Kako je metabolički sindrom povezan s dislipidemijom?

How is metabolic syndrome related to dyslipidemia?

Victor H. Blaton[1], Irena Korita[2], Anyla Bulo[2]

[1]KU-Leuven, Zavod za kliničku kemiju, Az St Jan, Brugge, Belgija

[1]KU-Leuven, Department of Clinical Chemistry, Az St Jan, Brugge, Belgium

[2]Laboratorijski odsjek, Klinički bolnički centar "Majka Tereza", Tirana, Albanija

[2]Laboratory Department, University Hospital Center "Mother Teresa", Tirana, Albania

Sažetak

Godine 1967. Crepaldi je prvi zapazio da se u mnogo ljudi istovremeno pojavljuju pretilost, dislipidemija, šećerna bolest i hipertenzija. Kasnih sedamdesetih godina dvadesetog stoljeća njemački su istraživači takvo nakupljanje stanja nazvali metaboličkim sindromom. Otada je taj sindrom opisivan pod nekoliko naziva kao „sindrom inzulinske rezistencije", „sindrom X", „plurimetabolički sindrom", te „metabolički sindrom". Sindrom zapravo predstavlja višekomponentnu bolest nastalu kombinacijom načina življenja i čimbenika okoline, s time da su neke populacije pokazale genetičku podložnost za razvoj tog sindroma.

Metabolički sindrom povećava rizik za kardiovaskularnu bolest i šećernu bolest tipa 2. Nacionalni program obrazovanja o kolesterolu - Panel liječenja odraslih III (engl. *National Cholesterol Education Program - Adult Treatment Panel* III, NCEP-ATP III) prepoznao je metabolički sindrom kao skup abnormalnih stanja koja povećavaju rizik, kako za kardiovaskularnu bolest (KVB), tako i za šećernu bolest tipa 2. Smjernice NCEP-ATP III također su istaknule središnju ulogu abdominalne pretilosti u razvoju tog sindroma.

Rastuća prevalencija sindroma ima važne zdravstvene implikacije. Svaka sastavnica metaboličkog sindroma predstavlja potvrđeni čimbenik rizika za KVB, no prisutnost mnogih komponenti rezultira većim rizikom nego zbroj rizika povezanih s pojedinačnim komponentama.

Dokazano je, primjerice, da su muškarci s istodobnom prisutnošću hiperinzulinemije nakon gladovanja, s povišenim koncentracijama apolipoproteina B, te povišenim udjelom malih LDL-čestica imali 20 puta veći rizik razvijanja KVB tijekom petogodišnjeg razdoblja praćenja u studiji, nego muškarci bez tog skupa netradicionalnih biljega rizika. Usto, rizik za KVB povezan s tom aterogenom metaboličkom trojkom ostao je značajan čak i nakon prilagodbe za tradicionalne rizične čimbenike kao što su koncentracije LDL-kolesterola, triglicerida i HDL-kolesterola.

Procjena rizika uključuje listu bioloških parametara u kojoj važnu ulogu imaju lipidi, posebice trigliceridi i HDL-čestice. Tradicionalni čimbenici povezani s metaboličkim sindromom su pretilost, inzulinska rezistencija, hiperglikemija, dislipemija, hipertenzija i mikroalbuminurija.

Ključne riječi: metabolički sindrom, dislipidemija, HDL-kolesterol

Abstract

The observation that obesity, dyslipidemia, diabetes and hypertension occur simultaneously in many people was first made by Crepaldi in 1967. In the late 1970s this clustering of conditions was termed "metabolic syndrome" by German researchers. Since then the syndrome has been described under a number of guises as "Insulin resistance syndrome", "Syndrome X", "Plurimetabolic syndrome" and the "Metabolic syndrome". The syndrome is a multi-component disease brought on by combination of lifestyle and environmental factors, with some populations exhibiting a genetic susceptibility for its development.

Metabolic syndrome increases the risk of cardiovascular disease and type 2 diabetes. The National Cholesterol Education Program - Adult Treatment Panel III (NCEP-ATP III) has recognized the metabolic syndrome as a cluster of abnormalities increasing the risk for both cardiovascular disease (CVD) and type 2 diabetes. The NCEP-ATP III guidelines have also underlined the central role of abdominal obesity in the development of this syndrome.

The escalating prevalence of the syndrome has important health implications. Each component of the metabolic syndrome is an established cardiovascular disease risk factor, and the presence of multiple components confer greater risk than the sum of the risks associated with the individual ones.

For instance, it has been shown that men with the simultaneous presence of fasting hyperinsulinemia, elevated apolipoprotein B concentration and an increased proportion of small LDL particles were characterized by a 20-fold increase in the risk for developing CVD over the 5-year follow-up period of the study, compared with men without this cluster of non-traditional risk markers. In addition, the risk of CVD associated with the atherogenic metabolic triad remained significant even after adjustment for traditional risk factors such as LDL-cholesterol, triglyceride and HDL-cholesterol levels.

Risk assessment includes a list of biological parameters wherein lipids play an important role, especially triglycerides and HDL-particles. The traditional factors associated with the syndrome are obesity, insulin resistance, hyperglycemia, dyslipemia, hypertension and microalbuminuria.

Key words: metabolic syndrome, dyslipemia, HDL-cholesterol

Pristiglo: 17. rujna 2007. *Received: September 17, 2007*

Prihvaćeno: 28. studenog 2007. *Accepted: November 28, 2007*

Biochemia Medica 2008;18(1):14–24

Metabolički sindrom: što sadrži sam naziv?

Za sada ne postoji suglasnost u definiciji metaboličkog sindroma iako se gomilanje metaboličkih abnormalnosti kao što su abdominalna pretilost, oštećeno podnošenje glukoze te šećerna bolest tipa 2, dislipidemija i hipertenzija bilježe u nekih bolesnika već mnogo godina. Nedavno je predloženo još nekoliko sastavnica tog sindroma koje uključuju vaskularnu upalu, hiperkoagulabilnost, hiperurikemiju i mikroalbuminuriju. Prema Reavenu (1), nužno je razlučiti metabolički sindrom kao dijagnostičku kategoriju te metabolički sindrom kao patofiziološki entitet koji označava skup srodnih metaboličkih abnormalnosti. Tijekom proteklih 25 godina studije su pružile dokaze da je inzulinska rezistencija na razini mišića i adipoznog tkiva uobičajena abnormalnost koja povećava vjerojatnost razvoja, ne samo šećerne bolesti tipa 2 u pojedinca, već i kardiovaskularne bolesti (KVB) (2). Pojam metaboličkog sindroma promatranog kao prethodnika, kako šećerne bolesti tipa 2, tako i KVB, progresivno se pojavio uz službeno prepoznavanje Svjetske zdravstvene organizacije (SZO) 1998. godine, te prepoznavanje NCEP ATP III 2001. godine koji je nedavno predložio službenu definiciju metaboličkog sindroma.

Sindrom je višekomponentna bolest nastala kombinacijom načina života i čimbenika okoline, s time da neke populacije pokazuju genetičku podložnost za razvoj metaboličkog sindroma. Tijekom godina objavljeno je nekoliko izvješća u kojima je ukazano da je zajednička sastavnica inzulinska rezistencija, za koju se nagađa da bi djelomice mogla biti posredovana porastom slobodnih masnih kiselina, praćenim pretjeranim stvaranjem čestica obogaćenih trigliceridima i povećanim malim gustim LDL-česticama.

