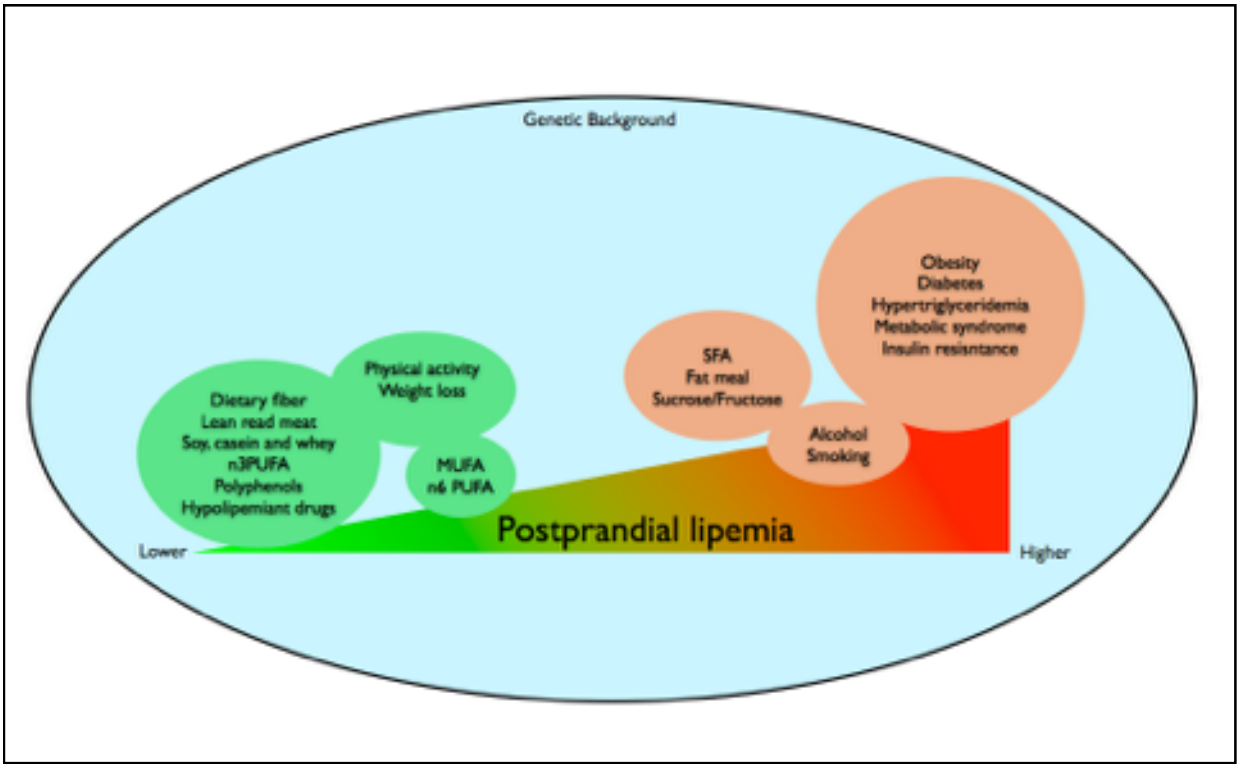

Figure 4. Factors affecting the postprandial lipemia response. Adapted from Klop B et al, Int J Vasc Med (2012)(51). Abbreviations: MUFA, Monounsaturated fatty acids; n3 PUFA, omega 3 polyunsaturated fatty acids; n6 PUFA, omega 6 polyunsaturated fatty acids; SFA, Saturated fatty acids.

2.1. Meal size and composition

Nutrition is the most important environmental factor that modulates PPL. Solid evidences support the fact that postprandial lipoprotein metabolism is modulated by dietary patterns and food composition(50, 52). The PPL is influenced by the amount and type of fat ingested(53-56). Likewise, repeated or consecutive fat meals have been reported to increase PPL due to TRLs accumulation from previous meals(57). This fact is even more important considering that PPL is a phenomenon that takes place during most of the day in developed countries, where the average content of fat is 20–40g/meal with 3-4 meals/ day(29, 58).

Additionally to the size of the meal, the type of fat is a clear determinant of PPL in the short (meal) or long (diet) basis. For example, regarding individual meals, previous studies have shown how fatty acid composition of a single fatty meal modulates TRL particle characteristics, such as their size, number, and apolipoprotein composition. Thus, a meal containing saturated fatty acids (SFAs) causes the most pronounced lipemia, followed by MUFA with polyunsaturated fatty acids (PUFA) causing the least pronounced effect(29, 30). The kinetics of the PPL, also, seems to be dependent on the main fat. MUFA induces higher TG peak with a faster clearance, whereas PUFA, and specially SFA, induce longer PPL time to baseline(30).

On the other hand, chronic intake and dietary supplementation of n-3 PUFA can lower the postprandial TG response as long as a high intake (2.7 – 4 g/d) are given(59). This effect has been related to an increase in endogenous LPL activity, causing in turn a decrease in

the production of TRLs(59-61) and an increased of TG clearance(62, 63). Studies with diets rich in MUFA or n-6 PUFA have been associated with a trend in lower postprandial lipid response compared with SFA(30, 52, 64).

Beyond fats, the amount and the nature of carbohydrates and proteins modulate postprandial lipid response. Diets rich in highly digestible carbohydrates have been related with higher postprandial response as a result of hepatic VLDL and CM remnants accumulation(65, 66). An amplification of the postprandial excursion of serum TG concentration has been reported in test meals with the addition of sucrose or fructose, but not with glucose(67, 68). In addition, in obese insulin-resistant subjects the ingestion of a high-glycaemic index mixed meal, compared with a low-glycaemic index one, has been linked to postprandial accumulation of ApoB-100 and ApoB-48 particles(69). Unlike the previous, lean red meat, soy protein, casein, whey protein and dietary fiber, in the form of oat bran, wheat fiber, wheat germ, or psyllium husk, have been associated with a reduced postprandial lipemic response(29, 30, 52, 70-72). Recent evidences suggest that the presence of certain micronutrients such as plant sterols and polyphenols positively influence fasting and postprandial TRLs in different ways such as reducing TG absorption (with consequent lower circulating apolipoprotein B/TRLs and remnant lipoproteins), lipogenesis, inflammation and oxidative stress(73-75).

2.2. Other Lifestyle factors

Physical activity

PPL can be attenuated by aerobic exercise(76). An acute bout of aerobic exercise significantly reduces PPL by 24 – 35% and increases LPL activity(77, 78). A higher affinity

of VLDL1 for LPL-mediated TG hydrolysis is one of the mechanism proposed to contribute to this TG-lowering effect of exercise(79).

Alternatively, the modulation of the lipemic response to a high fat and carbohydrate meal has been related to the intensity and/or energy expenditure of the preceding exercise(80). A review of 16 studies conducted by Murphy et al. revealed that exercise in short bouts was as effective as continuous exercise at reducing PPL(78). In addition, combining increased physical activity with n-3 PUFA supplementation has been found to have a synergistic effect in reducing PPL in active males(81).

Likewise, we have reported how compliance with moderate-to-high-intensity endurance training enhances the positive effects of a model of Mediterranean Diet on the regenerative capacity of endothelium and on the fitness of MetS patients(82).

Smoking and alcohol

Smoking and alcohol also affect the postprandial metabolism. Epidemiological studies show that low or moderate consumption of alcohol is related with lower postprandial TG concentrations in white population(58, 83). Paradoxically, single doses red wine induce an increased and prolonged postprandial response of TRLs when added to a test meal(84, 85), suggesting an increase in larger, TG-enriched chylomicron particles. However, it is currently unknown whether this unfavorable effect of wine is transient or not, as suggested by studies that have explored the effect of other alcohol drinks, such as vodka, where the effect on PPL was abolished at 12 hours after alcohol intake(86).

It is also stablished that habitual smokers have greater increases in postprandial plasma TGs than non-smokers, and that fact has been related to a defective clearance of CMs and their remnants induced by smoking(87). The exact mechanism that explain that fact is still unknown. Although some authors have proposed inflammation and insulin resistance as potential "drivers" for this phenomenon, studies where the effect of smoking on PPL was controlled by homeostatic model assessment of insulin resistance (HOMA-IR) and/or inflammatory parameters still showed smoking as a significant determinant(88) Some other proposed mechanisms include an increase in oxidative stress by components of cigarette smoke or a reduction in the binding of peroxisome proliferator activator receptors (PPAR) by fatty acids(88)

2.3. Physiological factors

Age

Age has been related to postprandial triglyceridemia. An early study (1988) performed in 22 non-diabetic subjects (9 males, 13 females, 22-79 yr old) showed a correlation between age and the postprandial TG response to a fatty meal (89). Later studies have reported that this fact may be resulting from a delayed clearance intestinally derived TRLs in older subjects by a decrease in LPL activity(90, 91). However, there are not many studies of age influence in PPL when other covariates are controlled. In most of those studies, subjects in the older groups exhibited some of the metabolic syndrome traits, which is logical if we take into account that the prevalence of metabolic syndrome clearly increases with age(92).

Gender

Gender modulates postprandial metabolism. Thus, several studies have demonstrated that men have higher levels of fasting and postprandial TG than premenopausal women(89, 93). Diurnal TG profiles had been shown to be lower in lean females than in lean males, with a mitigation on these gender differences in insulin resistance and overweight subjects(94). However, in MetS patients, men still have a more pronounced postprandial hypertriglyceridemia and seem to have delayed TG clearance(95).

This gender protective effect has been related to a higher clearance capacity in women caused by an oestrogen-induced increase in LPL activity(96), and softened in postmenopausal women(97).

2.4. Pathological conditions

Obesity

Obesity has been associated with several metabolic abnormalities including fasting and postprandial dyslipidemia(98). Furthermore, obese individuals have a greater postprandial TG response after a fat meal compared to non-obese control patients, even in the absence of fasting hypertriglyceridemia(64, 99). This effect is due to the lower level of activity of LPL and the diminished ability to remove remnant particles as BMI increases(100). However, recent evidences suggest that not all obese patients display the same metabolic and cardiovascular risk factors, and probably more studies that explore how the different phenotypes of obesity behave after fat overload are needed. Interestingly, some authors have proposed that inflammation state could be the link explaining that fact. In this regard, our group have reported previously how some variations on inflammatory genes (i.e. IL1b) regulate fasting and postprandial lipids and we have hypothesized that patients with those

gene variations may have a higher inflammatory status and may over-respond to the pro-inflammatory stimulus that represents a fatty meal(101).

Hypertriglyceridemia

The fact that fasting TG concentration is the main determinant of postprandial response is widely supported in the literature, and it has been described in many different populations(46-49). A reduction in LPL activity has been indicated as the cause to this exaggerated and prolonged postprandial lipid response(30). Moreover it has been proposed that in those situations where the liver induces overproduction of VLDL (such as central obesity, MetS, T2DM or familial combined hypercholesterolemia), VLDL and CMs catabolic mechanisms are saturated(51, 102, 103). This mechanism causes VLDL and CMs remnants accumulation, a lower concentration of high-density lipoprotein cholesterol (HDL-c) and the activation of leukocytes and endothelial cells by remnants and fatty acids(104).

Insulin resistance and type 2 diabetes mellitus

Two important conditions that modulate the postprandial metabolism are insulin resistance and T2DM. Both conditions are associated with increased PPL(105). Besides glucose impaired metabolism, insulin resistance leads to disturbed lipid metabolism, including elevated levels of fasting and postprandial TGs, low HDL-c levels and low LDL-particle diameter(106). Hyperinsulinaemia by itself delays and exacerbates postprandial accumulation of intestinally derived CMs in plasma and thus is involved in the regulation of apoB-48-TRL metabolism(107). Although the underlying mechanisms are not entirely understood, it has been also proposed an aberrant insulin-mediated suppression of hepatic VLDL production and fatty acid release from adipose tissue(108). Our group has recently

reported that prediabetic patients show higher postprandial TG response compared with those non-diabetic patients after a fat overload(109).

Thus, it seems clear that the PPL response increases progressively according as the glycemic control worsens, according to the scale non-diabetic > prediabetic > T2DM state, and it is higher in patients with liver insulin-resistance(109).

Chronic kidney disease

Although dyslipidemia in chronic kidney disease (CKD) is usually characterized by fasting hypertriglyceridemia, postprandial TG clearance is also impaired in adults with chronic kidney disease (CKD)(110). A decreased utilization or catabolism of VLDL and CM TG is generally considered as the more relevant mechanism explaining this fact. Recently, in a young group of subjects with primary CKD, postprandial TG and CM metabolism have been reported as deteriorated in direct proportion to the degree of CKD, in mechanism involving LPL activity via increased apoC-III concentration(111).

Drugs

The medications used for the management of dyslipidemia, diabetes and obesity also regulate PPL(112-114). The following paragraphs summarize the main effects described with these drugs:

- Statins reduce postprandial lipemia mainly by inhibiting the production of ApoB containing lipoproteins from the liver and thus increasing the clearance of TG and increase HDL-c levels(113).

- Fibrates enhance the LPL expression at the transcriptional level mediated by PPAR, inhibited the transcription of the apoC-III gene, decrease ApoB and VLDL production and increase fatty acid oxidation in the liver. As a consequence, fibrates induce hypolipidemic effect via reduced secretion of VLDL particles, together with the enhanced clearance of TG-rich particles(115, 116).
- Ezetimibe, a cholesterol uptake inhibitor that targets the Niemann-Pick C1-like 1 cholesterol transporter, not only inhibits cholesterol uptake, but it may also decrease postprandial apoB48-containing CMs particles(117).
- Supplementation with omega-3 fatty acids significantly suppresses postprandial elevation of TGs and remnant lipoprotein-cholesterol(118).
- Insulin treatment reduces fasting and postprandial total TGs as well as the TGs contained in VLDL, LDL and HDL particles(119, 120).
- Metformin interrupts mitochondrial oxidative processes in the liver and corrects abnormalities of intracellular calcium metabolism in insulin-sensitive tissues, reducing fasting plasma total cholesterol, total TG and VLDL cholesterol concentrations and increasing HDL-c levels. This last observation seems to be more pronounced in Whites and African Americans than in Hispanic populations. Nevertheless, the exact mechanism of action of metformin are only partially known(112).
- Sulfonylureas increase activity of LPL and HL, reduce postprandial free fatty acids (FFAs) and reduce postprandial CM and VLDL TGs(112).
- Meglitinides do not have high impact on PPL, however, in newly diagnosed T2DM patients repaglinide had more effects decreasing TGs and total cholesterol than gliclazide (121).
- Pioglitazone reduces postprandial FFAs, and unlike rosiglitazone, reduces fasting and postprandial TGs and increases HDL-c levels(122).

- Incretin-based therapies with GLP-1 receptor agonists and dipeptyl peptidase-4 inhibitors improve fasting and postprandial lipid parameters by reducing total-cholesterol, LDL-c and TGs concentrations, and increasing HDL-c values(123). The underlying mechanisms which lead to this phenomenon seem to be independent to gastric emptying(124).
- The sodium glucose co-transporter 2 (SGLT2) inhibitors have been associated with reductions in body weight and total fat mass and some placebo-controlled studies suggest that they may induce small reductions in TG levels, and small increases in LDL-c and HDL-c(114). The clinical relevance of such small changes affecting CVD risk has not yet been determined.

2.5. Genetic background

Thanks to the development in the last decade of Nutrigenetics, it has been possible the identification of multiple candidate genes as key players of the postprandial metabolism to explain individual differences in dietary response. As highlighted in previous studies, variants in the majority of the apoprotein genes (A1, A4, A5, B, C3 and E), fatty acid binding protein 2 (FABP2), LPL, HL, microsomal transfer protein and scavenger receptor class B1 have been associated with the magnitude of PPL response(50, 125, 126). More recently, other gene loci have been pointed as potentially important such as PPAR-alfa, PPAR-gamma, CETP, angiopoietin like protein 4 (ANGPLT4), perilipin (PLIN), SCARB1, IL-6, melanocortin-4 receptor (MC4R), ZNF259, TRIB, GCKR or TCF7L2(127-134).

Interestingly, some authors have described a new approach to explore novel genes involved in lipid metabolism by integrating epigenome-wide association studies (EWAS) and genome-wide association studies (GWAS) data with the lipidomic measurements, pointing

that integration of lipidomic and genomic data has the potential to identify new biomarkers of CVD risk(135). Thus, some SNPs in the sorbin and SH3 domain containing 1 (SORBS1) gene and PRIC285 (a co-activator of PPARa) have been highlighted in association with fat metabolism.

3. POSTPRANDIAL HYPERTRIGLYCERIDEMIA AND CARDIOVASCULAR DISEASE

The significance of fasting and postprandial TG in CVD has been debated since Zilversmit's proposal in 1979 that CMs and their remnants are atherogenic(136). Fasting TGs level have been identified as a risk factor in case–control studies, even after adjustment for total cholesterol (TC) or HDL-C(137-139). Large population studies have assessed the association between non-fasting TGs and the risk of CVD events, showing that postprandial TG levels are excellent markers of risk for coronary artery disease, peripheral vascular disease and cerebrovascular disease(24, 25, 27, 28, 140). In this regard, it has been proposed that non-fasting TG (442 mg/dL vs. < 88 mg/dL), marked an increased risk of myocardial infarction, ischemic stroke and early death in women and men in the general population(141).

Possible pathways linking TRL and CVD have been suggested, both in vitro and in vivo(50, 142). Atherogenic effects may be mediated directly by TRL particles or components of the particles: Postprandial CMs and VLDL and their remnants may enter the sub-endothelial space, become modified, and stimulate monocyte chemoattractant protein-1(MCP-1), promote the differentiation of monocytes into macrophages and be taken up by the macrophages to form foam cells(29, 50) (Figure 6).

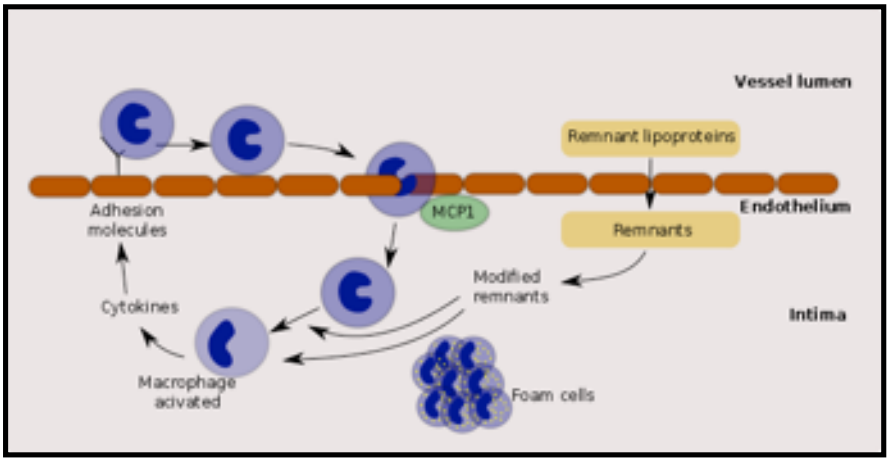

Figure 6. The effects of postprandial chylomicrons and VLDL on arterial endothelium. VLDL remnants and chylomicron remnants enter the subendothelial space, where they become modified, and the modified remnants stimulate Chemoattractant protein-1 (MCP-1), promote the differentation of monocytes into macrophages and are taken up by the macrophages to form foam cells. Adapted from Lopez-Miranda et al., Br J Nutr (2007)(50).

TRLs have been also reported as directly cytotoxic to endothelial cells in cell culture studies(143). They induce an elevation in the expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and tissue factor(144) and induce modification in LDL composition and size with generation of small and dense LDL(145). Finally, postprandial hyperlipemia has been associated with changes in haemostatic variables known to promote the risk of thrombotic events. Thereby, it has been associated with an increase in factor VII and tissue plasminogen activator activity(146-148), an increased platelet reactivity(149, 150), and by contrast, with a lower plasminogen activator inhibitor type-1 activity (PAI-1)(146-148). In our group, it has been reported that this higher thrombogenic state induced by postprandial lipemia may be partly regulated by the type of fat ingested(151) (Table 2).

Many clinical trials have reported that statin therapy targeting reduction of LDL-c decreases the risk of CVD, however many cases of them are not yet prevented and residual risk factors remain unaddressed(152). Although high TG levels are considered to be an important residual risk factor, there is a lack of randomized controlled trials showing a lower risk of cardiovascular events induced by a reduction in TG levels during the postprandial period in patients at high risk of atherosclerotic cardiovascular disease. A recent meta-analysis have showed that adding niacin, CETP inhibitors, n-3 fatty acid or fibrates to statin therapy in those patients have no clear clinical benefit(153). Probably more studies with other therapeutic options are needed to explore this issue.

Parameter	MUFA	Minor Compounds
Platelet aggregation (response to ADP/collagen) Platelet activity (measured by platelet activating factor)	Reduction compared with SAFA Inhibited compared with SAFA	Reduction compared with SAFA Inhibited compared with SAFA
Thromboxanes A2, B2	-	Lowered production and excretion both in fasting and postprandium. Reduction compared with low phenols Olive Oil
Von Willebrand Factor	Reduction compared with low-fat and PUFA in diabetics Reduction compared with low-fat and SAFA in healthy	-
Tissue Factor	Reduced expression in monocites. Reduction compared with SAFA	-
TFPI	Reduction compared with SAFA	-
PAI-1	Reduction compared with low-fat and SAFA	Reduction compared with low-fat and SAFA
Factor VII (Chronic Effect) (Acute Effect)	Reduction compared with SAFA and PUFA Reduction compared with SAFA	Reduction compared with other sources of MUFA Reduction compared with low phenols Olive Oil
Factor XII	Reduction compared with SAFA	-

Table 2. Differential Effects of Olive Oil Compounds Versus Other Sources of fat. Abbreviations: MUFA: Monounsaturated Fatty Acids. SAFA: Saturated Fatty Acids. PUFA: Polyunsaturated Fatty Acids. TFPI: Tissue Factor Pathway Inhibitor. PAI-1: Plasminogen Activator Inhibitor 1. All Cells Refer to Plasma Concentration if Otherwise is not Stated. Adapted from Delgado-Lista J at al, Current Pharmaceutical Design (2011)(154)

4. ASSESSMENT OF POSTPRANDIAL TG METABOLISM

Recent studies have reported that random non-fasting TG levels are good risk markers for CVD. However, the use of non-fasting TG values in clinical practice has been hampered by a lack of standardization of non-fasting TG measures, with respect to the time since last meal, and population-based reference values. Moreover, the measurement of TGs is complicated by its high intra-individual variability, reported on average as 22.5%(155), and the fact that they increase during the day(156). To evaluate changes and dynamics of postprandial lipoprotein metabolism, performing of an oral fat tolerance test (OFTT) has been used in research studies since several decades. Thus, TG response to an OFTT is commonly analyzed from sequential blood samples as the area under the curve (AUC) or incremental AUC (iAUC)(9). Postprandial TG levels increase for up to 3-4 h after the oral fat load and remain elevated for up to 6-8 hours. In healthy subjects the 4 h time-point after an oral load of 70-79 gr fat has been proposed as the most representative measurement of postprandial TGs in a recent meta-analysis(157). However, the postprandial response is higher and delayed in subjects with metabolic disorders or genetic variants causing hypertriglyceridemia(9).

Although there have been recent advances in the standardization of the postprandial assessment, currently there is no definitive consensus or enough evidence to sustain the further development of routine non-fasting/postprandial TGs measurements for clinical purposes. In this context, an Expert Panel of scientists and clinicians, together with a meta-analysis of 113 studies conducted in healthy white subjects (without clinical or physician-diagnosed CVD or metabolic disease, with baseline TGs <177 mg/dL, with body mass index <30 kg/m2 and not on chronic medication) has suggested that subjects with fasting

TGs between 89-180 mg/dL would benefit from the additional clinical information provided by an OFTT(158, 159). We have recently tested this issue in two large cohorts of patients (1,002 patients with CHD from the CORDIOPREV clinical trial, and 1,115 white US healthy subjects from the GOLDN study), validating the predictive values reported in previous consensus, and recommending that subjects with fasting TG between 89-180 mg/dL (1-2 mmol/L) should be tested in order to identify an exaggerated postprandial response and to treat them more agressively (Article in press in Journal of Clinical Lipidology, DOI:http://dx.doi.org/10.1016/j.jacl.2016.05.009).

Alternatively to performing an OFTT, different methods have been proposed to assess the postprandial TRLs metabolism, such as measurements of apoB48(160) (reflecting the number of CM particles), calculated non-fasting remnant cholesterol(161) (evaluating not only VLDL and CMs but also their remnants), measurements of remnant-like particles (RLP) (162) or performing ambulatory capillary TG profiles(163), but these techniques are much less extended and supported than OFTT.

B. HYPERGLYCEMIA, METABOLIC SYNDROME AND BODY SIZE PHENOTYPES AND THEIR RELATIONSHIP WITH CARDIOVASCULAR DISEASE

Hyperglycemia

The role of diabetes in the pathogenesis of CVD was indeterminate until Kannel et al. used data from the Framinghan Heart Study (FHS) in 1979 to identify diabetes as a major cardiovascular risk of CVD in women with diabetes compared to men with diabetes(164). In that study, diabetes seemed to double the risk of total CVD in men, and triple it in women. Furthermore, after age-adjustment relative risks were higher for women than for men for every end-point that the authors had considered in the study (CHF, IC, Stroke, CHD, CVD, and CVD deaths). Despite reductions in CVD mortality over the past few decades, re-examination of the contribution of diabetes became especially important since the definition of diabetes has changed since publication of the original study, and the prevalence of diabetes has increased seriously(165, 166). Furthermore, even though there has been a 50% reduction in the rate of CVD among participants with diabetes from the FHS, the relative risk of diabetes as a risk factor for CVD has remained unchanged(6).

In line with hypertriglyceridemia, it has been proposed that postprandial hyperglycemia may be a stronger risk factor for CVD than fasting hyperglycemia(167). In 2002 Meigs et al examined 3370 subjects from the Framingham Offspring Cohort and found post-challenge hyperglycemia as an independent risk factor for CVD(164). The mechanisms through which hyperglycemia induces atherosclerosis include:

- *Direct effect of elevated glucose concentrations*. High blood glucose levels have toxic effects on cell function, some of them occur rapidly (e.g., inducing oxidative stress via the generation of free radicals; expression of several inflammatory genes, including adhesion molecules that facilitate monocyte adhesion to endothelial cell), while others develop slowly in response to prolonged periods of hyperglycemia (e.g., nonenzymatic glycosylation of proteins exposed to glucose such cell membrane proteins; circulating proteins, such as lipoproteins; and structural proteins that form vessel walls)(168-170).

- *Insulin resistance and hyperinsulinemia*. It has been suggested that reduced beta cell function together with insulin resistance are responsible for development of hyperglycemia. Previous studies proposed that high levels of insulin was an important risk factor for isquemic heart disease(171, 172). A recent meta-analisis of prospective cohort studies has confirmed that hypothesis, and identified hyperinsulinemia as a risk factor for coronary heart disease(173). Various mechanisms have been suggested to explain how hyperinsulinemia may promote atherosclerosis, such as stimulating smooth muscle proliferation and vascular growth factor production(174), stimulating renal sodium and water retention(175), or increasing noradrenaline release through activation of the sympathetic nervous system(176). Additionally, in vitro studies have shown that insulin also stimulates cholesterol synthesis and the binding of LDL to cell membranes in monocytes(177, 178).

- *Association with other risk factors for CVD*. Hyperglycemia commonly occurs in association with other risk factors for CVD such as elevated blood pressure, dyslipidemia and insulin resistance. Hypertension occurs approximately twice as frequently in patients with diabetes compared with patients without it(179). Most patients with T2D present with a

cluster of lipoprotein abnormalities that include elevated fasting and postprandial TG levels, small-dense LDL-c levels, and decreased HDL-cholesterol levels(180, 181). The integrated epidemiological concept of metabolic syndrome discussed below was originated from the observation that several metabolic risk factors often co-occur in patients at high risk of CVD(182).

Metabolic syndrome

The prevalence of MetS is dependent on the population studied, determined by age, sex, race, or ethnicity, as well as on the definition used. In western countries the estimated prevalence of MetS is about one-fifth of the adult population, and the prevalence increases with age(183). As pointed previously, this entity is increasing its prevalence to epidemic proportions worldwide and the health care costs and burden are substantial. MetS in Europe in adults includes over 30%(15), and in Spain 31%(16).

Although to date there are no universally accepted definition criteria for MetS, one of the most widely accepted definitions is that provided by Grundy et al. (AHA/NHLBI, 2005)(184) that consider as criteria for clinical diagnosis of metabolic syndrome the presence of at least 3 of the following: Fasting blood glucose ≥ 100 mg/dl, High-density lipoprotein cholesterol <40 mg/dl in men and <50 mg/dl in women, Triglycerides ≥ 150 mg/dl, Waist circumference ≥ 102 cm in men and ≥ 88 cm in women and Hypertension (≥ 130/85 mmHg or specific treatment for this disorder).

The pathogenesis of MetS is complex and not well understood but a multi-factorial origin has been suggested, involving complex interactions among the genetic background and environmental factors such as nutrition. To date, obesity, insulin resistance, ectopic fat

accumulation, secretion of adipo-cytokines and an increased inflammatory and oxidative states are currently identified as key factors in MetS pathology(185, 186). Other factors such as chronic stress and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS), renin-angiotensin-aldosterone system activity and micro RNAs have been also related to its pathogenesis(187).

In a recent meta-analysis(18) carried out to assess the prognostic significance of MetS in CVD and which contained a population of 952.083 patients included in prospective observational studies, MetS was associated with a 2-fold increase in cardiovascular outcomes (CVD, cardiovascular mortality, myocardial infarction and stroke) and a 1.5-fold increase in all-cause mortality. In turn, excluding the influence of the presence of T2DM, this increased risk persisted for cardiovascular mortality, acute myocardial infarction and stroke. These data are consistent with previously published evidence(188). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial cohort, performed on diabetic population, the absolute risk of suffering a first cardiovascular event was higher in patients with more components of MetS(189).

For lack of conclusive studies, it is still unknown whether the prognostic significance of metabolic syndrome is greater than the risk associated with the sum of its individual components(190-194). Furthermore, as stated above, the complex interrelationships of some of the metabolic criteria makes really difficult to isolate the true risk conferred by a given component.

Body size phenotypes

High BMI is an important cardiovascular disease risk factor which has an outstanding impact on public health due to its increasing prevalence(5, 195). However, it has been suggested that individuals in the same BMI category can have substantial heterogeneity of metabolic features, such as lipid profile, glucose tolerance, blood pressure and waist circumference(19, 196). Thus, recently more attention has been paid to the different metabolic phenotypes of obesity (Figure 7). Previous studies have suggested that the cardiometabolic risk may change considerably among subjects with a similar BMI, depending on their metabolic profile(197).

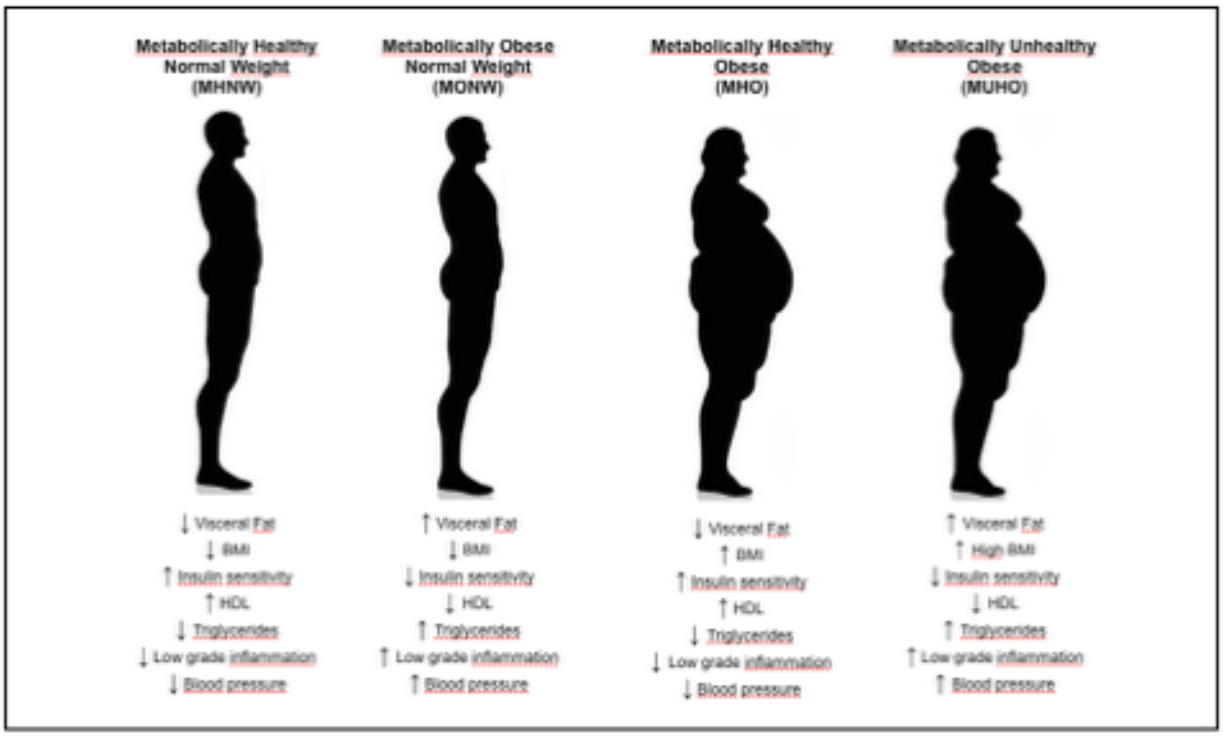

Figure 7. Cardiometabolic phenotypes according to body size. Abbreviations: BMI: Body Mass index. HDL: High-density Lipoprotein. Adapted and modified from Karelis AD et al, J Clin Endocrinol Metab (2004)(21).

Metabolically healthy obesity (MHO) is a clinical condition that describes the absence of any cardiometabolic disease (T2DM, hypertension and dyslipidemia) in subjects with a high BMI (>30 kg/m2)(198). In contrast, metabolically obese but normal-weight (MONW) subjects are a subgroup of individuals who have normal weight and BMI, but display a cluster of obesity-related abnormalities such insulin resistance, hyperinsulinemia or dyslipidemia, with a higher risk for developing T2DM and CVD(21). This fact may support the idea that obesity is a multisystemic disease with loss of flexibility in one or more metabolic processes involved(20).

Descriptive studies have pointed that up to 30% of obese people seem to be metabolically healthy(199), and there is a high prevalence of clustering of cardiometabolic abnormalities among normal-weight individuals. Although there is not yet a standardized definition of body size phenotypes, mostly authors agree with the cardiometabolic abnormalities to consider: elevated blood pressure, elevated TGs levels, decreased HDL-c level, insulin resistance and low grade inflammation (Table 3).

	Population studied	Diagnosis criteria	Prevalence (%)
St-Onge MP, 2004	N = 7602 ≥ 20 years (mean 43.3 yr) with BMI 18.5 – 24.9 kg/m2 USA (NHANES 1988-1994)	BMI 18.5-26.9 kg/m2 and MetS - ATP-III	MANW: 3% for BMI 18.5-20.9; 6.5% for BMI 21-22.9; 10% for BMI 23-24.9 MAOW (BMI 25- 26.9): 22.5% (data for white people)
Meigs JB, 2006	N = 2.902 ≥ 20 years without diabetes or cardiovascular disease (mean age 53.6 yr) USA (Framingham Offspring Study)	BMI < or ≥ 25 kg/m2 and presence or absence of the MetS - ATP-III or three lower quartiles of HOMA-IR	MANW: 7.1% (MetS) / 7.7% (HOMA-IR) MHO: 37% (MetS) / 44.3% (HOMA-IR)
Wildman RP, 2008	N = 5.400 ≥ 20 years (mean 45.0 yr) USA (NHANES 1999-2004)	BMI <25 kg/m2, 25-29.9 or ≥ 30 kg/m2 Metabolically healthy, 0 or 1 and metabolically abnormal, ≥ 2 cardiometabolic abnormalities of the following: MetS - ATP-III criteria (except waist circumference), hsCRP > 0.1 mg/L, HOMA-IR > 5.13	MANW: 23.5% MHOW: 51.3% MHO: 31.7%
Lee K, 2009	N = 5276 ≥ 20 years (mean 43.4 yr) Korea	BMI < or ≥ 25 kg/m2 and presence or absence of MetS - ATP-III adapted criteria (waist circumference ≥ 90 cm in men, ≥ 85 cm in women)	MANW: 12.7% MHOW + MHO: 47.9%
Kuk JL, 2009	N = 6.011 18-65 years USA (NHANES III)	BMI ≥ 30 kg/m2 and ≤ 1 MetS - ATP-III criteria (excluding waist) and/or HOMA-IR < 2.5	MHO: 30.2% (IR) / 38.4% (MetS) / 6% (IR and MetS)
Velho S, 2010	N = 5.360 35-75 years (mean 53.3 yr) Switzerland	BMI ≥ 30 kg/m2 Different combinations of the following criteria: waist; BP; total, HDL-c, LDL-c; TGs; FPG; HOMA-IR; hs-CRP; personal history of cardiovascular, respiratory or metabolic diseases.	MHO: 3.3 - 32.1% in men and 11.4 - 43.3% in women
Calori G, 2011	N = 2.011 mean age 55 years	BMI ≥ 30 kg/m2 and HOMA-IR < 2.5	MHO: 11%

Table 3. Prevalence and criteria used for the definition of metabolic phenotypes in population-based studies. Abbreviations: ATP-III: Adult Treatment Panel III. BMI: Body Mass Index. BP: Blood Pressure. FPG: Fasting Plasma Glucose. HDL-c: High-density Lipoprotein Cholesterol. HOMA-IR: Homeostatic Model Assessment of Insulin Resistance. hsCRP: High-sensitivity C-reactive Protein. IR: Insulin Resistance. LDL-c: Low-density Lipoprotein Cholesterol. MANW: Metabolically Abnormal but Normal Weight. MAOW: Metabolically Abnormal but Overweight. MetS: Metabolic Syndrome.