U prospektivnoj Kardiovaskularnoj münsterskoj studiji (PRO-CAM) ispitano je 4559 muških sudionika na kardiovaskularne rizične čimbenike koji su zatim bili promatrani radi bilježenja smrtnosti i kardiovaskularnih događaja, uključujući infarkt miokarda i moždani udar. Zanimljivo je da su rezultati pokazali da visoka koncentracija triglicerida uz odsutnost visokog LDL-a/niskog HDL-a ne povećava rizik za KVB.

Ako je, međutim, omjer LDL/HDL viši od 5, onda povišena koncentracija triglicerida predstavlja dodatni rizik (6). Nadalje, važnost trolista kojeg čine visoka koncentracija triglicerida, nizak HDL i povišeni LDL-kolesterol podržala je nedavna analiza Helsinške studije srca (7). Razmotreni zajedno, navedeni podatci ukazuju da sindrom hipertrigliceridemija/nizak HDL-kolesterol predstavlja snažan čimbenik rizika za nesmrtonosni infarkt miokarda ili smrt zbog koronarne bolesti koji se ne bi zapazio kad bi se utvrđivale samo koncentracije LDL-a. Za praktične se svrhe čini razumno temeljiti predviđanje rizika aterosklerozne koronarne bolesti te odluku o liječenju na potpunom

The metabolic syndrome: What's in the name?

There is currently no consensus definition of the metabolic syndrome, although the clustering of metabolic abnormalities, such as central obesity, impaired glucose tolerance and type 2 diabetes, dyslipidemia and hypertension has been noted in certain patients for many years. Recently more components of the syndrome have been proposed, including vascular inflammation, hypercoagulability, hyperuricemia and microalbuminuria. According to Reaven (1) it is necessary to make a distinction between metabolic syndrome as a diagnostic category and metabolic syndrome as a pathophysiologic entity designating a cluster of related metabolic abnormalities. Studies over the last 25 years have provided the evidence that insulin resistance at the level of muscle and adipose tissue was the common abnormality that increased the likelihood of an individual developing, not only type 2 diabetes, but also cardiovascular disease (2). The concept of the metabolic syndrome viewed as precursor to the development of both type 2 diabetes and cardiovascular disease has progressively emerged with a formal recognition by the World Health Organization (WHO) in 1998 and the National Cholesterol Education Program Adult Treatment Panel III in 2001 (NCEP ATP III), which has recently proposed a formal definition of the metabolic syndrome.

The syndrome is a multi-component disease brought on by combination of lifestyle and environmental factors, with some populations exhibiting a genetic susceptibility for its development. Over years several reports have been published indicating that the common ingredient has been insulin resistance, and it has been speculated that it may be mediated in part by an increase in FFA accompanied by an overproduction of triglyceride-enriched particles and increased small dense LDL particles.

In the prospective Cardiovascular Munster study (PROCAM), 4559 male participants were examined for cardiovascular risk factors and kept under observation to record mortality and cardiovascular events including myocardial infarction and stroke. Interestingly, the results showed that a high triglyceride level in absence of high LDL/low HDL does not increase the risk of CHD.

However if the LDL/HDL ratio is higher than 5, then an increased triglyceride level confers an additional risk (6). The importance of the triad of high triglycerides, low HDL and elevated LDL cholesterol has been further supported by recent analysis of the Helsinki Heart Study (7). Taken together, the above data suggest that hypertriglyceridemia/low HDL cholesterol syndrome constitutes a powerful risk factor for non-fatal myocardial infarction or coronary artery disease death that would escape attention if LDL levels alone were determined. For practical purposes, it appears advisable to base risk prediction of atherosclerotic coronary artery disease and treatment decision

spektru lipidnih pretraga, a ne samo na određivanju kolesterola ili LDL-kolesterola.

Za razumijevanje uloge hiperlipemije u razvoju KVB važno je ispitati zbivanja na razini endotela. LDL-kolesterol prolazi kroz endotel i modificira ga stimulirajući makrofagni kemoatraktantni protein-1 (MCP-1) radi privlačenja monocita, te također potiče diferencijacije u makrofagima koji izražavaju receptore čistača, a koji koriste lipide za pjenaste stanice. Pjenaste stanice stvaraju čimbenike rasta i proteinaze te također otpuštaju citokine koji potiču adhezijske proteine. HDL ima mnogostruke učinke i može spriječiti aterogeni proces na nekoliko razina. Najpoznatija je istjecanje kolesterola iz pjenastih stanica i sprječavanje nastanka pjenastih stanica. HDL također priječi oksidirajuću modifikaciju LDL-a unutar intime. Za HDL je dokazano da koči citokinima pobuđeni izražaj adhezijskih proteina te inhibira MCP-1. HDL je također antitrombotičan i antiapoptotičan. Opće je prihvaćeno da 1%-tni porast HDL-kolesterola pretkazuje 1%-tno smanjenje koronarnih događaja, i to neovisno o promjenama u koncentraciji LDL-kolesterola.

Nofer je dokazao da HDL ima mnoge protuupalne učinke, kao što je to objašnjeno na primjerima eksperimentalnih modela ateroskleroze te na stvarnim modelima upale. ApoA-I i lizosfingolipidi su odgovorni za mnoge protuupalne učinke HDL-a.

Oksidirani kolesterol lipoproteina male gustoće može povećati izražaj citokina (IL-1ß, TNF-α; IL-6 i IL-8) u endotelnim stanicama. Nakon tog događaja slijede vaskularne molekule stanične adhezije, VCAM-1 i ICAM-1. Izolirani HDL i uspostavljeni HDL inhibiraju izražaj tih citokina u izoliranim endotelnim stanicama. U tijeku su daljnje studije čiji je cilj razjašnjenje utjecaja HDL-a na izražaj adhezijskih molekula endotelnih stanica.

Inzulinska rezistencija i poremećaji lipida

Između nekoliko uzroka, nagađa se da bi inzulinska rezistencija mogla biti djelomice posredovana povećanjem slobodnih masnih kiselina (engl. *free fatty acids*, FFA) koje inhibiraju signaliziranje postinzulinskog receptora i time doprinose inzulinskoj rezistenciji. FFA mogu također biti važne odrednice metaboličkog sindroma, jer je njihova koncentracija u tom stanju općenito visoka (Slika 1). S obzirom da je rezistencija na djelovanje ili gubitak inzulina povezana s povećanom lipolizom, intraabdominalna masnoća, koja je metabolički vrlo aktivna, otpušta slobodne masne kiseline u portalni optok. Jetra pretvara slobodne masne kiseline u trigliceride, što može objasniti odnos između hipertrigliceridemije i metaboličkog sindroma. Povećana opskrba glukozom te prekomjerno stvaranje VLDL-a povisuje koncentraciju čestica obogaćenih trigliceridima i dovodi do uzajamne izmjene masnih kiselina: kolesterol-esteri se prenose na VLDL i hilomikronske

on a full lipid profile rather than cholesterol alone or LDL-cholesterol determination.

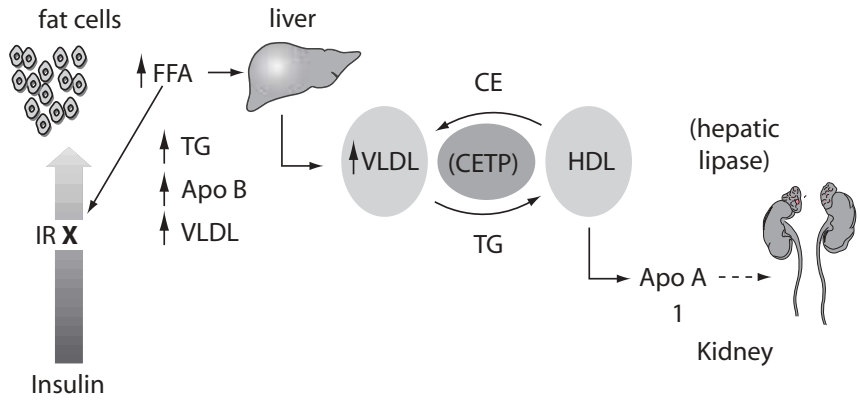
In order to understand the role of hyperlipemia in the development of CHD, it is important to examine what happens at the endothelial level. LDL-cholesterol passes across the endothelium and is modified by stimulating macrophage chemoattractant protein-1 (MCP-1) to recruit monocytes, and also by stimulating differentiation in macrophages which express scavenger receptors that take up lipid to make foam cells. The foam cells produce growth factors and proteinases and they also release cytokines to stimulate adhesion proteins. HDL has multiple effects and can block the atherogenic process at several levels. The best known is the efflux of cholesterol from foam cells and the prevention of foam cell formation. HDL also prevents the oxidizing modification of LDL within the intima. HDL has been shown to inhibit the cytokine-induced expression of adhesion proteins and to inhibit MCP-1. It is also anti-thrombotic and anti-apoptotic. It has been generally accepted that 1% increase in concentration of HDL-cholesterol predicts a 1% reduction in coronary events, independent of the changes in LDL-cholesterol.

Nofer has demonstrated that HDL exerts many anti-inflammatory effects, as illustrated in experimental models of atherosclerosis and in true models of inflammation. ApoA-I and lysosphingolipids can account for many of the anti-inflammatory effects of HDL.

Oxidized low-density lipoprotein cholesterol (ox-LDL) may increase cytokine expression (IL-1ß, TNF-α; IL-6 and IL-8) in endothelial cells. This event is followed by vascular cell adhesion molecules, VCAM-1 and ICAM-1. Isolated HDL and reconstituted HDL inhibit the expression of these cytokines in isolated endothelium cells. Further studies are underway to clarify the influence of HDL on the expression of endothelial cell adhesion molecules.

Insulin resistance and lipid disorders

Among several causes of insulin resistance, it has been speculated that it may be mediated in part by an increase in free fatty acids (FFA) that inhibits post-insulin receptor signalling and thus contributes to insulin resistance. FFA may also be an important determinant of the metabolic syndrome as their level is generally high in this condition (Figure 1). As resistance to insulin action or insulin deprivation is associated with increased lipolysis, intra-abdominal fat, which is metabolically very active, releases FFA into portal circulation. The liver converts FFA into triglycerides and this may explain the relationship of hypertriglyceridemia and the metabolic syndrome. Increased supply of glucose and overproduction of VLDL raises the concentration of triglyceride-enriched particles, leading to a reciprocal exchange of fatty acids: cholesterol-esters



Slika 1. Mehanizmi koji se odnose na inzulinsku rezistenciju i dislipidemiju

Figure 1. Mechanisms relating to insulin resistance and dyslipidemia

ostatke, a trigliceridi na LDL- i HDL-čestice radi stvaranja malih gustih LDL i HDL. Te su guste čestice dobro poznate po svom visokom aterogenom potencijalu.

Uz postojeće farmakološke lijekove koji se koriste za smanjenje inzulinske rezistencije, vježbanje i gubitak težine predstavljaju ključne korake jer su široko provedivi i prilično jeftini. Vježbanjem se povećavaju receptori GLUT-4 u skeletnim mišićima zbog uporabe glukoze, uz smanjenje inzulinske rezistencije.

Nekoliko čimbenika rasta i citokina može modulirati signaliziranje inzulinskog postreceptora. Dok IGF-1 povećava djelovanje inzulina posredovano staničnim receptorom, čini se da slobodne masne kiseline, TNF-α (proupalni citokin kojega uglavnom stvaraju aktivirani makrofagi), te leptin imaju suprotan učinak (8). TNF-α oslabljuje inzulinsko signaliziranje serinskom fosforilacijom IRS-1 te inhibira aktivnost inzulinskog receptora tirozinske kinaze, što pak dovodi to oslabljenog daljnjeg signaliziranja (5). Kako je koncentracija TNF-α u plazmi povišena kod pretilosti, sepse i karcinoma, ta činjenica može djelomice objasniti zašto bolesnici koji se prijavljuju s tim stanjima često imaju abnormalan metabolizam glukoze. Čini se da je djelovanje leptina na odlaganje glukoze neodređenije u povećanju fosforilacije IRS-1 i IRS-2; s druge strane, čini se da je leptin povezan s inzulinskom rezistencijom, jer je u pretilih ljudi utvrđena snažna korelacija između koncentracija leptina u plazmi i inzulinske rezistencije, koja inhibira signaliziranje i djelovanje postreceptorskog inzulina.

Lipidni čimbenici rizika

Jedan od glavnih rizičnih čimbenika u metaboličkom sindromu jest dislipidemija koja može biti povezana s promijenjenim spektrom lipoproteina i s modificiranim lipoproteinima.

are transferred to VLDL and chylomicron remnants, while triglycerides are transferred to LDL and HDL particles to form small-dense LDL and HDL. These dense particles are well known for their high atherogenic potential.

Besides available pharmacological remedies used to decrease insulin resistance, exercise and weight loss represent the key steps as they are widely implemental and rather inexpensive. Exercising increases GLUT-4 receptors in skeletal muscles using glucose with a reduction in insulin resistance.

Several growth factors and cytokines can modulate insulin post-receptor signaling. While IGF-1 enhance insulin action mediated by its cellular receptor, FFA, TNF-α (a pro-inflammatory cytokine mainly produced by activated macrophages), and leptin seem to have the opposite effect (8). TNF-α impairs insulin signaling by serine phosphorylation of IRS-1 and inhibits insulin receptor tyrosine kinase activity, which leads to impaired downstream signaling (5). As TNF-α plasma concentration is increased in obesity, sepsis and cancer, this may in part explain why patients presenting with these conditions often exhibit abnormal glucose metabolism. The action of leptin on glucose disposal seems to be more equivocal in increasing phosphorylation of IRS-1 and IRS-2; on the other hand, leptin seems to be associated with insulin resistance as a strong correlation has been found between plasma leptin levels and insulin resistance in obese people, inhibiting post-receptor insulin signaling and action.

Lipid risk factors

One of the major risk factors in metabolic syndrome is dyslipidemia which can be related to a changed lipoprotein spectrum and to modified lipoproteins.