MHO: Metabolically Healthy but Obese. MUFA: Monounsaturated Fatty Acids. SAFA: Saturated Fatty Acids. PUFA: Polyunsaturated Fatty Acids. TGs: Triglycerides. Adapted from Gomez-Huelgas R et al, Endocr Pract (2013)(197).

From a clinical point of view, there is a debate over whether or not different body size cardiometabolic phenotypes have an increased risk of metabolic complications, with conflicting results published. Some prospective cohort studies have reported that, unlike MHO subjects, MONW individuals exhibited greater all-cause mortality during follow-up(200, 201). On the other hand, a recent meta-analysis has shown that MHO individuals are at increased risk for all-cause mortality and CV events over the long term compared with metabolically healthy normal-weight persons. This study has also reported that obese individuals have an increased risk for death and CV events over the long-term regardless of metabolic status(202). More prospective and intervention studies need to be carried out to clarify and to answer these questions, but at present, identifying certain types of the metabolic phenotypes of obesity may be a good strategy for personalized treatment.

At this point, it is important to understand the underlying causes for those phenotypic differences associated to obesity and to explore whether obesity in its various forms may influence different biomarkers. Two of these biomarkers could be postprandial triglyceride response and inflammation state related to insulin resistance.

C. SUMMARY

In this introduction we have reviewed the current knowledge about factors affecting PPL in physiologic and pathologic conditions.

Among physiologic, behavior associated factors, like diet, smoking or exercise seem to have a well supported basis. This may be helped by the fact that they are factors that can be easily identified and measured. However, there are other not modifiable markers, like age, which real contribution to PPL are not clearly stated. There is a paucity of studies evaluating PPL in the aged compared to young or middle aged persons, and most of them have methodological limitations. The fact that aging and metabolic syndrome traits are intimately overlapped makes that the adequate matching of the two populations to study is crucial for studying this phenomenon, because otherwise, some of the effects attributed to age could be really due to fasting lipids, obesity, inflammation or high blood pressure.

Among the pathological factors affecting PPL, we reviewed how T2DM have an worsen PPL due to biochemical and inflammatory factors derived/favored from hyperglycemia. However, the intermediate metabolic statuses from health to T2DM are still to be studied, and the relevance of factors defining altered metabolic balance (well metabolic syndrome or altered metabolic phenotypes) may be better characterized.

Chapter 3

OBJECTIVES

III. Chapter 3: OBJECTIVES

Main objective

- To determine whether MetS traits influence the PPL of coronary patients, and whether this influence depends on the number of MetS criteria (Chapter 4.B).

Secondary objectives

- To investigate whether the number of criteria of metabolic syndrome may predict the degree of postprandial response in patients with normal fasting TGs (Chapter 4.B).

- To determine the exact contribution of the presence of MetS to age-associated enlarged PPL (Chapter 4.A).

- To explore the phenotypic flexibility of high risk patients, measured with an OFTT, according to different cardio-metabolic abnormalities and BMI (Chapter 4.C).

Chapter 4

PUBLICATIONS DERIVED FROM THIS THESIS

IV. Chapter 4: PUBLICATIONS DERIVED FROM THIS THESIS

A. LIPID METABOLISM AFTER AN ORAL FAT TEST MEAL IS AFFECTED BY AGE-ASSOCIATED FEATURES OF METABOLIC SYNDROME, BUT NOT BY AGE.

Perez-Caballero AI*, **Alcala-Diaz JF***, Perez-Martinez P, Garcia-Rios A, Delgado-Casado N, Marin C, Yubero-Serrano E, Camargo A, Caballero J, Malagon MM, Tinahones FJ, Perez- Jimenez F, Lopez-Miranda J, Delgado-Lista J. **Atherosclerosis. 2013 Jan;226(1):258-62**. doi: 10.1016/j.atherosclerosis. 2012.10.052. Epub 2012 Oct 31. *Perez-Caballero AI and Alcala-Diaz JF contributed equally to this article.

Journal Impact Factor (2013): 3.971. Q1, Peripheral Vascular Disease.

B. HYPERTRIGLYCERIDEMIA INFLUENCES THE DEGREE OF POSTPRANDIAL LIPEMIC RESPONSE IN PATIENTS WITH METABOLIC SYNDROME AND CORONARY ARTERY DISEASE: FROM THE CORDIOPREV STUDY.

Alcala-Diaz JF, Delgado-Lista J, Perez-Martinez P, Garcia-Rios A, Marin C, Quintana-Navarro GM, Gomez-Luna P, Camargo A, Almaden Y, Caballero J, Tinahones FJ, Ordovas JM, Perez-Jimenez F, Lopez-Miranda J. **PLoS One. 2014 May 6;9(5):e96297**. doi: 10.1371/journal.pone.0096297. eCollection 2014.

Journal Impact Factor (2014): 3.23. Q1, Multidisciplinary Sciences.

C. ***METABOLIC PHENOTYPES OF OBESITY INFLUENCE TRIGLYCERIDE AND INFLAMMATION HOMOESTASIS.***

Perez-Martinez P**, **Alcala-Diaz JF****, Delgado-Lista J, Garcia-Rios A, Gomez-Delgado F, Marin-Hinojosa C, Rodriguez-Cantalejo F, Delgado-Casado N, Perez-Caballero AI, Fuentes-Jimenez FJ, Camargo A, Tinahones FJ, Ordovas JM, Perez-Jimenez F, Lopez-Miranda J. **Eur J Clin Invest. 2014 Nov;44(11):1053-64.** doi: 10.1111/eci.12339. **Perez-Martinez P and Alcala-Diaz JF contributed equally to this article.

Journal Impact Factor (2014): 2.734. Q1, Medicine, General & Internal.

A. Lipid Metabolism After An Oral Fat Test Meal Is Affected By Age-Associated Features Of Metabolic Syndrome, But Not By Age.

Perez-Caballero AI*, **Alcala-Diaz JF***, Perez-Martinez P, Garcia-Rios A, Delgado-Casado N, Marin C, Yubero-Serrano E, Camargo A, Caballero J, Malagon MM, Tinahones FJ, Perez-Jimenez F, Lopez-Miranda J, Delgado-Lista J. *Perez-Caballero AI and Alcala-Diaz JF contributed equally to this article.

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Lipid metabolism after an oral fat test meal is affected by age-associated features of metabolic syndrome, but not by age

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ABSTRACT

Objective: Postprandial lipemia influences the development of atherosclerosis. Age has been defined as a regulating factor of the extent of postprandial lipemia, but its independence of other age-associated phenotypic features, such as metabolic syndrome, has not been fully elucidated.
Methods: To investigate if age is an independent factor influencing postprandial lipemia, we compared the lipemic response to a rich fatty meal (60% fat) of 88 healthy young men (<30 years old) and 97 older participants (77 metabolic syndrome patients aged > 40; and 20 healthy people > 65) (all ApoE3/E3), at fasting state and at 2nd and 4th postprandial hours.
Results: We didn't find differences between the healthy young men and the healthy elderly. The metabolic syndrome patients displayed a higher postprandial TG area below the curve than the other two cohorts $p < 0.001$. ANOVA for repeated measurements confirmed that these differences were significant at every time-point (fasting, 2 h and 4 h). Concomitant higher responses for Large and Small TRL-carried TG and Chol were found in these metabolic syndrome patients. Interestingly, the most significant differences were found for Small-TRL-carried particles, which suggest that this fact may be mainly due to impaired lipid clearance.
Conclusion: Metabolic syndrome may account for the differences in postprandial lipemia that have been attributed to age. In our study, there were no significant differences in postprandial lipemia between a young population (mean age 22.6 years) and a healthy people >65 years one (67.2 years) without metabolic syndrome.

Postprandial lipemia following a fat-rich meal is a situation characterized by the generation of an atherogenic environment in the bloodstream, derived by the conjunction of the direct atherogenic properties of some lipid particles, especially those carried in the triglyceride-rich lipoproteins, and by the activation of the inflammatory and hemostatic systems [1,2]. These last features may be influenced by the amount and type of fat in the meal [1,3–5], and are partly mediated by mononuclear cells, which respond directly to the increase in triglyceride remnants in the blood, to the apparition in the blood of proteins of bacterial origin (like LPS) and to stimuli secreted from intestinal endothelial cells (via TLR4 receptors) [6–8]. Furthermore, endothelial vasodilatory capacity is transiently impaired after a high-fat meal, a fact linked to the nitric oxide synthase pathway [9]. All these features turn the postprandial state into an atherogenic environment, and the extent of this period has been related to increased atherosclerosis [10].

Dietary background has been identified as the main extrinsic factor influencing postprandial lipemia [11,12], while genetics, sex,

Abbreviations: ApoB, apolipoprotein B; Chol, cholesterol; HDL-C, high density lipoproteins cholesterol; MetS, metabolic syndrome; SFA, saturated fatty acids; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus; TG, triglycerides; TRL, triglyceride-rich lipoproteins.

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and age have been identified as potent intrinsic modifiers of postprandial lipemia [11,13,14]. For example, ApoE genotyping is a clearly established modifier of triglyceride levels, and the ApoE3/E3 genotype (the commonest) identifies those persons with an average response to diet and lipid overload [15–19]. However, although the influence of sex and genetics is easier to isolate from other interfering factors, age is often associated to related conditions, such as the appearance of metabolic syndrome. In developed countries, up to 35% of the general population suffers from metabolic syndrome [20]. The exact contribution of the presence of metabolic syndrome to age-associated enlarged postprandial lipemia has not been explored much.

1. Subjects and methods

1.1. Subjects

We evaluated one hundred and eighty-five persons who participated in three different studies performed in our unit: Eighty-eight healthy young men (<30 years old), and ninety-seven persons ranging from 40 to 70 years old, and who, in turn, came from two studies: 77 participants with metabolic syndrome from the LIPGENE study, and 20 healthy participants from the coenzyme Q and age study. Detailed information of these three studies has been published elsewhere [12,21–24]. The metabolic syndrome in the LIPGENE study was determined using a modified version of the NCEP criteria for MetS [25], where subjects were required to fulfill at least three of the following five criteria: waist circumference > 102 cm (men) or >88 cm (women); fasting glucose 5.5–7.0 mmol/L; TAG ≥ 1.5 mmol/L; HDL cholesterol < 1.0 mmol/L (men) or < 1.3 mmol/L (women); blood pressure ≥ 130/85 mmHg or treatment of previously diagnosed hypertension. Notably, all participants were previously genotyped for the ApoE genotype, and only ApoE3/E3 subjects were selected. None of the 88 healthy young males had any criteria of metabolic syndrome. All participants provided their written informed consent before enrolling for the study, according to the Declaration of Helsinki II. The study was approved by the local committee for scientific ethics.

1.2. Protocol

More detailed protocols have been published previously [12,21–24]. To sum up, participants fasted for 12 h, and then received a fatty meal, containing 1 g fat and 7 mg cholesterol per kg of body weight in the case of the healthy young men, and 0.7 g fat and 5 mg cholesterol per kg of body weight in the case of the other two cohorts. The meals contained 60–65% of energy as fat, 10–15% of energy as protein, and 25% of energy as carbohydrates, and were consumed in 20 min. We measured the lipid particles at the fasting state, and via blood drawn performed at different time-points in the postprandial state: with the young cohort, we assessed these fractions every hour until the sixth, and then every 2.5 h until 11th. With the metabolic syndrome patients, we measured them twice-hourly for 8 h, while with the cohort of persons above 65 years, for ethical reasons, we performed a short lipemia study (twice-hourly until the 4th hour). In the present study, only the common time-points (fasting, 2nd and 4th postprandial hour measurements) of all cohorts are considered.

1.3. Blood test

Extensive laboratory methodology has been published for the three populations elsewhere [22,24,26]. Blood drawn and TRL (Large and Small) isolation were performed by standard methodology, as published previously [24]. In short, plasma was separated from red cells by centrifugation at 1500× *g* for 15 min at 4 °C. The chylomicron fraction of TRL (Large-TRL) was isolated from 4 mL of plasma overlayered with 0.15 mol/L NaCl, 1 mmol/L EDTA (pH 7.4, $d < 1.006$ kg/L) by a single ultracentrifugal spin (36,200 *g*, 30 min, 4 °C) in a 50-type rotor (Beckman Instruments, Fullerton, CA). Large-TRL, contained in the top layer, was removed by aspiration after cutting the tubes, and the infranatant was centrifuged at a density of 1.019 kg/L for 24 h at 183,000 *g* in the same rotor. The non-chylomicron fraction of TRL (also referred to as Small-TRL) was removed from the top of the tube. All operations were done in subdued light. Large and Small TRL fractions were stored at −70 °C until biochemical determinations were performed. Total cholesterol (Chol) and triglycerides (TG) in plasma and lipoprotein fractions were assayed by enzymatic procedures. APOA1 and APOB were determined by turbidimetry [27]. HDL-C was measured by the dextran sulfate-Mg^{2+} method, as described in Ref. [28]. LDL-C levels were estimated using the Friedewald formula [29].

1.4. Statistics

In the baseline fasting comparisons, we used univariate ANOVA. We tested the size of the postprandial lipid fractions by univariate ANOVA for the area below the curve (AUC), defined as the area within the plasma concentration-versus-time curve, using the trapezoidal rule and treating the cohort as independent variable, with each of the lipid fractions as dependent variables. Then, to estimate if the influence was regular throughout the lipemia, we performed repeated ANOVA measurements, testing all the time-points (fasting, 2nd hour and 4th hour) in the Post-Hoc analysis, using Bonferroni's corrections. Additionally, we tested if the postprandial findings were influenced by fasting values by relativizing each tested variable to its fasting figures. For any given variable, fasting value was set to 1, and the relativized values for the 2nd and 4th hours were calculated as the quotient of the real value of that given variable in the studied time-point by that of the fasting value.

To infer if the short lipemia (4 h) was suitable to represent the whole postprandial lipemia, we used our existing data with the complete set of extracted blood for the young men's cohort (hourly after the meal for 6 h, then every 2.5 h until 11th) and the metabolic syndrome cohort (twice-hourly for 8 h), and we performed the correlation tests to obtain the R^2 index and the equation for the linear regression function.

A *p*-value of less than 0.05 was considered significant. All the data presented in the text and tables are expressed as mean ± SE unless otherwise specified. SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical comparisons.

2. Results

Baseline characteristics of the groups are described in Table 1. Young men differed from the metabolic syndrome and the healthy >65 years old cohorts in BMI, total cholesterol, and LDL cholesterol (all $p < 0.001$, Table 1). Metabolic syndrome patients exhibited higher baseline TG levels, while healthy >65 years patients showed higher HDL concentrations than the other two groups (Table 1).

The metabolic syndrome cohort showed a higher postprandial TG response than the other two groups ($p < 0.001$), both in AUC (Fig. 1) and in repeated ANOVA measurements, with differences found at the baseline and at every time-point blood drawn (Fig. 2).

When evaluating the different postprandial lipid fractions, we found differences for metabolic syndrome patients versus the other two groups for Large-TRL-TG ($p < 0.05$), Small-TRL-Chol ($p < 0.001$) and Small-TRL-TG ($p < 0.001$), both in AUC (Fig. 1) and in the repeated ANOVA measurements (Fig. 3). When evaluating the differences in the different time-points, we found higher Large-TRL-TG in metabolic syndrome patients than in the other two

Table 1
Baseline characteristics.

	Healthy young men ($n = 88$)	MetS ($n = 77$)	Healthy subjects >65 years old ($n = 20$)	p
Age (years)	22.2 ± 0.31^a	56.55 ± 0.84^b	67.25 ± 1.15^c	<0.001
BMI (kg/m^2)	25.38 ± 0.38^a	34.9 ± 0.4^b	31.63 ± 1.3^c	<0.001
TG (mg/dL)	80.4 ± 3.72^a	148.73 ± 9.59^b	102.53 ± 7.64^a	<0.001
Total Chol (mg/dL)	151.53 ± 2.45^a	196.51 ± 4.48^b	201.3 ± 6.51^b	<0.001
HDL (mg/dL)	46.29 ± 1.08^a	43.62 ± 1.13^a	54.01 ± 2.75^b	<0.001
LDL (mg/dL)	89.16 ± 2.28^a	136.86 ± 3.8^b	126.79 ± 5.21^b	<0.001
Glucose (mg/dL)	84.15 ± 3.56^a	114.78 ± 2.16^b	97.3 ± 3.39^c	<0.001
Blood pressure (mm Hg). systolic	107 ± 3^a	140 ± 15^b	147 ± 16^b	<0.001
Blood pressure (mm Hg). diastolic	70 ± 7^a	85.6 ± 16^b	87.5 ± 16^b	<0.001
Waist circumference (cm)	91.60 ± 6.55^a	108.33 ± 8.76^b	100.98 ± 12.88^b	<0.001
Female (%)	0	63.6	50	–

The p-value in the last column corresponds to univariate ANOVA, with each age group as an independent factor and each phenotypic variable as a dependent factor. Within each row, values with different superscript letters are different at $p < 0.05$. All values are means ± SE.

groups at time-point 2 h, and at 4 h than the young men (all $p < 0.05$). Metabolic syndrome patients also showed higher Small-TRL measurements (Small-TRL-TG and Small-TRL-Chol), at every time-point (fasting, 2nd and 4th hours) than the other two groups (all $p < 0.001$) (Fig. 3). We did not find any differences between the young men's cohort and the cohort of healthy persons >65 years, although a trend towards higher TG in this last population at the fasting state versus the young men cohort ($0.05 < p < 0.10$) was found. All significant differences were influenced by the fasting values, as these differences disappeared when relativizing the figures to the fasting values.

We also evaluated the concordance of the short lipemia method (by assessing the AUC of TG in the first 4 h) which was used in the evaluation of the three groups in this work, with the additional data that we had from the young men (11 postprandial hours) and the metabolic syndrome patients (8 postprandial hours). When comparing these AUCs of TG, the short lipemia reproduced the long lipemia data with accuracy: in the young men's cohort, R^2 for the 4 h versus the 11 h was 0.928, while in metabolic syndrome patients, the 4 h lipemia versus the 8 h lipemia resulted in a R^2 of 0.9059.

3. Discussion

In our study, healthy people above 65 years do not present higher postprandial lipemia than healthy young men in response to a single fat meal. However, in our study, the existence of metabolic syndrome is a clear determinant of postprandial lipemia. Age has been proposed as a determinant of postprandial lipemia in previous reports [11], based in original works [30–32]. Two main underlying causes have been proposed: a decrease in postprandial lipid clearance, and an increase in VLDL ApoB-100 production in elderly persons [31]. However, in most of these studies, subjects in the older groups exhibited some of the features of metabolic syndrome, which is logical if we take into account that the prevalence of metabolic syndrome clearly increases with age [30–32].

Other factors, like genetics, may interact with age to determine postprandial lipids [13,14]. In a recent report, it has been shown that the impact of ApoE gene variations (clear modifiers of triglyceride metabolism) on postprandial lipemia are closely linked to age, and are more important in the elderly than in young persons [33]. To rule out the possibility of our results being influenced by ApoE, we only included ApoE3/E3 subjects. This phenotype (ApoE3/E3) is the commonest in the general population, and the one that has a more physiological TG response to fat overload [15–18].

The most important aspects of this study are not the differences of postprandial lipemia between the metabolic syndrome patients and the healthy persons, which has been previously stated [20], but the following two main premises. First, the lack of differences between young and healthy persons above 65 years, and second, that the main particles affected in postprandial lipemia in the metabolic syndrome are those carried by Small-TRL (Small-TRL-carried Chol and TG), reflecting a delayed clearance of lipids. In fact, these lipid fractions were already higher at fasting levels, which implies that these patients are not able to clear their postprandial lipid fractions sufficiently after 12 h fasting. In our study, the cohort of participants >65 years old had higher baseline HDL concentrations that the other two cohorts, which may partly explain our findings. HDL serves as a donor of ApoC, apo E and other apoproteins during postprandial state [34]. However, pointing to additional underlying mechanisms, the findings were more evident in the markers of the late postprandial state (Small-TRL particles), while HDL and ApoA1 influence is better documented in the

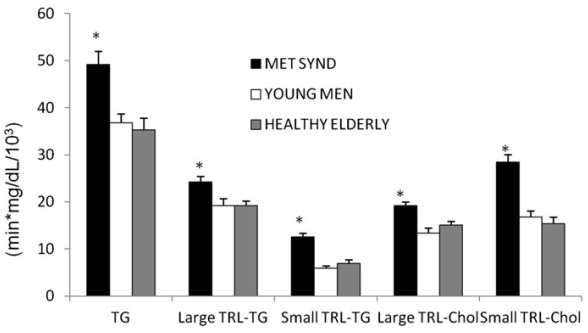

Fig. 1. Area below the curve (AUC) of the different postprandial lipid fractions during lipemia. *$p < 0.05$ MetS versus the young men and the healthy >65 years old cohorts. Figures of Large-TRL-Chol and Small-TRL-Chol have been multiplied by 10 for visual purposes.

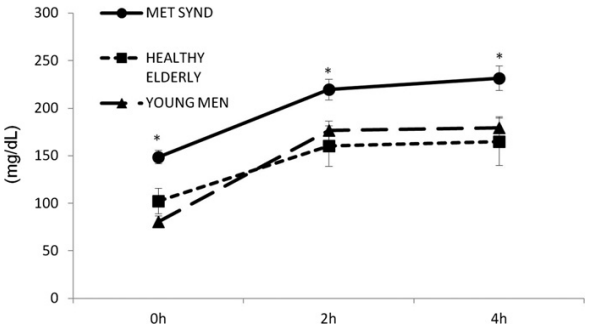

Fig. 2. Evolution of TG (mg/dL) depending on the distinct phenotype group. Means ± SE. *$p < 0.05$ MetS versus the other two groups.

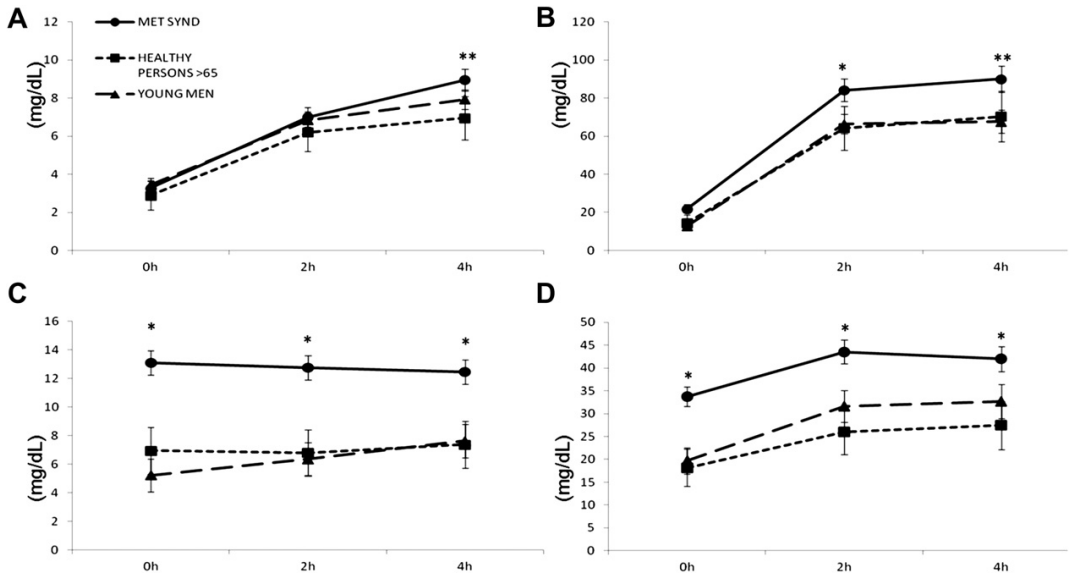

Fig. 3. Evolution of Large-TRL-chol (Panel A), Large-TRL-TG (Panel B), Small-TRL-chol (Panel C) and Small-TRL TG (Panel D). All values are mg/dL. Means ± SE. $^{*}p < 0.05$ MetS versus the other two groups. $^{**}p < 0.05$ MetS versus participants above 65 years.

markers of early postprandium (Large-TRL), [34–36]. The effect of BMI on postprandial lipemia has also been reported [37]. In our sample, the metabolic syndrome patients had higher BMI than the young men, and this could somehow explain the findings; however, there were no differences between the metabolic syndrome and the healthy persons above 65 years old in terms of BMI, which replicates previous findings pointing to the fact that it is metabolically active obesity which impairs energy homeostasis [38], which may explain the lack of differences between the persons above 65 years old and the healthy young men.

When we tested the influence of the fasting values in the postprandial lipids we observed that the postprandial differences were parallel to the differences in fasting values. Relativizing the values by the fasting figures resulted in the absence of differences in any of the significant differences found when using the real values. This fact reveals that the concentration of the different tested variables of the postprandial lipemia in metabolic syndrome patients are shifted towards up.

Our study comprises the first four postprandial hours of the postprandial lipemia, which show the initial and maximal peaks for the postprandial lipid particles, and which have been identified as the most accurate predictor of total postprandial lipemia [39,40]. Furthermore, one of the problems when trying to translate postprandial lipemia into clinical practice is to find a tolerable methodology to be performed in the real setting of usual care practice. Periods of waiting longer than 4 h are difficult to be implemented in the real setting. Supporting our model, in the Women's Health Study, TGs calculated in the 2nd to 4th postprandial hours had the strongest association with CVD events fully adjusted hazard ratio [95% CI] for highest versus lowest tertiles of levels, 4.5 [2.0–10.2] and this association progressively decreased with longer periods of sampling [41]. Additionally, we subsequently performed an internal control of the correlation between the lipemia in the first 4 h versus the complete data that we had for metabolic syndrome (8 h) and for the young men (11 h). In the two cases, the correlations (R^2) were higher than 0.90 (Fig. 4).

Although all studies which are presented in the present manuscript have been carried out in our Unit, and we try a standardized methodology, our results may be taken with caution, and require confirmatory studies prior to be fully extrapolated. The fact that the populations are pooled together but the experiments were not performed at the same time is a limitation of our study. Other point to be considered when extracting conclusions from the present study is the fact that, while the two older cohorts (metabolic syndrome and healthy persons aged >65) received a fat load with 0.7 g of fat per kg body weight, the healthy young men received 1 g/kg. This fact was due to that previous data indicated that optimal fat loads to ascertain postprandial lipemia were inferred to be 70–79 g/fat. Participants in the latter cohorts were expected to be obese when these studies were projected. In fact, 70 and 80% of the healthy >65 and the

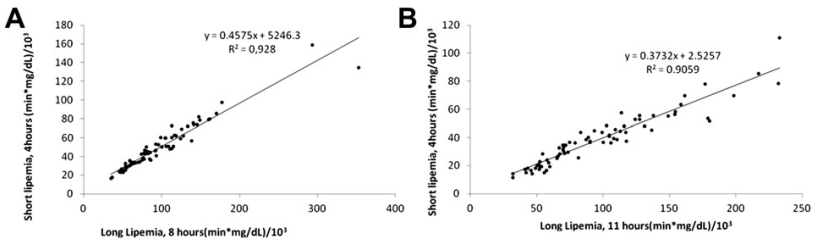

Fig. 4. Correlation and linear regression data of postprandial AUC of TG for short versus long lipemia in MetS patients (Panel A) and healthy young men (Panel B).

metabolic syndrome patients cohorts were weightier than 80 kg. Nevertheless, the meals provoked a postprandial lipemia that was not correlated to the grams of fat ingested (Suppl. Fig. 1).

To conclude, we have tested the effects of a single meal on three populations: young men, middle-aged metabolic syndrome patients and healthy subjects >65 years. We did not find any differences between the healthy men and the healthy persons >65 years, which may be linked to the absence of metabolic syndrome traits and the fact that we limited the effects of confounding factors, such as the existence of ApoE gene variations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2012.10.052.

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B. Hypertriglyceridemia Influences The Degree Of Postprandial Lipemic Response In Patients With Metabolic Syndrome And Coronary Artery Disease: From The Cordioprev Study.

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Hypertriglyceridemia Influences the Degree of Postprandial Lipemic Response in Patients with Metabolic Syndrome and Coronary Artery Disease: From the Cordioprev Study

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Abstract

Objective: To determine whether metabolic syndrome traits influence the postprandial lipemia response of coronary patients, and whether this influence depends on the number of MetS criteria.

Materials and Methods: 1002 coronary artery disease patients from the CORDIOPREV study were submitted to an oral fat load test meal with 0.7 g fat/kg body weight (12% saturated fatty acids, 10% polyunsaturated fatty acids, 43% monounsaturated fatty acids), 10% protein and 25% carbohydrates. Serial blood test analyzing lipid fractions were drawn at 0, 1, 2, 3 and 4 hours during the postprandial state. Total and incremental area under the curves of the different postprandial parameters were calculated following the trapezoid rule to assess the magnitude of change during the postprandial state

Results: Postprandial lipemia response was directly related to the presence of metabolic syndrome. We found a positive association between the number of metabolic syndrome criteria and the response of postprandial plasma triglycerides ($p< 0.001$), area under the curve of triglycerides ($p<0.001$) and incremental area under the curve of triglycerides ($p<0.001$). However, the influence of them on postprandial triglycerides remained statistically significant only in those patients without basal hypertriglyceridemia. Interestingly, in stepwise multiple linear regression analysis with the AUC of triglycerides as the dependent variable, only fasting triglycerides, fasting glucose and waist circumference appeared as significant independent ($P<0.05$) contributors. The multiple lineal regression (R) was 0.77, and fasting triglycerides showed the greatest effect on AUC of triglycerides with a standardized coefficient of 0.75.

Conclusions: Fasting triglycerides are the major contributors to the postprandial triglycerides levels. MetS influences the postprandial response of lipids in patients with coronary heart disease, particularly in non-hypertriglyceridemic patients.

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Introduction

The postprandial state is the period from food intake to post-absorptive state, defined in terms of extent and duration of increased plasma triglycerides (TG) in response to fat intake. It is a dynamic condition, with a continuous fluctuation in the degree of lipemia and glycemia over the day, in which there is a rapid continuous remodeling of the lipoprotein and a host of other metabolic adaptations compared to the relatively stable conditions in the fasting state. Over the last decade, postprandial triglyceride metabolism has taken on more importance, since fasting is not the typical physiological state of humans in modern society, who spend most of the time in the postprandial state. In this context, the evaluation of the postprandial lipemic response may be more important to identify disturbances in lipid metabolism than measurements taken in the fasting state. In fact, large population studies (e.g. Women's Health Study and the Copenhagen City Heart Study) have assessed the association between non-fasting triglycerides and the risk of cardiovascular disease (CVD) events. Data from these studies have clearly documented that postprandial TG levels are excellent markers of risk for coronary artery disease, peripheral vascular disease and cerebrovascular disease [1–5]. In this regard, it has been proposed that non-fasting TG (5 mmol/L *vs.* <1 mmol/L) marked a 17- and 5-fold increased risk of myocardial infarction, a 5-and 3-fold increased risk of ischemic stroke, and a 4- and 2-fold increased risk of early death in women and men in the general population [1–4].

Moreover, several studies have linked the extent of postprandial lipemia to the incidence of coronary heart disease and it has been proposed that postprandial lipoprotein metabolism is modulated by dietary patterns, food composition, conditions associated with lifestyle (physical activity, smoking and alcohol consumption), physiological factors (age, gender, genetic background and postmenopausal status) and cardiometabolic conditions such as fasting triglycerides levels [6–10], type 2 diabetes (T2DM), insulin resistance and obesity [11–13].

The importance of Metabolic Syndrome (MetS) lies in its close association with the risk of CVD and T2DM. Unfortunately its prevalence is increasing to epidemic proportions and the health care costs and burden are substantial. One of the most widely accepted definitions is that provided by the National Cholesterol Education Program guidelines, revised in 2004 (rNCEP) [14]. In a recent meta-analysis [15], including 952.083 patients and carried out to assess the prognostic significance of MetS in cardiovascular disease, it was shown that MetS was associated with a 2-fold increase in cardiovascular outcomes (cardiovascular disease, cardiovascular mortality, myocardial infarction and stroke) and a 1.5-fold increase in all-cause mortality. In turn, excluding the influence of the presence of T2DM, this increased risk persists for cardiovascular mortality, acute myocardial infarction and stroke. These data confirmed previously published evidence [16]. From a clinical point of view, there is a debate as to whether the MetS alone or its associated conditions are more important for CVD incidence and mortality or whether prevention and/or treatment of the MetS will reduce CVD incidence and mortality. In this regard, previous observations have reported that the presence of more components of MetS was associated with an increase in subclinical atherosclerosis, and incidence and mortality of coronary heart disease [17–21]. In the same context, it has been suggested that, in healthy people, there is a relationship between MetS components and exacerbated postprandial lipemia [22], but there is still a lack of data in patients with CVD.

Based on this previous evidence, our objective was to determine if MetS traits influence the postprandial lipemia of coronary patients, and whether this influence depends on the number of MetS criteria.

Materials and Methods

Ethics Statement

Patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the Ethics Committee from Reina Sofia University Hospital, following the Declaration of Helsinki (2008) of the World Medical Association.

Population

The current work was conducted within the framework of the CORDIOPREV study. The CORDIOPREV study is an ongoing prospective, randomized, opened, controlled trial including 1002 patients with coronary heart disease (CHD), who had their last coronary event more than six months before enrollment in two different dietary models (Mediterranean and low-fat) over a period of five years, in addition to conventional treatment for CHD.

Patients were recruited from November 2009 to February 2012, mostly at the Reina Sofia University Hospital (Cordoba, Spain), but patients from other hospital centers from the Cordoba and Jaen provinces were also admitted.

Inclusion and exclusion criteria are shown in **Table 1**. In summary, patients were eligible if they were between 20 and 75, had established CHD without clinical events in the last six months, were thought to follow a long-term dietary intervention and had no severe diseases or an expected life expectancy of under five years. Patients were categorized depending on the presence or not of MetS and number of its criteria, defined by the rNCEP criteria [14].

Study design

Before participants were enrolled in the two different dietary models from CORDIOPREV study, they received an oral fat tolerance test using a weight-adjusted meal (0.7 g fat and 5 mg cholesterol per kg body weight) with 12% saturated fatty acids (SFA), 10% polyunsaturated fatty acids (PUFA), 43% monounsaturated fatty acids (MUFA), 10% protein and 25% carbohydrates (CHO). The meal composition was designed by a group of nutritionists with olive oil, skimmed milk, white bread, cooked egg yolks and tomatoes.

Methodology of the oral fat tolerance test

Before starting the test, the patients had fasted (food/drugs) for 12 hours and were asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 7 days. They were also asked to avoid strenuous physical activity the day before the test was given. The patients arrived at the clinical center at 08:00 h. We measured anthropometric (weight, height, waist circumference, Body mass index (BMI) and blood pressure) and biochemical measurements, took a fasting blood sample and under supervision, the patients ingested the fatty food meal. The breakfast was eaten in 20 min. After the meal, the volunteers rested and consumed no food for 5 hours, but were allowed to drink water.

Blood samples for biochemical testing were collected before the meal and every hour during the next 4 hours, following recommendations for an oral fat tolerance test proposed by Mihas et al. in a recent meta-analysis [23].

Table 1. Inclusion and exclusion criteria for the CORDIOPREV study.