A first step in separation and identification of serum lipoprotein classes was ultracentrifugation. Goffman and

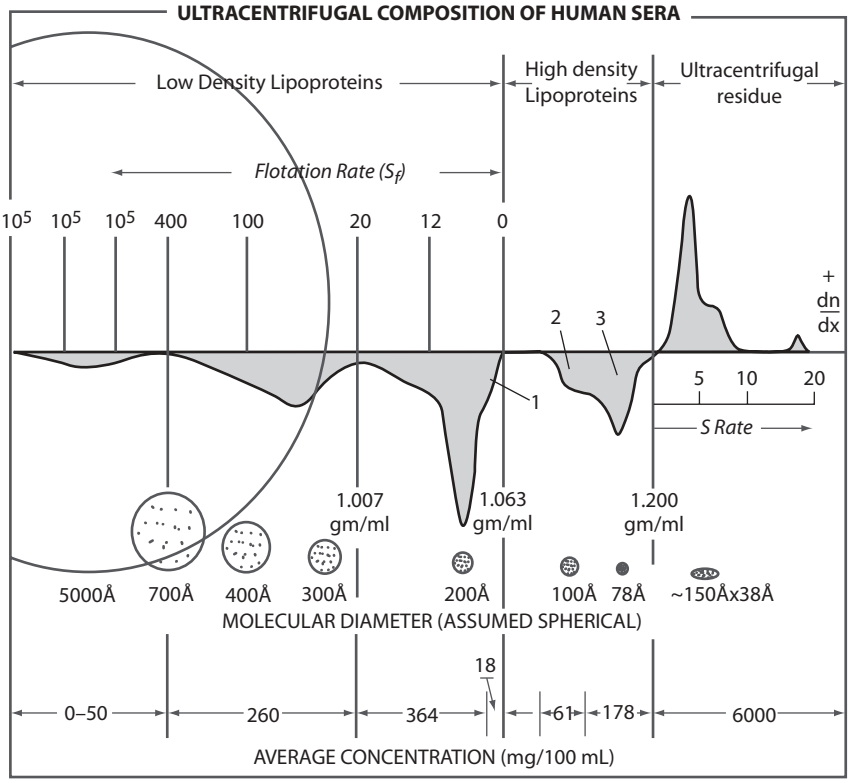
Prvi korak u odvajanju i identifikaciji razreda lipoproteina u serumu bio je ultracentrifugiranje. Goffman i Lindgren prvi su odvojili serumske lipoproteine u različite razrede prema gustoći, a na temelju gradijenata gustoće (Slika 2). Oni su uspjeli karakterizirati veličine čestica i povezati ih s rizikom kod koronarnih događaja. Trebalo je čekati sve do današnjeg doba da bismo što bolje razumjeli odnos između fizičkih parametara molekula i rizika bolesti. Izazov u budućnosti jest istražiti utjecaj fizičkih parametara neke čestice na razvoj bolesti, pronaći metode za dijagnostičke mogućnosti, te ispitati načine liječenja.

Ultracentrifugiranje koje su pedesetih godina dvadesetog stoljeća (tj. 1952.) proveli Goffman i Lindgren, a uveo Svedberg, postalo je referentnom metodom za razdvajanje lipoproteina te još uvijek predstavlja zlatni standard za separaciju, identifikaciju i klasifikaciju lipoproteina. Lipoproteini se karakteriziraju prema veličini čestica (Å), rasponu gustoće (gm/mL), brzini flotacije (Sf), te koncentracijama (mg/mL). Od uvođenja te metode znamo da su LDL-lipoproteini prisutni u većini kardiovaskularnih događaja, a za HDL se smatra da štiti od kardiovaskularne bolesti.

Lindgren were the first to separate serum lipoproteins in different density classes based on density gradients (Figure 2).

They were able to characterize particle sizes and relate them to the risk in coronary heart events. We have had to wait till now to understand more about the relationship between physical parameters of molecules and the risk for disease. It is a challenge for the future to explore the influence of physical parameters of a particle on the development of a disease, to find methods for diagnostic possibilities and also to explore the ways of treatment.

Ultracentrifugation performed by Gofman and Lindgren in the 1950s (i.e. in 1952) and introduced by Svedberg became the reference method for lipoprotein separation and is still the golden standard for lipoprotein separation, identification and classification. They are characterized by the size of particles (Å), density range (gm/mL), flotation rate (Sf) and concentrations (mg/mL). Since the introduction of this method, we have known that LDL lipoproteins are present in most cardiovascular events and that HDLs are considered protective against CVD.



Slika 2. Prikaz lipoproteina u plazmi nakon ultracentrifugiranja, karakterizacija prema veličini (Å), rasponu gustoće (g/mL), koncentraciji (mg/100 mL) i postotku flotacije (Sf).

Figure 2. Ultracentrifugation image of plasma lipoproteins, Characterized in size (Å), density range (g/mL), concentration (mg/100 mL) and flotation rate (Sf).

Ukupni kolesterol ne predviđa točno rizik od kardiovaskularne bolesti; odluka o liječenju temelji se na LDL-kolesterolu, no heterogenost LDL-a također treba uzeti u obzir. Male guste LDL-čestice su aterogenije nego velike, lagane LDL-čestice, dok oksidirane LDL čestice (ox-LDL) također povećavaju aterogenost. Dimenzije čestica uskoro će postati dijagnostičkim pomagalom. Gel-elektroforeza u gradijentu bez primjene denaturirajućih uvjeta uobičajeno se koristi za karakterizaciju razdiobe veličine čestica. Gel-filtracijska kromatografija visoke učinkovitosti te nuklearna magnetna rezonancijska (NMR) spektroskopija odnedavno se koriste za određivanje veličine LDL-čestica. Različite metode ultracentrifugiranja radi gradijenta gustoće u uporabi su za karakterizaciju postotka flotacije LDL-a, dok se nekoliko metoda koristi na nepovezanim gradijentima soli radi određivanja LDL-podrazreda na temelju gustoće. Različite metode određivanja LDL-podrazreda pokazuju visoki stupanj korelacije unatoč tome što mjere različita fizikalna svojstva LDL-a.

Novi postupak iskorištava ono što se čini prirodnim, no čemu se općenito nije pridavalo dovoljno pozornosti, tj. protonske NMR spektroskopske razlike koje iskazuju lipoproteinske čestice različitih veličina. Taj je novi proces do sada uvelike završen. Primjenom NMR-analizatora sa zasebnim poljem srednje jakosti (360 MHZ) postignuta je rutinska kvantifikacija 15 različitih podrazreda VLDL, LDL i HDL unutar oko jedne minute. U Europskom prospektivnom istraživanju karcinoma i prehrane (studija EPIC) (9) istraživan je, pomoću NMR-a, odnos između broja i veličina LDL-čestica, zajedno s koncentracijom LDL-kolesterola i rizikom za buduću koronarnu bolest. Veličina LDL čestica bila je povezana s koronarnom bolešću također nakon prilagodbe za koncentraciju LDL-kolesterola. Na slici 3. NMR-profili razdiobe lipoproteina u dva bolesnika srednje dobi (A i B) ilustriraju koliko različiti mogu biti temeljni metabolički status i povezani rizik za KVB kod dvoje ljudi s praktički istovjetnim koncentracijama LDL- i HDL-kolesterola, no s različitim rizikom za KVB (Slika 3).

Nuklearna magnetna rezonancijska spektroskopija mjeri koncentraciju lipida u plazmi za većinu lipoproteina te se može koristiti za procjenu koncentracije čestica. Tom se tehnikom također mjeri veličina lipoproteinske čestice. U kohorti žena srednje dobi, u studiji ženskog zdravlja, dokazana je visoka značajnost kardiovaskularnih događaja i broja čestica čak i nakon prilagodbe za kolesterol/HDL i trigliceride; međutim, za samu veličinu čestice nije utvrđeno da doprinosi rizičnim događajima. Unatoč tome, postoji izravan odnos između veličine LDL-čestice i postotka ox-LDL-a (10).

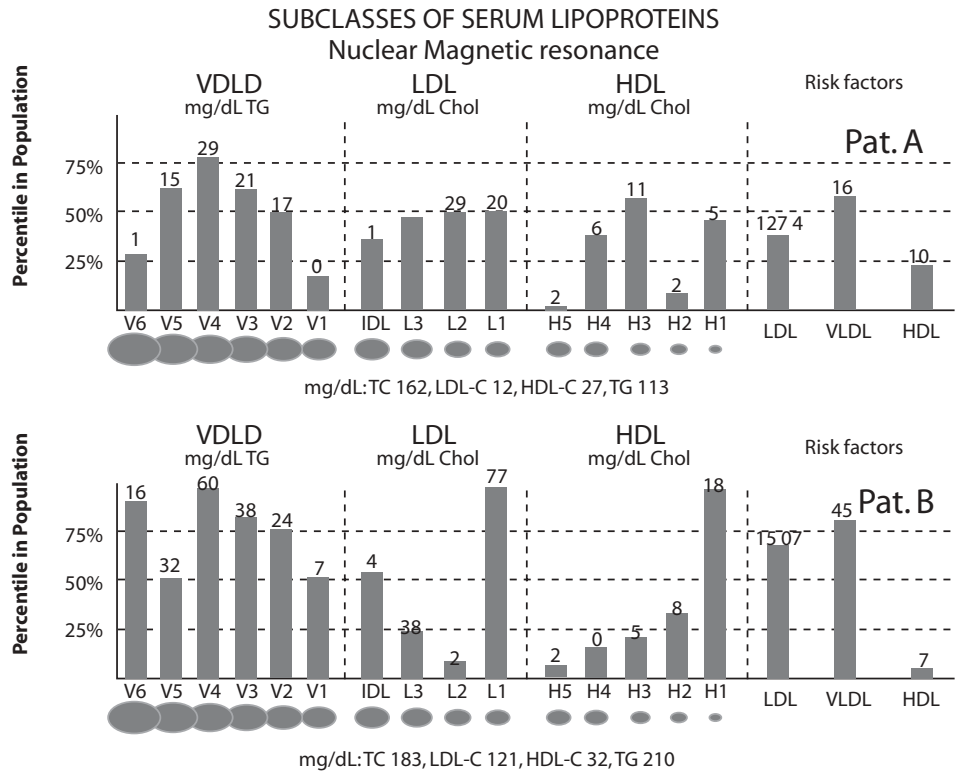
Male LDL-čestice su podložnije oksidaciji, a ox-LDL je neovisan rizični čimbenik za KVB. Odnos između ox-LDL-a i različitih sastavnica metaboličkog sindroma ispitan je u populacijskoj studiji koja je obuhvatila 3030 osoba s normalnim funkcijama starih između 30 i 75 godina. Također

Total cholesterol does not accurately predict the risk of CVD, the decision on treatment is based on LDL-cholesterol, but LDL heterogeneity may also be taken into account. Small dense LDL particles are more atherogenic than large, buoyant LDL particles, and ox-LDL also increases atherogenicity. Particle dimensions are very soon to become a diagnostic tool. Gradient gel electrophoresis without the use of denaturing conditions is commonly applied to characterize particle size distribution. High-performance gel-filtration chromatography and nuclear magnetic resonance (NMR) spectroscopy have been recently applied for determinations of LDL particle size. Different methods of density gradient ultracentrifugation have been used to characterize LDL flotation rates, and several methods have been employed on discontinuous salt gradients to determine LDL subclasses based on density. Various methods of determining LDL subclasses show a high degree of correlation despite the fact that they measure different physical properties of LDL.

The new procedure exploits what appears to be natural but has generally been unappreciated, i.e. proton NMR spectroscopic differences exhibited by lipoprotein particles of different sizes. The new process has now largely been complete. Using a dedicated intermediate-field (360 MHZ) NMR analyzer, routine quantification of 15 different subclasses of VLDL, LDL and HDL has been achieved in about one minute. In the European Prospective Investigation into Cancer and Nutrition (EPIC Study) (9), the relationship between LDL particle number and sizes were studied by NMR, together with LDL-cholesterol concentration and the risk of future coronary artery disease. LDL particle number was related to CAD also after adjustment for LDL-cholesterol concentration. In figure 3, NMR profiles of lipoprotein distribution of two middle-aged patients (A and B) illustrate how different the underlying metabolic status and associated risk of CHD can be for two people who have virtually identical LDL and HDL cholesterol levels with, however, differences in CVD risk (Figure 3).

Nuclear magnetic resonance spectroscopy measures the plasma concentration of lipids in most lipoproteins, and it can be used to estimate particle concentration. The technique also measures the size of the lipoprotein particle. In a cohort of middle-aged women in Women's Health study, high significance between cardiovascular events and particle numbers has been shown also after adjusted cholesterol/HDL and triglycerides; however, the particle size alone was not determined to be a contributor to risk events. Nevertheless, there is a direct relation between the size of the LDL particle and the rate of ox-LDL (10).

Small LDL particles are more susceptible to oxidation and ox-LDL is an independent risk factor for CVD. The relationship between ox-LDL and different components of metabolic syndrome has been examined in a population study of 3.030 normal functioning individuals between 30 and

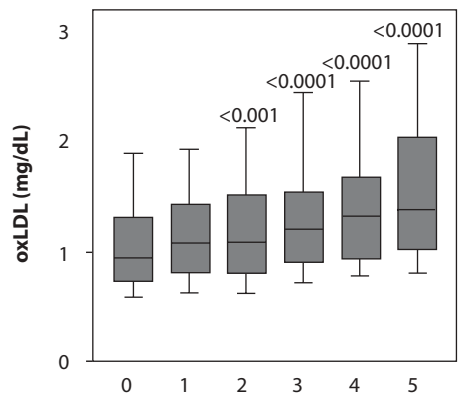


Slika 3. NMR-profili razdiobe lipoproteina u bolesnika A i B

Figure 3. NMR profiles of lipoprotein distribution of patients A and B

je procijenjena težina pojedinačnih komponenti sindroma (11). Ox-LDL se mjeri u odnosu na omjer vjerojatnosti nastanka kardiovaskularne bolesti. Koncentracije ox-LDL-a porasle su, ne samo kao funkcija broja sastavnica

75 years of age. Also, the severity of individual components was evaluated (11).
Ox-LDL was measured against ODS ratio of cardiovascular disease. The ox-LDL levels increased not only in function



Slika 4. Ox-LDL u funkciji sastavnica metaboličkog sindroma. 0 = kontrolna skupina ispitanika; 1 = ispitanici s jednom komponentom metaboličkog sindroma (npr. visoki LDL-kolesterol); 2 = ispitanici s dvije komponente metaboličkog sindroma (npr. visoki LDL-kolesterol i hipertenzija); 3 = ispitanici s tri komponente metaboličkog sindroma (npr. visoki LDL-kolesterol, hipertenzija i šećerna bolest) itd.