Ages Eligible for Study	**20 to 75**
Genders Eligible for Study	**Both**
Inclusion Criteria	Unstable Coronary Disease Chronic
	Acute Myocardial Infarction
	Unstable Angina
	Chronic Coronary Disease with high risk of event
Exclusion Criteria	Age <20 or >75 (or life expectancy below 5 years)
	Patients already scheduled for revascularization Patients submitted to revascularization in the last 6 months Grade II/IV Heart failure
	Left ventricle dysfunction with ejection fraction lower than 35%
	Patients unable to follow a protocol
	Patients with severe uncontrolled Diabetes Mellitus, or those with Renal Insufficiency with permanent plasma creatinine higher than 2 mg/dl, or cerebral complications of Diabetes Mellitus
	Other chronic diseases: Psychiatric diseases, Chronic Renal Insufficiency, Chronic Hepatopathy, Active Malignancy, Chronic Obstructive Pulmonary Disease, Diseases of the digestive tract, Endocrine disorders, Patients participating in other clinical trials (at the time of enrollment or 30 days before)

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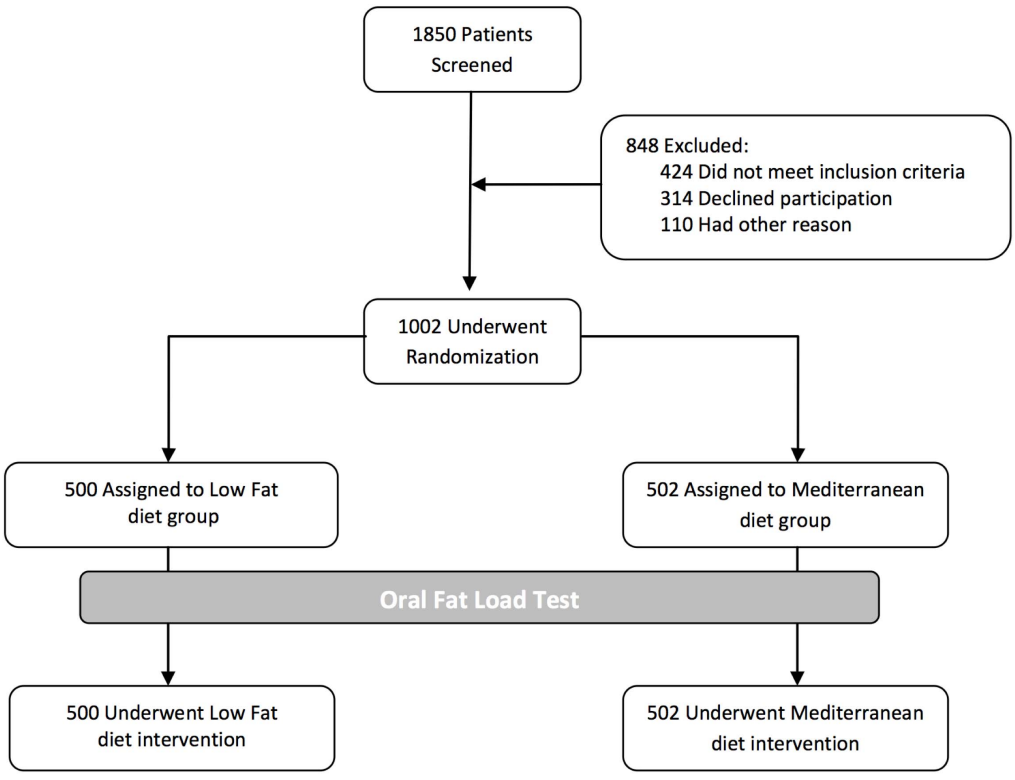

Figure 1. Flow-chart of CORDIOPREV study. Before participants were enrolled in the two different dietary models from CORDIOPREV study, they received an oral fat tolerance test using a weight-adjusted meal (0.7 g fat and 5 mg cholesterol per kg body weight) with 12% saturated fatty acids (SFA), 10% polyunsaturated fatty acids (PUFA), 43% monounsaturated fatty acids (MUFA), 10% protein and 25% carbohydrates (CHO).
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Table 2. Baseline characteristics of the patients.

	All patients	Metabolic Syndrome	Non-Metabolic Syndrome	p-value
	(n = 1002)	(n = 581)	(n = 421)	
Age (Years)	59.5±0.2	60.0±0.3	58.9±0.4	NS
Male/Female	837/165	470/111	367/54	<0.001
Weight (Kg)	85.1±0.4	88.8±0.6	80.1±0.6	<0.001
Waist circumference	105.1±0.3	108.7±0.4	100.1±0.5	<0.001
BMI (kg/m2)	31.1±0.1	32.4±0.1	29.3±0.2	<0.001
HDL-c (mg/dL)	42.2±0.3	38.6±0.4	47.1±0.5	<0.001
Fasting Plasma Glucose (mg/dL)	113.7±1.2	125.9±1.8	97.1±1.0	<0.001
TG (mg/dL)	135.4±2.2	159.9±3.1	102.1±2.2	<0.001
APO-A1 (mg/dL)	129.6±0.7	124.9±0.8	136.1±1.1	<0.001
Total Cholesterol (mg/dL)	159.0±0.9	158.6±1.3	159.5±1.4	NS
APO-B (mg/dL)	73.6±0.5	76.1±0.8	70.2±0.8	<0.001
LDL-c (mg/dL)	88.5±0.8	86.37±1.1	91.5±1.2	<0.001
Lipid lowering drugs:				
Statins (%)	85.6	85.7	85.5	NS
Fibrates (%)	1.6	2.4	0.5	0.01
Other[1](%)	4.8	4.6	4.9	NS

Values are means ±SEM. Continuous variables were compared using the analysis of variance (ANOVA). Qualitative variables were compared using Chi Square test. BMI = Body mass index. HDL-c = High density lipoprotein cholesterol. TG = Triglycerides. LDL-c = Low density lipoprotein.
[1]Other lipid lowering drugs: Ezetimibe and Nicotinic acid.
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Laboratory test

Venous blood was sampled from the antecubital vein and collected into tubes with no anticoagulant and EDTA, and immediately transferred to 4°C. To minimize proteolytic degradation, plasma was supplemented with protease inhibitor cocktail 40 µL per mL of plasma. Plasma and serum samples were frozen at −80°C for further biochemical analysis.

Serum parameters were measured using spectrophotometric techniques (enzymatic colorimetric methods): hexokinase method for glucose, and oxidation-peroxidation for total cholesterol, HDL-

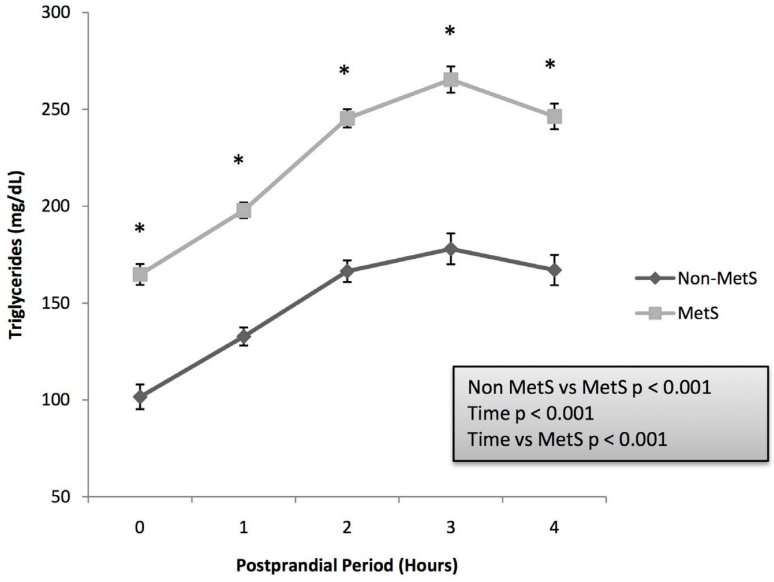

Figure 2. Plasma levels of triglycerides (mg/dL) during postprandial period in patients with and without MetS. MetS patients showed higher plasma levels of triglycerides in each of the time points performed during the postprandial period (p<0.001). Results are plotted as Mean±SE. Variables were compared using repeated measured ANOVA, with sex and age as covariates.
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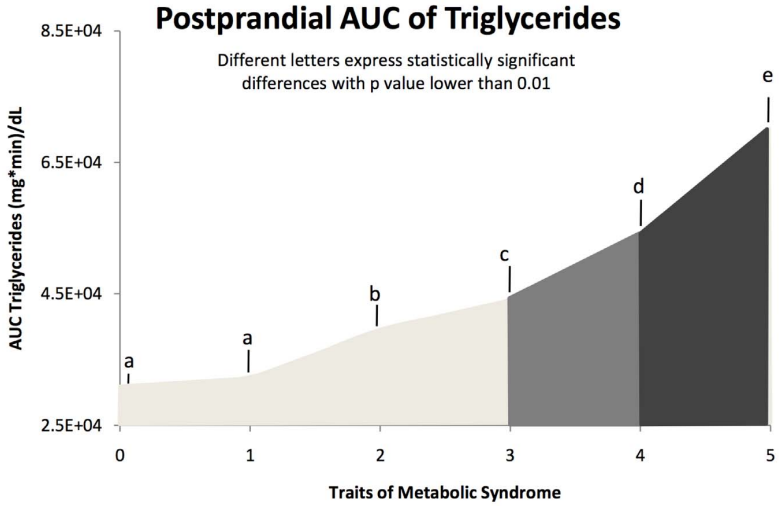

Figure 3. Postprandial AUC of triglycerides in relation to Mets traits. The magnitude of the AUC of postprandial TG increased in the sequence 0, 1<2 criteria <3 criteria <4 criteria <5 criteria. Variables were compared using ANOVA with sex and age as covariates. Different letters express statistically significant differences with a p value below 0.01.
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cholesterol and triglycerides. The LDL-cholesterol was calculated using the Friedewald formula (provided the triglyceride level was less than 300 mg/dl). Apolipoprotein A1 and apolipoprotein B were determined by immunoturbidimetry by means of mouse specific antibodies for every magnitude.

TG-rich lipoprotein fraction (TRL) containing chylomicrons and VLDL was removed from plasma by ultracentrifugation performed in a 70Ti fixed-angle rotor at 30.000 rpm and 4°C for 30 min. at d<1.006 g/mL.

Statistical

All statistical analyses were made with PASW Statistics software, version 18.0.0. Continuous variables were compared using Student's "t" and the analysis of variance (ANOVA) depending on the existence of two or more groups in each comparison. When these variables did not follow a normal distribution, the required transformation of the data was used for analysis. We used total (AUC) and incremental (iAUC) area under the curves of the different postprandial parameters following the trapezoid rule to assess the magnitude of change during the postprandial state, as in previous works by our group [24]. The units used for the AUC and iAUC were (mg * min * dL^{-1}). To determine the influence of metabolic syndrome in the postprandial metabolism, we used a general linear model of repeated measures of each postprandial parameter, with presence or not of metabolic syndrome as a between-subjects variable, blood drawing time as a within-subject variable and gender and age as covariates. Bonferroni's correction was used for multiple comparisons. Pearson's correlation or Spearman rank order correlation analyses were performed to examine the correlations between the levels of metabolic syndrome traits (Systolic blood pressure, Diastolic Blood Pressure, HDL-c, TG, Glucose and waist circumference), treatment (statins and fibrates) and AUC of postprandial parameters. The values of fasting triglycerides, fasting glucose, fasting HDL-c, waist circumference and both systolic and diastolic blood pressure were tested in a stepwise multiple linear regression to predict the AUC of triglycerides and determine their individual effect on it.

Results

Baseline characteristics

A total of 1002 participants with coronary artery disease were included in the CORDIOPREV study (**Figure 1**), of which 581 had MetS criteria.

Table 2 shows the baseline characteristics. The mean age was 59.5 years for all the population. They were mostly males (83.4%) with a mean body mass index of 31.1 Kg/m^2. Patients with MetS showed significant differences compared with patients without MetS, with greater weight, waist circumference, body mass index, plasma glucose, and higher levels of fasting TG and ApoB (all, p< 0.05). MetS patients showed lower levels of fasting HDL-c, ApoA1 and LDL-c (all, p<0.05).

Postprandial parameters and Metabolic Syndrome

After the intake of the fat load test, we found differences between patients with and without MetS during the postprandial period. MetS patients showed higher plasma levels of TG (**Figure 2**) and ApoB as well as lower HDL-c and ApoA1 in each of the time points performed during the postprandial period (all, p≤0.001). No differences were detected for total cholesterol between groups. Furthermore, MetS patients showed lower AUC of postprandial HDL-c (8375.6±84.6 vs 9940.5±99.8, p<0.001) and ApoA1 (28236.1±205.7 vs 30546.9±242.9, p<0.001), with higher AUC of postprandial TG (55048.74±1186.7 vs 36373. 6±1398.3, p<0.001), TRL (22389.3±606.9 vs 13983.4±703.1, p<0.001), ApoB (16697.4±176.8 vs 15289.7±207.7, p<0.001) and glucose (36936.7±568.4 vs 27546.3±669.5, p<0.001). We also analyzed the incremental (iAUC) of area under postprandial parameters curve. MetS patients showed higher iAUC of TG (15298.9±540.1 vs 12296.2±636.4, p<0.001), TRL (12673.1±353.6 vs 9322.5±409.6) and glucose (5608.0±286.7 vs 2131.9±337.6, p<0.001), with higher negative iAUC of ApoB (−743.2±68.4 vs −490.2±80.3, p<0.001) and total cholesterol (−1143.3±92.9 vs −828.5±109.8, p<0.001).

Table 3. Correlations between Mets traits and area under the curve of postprandial parameters.

	Triglycerides	TRL-TG	TC	HDL-c	Apo-A1	Apo-B	Glucose
	r[1]						
	(p values)						
SBP, mmHg	0.03	0.01	0.07	−0.01	0.02	0.09	0.17
	(0.30)	(0.67)	(0.03)	(0.96)	(0.40)	(<0.01)	(<0.01)
DBP, mmHg	0.11	0.13	0.13	0.01	0.01	0.14	−0.02
	(<0.01)	(<0.01)	(<0.01)	(0.92)	(0.58)	(<0.01)	(0.52)
HDL-C, mg/dL	−0.27	−0.25	0.24	0.82	0.68	−0.02	−0.18
	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(0.47)	(<0.01)
TG, mg/dL	0.77	0.58	0.30	−0.31	−0.14	0.39	0.17
	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
Glucose, mg/dL	0.20	0.16	0.02	−0.12	−0.09	0.06	0.80
	(<0.01)	(<0.01)	(0.44)	(<0.01)	(<0.01)	(0.04)	(<0.01)
Waist circumference, cm	0.20	0.15	−0.01	−0.15	−0.10	0.03	0.24
	(<0.01)	(<0.01)	(0.87)	(<0.01)	(<0.01)	(0.31)	(<0.01)

[1]Values are shown as correlation coefficients and (p values). DBP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, Total cholesterol; TG, triglycerides.

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Table 4. Multiple linear regression coefficients[1] to predict AUC of triglycerides.

Parameter	Unstandardized Coefficients		Standardized Coefficients	Sig.
	B	Std. Error	Beta	
Fasting TG	228.49	6.68	0.752	<0.001
Fasting Glucose	29.41	11.05	0.059	0.008
Waist Circumference	85.78	37.43	0.051	0.02

Predictive variables tested by stepwise method: fasting triglycerides (mg/dL), fasting glucose (mg/dL), waist circumference (cm), fasting HDL-c (mg/dL), systolic and diastolic Blood (mmHg).
[1](Constant) = 2163.28. (R^2) = 0.602.
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Components of Metabolic Syndrome and postprandial response

Postprandial lipemia response was directly related to the number of MetS components. Specifically, we found a positive association between the number of MetS criteria and the response of postprandial plasma TG ($p<0.001$), AUC of TG ($p<0.001$) and iAUC of TG ($0<0.001$). Interestingly, the magnitude of the AUC of TG increased in the sequence 0, 1<2 criteria <3 criteria <4 criteria <5 criteria, as shown in **Figure 3** ($p<0.001$). For the iAUC of TG, this sequence differs as follows: 0, 1<2, 3<4<5 (10179±1862, 10501±828<13746±641, 13774±633< 15886±653<19006±1070, respectively). A similar fashion was detected for AUC of plasma glucose and ApoB. In contrast, a negative relationship in the number of criteria of MetS with AUC of HDL-c and ApoA1 was observed (data not shown).

The correlations between the levels of MetS traits and postprandial parameters (AUCs of TG, TRL, total cholesterol, HDL-c, ApoA1, ApoB and glucose) are shown in **Table 3**. Specifically, postprandial AUC of TG were significantly correlated with the values of diastolic blood pressure ($r = 0.11$, $p<0.01$), HDL-c ($r = -0.27$, $p<0.01$), fasting glucose ($r = 0.20$, $p<0.01$), TG ($r = 0.77$, $p<0.01$) and waist circumference ($r = 0.20$, $p<0.01$). Moreover, levels of postprandial AUC of TRL were significantly correlated with the values of diastolic blood pressure ($r = -0.13$, $p<0.01$), HDL-c ($r = -0.25$, $p<0.01$), TG ($r = 0.58$, $p<0.01$), fasting glucose ($r = 0.16$, $p<0.01$) and waist circumference($r = 0.15$, $p<0.01$). Hypolipidemic drugs (statins or fibrates) were not significantly correlated with fasting TG and the AUC of TG (all $p>0.05$).

In stepwise multiple linear regression analysis with the AUC of triglycerides as the dependent variable, only fasting triglycerides, fasting glucose and waist circumference appeared as significant ($P<0.05$) contributors. The multiple regression (R) was 0.77, and fasting triglycerides showed the greatest effect on AUC of triglyceride (**Table 4**).

To explore the effect of basal hypertriglyceridemia on postprandial metabolism, patients were divided into two groups according to the presence or abscence of basal hypertriglyceridemia. In patients with high fasting triglycerides (TG ≥150 mg/dL), the AUC and iAUC of TG were significantly greater (64164±1169 vs 36403±501, $p<0.001$; and 17548±1083 vs 12229±324, $p = 0.001$, respectively) than in the group of patients with fasting TG<150 mg/dL.

The influence of the different MetS factors still remained statistically significant ($p<0.001$) when we analyzed the AUC of TG on those patients without high TG at the basal point, but not on those patients with basal hypertriglyceridemia (**Table 5**).

Discussion

In the present study we investigated the effect of a fatty meal on postprandial lipid metabolism in patients with coronary artery disease. We showed that MetS and the number of its components influence the degree of postprandial lipemic response. Specifically, postprandial AUC of TG showed a progressively unfavorable increase from one component to five in our population. However, this effect was attenuated when the population was divided into two groups according to the presence or absence of basal hypertriglyceridemia. Thus, only those patients without high fasting TG remained a statistically significant influence.

Recently, it has been established that the presence of higher number of components of MetS is associated with an increase in subclinical atherosclerosis, and incidence and mortality of CHD. Teramura et al. reported that intima-medial thickness was significantly higher in subjects with MetS and increased with the number of coexisting components of MetS, compared with those without MetS [17]. Furthermore, a prospective cohort study including 6255 subjects, showed how CHD and CVD mortality were both influenced by the number of MetS components [20]. In the same context, Sattar et al. observed that men presenting four or five MetS traits had a 3.7-fold increase in risk for CHD and a 24.5-fold increase for diabetes compared with men with none [19]. However, the mechanisms underlying this fact are still unknown. Although it is generally accepted that the main pathogenic mechanism underlying the development of cardiometabolic changes in patients with MetS relies on insulin resistance, other mechanisms could influence the increased risk of CVD associated to MetS. While the independence of the association and causality has not been fully established, postprandial TG concentrations have emerged as a clinically significant CVD risk factor following the results of several prospective studies [25]. In our study, patients with an increased number of MetS components showed higher levels of postprandial TG, confirmed by AUC and iAUC of TG. This deterioration in postprandial lipid metabolism associated with the increase number of MetS components may favor a higher risk of atherogenesis.

Previous studies have explored the mechanisms underlying the relation between postprandial lipid metabolism and the increased risk of atherogenesis [26,27]. High levels of postprandial triglycerides have been reported to correlate with high remnant cholesterol in individuals in the general population [4], and, in addition, it has been proposed that in those situations where the liver induces an overproduction of VLDL, such as central obesity, metabolic syndrome, type 2 diabetes mellitus and familial combined hypercholesterolemia, VLDL and chylomicrons catabolic mechanisms are saturated [12,28–30]. These mechanisms cause the accumulation of VLDL and chylomicron remnants [31–

Table 5. Area under the curve of postprandial triglycerides in patients with and without basal hypertriglyceridemia in relation to Metabolic Syndrome components.