Figure 4. Ox-LDL in function of the metabolic syndrome components. 0 = control group of subjects; 1 = subjects with one component of the metabolic syndrome (e.g. high LDL-cholesterol); 2 = subjects with two components of the metabolic syndrome (e.g. high LDL-cholesterol and hypertension); 3 = subjects with three components of the metabolic syndrome (e.g. high LDL-cholesterol, hypertension and diabetes mellitus) etc.

metaboličkog sindroma, već i u funkciji težine pojedinačnih sastavnica (Slika 4). Monoklonsko antitijelo mAB-4E6, koje se stvara radi neoepitopa u aldehidu supstituiranom s apo-B 100 u miševa, koristi se u ELISA-kompeticiji za određivanje cirkulirajućeg ox-LDL-a. Možemo zaključiti da se ox-LDL može koristiti kao biljeg za analizu kardiovaskularnog rizika u metaboličkom sindromu (11).

Metabolički sindrom: uloga i važnost lipidnih sastavnica

Nedavni su dokazi pokazali da je prisutnost metaboličkog sindroma povezana s povećanim rizikom od razvoja koronarne bolesti (KB), infarkta miokarda, te moždanog udara u oba spola. Takav znatno povišen rizik kardiovaskularnog pobola i smrtnosti povezanih s prisutnošću metaboličkog sindroma čini se neovisnim o drugim značajnim, potencijalno zbunjujućim čimbenicima kao što su pušenje, koncentracije LDL-kolesterola u plazmi ili uzimanje alkohola. Dislipidemija je sastavni dio metaboličkog sindroma, jer obje definicije uključuju hipertrigliceridemiju (definiranu kao koncentracija triglicerida u serumu ≥ 150 mg/dL) i niske koncentracije HDL-kolesterola (definirane prema NCEP ATP III kao HDL-kolesterol < 40 mg/dL za muškarce i < 50 mg/dL za žene, te prema SZO kao HDL-kolesterol < 35 mg/dL za muškarce i < 40 mg/dL za žene) kao obilježja sastavnica sindroma. Osobe s metaboličkim sindromom, osobito one s abdominalnom pretilošću, pokazuju visoko aterogeni profil lipida koji može biti odgovoran za visok rizik za KVB. I središnje nakupljanje masnoća, kao i prisutnost inzulinske rezistencije, povezani su sa skupom dislipidemijskih svojstava, tj. povišenom koncentracijom triglicerida u plazmi, porastom lipoproteina vrlo niske gustoće (VLDL) te lipoproteina srednje gustoće (IDL), prisutnošću malih gustih LDL-čestica i smanjenim HDL-kolesterolom. Za te je abnormalnosti metabolizma lipoproteina vjerojatnije da će se pojaviti zajedno, a ne odvojeno, te one čine ključna obilježja sastavnica metaboličkog sindroma.

Nedavne su prospektivne studije ukazale da su povišeni trigliceridi neovisan čimbenik rizika u KVB. Hipertrigliceridemija je povezana s nekoliko aterogenih čimbenika, uključujući povišene koncentracije trigliceridima bogatih lipoproteina i aterogeni lipoproteinski fenotip, koji se sastoji od malih gustih LDL-čestica te niskog HDL-kolesterola. Čimbenici koji doprinose hipertrigliceridemiji u općoj populaciji obuhvaćaju pretilost, prekomjernu težinu, fizičku neaktivnost, pretjerani unos alkohola, prehranu bogatu ugljikohidratima, šećernu bolest tipa 2, te neke druge bolesti (npr. kronično zatajivanje bubrega, nefrotski sindrom), određene lijekove (npr. kortikosteroide, estrogene, retinoide, visoke doze adrenergičkih blokirajućih agenasa), te genetičke poremećaje (obiteljska kombinirana hiperlipidemija, obiteljska hipertrigliceridemija

of the number of metabolic syndrome components but also in function of the severity of individual components (Figure 4). Monoclonal antibody mAB-4E6 formed against a neo-epitope in the aldehyde substituted by apo-B 100 in mice is used in the ELISA competition for determination of circulating ox-LDL. As a conclusion, ox-LDL can be used as a marker for the analysis of cardiovascular risk in metabolic syndrome (11).

Metabolic syndrome: the role and importance of lipid components

Recent evidence has shown that the presence of metabolic syndrome is associated with an increased risk of coronary heart disease (CHD), myocardial infarction, and stroke in both sexes. This substantially increased risk of CV morbidity and mortality associated with the presence of metabolic syndrome appears independent of other significant, potentially confounding factors such as smoking, plasma LDL cholesterol levels or alcohol consumption. Dyslipidemia is an integral part of metabolic syndrome since both definitions include hypertriglyceridemia (defined as serum triglycerides ≥ 150 mg/dL) and a low HDL cholesterol concentration (defined as HDL-cholesterol < 40 mg/dL for men and < 50 mg/dL for women by NCEP ATP III, or HDL-cholesterol < 35 mg/dL for men and < 40 mg/dL for women by WHO) as components. Individuals with metabolic syndrome, particularly those with abdominal obesity, exhibit a highly atherogenic lipid profile which may account for their high risk of CVD. Central fat accumulation and the presence of insulin resistance have both been associated with a cluster of dyslipidemic features, i.e., elevated plasma triglyceride level, an increase in very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL), the presence of small dense LDL particles, and a decrease in HDL-cholesterol. These abnormalities of lipoprotein metabolism are more likely to occur together than separately and constitute the key component traits of the metabolic syndrome.

Recent prospective studies indicate that elevated triglycerides are an independent risk factor in CHD. Hypertriglyceridemia is associated with several atherogenic factors including increased concentrations of triglyceride-enriched lipoproteins and the atherogenic lipoprotein phenotype consisting of small dense LDL particles and low high-density lipoprotein (HDL) cholesterol. The factors contributing to hypertriglyceridemia in general population include obesity, overweight, physical inactivity, excess alcohol intake, high-carbohydrate diet, type 2 diabetes, and some other diseases (e.g. chronic renal failure, nephrotic syndrome), certain drugs (e.g. corticosteroids, estrogens, retinoids, high doses of adrenergic blocking agents), and genetic disorders (familial combined hyperlipidemia, familial hypertriglyceridemia, and

te obiteljska disbetalipoproteinemija). U svakodnevnoj se praksi povišena koncentracija triglicerida u serumu poglavito zapaža u osoba s metaboličkim sindromom. Mnoge su prethodne studije ukazale da je hipertrigliceridemija snažno povezana sa svim sastavnicama metaboličkog sindroma. Bolesnici s metaboličkim sindromom koji imaju hipertrigliceridemiju najčešće pokazuju povišenu koncentraciju lipoproteina bogatih trigliceridima koji se smatraju aterogenima. To su djelomično razgrađeni VLDL koji se uobičajeno nazivaju „ostatnim lipoproteinima". VLDL je u kliničkoj praksi najlakše dostupna mjera aterogenih ostatnih lipoproteina. Zbog toga VLDL-kolesterol može biti cilj terapije za sniženje kolesterola. U nedavnim je smjernicama ustanovljen zbroj LDL + IDL + VLDL-kolesterola (nazvan „ne-HDL-kolesterol" [ukupan kolesterol - HDL-kolesterol]) kao sekundarni cilj terapije u osoba s hipertrigliceridemijom (Tablica 1).