TG<150 mg/dL							
	Traits of Metabolic Syndrome						
	0	**1**	**2**	**3**	**4**	**5**	**global p value**
AUC TG	$31249.9^{3,4}$	$32162.0^{2,3,4}$	$36180.3^{1,4}$	$37815.1^{0,1,4}$	$41472.0^{0,1,2,3}$	-	1.01×10^{-7}
	(2364.3)	(1060.3)	(881.2)	(920.7)	(1219.2)		
TG≥150 mg/dL							
	Traits of Metabolic Syndrome						
	0	**1**	**2**	**3**	**4**	**5**	**global p value**
AUC TG	-	63198.2	58217.3^{5}	62995.1	64265.9	67544.7^{2}	NS
		(19253.4)	(3362.4)	(2607.4)	(1852.1)	(2300.4)	

Area under the curve of triglycerides is expressed as mg*min*dL^{-1}. Values are shown as Mean (SEM) and were compared using ANOVA and Bonferroni multiple comparison post hoc test with sex and age as covariates. Superscript characters indicate differences between groups (0, 1, 2, 3, 4, 5) within the same row.

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33], a lower concentration of HDL-c and the activation of leukocytes and endothelial cells by the remnants and fatty acids [34,35]. At this stage, postprandial remnant lipoproteins would penetrate the vessel wall and monocytes would catch them, inducing the formation of foam cells [36]. Although it is generally accepted that the main pathogenic mechanism underlying the development of metabolic changes in patients with MetS relies on insulin resistance, a large body of evidence supports the concept that increased oxidative stress and a state of chronic low-level inflammation may have important roles in MetS-related manifestations [37]. In this way, the formation of oxidized reactive species and oxidized remnant lipoproteins would also contribute to endothelial dysfunction and the development of coronary artery disease [37]. In this regard, we have recently demonstrated the relationship between the number of MetS components and the degree of oxidative stress in MetS patients [38].

Previous evidence carried out in healthy population has suggested a significant linear trend between increasing numbers of MetS components and magnitude of postprandial lipemia in 112 healthy subjects [25]. Nevertheless, to our knowledge, our study is the first one to show that in non-hypertriglyceridemic coronary patients. Another important feature underlying MetS is atherogenic dyslipidemia, defined as a rise in triglycerides and small LDL particles and low HDL-c [39]. In this regard, we have observed that patients with at least three MetS components have higher ApoB plasma levels and lower HDL-c and ApoA1 plasma levels in all blood drawn during the postprandial state, as well as a positive relationship with AUC of ApoB and a negative relationship with AUC of HDL-c and ApoA1. All of these abnormalities have been implicated as being independently atherogenic [14].

Although baseline TG has been previously proposed in different studies as the major determinant of postprandial lipemia [7–10], in our study the involvement of other factors were also statistically significant. Thereby, the stepwise multiple linear regression analysis with the AUC of triglycerides as the dependent variable, showed that fasting TG, fasting glucose and waist circumference appeared as significant independent contributors, with fasting TG as the major contributors (see Table 4).To avoid the influence of high levels of fasting TG on postprandial response, patients were divided in our study into two groups on the basis of their fasting TG concentrations. In hypertriglyceridemic patients, the AUC and iAUC of TG were significantly greater than in the group of normotriglyceridemic patients, according to previous data reported [40]. Besides, the influence of number of MetS components on AUC of TG remained statistically significant in those patients without high fasting TG but not in those patients with basal hypertriglyceridemia. This feature may be related to the fact that in an already disturbed background, as suggested by a fasting hypertriglyceridemia, the postprandial lipid metabolism is altered, and cannot be impaired further by the presence of MetS traits. However, in patients that are not hypertriglyceridemic, the addition of different metabolic syndrome criteria can progressively worsen the efficient management of a fat meal, suggesting, from a clinical point of view, that MetS subjects with normotriglyceridemia would obtain a higher benefit on the size of postprandial lipemia controlling MetS components than those with hypertriglyceridemia.

Despite the great strength of our study given the population size and the standardized methodology used, there were some limitations. The cross-sectional study design limited our ability to make an inference about the casual relationship between MetS components and postprandial parameters. However, it will be possible to evaluate this point in the future taking in consideration

that the CORDIOPREV study is an ongoing prospective, randomized, opened and controlled trial with a mean proposed follow-up of 5 years. Moreover, it would be interesting to study whether those patients with an increased number of MetS components and higher postprandial lipemia have more cardiovascular events in the future.

In summary, our study shows that the existence of MetS influences the postprandial response of carbohydrates and lipids in patients with coronary heart disease. In non-hypertriglyceridemics patients, the magnitude of postprandial response is related to the number of MetS components altered. Fasting triglycerides are the major contributors to the postprandial triglycerides levels. Our findings imply the need for intensive control of MetS components to decrease the cardiovascular risk.

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Author Contributions

Conceived and designed the experiments: FP-J JL-M. Performed the experiments: JFA-D JD-L PP-M AG-R CM GMQ-N PG-L AC JC JA YA. Analyzed the data: JFA-D JD-L PP-M FP-J JL-M. Wrote the paper: JFA-D JL-M. Provided critical revision of the paper for important intellectual content: JMO FJT.

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C. *Metabolic Phenotypes Of Obesity Influence Triglyceride And Inflammation Homoestasis.*

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ORIGINAL ARTICLE

Metabolic phenotypes of obesity influence triglyceride and inflammation homoeostasis

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ABSTRACT

Background We examined the degree of postprandial triglyceride (TG) response over the day, representing a highly dynamic state, with continuous metabolic adaptations, among normal-weight, overweight and obese patients, according to their metabolically healthy or abnormal status.

Materials and methods A total of 1002 patients from the CORDIOPREV clinical trial (NCT00924937) were submitted to an oral fat load test meal with 0·7 g fat/kg body weight (12% saturated fatty acids (SFA), 10% polyunsaturated fatty acids (PUFA), 43% monounsaturated fatty acids (MUFA), 10% protein and 25% carbohydrates). Serial blood test analysing lipid fractions and inflammation markers (high-sensitivity C-reactive protein (hs-CRP)) were drawn at 0, 1, 2, 3 and 4 h during postprandial state. We explored the dynamic response according to six body size phenotypes: (i) normal weight, metabolically healthy; (ii) normal weight, metabolically abnormal; (iii) overweight, metabolically healthy; (iv) overweight, metabolically abnormal; (v) obese, metabolically healthy; and (vi) obese, metabolically abnormal.

Results Metabolically healthy patients displayed lower postprandial response of plasma TG and large triacylglycerol-rich lipoproteins (TRLs)-TG, compared with those metabolically abnormal, independently whether or not they were obese ($P < 0{\cdot}001$ and $P < 0{\cdot}001$, respectively). Moreover, the area under the curve (AUC) of TG and AUC of large TRLs-TG were greater in the group of metabolically abnormal compared with the group of metabolically healthy ($P < 0{\cdot}001$ and $P < 0{\cdot}001$, respectively). Interestingly, metabolically abnormal subjects displayed higher postprandial response of plasma hs-CRP than did the subgroup of normal, overweight and obese, metabolically healthy patients ($P < 0{\cdot}001$).

Conclusions Our findings showed that certain types of the metabolic phenotypes of obesity are more favourable modulating phenotypic flexibility after a dynamic fat load test, through TG metabolism and inflammation homoeostasis. To identify, these phenotypes may be the best strategy for personalized treatment of obesity.

Keywords CORDIOPREV study, hs-CRP, metabolically healthy obesity, phenotypic flexibility, postprandial lipaemia, triglycerides.

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Introduction

Obesity is a chronic disease which has an outstanding impact on public health due to its increasing prevalence and to the high impact on cardiometabolic diseases. Recent evidence suggests that not all obese subjects display a clustering of metabolic and cardiovascular risk factors, and, likewise, not all lean subjects present a healthy metabolic and disease-free profile [1,2]. Thus, recently more attention has been paid to the different metabolic phenotypes of obesity. Metabolically

[1]The first two authors contributed equally to this study.

healthy obesity (MHO) describes the absence of any cardiometabolic disease (type 2 diabetes mellitus (T2DM), hypertension and dyslipidaemia) in subjects with a body mass index (BMI) >30 kg/m^2. In contrast, metabolically obese status with normal-weight subjects is characterized by hyperinsulinism and insulin resistance, which are susceptible to develop T2DM and cardiovascular disease (CVD), even if they are not obese [3,4]. This fact could support the hypothesis to consider obesity as a systems disease with loss of flexibility in one or more metabolic processes involved [5]. Therefore, the capacity to adapt in time and location to alterations in external factors, such as environmental conditions, is called phenotypic flexibility.

At this point, there is an intriguing debate over whether or not MHO and metabolically obese status with normal-weight individuals have an increased risk of metabolic complications. In this regard, and based in clinical implications, it is important to distinguish between these phenotypes for minimizing or delaying the comorbidities associated with obesity [6]. Moreover, several processes and mechanisms involved in phenotypic flexibility include triglyceride metabolic regulation, glucose regulation, optimal inflammatory balance, oxidative stress regulation, muscle metabolic flexibility and many others. Interestingly, most of these mechanisms are disrupted in obese patients, and it requires a high degree of flexibility, in order to adjust the parameters to fit the situation. One biomarker of this phenotypic inflexibility is the degree of postprandial triglyceride response over the day, representing a highly dynamic state, with continuous metabolic adaptations [7]. Moreover, postprandial lipaemia is considered as a factor in the development of cardiometabolic diseases [8]. Specifically, the effect of triglyceride metabolism on all rapidly changing parameters related to the phenotypes of obesity and the extent to which the human body is able to flexibly react to such challenges can be used to quantify many aspects of phenotypic flexibility. In this regard, the oral fat load test is a classic example of a challenge test [9].

Based on this knowledge, it is important to understand the underlying causes for phenotypic inflexibility and whether obesity in its various forms may influence the maintenance of overall triglycerides homoeostasis and inflammation state. The CORDIOPREV study is an ongoing prospective, controlled trial with a mean follow-up of 5-year duration, including 1002 patients with high-risk coronary disease. In this cohort of high-risk patients, we examined the phenotypic flexibility, measured with the fat tolerance test, among normal-weight, overweight and obese patients, according to their metabolically normal or abnormal status, from the CORDIOPREV clinical trial (NCT00924937). In a next step, we investigated whether several cardiometabolic abnormalities may influence the postprandial lipaemic response.

Material and methods

Population

This work was conducted within the framework of the CORDIOPREV study. The CORDIOPREV study is an ongoing prospective, randomized, opened, controlled trial including 1002 patients with coronary heart disease (CHD), which had their last coronary event more than 6 months before the enrolment in two different dietary models (Mediterranean diet and low-fat diet) over a period of 5 years in addition to conventional treatment for CHD (Figure S1).

Patients were recruited from November 2009 to February 2012, mostly at the Reina Sofia University Hospital (Cordoba, Spain), but other centres from the Cordoba and Jaen provinces were also included. Inclusion and exclusion criteria are exposed in Table 1. In summary, patients were eligible if they were older than 20 years, but younger of 75, had established CHD without clinical events in the last 6 months, were thought to follow a long-term dietary intervention and did not have severe diseases or expected life expectancy lower than 5 years. Patients were categorized depending on the presence or not of several cardiometabolic abnormalities in six different body size phenotypes. To homogenize the analysis, only subjects with all plasma variables available were included (n = 992).

Cardiometabolic abnormalities

Cardiometabolic abnormalities were considered according to body size phenotype definitions proposed by Wildman *et al.* [10]. For homoeostasis model assessment of insulin resistance (HOMA-IR), we used the cut-off points of insulin resistance for the Spanish population [11], and for the high-sensitivity C-reactive protein (hs-CRP) levels, we used the cut-off point suggested for use by the CDC/AHA guidelines to define high-risk levels [12]:

1 Elevated blood pressure: systolic/diastolic blood pressure ≥130/85 mmHg or antihypertensive medication use;
2 Elevated triglyceride level: fasting triglyceride level ≥150 mg/dL;
3 Decreased HDL-C level: HDL-C level <40 mg/dL in men or <50 mg/dL in women or lipid-lowering medication use;
4 Elevated glucose level: fasting glucose level ≥100 mg/dL or antidiabetic medication use;
5 Insulin resistance: HOMA-IR >2·6;
6 Systemic inflammation: hs-CRP level ≥3 mg/L.

Criteria for body size phenotypes [10]

1 Normal weight, metabolically healthy: BMI < 25·0 and <2 cardiometabolic abnormalities;

Table 1 Inclusion and exclusion criteria for CORDIOPREV study

Ages Eligible	20–75 years
Genders Eligible	Both
Inclusion Criteria	Unstable coronary disease
	Acute myocardial infarction
	Unstable angina
	Chronic coronary disease at high risk for event
Exclusion Criteria	Age <20 or >75 years (or life expectancy lower than 5 years)
	Patients already planned for revascularization
	Patients submitted to revascularization in the last 6 months
	Grade II/IV Heart failure
	Left ventricle dysfunction with ejection fraction lower than 35%
	Patients unable to follow a protocol
	Patients with severe uncontrol of diabetes mellitus, or those with renal insufficiency with permanent plasma creatinine higher than 2 mg/dL, or cerebral complications of diabetes mellitus
	Other chronic diseases:
	Psychiatric diseases
	Chronic renal insufficiency
	Chronic hepatopathy
	Active malignancy
	COPD
	Diseases of the digestive tract
	Endocrine disorders
	Patients participating in other clinical trials (in the enrolment moment or 30 days prior)

2 Normal weight, metabolically abnormal: BMI < 25·0 and ≥2 cardiometabolic abnormalities;
3 Overweight, metabolically healthy: BMI 25·0–29·9 and <2 cardiometabolic abnormalities;
4 Overweight, metabolically abnormal: BMI 25·0–29·9 and ≥2 cardiometabolic abnormalities;
5 Obese, metabolically healthy: BMI ≥ 30·0 and <2 cardiometabolic abnormalities;
6 Obese, metabolically abnormal: BMI ≥ 30·0 and ≥2 cardiometabolic abnormalities.

All patients gave written informed consent to participate in the study. The trial protocol and all amendments were approved by the local ethics committees, following the Helsinki declaration and the good clinical practices.

Study design

Before participants were enrolled in two different dietary models (Mediterranean diet and low-fat diet) from CORDIOPREV study, they received an oral fat tolerance test using a weight-adjusted meal (0·7 g fat and 5 mg cholesterol per kg body weight) with 12% saturated fatty acids (SFA), 10% polyunsaturated fatty acids (PUFA), 43% monounsaturated fatty acids (MUFA), 10% protein and 25% carbohydrates (CHO). Meal preparation was performed by a group of nutritionists with olive oil, skimmed milk, white bread, cooked egg yolks and tomatoes.

Methodology of the oral fat tolerance test

Previously, to the starting of the test, the patients had been fasting for 12 h and were asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 7 days. They were also asked to avoid strenuous physical activity the day before the test given. At 8:00 a.m., patients presented in the laboratory, completed anthropometric (weight, height, waist circumference, BMI and blood pressure) and biochemical measurements, donated a fasting blood sample and under supervision, ingested the fatty food meal. The breakfast was eaten in 20 min. After the meal, volunteers were resting and consumed no food for 5 h, but were allowed to drink water.

Blood samples for biochemical testing were collected before the meal and every hour during the next 4 h, following recommendations for an oral fat tolerance test proposed by Mihas *et al.* [13] in a recent meta-analysis.

Laboratory test

Venous blood was sampled from the antecubital vein and collected into Vacutainer tubes with no anticoagulant and to tubes containing EDTA, and immediately transferred to 4 °C. To minimize proteolytic degradation, plasma was supplemented with protease inhibitor cocktail (Roche Diagnostic, Mannheim, Germany) 40 μL per mL of plasma. Plasma and serum samples were frozen at −80 °C for further biochemical analysis. Serum parameters were measured in Architect c16000 analysers (Abbott®, Chicago, IL, USA) by spectrophotometric techniques (enzymatic colorimetric methods): hexokinase method for glucose, and oxidation–peroxidation for total cholesterol, HDL-C and triglycerides (TG). Apolipoprotein A-1 and apolipoprotein B were determined by immunoturbidimetry by means of mouse-specific antibodies for every magnitude. Plasma levels of insulin were measured by chemiluminescent microparticle immunoassay using an analyser (i-2000Abbott Architect ®,

Chicago, IL, USA). HOMA-IR was derived from fasting glucose and insulin levels [(fasting plasma glucose x fasting serum insulin)/22·5]. As HOMA-IR takes into account both insulin and glucose levels, it may be a more complete index than plasma insulin. The hs-CRP is a strong independent risk factor for cardiovascular events, and levels of hs-CRP ≥3 mg/L have been suggested to define high-risk group [12]. Thus, plasma concentrations of hs-CRP were determined by high-sensitivity ELISA (BioCheck, Inc., Foster City, CA, USA) at the University College Dublin. Large triacylglycerol-rich lipoproteins fraction (TRL) containing chylomicrons and VLDL was removed from plasma by ultracentrifugation performed in a 70Ti fixed-angle rotor (Beckman Instruments, Fullerton, CA, USA) at 82 508 gpm and 4 °C during 30 min at density <1·006 g/mL.

Statistical analysis

All statistical analyses were made with PASW Statistics software, version 18.0.0. Continuous variables were compared using Student's '*t*-test' and the analysis of variance (ANOVA) depending on the existence of two or more groups in each comparison. When these variables did not follow a normal distribution, the required transformation of the data was used for analysis. Data are presented as means ± standard error (SE) for continuous variables and as frequencies or percentages for categorical variables. To determine the influence of body size phenotypes in the postprandial metabolism, we used a general linear model of repeated measures of each postprandial parameter, with the different phenotype as between-subjects variable, blood drawn time as within-subject variable, and gender and age as covariates. We used total area under the curve (AUC) and delta (Δ) AUC of the different postprandial parameters following the trapezoid rule to assess the magnitude of change during postprandial state, as in previous works of our group [14]. Pearson's correlation was performed to examine the correlations between the levels of cardiometabolic abnormalities (systolic blood pressure, diastolic blood pressure, HDL-C, TG, glucose, HOMA-IR and hs-CRP) and postprandial parameters (AUC TG and AUC hs-CRP). Bonferroni's test was used in the *post hoc* analysis. All the analyses were adjusted for potential confounders, and $P < 0·05$ was considered to be significant.

Results

Baseline demographic and metabolic characteristics according to several body size phenotypes are presented in Table 2.

TG metabolism response during fat load test

We explored the dynamic response during the fat load test according to the same body size phenotypes. Thus, 'metabolically healthy' patients showed lower postprandial TG concentration, compared with those 'metabolically abnormal' ($P < 0·001$), independently whether or not they were obese (Fig. 1a). No significant differences were observed within the group of metabolically healthy (among normal, overweight and obese) or the group of metabolically abnormal (among normal, overweight and obese) (Fig. 1a). Specifically, we observed that overweight and obese, metabolically abnormal subjects showed a higher TG postprandial response compared with the subgroup of normal, overweight and obese metabolically healthy patients ($P < 0·05$ for all comparisons) (Fig. 1a). The same effect was observed in normal-weight but metabolically abnormal subjects compared with normal-weight but metabolically healthy subjects ($P = 0·039$) (Fig. 1a). Consistently, metabolically healthy patients displayed lower postprandial response of large TRL-TG, compared with those metabolically abnormal, independently whether or not they were obese ($P < 0·001$) (Fig. 1b). The area under the postprandial curve in the study participants according to the body size phenotypes was analysed; significant differences were observed between subgroups (Fig. 2). AUC of TG and AUC of large TRL-TG were greater in the group of metabolically abnormal compared with the group of metabolically healthy ($P < 0·001$ and $P < 0·001$, respectively) (Fig. 2). Moreover, the ΔAUC of TG confirmed these results ($P = 0·008$, 14555·89 ± 455·04 vs. 11686·81 ± 972·93 metabolically abnormal vs. metabolically healthy). A gender-stratified analysis of AUC of TG and AUC of TRL-TG is shown in Table S1. In this analysis, significant differences remained between subgroups for AUC of TG in both men and women. For AUC of TRL, only the TG differences in men remained significant.

Hs-CRP response during fat load test

We also measured the postprandial serum hs-CRP levels and the effect of the different body size phenotypes. Patients with very high levels of hs-CRP (≥10 mg/L) were excluded from the analysis to avoid nonspecific inflammation. We observed that metabolically abnormal subjects displayed a higher hs-CRP postprandial response compared with the subgroup of metabolically healthy patients ($P < 0·001$). Specifically, we observed that overweight and obese, metabolically abnormal subjects showed a higher hs-CRP postprandial response compared with the subgroup of normal, overweight and obese metabolically healthy patients ($P < 0·05$ for all comparisons) (Fig. 3). In addition, the subgroup of normal weight but metabolically abnormal had a higher postprandial hs-CRP response compared with the obese but metabolically normal ($P = 0·012$) (Fig. 3). Our results remained significant even after adjustment made by fasting hs-CRP (Fig. 4).

Influence of other potential factors on the postprandial response

Finally, we explored the influence of several potential factors on the postprandial response. The correlations between the

Table 2 Demographic and metabolic characteristics of CORDIOPREV study by body size phenotype[a]

Demographic and metabolic characteristics	Overall	Metabolically healthy			Metabolically abnormal			P global
		Normal weight[(a)]	Overweight[(b)]	Obese[(c)]	Normal weight[(d)]	Overweight[(e)]	Obese[(f)]	
Prevalence, % (population frequency)	100 (992)	2·6 (26)	8·5 (84)	7·5 (74)	3·9 (39)	28·3 (281)	49·2 (488)	
Age, year	59·5 (0·3)	59·0 (1·7)	58·4 (1·1)	61·5 (1·1)	60·9 (1·4)	59·3 (0·5)	59·5 (0·4)	NS
Men, %	83·9	80·8	89·3	83·4	76·9	85·4	81·9	NS
SBP, mmHg	138·7 (0·6)	128·0 (3·6)^{d,e,f}	127·8 (1·9)^{d,e,f}	126·8 (2·0)^{d,e,f}	144·6 (2·9)^{a,b,c}	140·2 (0·9)^{a,b,c}	141·7 (0·9)^{a,b,c}	<0·001
DBP, mmHg	77·2 (0·3)	70·2 (1·9)^{d,e,f}	74·3 (1·0)^{f}	70·9 (1·1)^{d,e,f}	78·3 (1·4)^{a,c}	78·1 (0·6)^{a,c}	78·4 (0·5)^{a,b,c}	<0·001
MetS, %	58·1	0	0	0	28·2	58·7	81·7	<0·001
Elevated blood pressure (SBP> 130 mmHg and/or DBP>85 mmHg), %	68·5	36	33·7	32·4	84·6	76·5	75·8	<0·001
Total cholesterol, mg/dL	160·8 (1·59)	163·1 (6·0)	156·9 (3·4)	159·2 (3·6)	167·8 (5·0)	159·1 (1·8)	158·5 (1·4)	NS
HDL-C, mg/dL	42·2 (0·3)	50·1 (2·3)^{e,f}	48·8 (1·1)^{e,f}	48·7 (1·1)^{e,f}	45·6 (1·8)^{f}	41·1 (0·6)^{a,b,c}	39·9 (0·4)^{a,b,c,d}	<0·001
HDL-C <40 mg/dL for men or <50 mg/dL for women, %	54·7	19·3	19·0	10·8	48·7	60·1	66·7	<0·001
Triglycerides, mg/dL	132·9 (2·1)	79·8 (5·1)^{e,f}	92·2 (3·2)^{e,f}	91·6 (3·2)^{e,f}	113·5 (6·3)^{f}	136·9 (4·1)^{a,b,c}	148·6 (3·0)^{a,b,c,d}	<0·001
Triglycerides >150 mg/dL, %	31·7	0·0	2·4	2·7	23·1	36·3	40·9	<0·001
Apo-B, mg/dL	72·0 (0·9)	69·3 (3·5)^{d}	65·4 (2·0)^{d,e,f}	68·7 (2·1)^{d,f}	79·4 (2·9)^{a,b,c}	73·2 (1·1)^{b}	75·8 (0·8)^{b,c}	<0·001
Apo-A1, mg/dL	135·4 (1·1)	147·5 (4·0)^{b,d,e,f}	137·8 (2·2)^{a,d,e,f}	139·0 (2·3)^{e,f}	133·7 (3·3)^{a,f}	127·7 (1·2)^{a,b,c}	126·6 (0·9)^{a,b,c,d}	<0·001
Glucose, mg/dL	110·7 (1·1)	88·4 (2·4)^{e,f}	89·1 (1·1)^{e,f}	91·0 (1·2)^{e,f}	107·6 (7·1)	113·8 (1·9)^{a,b,c,f}	123·5 (2·9)^{a,b,c,e}	<0·001
Glucose >100 mg/dL and/or antidiabetic medication use, %	60·6	15·3	11·9	20·2	43·5	69·3	73·9	<0·001

Table 2 *Continued*

Demographic and metabolic characteristics	Overall	Metabolically healthy			Metabolically abnormal			P global
		Normal weight[(a)]	Overweight[(b)]	Obese[(c)]	Normal weight[(d)]	Overweight[(e)]	Obese[(f)]	
Insulin, μU/mL	11·0 (0·3)	4·2 (0·5)^{f}	5·4 (0·3)^{e,f}	7·2 (0·4)^{f}	5·9 (0·6)^{f}	9·8 (0·4)^{b,f}	14·0 (0·3)^{a,b,c,d,e}	<0·001
HOMA-IR	2·9 (0·1)	1·0 (0·1)^{e,f}	1·3 (0·1)^{e,f}	1·7 (0·1)^{e,f}	1·7 (0·2)^{f}	2·7 (0·1)^{a,b,c,f}	3·6 (0·1)^{a,b,c,d,e}	<0·001
HOMA-IR >2·6, %	45·6	0	2·7	10·4	22·2	42·9	65·2	<0·001
BMI	31·1 (0·1)	23·3 (0·3)^{b,c,e,f}	27·5 (0·1)^{a,c,d,f}	32·6 (0·3)^{a,b,d,e,f}	23·7 (0·2)^{b,c,e,f}	28·0 (0·1)^{a,c,d,f}	34·3 (0·2)^{a,b,c,d,e}	<0·001
Waist circumference, cm	105·1 (0·3)	90·9 (1·7)^{b,c,e,f}	97·4 (0·8)^{a,c,d,f}	107·1 (0·9)^{a,b,d,e,f}	90·1 (1·4)^{b,c,e,f}	98·5 (0·4)^{a,c,d,f}	111·9 (0·4)^{a,b,c,d,e}	<0·001
CRP,mg/L	2·4 (0·1)	0·9 (0·1)^{e,f}	1·4 (0·1)^{e,f}	1·7 (0·1)^{f}	2·4 (0·3)	2·3 (0·1)^{a,b,f}	2·9 (0·1)^{a,b,c,e}	<0·001
CRP >3 mg/L, %	54·6	3·8	9·5	10·8	43·6	34·4	42·4	<0·001
AUC Postprandial Triglycerides	45514·5 (684·2)	27598·6^{d,e,f} (1915·7)	34358·1 (1532·0)^{d,e,f}	33584·1 (1390·2)^{d,e,f}	43653·7 (3002·6)^{a,b,c}	45486·5 (1304·8)^{a,b,c,f}	50324·1 (995·6)^{a,b,c,e}	<0·001

Data are given as mean (SE) unless otherwise specified. Bonferroni correction was used as *post hoc* test for comparison between groups. Superscript letters indicate statistically significant differences between groups with $P < 0·05$.
BMI, body mass index (calculated as weight in kilograms divided by height in metres squared); DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; MetS, metabolic syndrome; SBP, systolic blood pressure.
Cardiometabolic abnormalities considered: (i) Elevated blood pressure: systolic/diastolic blood pressure ≥130/85 mmHg or antihypertensive medication use; (ii) Elevated triglyceride level: fasting triglyceride level ≥150 mg/dL; (iii) Decreased HDL-C level: HDL-C level <40 mg/dL in men or <50 mg/dL in women or lipid-lowering medication use; (iv) Elevated glucose level: fasting glucose level ≥100 mg/dL or antidiabetic medication use; (v) Insulin resistance: HOMA-IR >2·6; 6. Systemic inflammation: hs-CRP level >3 mg/L.
Criteria for body size phenotypes [10]: (i) Normal weight, metabolically healthy: BMI <25·0 and <2 cardiometabolic abnormalities; (ii) Normal weight, metabolically abnormal: BMI <25·0 and ≥2 cardiometabolic abnormalities; (iii) Overweight, metabolically healthy: BMI 25·0–29·9 and <2 cardiometabolic abnormalities; (iv) Overweight, metabolically abnormal: BMI 25·0–29·9 and ≥2 cardiometabolic abnormalities; (v) Obese, metabolically healthy: BMI ≥30·0 and <2 cardiometabolic abnormalities; (vi) Obese, metabolically abnormal: BMI ≥30·0 and ≥2 cardiometabolic abnormalities.

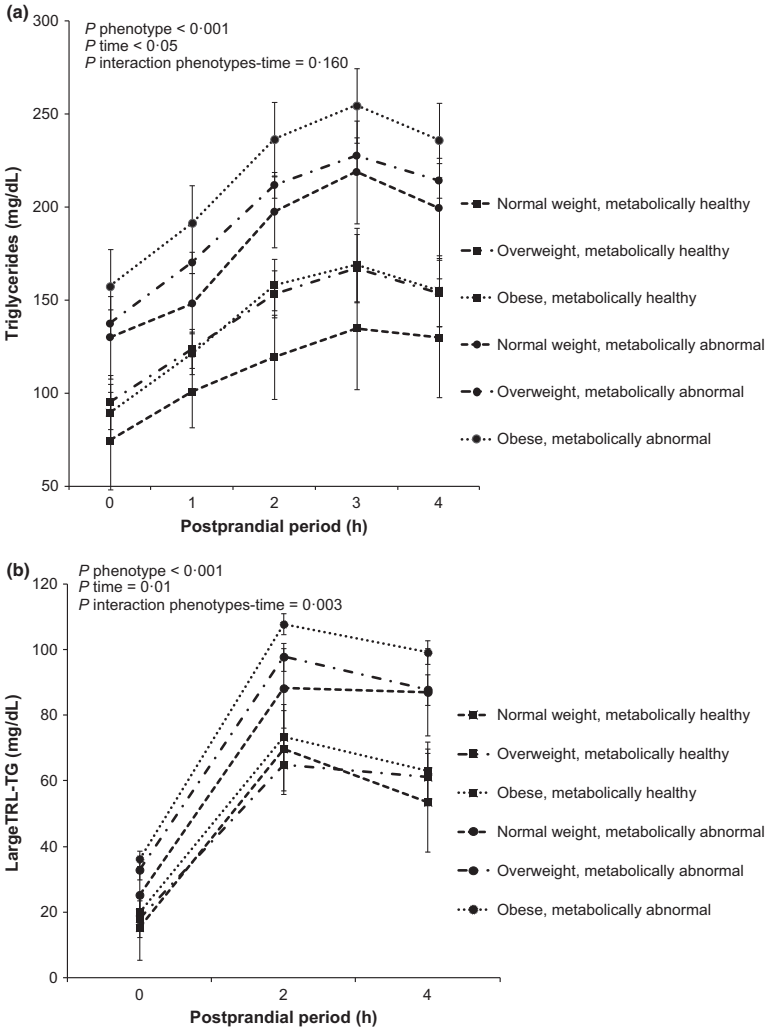

Figure 1 Evolution of (a) triglycerides (TG) and (b) large triacylglycerol-rich lipoproteins (TRLs)-TG after the oral fat tolerance test according to the body size phenotypes [10] defined as follows: (i) Normal weight, metabolically healthy: BMI < 25·0 and <2 cardiometabolic abnormalities (n = 26); (ii) Normal weight, metabolically abnormal: BMI < 25·0 and ≥2 cardiometabolic abnormalities (n = 84); (iii) Overweight, metabolically healthy: BMI 25·0–29·9 and <2 cardiometabolic abnormalities (n = 74); (iv) Overweight, metabolically abnormal: BMI 25·0–29·9 and ≥2 cardiometabolic abnormalities (n = 39); (v) Obese, metabolically healthy: BMI ≥ 30·0 and <2 cardiometabolic abnormalities (n = 281); (vi) Obese, metabolically abnormal: BMI ≥ 30·0 and ≥2 cardiometabolic abnormalities (n = 488). Results are plotted as mean ± SE. Variables were compared using repeated measured ANOVA, with sex and age as covariates.

levels of cardiometabolic abnormalities and postprandial parameters are shown in Table S2. The levels of postprandial AUC of TG were significantly correlated with the values of diastolic blood pressure (r = 0·11, P < 0·01), HDL-C (r = −0·27, P < 0·01), fasting glucose (r = 0·20, P < 0·01), TG (r = 0·77, P < 0·01), HOMA-IR (r = 0·23, P < 0·01) and hs-CRP (r = 0·15, P < 0·01). The levels of postprandial AUC of hs-CRP were significantly correlated with the values of HDL-C (r = −0·11, P<0·01), TG (r = 0·07, P = 0·02), fasting glucose (r = 0·09, P < 0·01) and hs-CRP (r = 0·54, P < 0·01).

Discussion

Our findings support the hypothesis that metabolically healthy patients, even if they were overweight or obese, showed a higher degree of flexibility sustained by an improvement in the postprandial TG response and the inflammation status, compared with those metabolically abnormal, independently whether or not they were obese. Thus, we have identified some inflexibility-risk phenotypes (i.e. normal weight, metabolically abnormal and overweight, metabolically abnormal) and other

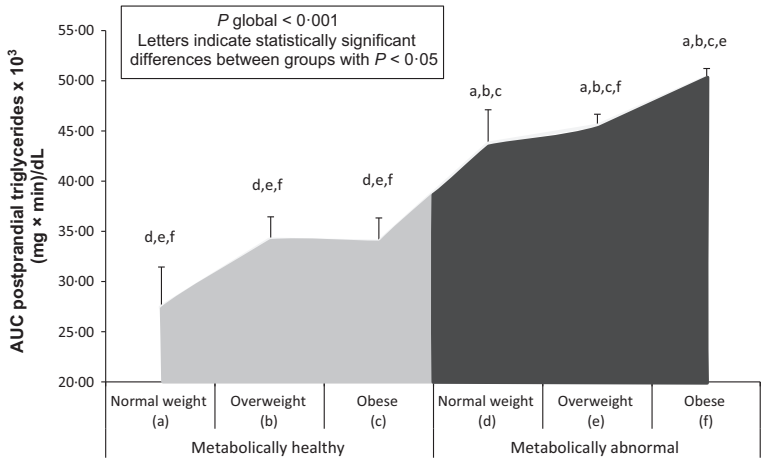

Figure 2 Postprandial area under the curve (AUC) of triglycerides (TG) according to the body size phenotypes [10] defined as follows: (i) Normal weight, metabolically healthy: BMI < 25·0 and <2 cardiometabolic abnormalities (n = 26); (ii) Normal weight, metabolically abnormal: BMI < 25·0 and ≥2 cardiometabolic abnormalities (n = 84); (iii) Overweight, metabolically healthy: BMI 25·0–29·9 and <2 cardiometabolic abnormalities (n = 74); (iv) Overweight, metabolically abnormal: BMI 25·0–29·9 and ≥2 cardiometabolic abnormalities (n = 39); (v) Obese, metabolically healthy: BMI ≥ 30·0 and <2 cardiometabolic abnormalities (n = 281); (vi) Obese, metabolically abnormal: BMI ≥ 30·0 and ≥2 cardiometabolic abnormalities (n = 488). Variables were compared using ANOVA with sex and age as covariates. Different letters express statistically significant differences with P value lower than 0·05.

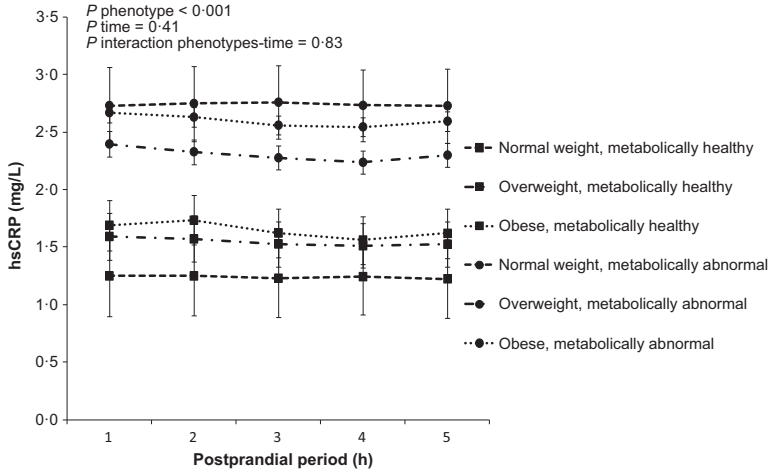

Figure 3 Evolution of high-sensitivity C-reactive protein (hs-CRP) after the oral fat tolerance test according to the body size phenotypes [10] defined as follows: (i) Normal weight, metabolically healthy: BMI <25·0 and <2 cardiometabolic abnormalities (n = 26); (ii) Normal weight, metabolically abnormal: BMI <25·0 and ≥2 cardiometabolic abnormalities (n = 84); (iii) Overweight, metabolically healthy: BMI 25·0–29·9 and <2 cardiometabolic abnormalities (n = 74); (iv) Overweight, metabolically abnormal: BMI 25·0–29·9 and ≥2 cardiometabolic abnormalities (n = 39); (v) Obese, metabolically healthy: BMI ≥ 30·0 and <2 cardiometabolic abnormalities (n = 281); (vi) Obese, metabolically abnormal: BMI ≥30·0 and ≥2 cardiometabolic abnormalities (n = 488). Results are plotted as mean ± SE. Variables were compared using repeated measured ANOVA, with sex and age as covariates.