Nizak HDL-kolesterol

Niske koncentracije HDL-kolesterola povezane su s povećanim rizikom koronarne bolesti. Taj je odnos zapažen bez obzira na dob, vrijednost krvnog tlaka, pretilost, te koncentracije ukupnog ili LDL-kolesterola. Izraz „izolirani niski HDL" korišten je za opisivanje situacije u kojoj se ukupan ili LDL-kolesterol smatra normalnim, no HDL-kolesterol je nizak. Dugotrajno praćenje ispitanika s niskim HDL kolesterolom pokazalo je da je njihov rizik razvijanja koronarne bolesti sličan riziku u ispitanika s povišenim ukupnim ili LDL-kolesterolom. Nizak HDL-kolesterol je najsnažniji pretkazatelj nadolazećih kardiovaskularnih događaja u bolesnika s angiografski potvrđenom koronarnom bolešću te koncentracijama ukupnog kolesterola unutar normalnog raspona. Prema sadašnjim smjernicama, prisutnost niskog HDL-kolesterola treba smatrati glavnim čimbenikom rizika za razvoj koronarne bolesti, koji modificira cilj terapije sniženja LDL-a i koristi se kao rizični čimbenik za procjenu 10-godišnjeg rizika za koro-

familial dysbetalipoproteinemia). In daily practice, elevated serum triglycerides are predominantly observed in persons with metabolic syndrome. Many previous studies have indicated that hypertriglyceridaemia is strongly associated with all metabolic syndrome components. Patients with metabolic syndrome who have hypertriglyceridemia most often exhibit elevated level of triglyceride-enriched lipoproteins which are considered atherogenic. Actually, those are partially degraded VLDL, commonly called "remnant lipoproteins". In clinical practice, VLDL cholesterol is the most readily available measure of atherogenic remnant lipoproteins. Thus, VLDL cholesterol can be a target of cholesterol-lowering therapy. Recent guidelines have identified the sum of LDL + IDL + VLDL cholesterol (termed "non-HDL cholesterol" [total cholesterol minus HDL cholesterol]) as a secondary target of therapy in persons with hypertriglyceridaemia (Table 1).

Low HDL-cholesterol

Low levels of HDL-cholesterol are associated with increased risk of coronary artery disease (CAD). This relationship has been observed irrespective of age, blood pressure level, obesity, total cholesterol or LDL-cholesterol levels. The term "isolated low HDL" has been used to describe the situation where total cholesterol or LDL-cholesterol are considered normal but HDL-cholesterol is low. Long-term follow-up of subjects with low HDL-cholesterol has demonstrated that their risk of developing CAD is similar to the risk in subjects with elevated total cholesterol or LDL-cholesterol. Low HDL-cholesterol is the strongest predictor of subsequent cardiovascular events in patients with angiographically confirmed CAD and the levels of total cholesterol within normal range. According to current guidelines, the presence of low HDL-cholesterol should be considered a major cardiovascular risk factor which modifies the goal for LDL-lowering therapy and is used as a risk factor to estimate the 10-year risk for CHD.

Tablica 1. Abnormalnosti povezane s hipertrigliceridemijom u metaboličkom sindromu.

Table 1. Abnormalities associated with hypertriglyceridemia in metabolic syndrome.

Hypertriglyceridemia and CHD Risk: Associated abnormalities
• Accumulation of chylomicron remnants
• Accumulation of VLDL remnants
• Generation of small, dense LDL-cholesterol
• Association with low HDL-cholesterol
• Increased coagulability
– ↑ plasminogen activator inhibitor (PAI-1)
– ↑ factor VIIc
– Activation of prothrombin to thrombin

narnu bolest. Postoji više uzroka niske koncentracije HDL-kolesterola, od kojih je nekoliko povezano s inzulinskom rezistencijom, tj. povišenim trigliceridima, prekomjernom težinom i pretilošću, fizičkom neaktivnošću te šećernom bolesti tipa 2. Kombinacija niskog HDL-kolesterola i povišene koncentracije triglicerida u plazmi stoga se smatra inzulinsko-rezistentnim stanjem. Treba napomenuti da određeni lijekovi također snižavaju koncentraciju HDL-kolesterola (npr. beta-blokatori, anabolični steroidi, progestacijski agensi). Unatoč tome, niski HDL-kolesterol je važno obilježje sastavnica metaboličkog sindroma i zaslužuje pomnu kliničku pozornost i obradu jer bolesnici s tim obilježjem imaju visok rizik za KVB. Omjer ukupni-/HDL-kolesterol je dobro poznati pretkazatelj rizika za KVB. Nedavno smo pokazali da su muškarci s hipertrigliceridemičnim fenotipom struka imali znatno povišen omjer ukupnog-/HDL-kolesterola u usporedbi s muškarcima bez tog fenotipa. U toj je studiji samo 3% muškaraca s opsegom struka < 90 cm i koncentracijama triglicerida < 2,0 mmol/L imalo omjer ukupnog-/HDL-kolesterola 6 ili više. Međutim, kod gotovo 50% ispitanika s hipertrigliceridemičnim fenotipom struka taj je omjer bio iznad 6. Slični su zaključci postignuti i za druge ispitivane populacije. Broj i veličina HDL-čestica također imaju važnu ulogu povezanu s rizikom koronarne bolesti u metaboličkom sindromu. Za molekule HDL-a dokazana je najznačajnija uloga u dislipidemiji, no postoje još uvijek mnoga neodgovorena pitanja: što regulira koncentraciju, subpopulacije i funkcije HDL-a? Kardioprotektivna funkcija HDL-a je nepoznata, kao i funkcija HDL-koncentracije. Koji je relativan doprinos istjecanja kolesterola, antioksidacijskih osobina, te uloga protuupalnih karakteristika podvrsta HDL-a? Nadalje, ne postoje podatci kojima bi se objasnio odnos između prekomjerne težine i pušenja te niskog HDL-a.

Zaključak

Metabolički sindrom predstavlja skupljanje čimbenika kardiovaskularnog rizika koji su povezani s inzulinskom rezistencijom (12). S obzirom da je inzulinska rezistencija neovisan rizični čimbenik kardiovaskularne bolesti, njena prisutnost može dovesti do makrovaskularnih komplikacija mnogo prije očitovanja ostalih obilježja metaboličkog sindroma.