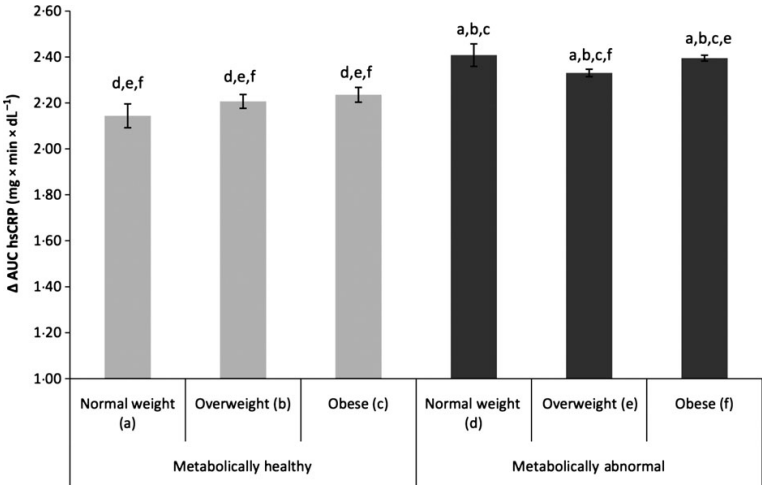

Figure 4 Postprandial incremental (Δ) area under the curve (AUC) of hs-CRP according to the body size phenotypes.

flexibility-risk phenotypes (overweight, metabolically healthy and obese, metabolically healthy). Previous evidences have linked the postprandial TG response to the incidence of coronary artery disease and stroke [15,16]. Thus, to identify these inflexibility-risk phenotypes with an exaggerated postprandial TG and inflammation response may be important in terms of early identification of those at greatest risk who should be prioritized for pharmacological and lifestyle intervention. It is noteworthy that we did not find significant differences within the group of metabolically healthy (among normal, overweight and obese) or the group of metabolically abnormal (among normal, overweight and obese).

Despite the increase in the prevalence of obese individuals, less is known about the factors involved in the development of phenotypic inflexibility in this population. It looks clear that the more efficient an organism is in adjusting its phenotype to a new situation, the more stable and healthy it remains. In this regard, our study has identified how the different metabolic phenotypes of obesity can adapt their response to a stressful dynamic test according to their triglyceride homoeostasis. Interestingly, this response was accompanied by differences in the inflammation system. Based on our results, the question then arises whether some normal weight but metabolically abnormal phenotypes are unhealthier than some obese metabolically healthy phenotypes. Mechanisms underlying the different metabolic phenotypes of obesity and the development of cardiometabolic diseases have been poorly examined to date. Several hypotheses have been proposed in an attempt to explain the role of the adipose tissue in the metabolic dysfunction associated with obesity to better understand the difference between different groups. Interestingly, most of mechanisms disrupted in obese individuals, such as glucose regulation, optimal inflammatory balance, oxidative stress regulation and triglyceride metabolic regulation, require a high degree of flexibility, in order to adjust the parameters to fit the situation. Other hypotheses suggest the contribution of environmental and genetic and/or epigenetic factors to metabolically abnormal phenotypes risk, although these hypotheses should be investigated in depth.

In obesity, adipose tissue dysfunction will eventually lead to abnormalities in lipid metabolism, such as hypertriglyceridaemia (due to decreased TG hydrolysis and increased hepatic very-low-density lipoprotein production), small dense LDL particles, remnant lipoproteins and low HDL-C levels, all associated with a higher risk for the development of cardiovascular diseases [17–20]. Adipocyte hypertrophy leads to many changes in adipocyte function and production of anti- and pro-inflammatory cytokines. By secreting adipokines and other proteins (such as lipoprotein lipase and cholesteryl ester transferase protein), adipose tissue affects TG metabolism. In this context, we observed that obese, metabolically abnormal subjects showed a higher triglyceride postprandial response compared with the subgroup of normal, overweight and obese metabolically healthy patients. Moreover, several studies support the concept that the levels of circulating TRL after meals are significantly associated with the development of atherosclerosis [21,22]. Therefore, we observed that metabolically healthy patients displayed lower postprandial response of large TRL-TG, compared with those metabolically abnormal, independently whether or not they were obese. The mechanisms that might explain our findings are complex and could reflect differences in chylomicron synthesis, secretion or clearance.

However, from a clinical point of view, it is important to identify the higher and undesirable postprandial TG response related to particular obese phenotypes that could be strongly treated by modifying eating habits, (increasing fish consumption and consideration of n-3 supplements), weight loss, smoking cessation and increased physical activity [23,24].

In the last years, van Oostrom and others have provided evidence suggesting that postprandial triglyceridaemia is related to the proinflammatory state due to the high expression of the activation markers in neutrophils and monocytes [25]. Obesity is now considered to be a condition that facilitates the development of a low-grade inflammatory state, characterized by increased plasma levels of proinflammatory cytokines such as tumour necrosis factor (TNF)-alpha, interleukins and adipokines [26,27]. Furthermore, previous studies have confirmed that fat consumption induced the activation of inflammatory markers during the postprandial phase [28,29]. Nevertheless, whether obesity in its various forms has the same state of subclinical inflammation is still a matter of debate. Recently, in fasting state, we have observed that metabolically abnormal individuals displayed a more proinflammatory (higher hs-CRP and leptin), prothrombotic (higher plasminogen activator inhibitor-1 (PAI-1)), and proatherogenic (higher leptin/adiponectin ratio) metabolic profile relative to the metabolically healthy group [30]. In this study, as expected, obese, metabolically abnormal subjects displayed a higher hs-CRP postprandial response compared with the subgroup of normal, overweight and obese metabolically healthy patients. Interestingly, we also observed that levels of postprandial AUC of hs-CRP were strongly correlated with the values of hs-CRP at baseline. These findings confirm that not all obese individuals exhibit increased risk of inflammation and not all normal-weight individuals are inflammatory healthy. Our dynamic results confirm our previous and other fasting data, indicating that postmenopausal women displaying the metabolically healthy obesity phenotype also have a favourable inflammation profile as shown by lower CRP and alpha-1 antitrypsin levels compared with insulin-resistant women. In this context, we have previously demonstrated that after long-term consumption of the high MUFA diet and 4 h after the fat overload, induced a postprandial decrease in NF-kB activation and in the nuclear p65 protein levels in metabolic syndrome (MetS) patients [31]. Moreover, a postprandial increased in the transcription of PBMC IkB-gene and the reduced transcriptional activity of PBMC TNF- and MMP-9 after HMUFA diet, as reflected in decreased mRNA levels, is consistent with decreased NF-kB binding and also with an improvement in the pro-inflammatory state of MetS patients [31]. Therefore, our data suggested a different degree of flexibility between metabolically healthy and unhealthy obese individuals regarding the obesity-associated inflammatory mediators. This point is interesting because insulin resistance has been shown to be the most important, associated with a chronic state of subclinical inflammation and characterized by increased serum concentrations of hs-CRP [32]. It is very interesting to know how these potential mechanisms can modify the natural history of obesity, and how the metabolically healthy obesity converts into metabolically unhealthy obesity. Although there are other several inflammatory markers, hs-CRP is the only marker of inflammation used routinely in clinical practice.

In summary, our findings showed that certain types of the metabolic phenotypes of obesity are more favourable modulating phenotypic flexibility after a dynamic fat load test, through TG metabolism and inflammation homoeostasis. To identify, these phenotypes may be the best strategy for personalized treatment will help physicians in treating the right cohort of at high-risk patients.

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Conflict of interest

None of the authors has any conflict of interest that could affect the performance of the work or the interpretation of the data.

Contributions

Prof. Lopez-Miranda and Prof. Perez-Jimenez had full access to all of the data in the study and take responsibility for the

integrity of the data and the accuracy of the data analysis. Conception and design of the study: P.P-M, J.FA-D and J.L-M. Provision of study materials or subjects: J.D-L, A.G-R, F.G-D, C.M-H, F.R-C, A.C, FJ.F-J, AI.P-C, N.D-C and J.L-M. Collection and assembly of data: J.D-L., J.FA-D, FJ.F-J, AI.P-C and A.C. Analysis and interpretation of the data: P.P-M, J.FA-D, JM.O, FJ.T, F.P-J and J.L-M. Statistical expertise: J.FA-D, J.D-L and A.G-R. Drafting of the manuscript: P.P-M, J.FA-D and J.L-M. Critical review of the manuscript for important intellectual content: P.P-M, J.FA-D, JM.O and J.L-M. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Chapter 5

SUMMARY OF RESULTS AND DISCUSSION

V. Chapter 5: SUMMARY OF RESULTS AND DISCUSSION

In the last years there has been an increasing awareness on the importance of postprandial events in the development and exacerbation of atherosclerosis, since fasting is not the typical physiological state of human in western countries. PPL following a fat-rich meal is a situation characterized by the generation of an atherogenic environment in the bloodstream, derived by the conjunction of the direct atherogenic properties of some lipid particles, especially those carried in the TRLs, and by the activation of the inflammatory and hemostatic systems(22, 203). Results form large population studies have assessed that postprandial TG levels independently predict the risk for coronary artery disease, peripheral vascular disease and cerebrovascular disease, and are possibly even better predictors of CVD than fasting TG(24-28, 204).

As previously reviewed in **Chapter 2** ("**FACTORS AFFECTING THE POSTPRANDIAL RESPONSE")**, postprandial lipoproteins metabolism is modulated by dietary patterns, food composition, conditions associated with lifestyle (physical activity, smoking and alcohol consumption), physiological factors (age, gender, genetic background and postmenopausal status) and cardiometabolic conditions such as fasting levels, T2DM, inflammation, insulin resistance, obesity and MetS(45-52, 205). However, despite the broad existing knowledge, there are still significant gaps to fully understanding of postprandial metabolism and its regulating factors. Some of them have been addressed in the present thesis.

Thus, age has been defined as a regulating factor of the extent of postprandial lipemia(32, 33, 50, 206), but its independence of other age-associated phenotypic features, such as metabolic syndrome, has not been fully elucidated. In **Chapter 4.A. ("LIPID METABOLISM AFTER AN ORAL FAT TEST MEAL IS AFFECTED BY AGE-ASSOCIATED FEATURES OF METABOLIC SYNDROME, BUT NOT BY AGE")** we have explored whether age is an independent factor influencing postprandial lipemia on three different populations (88 healthy young men, 77 middle-age metabolic syndrome patients and 20 healthy subjects >65 years) at fasting state and at 2nd and 4th postprandial hours. We did not find any differences in PPL response between the healthy young men and the healthy persons >65 years, which may be linked to the absence of MetS traits and the fact that we limited the effects of confounding factors, such as the existence of ApoE gene variations. An early study performed in 22 non-diabetic subjects (9 males, 13 females, 22-79 yr old) showed a correlation between age and the postprandial TG response to a fatty meal (89). Later studies have reported that this fact may be resulting from a delayed clearance intestinally derived TRLs in older subjects by a decrease in LPL activity(90, 91). However, there are not many studies of age influence in PPL when other covariates are controlled. In most of those studies, subjects in the older groups exhibited some of the MetS traits, according to the high prevalence of MetS in older people(92). Our results show no differences in the evolution of postprandial TG between the healthy young men and the healthy persons >65 years. Although the evaluation of the increase in plasma TG concentrations only during the early part of the postprandial period may not accurately describe the complete effects of fat meal ingestion on the perturbation of plasma lipids induced in older people as some authors have argued(206), the study of the first four postprandial hours have been identified as the most accurate predictor of total PPL(158, 159). Also, we additionally performed an internal control of the correlation between the lipemia in the first 4 h versus the complete data that

we had for MetS (8 h) and for the young men (11 h), showing in the two cases high correlation indices (R^2 higher than 0.90). Nevertheless, confirmatory studies with standardized methodology and where covariates were controlled are needed before our results were fully extrapolated.

On the other hand, although baseline TG has been previously proposed in different studies as the major determinant of PPL(46-48), the involvement of other traits of the MetS and whether this influence depends on the number of MetS criteria has not fully established. In **Chapter 4.B. ("HYPERTRIGLYCERIDEMIA INFLUENCES THE DEGREE OF POSTPRANDIAL LIPEMIC RESPONSE IN PATIENTS WITH METABOLIC SYNDROME AND CORONARY ARTERY DISEASE: FROM THE CORDIOPREV STUDY")**, we have explored the effect of a fatty meal on PPL metabolism in 1002 patients with coronary artery disease. We showed not only that fasting TGs are clearly the major contributors to the postprandial TGs levels but also that MetS and the number of its components influence the postprandial response of lipids in patients with CHD, particularly in non-hypertriglyceridemic patients. Postprandial AUC of TG showed a progressively unfavorable increase from one component to five in our population. However, this effect was attenuated when the population was divided into two groups according to the presence of basal hypertriglyceridemia. Thus, only those patients without high fasting TG (<150 mg/dL) remained a statistically significant influence of MetS traits. Our results agree with previous reports that have established that the presence of higher number of components of MetS is associated with an increase in subclinical atherosclerosis, and incidence and mortality of CHD(207, 208). In that context, it has been observed that men presenting four or five MetS traits had a 3.7-fold increase in risk for CHD and a 24.5-fold increase for diabetes

compared with men with none(209). However, the mechanisms underlying this fact are still unknown. Although it is generally accepted that the main pathogenic mechanism underlying the development of cardiometabolic changes in patients with MetS relies on insulin resistance, other mechanisms could influence the increased risk of CVD associated to MetS. In our study, patients with an increased number of MetS components showed higher levels of postprandial TG, confirmed by AUC and iAUC of TG. This deterioration in postprandial lipid metabolism associated with the increase number of MetS components may favor a higher risk of atherogenesis. Previous evidence carried out in healthy population has suggested a significant linear trend between increasing numbers of MetS components and magnitude of postprandial lipemia in 112 healthy subjects(210). Nevertheless, to our knowledge, our study was the first one to show that in non-hypertriglyceridemic coronary patients. Another important feature underlying MetS is atherogenic dyslipidemia, defined as a rise in triglycerides and small LDL particles and low HDL-c. In this regard, we have observed that patients with at least three MetS components have higher ApoB plasma levels and lower HDL-c and ApoA1 plasma levels in all blood drawn during the postprandial state, as well as a positive relationship with AUC of ApoB and a negative relationship with AUC of HDL-c and ApoA1. All of these abnormalities have been implicated as being independently atherogenic(211).

Finally, recently more attention has been paid to the different metabolic phenotypes of obesity due to evidences that suggest that not all obese subjects display the same clustering of metabolic and cardiovascular risk factors, and, likewise, not all lean subjects present a healthy metabolic and disease-free profile(19, 196). The degree of postprandial TG response could be an interesting field of study to explore the underlying causes for that phenotypic differences. In **Chapter 4.C. (“METABOLIC PHENOTYPES OF OBESITY**

INFLUENCE TRIGLYCERIDE AND INFLAMMATION HOMOEOSTASIS"), we have reported in 1002 coronary patients that certain types of metabolic phenotypes are more favorable modulating their response to a fat load test. Specifically, metabolically healthy patients displayed lower postprandial response of plasma TG and large TRLs, compared with those metabolically abnormal, independently whether or not they were obese. Although there is not yet a standardized definition of body size phenotypes(199, 201, 212, 213), we have explored those including inflammation and insulin resistance parameters to the classical definition of MetS as the most related to pathophysiological factors involved in the development of atherosclerosis and easier to measure than other proposed(214). As reviewed previously in the introduction section, descriptives studies have pointed that up to 30% of obese people in general population seem to be metabolically healthy (MHO)(199), and there is a high prevalence of clustering of cardiometabolic abnormalities among normal-weight individuals. In our cohort of coronary patients from the CORDIOPREV study, more than 80% of the patients showed metabolically abnormal criteria, with a ratio of MHO of 7.5%. On the other hand, from a clinical point of view, there is a debate over whether or not different body size cardiometabolic phenotypes have an increased risk of metabolic complications, with conflicting results published(200-202). Although larger prospective studies are needed to reach final conclusions, in our study we have focused the attention in the identification of metabolical phenotypes of obesity as a good strategy to identify subjects at higher risk and drive personalized treatment.

Chapter 6

CONCLUSIONS

VI. Chapter 6: CONCLUSIONS

1. In patients with coronary heart disease, PPL response was directly related to the presence of MetS. We found a positive association between the number of MetS criteria and the response of postprandial plasma triglycerides (Chapter 4.B).

2. Metabolically healthy patients displayed lower postprandial response of plasma TGs and TRLs, compared with those metabolically abnormal, independently whether or not they were obese (Chapter 4.C).

3. MetS interacts with age to determine PPL (Chapter 4.A).

Chapter 7

ADDITIONAL CONTRIBUTIONS ACHIEVED DURING THE PH. D. PROGRAM

VII. Chapter 7: ADDITIONAL CONTRIBUTIONS ACHIEVED DURING THE PH. D. PROGRAM

Other publications in which the author of the thesis has participated during the realization of its doctoral program are listed below (only those in which he is included among the top three authors):

1. Yubero-Serrano EM, Delgado-Lista J, Alcala-Diaz JF, et al. A dysregulation of glucose metabolism control is associated with carotid atherosclerosis in patients with coronary heart disease (CORDIOPREV-DIAB study). Atherosclerosis 2016.

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Chapter 8

CURRICULUM VITAE

VIII. Chapter 8: CURRICULUM VITAE

The author of this thesis, Juan Francisco Alcalá Díaz, was born on 26 May 1982 in Torredonjimeno (Jaén, Spain). He started medical school at the University of Granada in 2000, obtaining his medical degree in 2006. He completed his specialty training in Internal Medicine at the Reina Sofia University Hospital (Córdoba) in 2012. As internal medicine resident he started the research described in this thesis at the department of Internal Medicine, Lipids and Atherosclerosis Unit at the Reina Sofia University Hospital in Cordoba under the supervision of Prof. Jose López Miranda and Dr. Javier Delgado Lista. Before he obtained his title as Internal Medicine specialist, he spent 3 months at the Diabetes and Vascular Center, Sint Franciscus Gasthuis (Rotterdam, The Netherlands) under the supervision of Prof. M. Castro Cabezas, where he collaborated investigating the role of manose binding leptin in cardiovascular disease. In 2013, the author obtained a Rio Hortega contract (Instituto de Salud Carlos III) and was included as researcher at the 'Instituto Maimónides de Investigación Biomédica de Córdoba' (IMIBIC). As part of his training in research, he completed a research stay at Instituto Madrileño de Estudios Avanzados en Alimentacion (IMDEA-Alimentación) under the supervision of Prof. José María Ordovás from October 2013 to April 2014.

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Juan Francisco is married to María José since September 2014. On 21 April 2016 they got a son who they named Juan.

Chapter 9

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IX. Chapter 9: REFERENCES

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