Vrlo je važno prepoznati da visoki LDL ili visoki ukupni kolesterol nisu sastavnice metaboličkog sindroma. U stvari, vrijednosti LDL-a često su ispodprosječne u bolesnika s metaboličkim sindromom. Liječnici stoga moraju biti svjesni da bolesnici još uvijek mogu imati visoki rizik kardiovaskularne bolesti čak i ako imaju nizak LDL ili ukupni kolesterol. U biti, metabolički se sindrom može formulirati kao „ne-LDL-na" vrsta rizika, no uz istu važnost u prepoznavanju i liječenju. Pojavnost metaboličkog sindroma je u porastu u čitavoj Europi i Sjevernoj Americi usporedo s

A low HDL-cholesterol level has several causes, some of which are associated with insulin resistance, i.e. elevated triglycerides, overweight and obesity, physical inactivity, and type 2 diabetes. The combination of a low HDL-cholesterol with elevated plasma triglyceride level has therefore been considered an insulin-resistant state. It should be noted that certain drugs also reduce the level of HDL-cholesterol (e.g. beta-blockers, anabolic steroids, progestational agents). Nevertheless, low HDL-cholesterol is an important component trait of metabolic syndrome and deserves close clinical attention and management since patients with this trait are at a high risk for CVD. The total cholesterol/HDL-cholesterol ratio is a well known predictor of CHD risk. We have recently shown that the men characterized by the hypertriglyceridemic waist phenotype had a substantially elevated total cholesterol/HDL-cholesterol ratio compared with those without this phenotype. In this study, only 3% of men with waist circumference < 90 cm and triglyceride levels < 2.0 mmol/L had a total cholesterol/HDL-cholesterol ratio of 6 or higher. However, almost 50% of subjects characterized by the hypertriglyceridemic waist phenotype had a ratio above 6. Similar conclusions have been reached in other study populations. The number and size of the HDL particles also play important roles in the risks of cardiovascular events in metabolic syndrome. HDL molecules have demonstrated the most important role in the dyslipidemia, yet there are many unanswered questions: what regulates HDL concentration, subpopulations and functions? The cardioprotective function of HDL and the function of HDL concentration is unknown. What is the relative contribution of cholesterol efflux, of antioxidant properties and the role of the anti-inflammatory characteristics of HDL subtypes? Further, there is no data to explain the relationship between overweight and smoking and low HDL.

Conclusion

Metabolic syndrome represents a clustering of cardiovascular risk factors linked through their association with insulin resistance (12). Since insulin resistance is an independent risk factor for cardiovascular disease, its presence can lead to macrovascular complications long before other features of metabolic syndrome are evident.

It is very important to recognize that high LDL or high total cholesterol are not components of the metabolic syndrome. In fact, LDL is often below average in patients with the metabolic syndrome. Thus, physicians must be aware that patients can still have a high risk of cardiovascular disease even if they have low LDL or total cholesterol. In essence, one can conceptualize metabolic syndrome as a "non-LDL" type of risk, and just as important as LDL to recognise and to treat. The incidence of metabolic syndrome has been increasing throughout Europe and North

porastom pretilosti, prekomjerne težine i šećerne bolesti (13). Kako je LDL sasvim učinkovito istaknut u smjernicama za liječenje, sada je vrijeme za skretanje pozornosti kliničara na metabolički sindrom. Ako se spomenuta epidemija prekomjerne težine i šećerne bolesti ne zaustavi, kardiovaskularna bolest će i dalje biti u porastu, a mi ćemo potratiti napredak koji smo postigli tijekom proteklih 20 godina. Metabolički sindrom iziskuje multifaktorski pristup liječenju jer sve njegove sastavnice u kombinaciji povećavaju rizik kardiovaskularne bolesti. Prehranom i vježbanjem ponajprije se poboljšavaju sve sastavnice snižavanjem triglicerida, glukoze i krvnog tlaka te povećanjem HDL-a. Zapravo, većina se metaboličkog sindroma može pripisati „pretjeranoj prehrani". Drugo, farmakološka terapija za poboljšanje sastavnica metaboličkog sindroma treba biti individualizirana za svakog bolesnika. Izazovi koji preostaju kod identificiranja visokorizičnih osoba uključuju uvođenje kliničkih biljega inzulinske rezistencije, integriranje koncentracija glukoze i lipida, te bolja definicija uloge upalnih, protrombotičkih i genetičkih čimbenika (14). Potrebno je bolje razumijevanje rizičnih čimbenika metaboličkog sindroma te provedba kliničkih ispitivanja terapijskih intervencija specifično usmjerenih na taj sindrom.

America, in parallel with an increase in overweight, obesity, and diabetes (13). Since LDL has been emphasised in treatment guidelines quite effectively, it is now time to turn the attention of clinicians to metabolic syndrome. If this epidemic of overweight and diabetes is not stopped, cardiovascular disease will increase and we will waste the progress we have made in the past 20 years. Metabolic syndrome requires a multi-factorial approach to treatment since all of its components combine to increase the risk of cardiovascular disease. Firstly, diet and exercise will improve all components by lowering triglycerides, glucose and blood pressure, and raising HDL. In fact, much of the metabolic syndrome can be attributed to "over nutrition". Secondly, pharmacological therapy for improving the components of metabolic syndrome should be individualized in each patient. Challenges remaining in the identification of high-risk persons include the introduction of clinical markers of insulin resistance, integration of post challenge glucose and lipid concentrations, and better definition of the role of inflammatory, prothrombotic, and genetic factors (14). Improved understanding of the risk factors for metabolic syndrome is required, and clinical trials of therapeutic interventions specifically targeted to this syndrome need to be conducted.

Adresa za dopisivanje:

Prof. Victor Blaton
KU-Leuven
Department of Clinical Chemistry
Az St Jan
Ruddershove 10
8.000 Brugge
Belgium
e-pošta: victor.blaton@skynet.be

Corresponding author:

Prof. Victor Blaton
KU-Leuven
Department of Clinical Chemistry
Az St Jan
Ruddershove 10
8.000 Brugge
Belgium
e-mail: victor.blaton@skynet.be

Literatura/References

1. *Reaven GM. The metabolic syndrome: What is in a name? Clin Chem 2006;52:5.*
2. *Reaven GM. The metabolic syndrome or the insuline resistance syndrome? Different names, different concepts, and different goals. Endorcinal Metab Clin N Am 2004;33;283-303.*
3. *Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.*
4. *Despreés JP, Lemieux I, Prud'homme D. Treatment of obesity: need to focus on high risk abdominally obese patients. BMJ 2001;322:716-20.*
5. *Lamarche B, Tchernof A, Maurieége P, Cantin B, Dagenais GR, Lupien PJ, Despres JP. Fasting insulin and apolipoprotein B levels and low-density lipoprotein particle size as risk factors for ischemic heart disease. JAMA 1998;279:1955-61.*
6. *Assmann G, Schulte H. Relation of high density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience) Am J Cardiol. 1992:70,733-7.*
7. *Mannienen V, Tekanen L, Koskinen P, Huttunen JK, Mänttäri M, Heinonen OP, Frick MH. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. Circulation 1992;85:37-45*
8. *Donahue RP, Prineas RJ, Donahue RD, Zimmet P, Bean JA, De Courten M, et al. Is fasting leptin associated with insulin resistance among nondiabetic individuals? Diabetes Care 1999;22:1092-6.*
9. *El Harchaoul K, van der Steeg WA, Stroes J, Kastelein J. Value of low-density lipoprotein particle number and size as predictors of coronary artery disease in apparently healthy men and women: the EPIC-Norfolk Prospective population study. J Am Coll Cardiol 2007;49(5):547-53.*
10. *Sacks FM. The apolipoprotein story. Atherosclerosis Supplements 2006;7:23-7.*
11. *Blaton V. Ox-LDL in function of metabolic syndrome components. Belgian Lipid Club 2005;6:4-5.*
12. *Haffner S. Diabetes and the metabolic syndrome - When is it best to intervene to prevent. Atherosclerosis supplements 2006;7:3-10.*
13. *Hu FB, Stampfer MJ, Haffner MJ. Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. Diabtes Care 2002;25:1129-34.*
14. *Alexander CM, Haffner SM. NCEP defined metabolic syndrome, diabetes and prevalence of coronary heart disease. Diabetes 2003; 52(5); 1210-4.*