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Chapter

Acute Changes in Lipoprotein-Associated Oxidative Stress

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Abstract

As inflammatory and oxidative stress are associated with cardiometabolic diseases, detection of abnormal fasting levels of inflammatory and oxidative biomarkers are indicative disease presence and may be too late for any preventive management. Metabolic flexibility refers to the ability of various metabolic processes to compensate for these acute changes and return all metabolites to baseline levels. By monitoring responses of key biomarkers to a standardized physiologic challenge, it is possible to assess the ability of the body to restore homeostasis, that is a measure of metabolic flexibility. Acute changes in lipoprotein-associated biomarkers of oxidative stress have been demonstrated following meal consumption. These include changes in circulating levels of oxidized low-density lipoproteins (LDL), levels of autoantibodies to malondialdehyde-modified LDL, as well as the oxidative susceptibility of isolated plasma LDL. These responses depend on the type and amount of dietary fats in the meal. Management with certain lipid-lowering drugs could also be shown to affect these meal-induced changes. However, plasma levels may be underestimated as we can demonstrate a spike in lipoprotein-associated biomarkers of oxidative stress resulting from the release oxidatively modified epitopes from the arterial wall by an intravenous bolus of heparin.

Keywords: lipoproteins, low-density lipoproteins, autoantibodies, MDA-LDL, postprandial, metabolic flexibility, heparin

1. Introduction

Cardiometabolic diseases (CMD), specifically cardiovascular diseases and type 2 diabetes mellitus account for 17.5 and 1.5 million annual deaths, respectively [1, 2]. These noncommunicable diseases arise from metabolic abnormalities that should be preventable, especially if they can be identified at early stages. Current understanding would suggest that the onset and progression of CMD are the result of cumulative disturbances in cardiometabolic pathways [3]. Ongoing clinical diagnoses are typically based on cut-off values of various metabolites that have been associated with advanced diseases. Although these cut-off values have been established by major studies in a large number of participants, they are typically measured after
8 h of fasting. Since a typical individual may be consuming 2–3 meals daily, it is evident that metabolic processes are carried out in a non-fasting state throughout the day. It has been estimated that an individual adhering to the typical western diet may have triglyceride levels above fasting levels throughout 75% of the day [4]. Furthermore, some individuals may be exposed to a significant level of physical activity as part of their daily routine. Both meal consumption [5, 6] and physical activity [7, 8] are associated with transient spikes in oxidative stress. Indeed, while regular physical activity is believed to be associated with health benefits, excessive exercise has been reported to result in elevated biomarkers of oxidative damage in skeletal muscle and blood [8].

Metabolic flexibility or phenotypic flexibility is a term used to describe a battery of metabolic/physiologic processes that allow the body to regain homeostasis after various types of physiologic stresses [9, 10]. For instance, the oral glucose tolerance test is a widely accepted physiologic challenge to identify individuals with disturbed glucose metabolism (i.e. impaired flexibility) despite having normal levels of fasting glucose [11]. High-fat challenge tests have also been used to study lipid metabolism and metabolic flexibility [12]. The mode and intensity of acute exercise have also been reported to result in transient changes in several markers of chronic systemic inflammation, including C-reactive protein (hsCRP) and interleukin 6 (IL-6) [13].

While regulated oxidative stress is necessary for normal cellular metabolism, imbalance between reactive oxygen species (ROS) generation and ROS scavenging is recognized as a central step in the initiation and progression of CMD. However, direct measurement of ROS in the circulation is not feasible in view of their short biological half-lives which is of particular importance as all substrates in the body are potential targets of oxidative modification caused by ROS. A prime example of oxidation targets is polyunsaturated fatty acids (PSF) which are characterized by the multiple double bonds in their hydrocarbon chain [14]. Among other potential circulating biomarkers, plasma lipoproteins, and in particular, triglyceride-rich lipoproteins, come in direct contact with endothelial cells along the vasculature as part of their normal function to transport lipid throughout the body [15]. Free fatty acids, a key by-product of triglyceride metabolism, are highly susceptible to oxidative modification, move readily in and out of the sub-endothelium and are taken up by various tissues. Oxidative modification of lipids results in the formation of reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) [16]. MDA and HNE-modified metabolites can be detected in plasma [17].

2. Metabolism of triglyceride-rich lipoproteins and free fatty acids

Triglycerides (TG) and their structural component free fatty acids (FFA) represent the key source of energy in humans. In the fasting state, triglycerides are continuously synthesized and secreted by the liver after packaging in very-low-density lipoprotein (VLDL) particles for distribution and storage throughout the body. VLDL unload their cargo of triglycerides by attaching themselves to the endothelium with the subsequent release of free fatty acids and monoglycerides. The neutral and oxidized FFA released by the hydrolysis of triglyceride-rich VLDL can induce endothelial inflammation [18]. In the same process, any excess ROS generated by activated macrophages in the arterial wall could diffuse through the endothelium and seed lipoproteins while they are still in circulation [19]. The residual product after the delivery of TG is the cholesterol-rich low-density lipoprotein (LDL) particles (Figure 1A).
In addition to the daily flux of endogenous TG secreted by the liver in the form of VLDL, approximately 90 g of fat are absorbed each day and packaged by the intestine within chylomicrons (Chylo) to transport newly ingested dietary fat [4]. For individuals with normal fasting TG levels (< 1.70 mmol/l, < 150 mg/dl), postprandial TG may be increased by 1.20 mmol/l (106 mg/dl), or approximately 70% [20]. VLDL and Chylo are commonly referred to as TG-rich lipoproteins (TRL).

Three key enzymes are responsible for the metabolism of circulating TG and FFA. They include heparin-releasable lipases, lipoprotein lipase (LpL) and hepatic lipase (HL), and hormone-sensitive lipase (hsL). Both TRL (VLDL and Chylo) share a common pathway requiring LpL-induced conversion of TG into non-esterified fatty acids (free fatty acids or FFA), that are taken up by cells and re-assembled as TG for storage in various tissues [15]. HL is primarily responsible for the breakdown of TG from the partially hydrolyzed TRL [15]. During fasting, when plasma insulin level is low, hsL is actively breaking down stored TG with the release of FFA back into the circulation to be transported back to the liver by albumin [21]. During postprandial lipemia, hsL is inhibited and there is a net increase in LpL-induced FFA release from Chylo for storage [21]. These metabolic processes are schematized in Figure 1.

LpL and HL are anchored to the luminal surface of vascular endothelial cells via membrane-bound heparan sulfate proteoglycans and could be released into the bloodstream by intravenous heparin [22]. Plasma TG levels are significantly reduced by as much as 80% following the release of the lipases by heparin [23, 24]. This acute reduction in plasma TG was explained by the failure of released FFA to inhibit LpL activity in the circulation [25]. LpL was originally described as the “clearing factor” [26, 27] for its ability to clear the turbidity due to elevated TG in plasma.

The residual product following the hydrolysis of VLDL by LpL is the cholesterol-rich LDL [28] that has been implicated in the initiation and progression of atherosclerosis [29]. According to the oxidation hypothesis of atherosclerosis [30, 31], only
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oxidatively modified LDL can be taken up by macrophages subsequently leading to the formation of foam cells, endothelium dysfunction, and the development and/or progression of atherosclerosis [32, 33]. As such, research on lipoprotein-associated oxidative stress has predominantly focused on oxidatively modified LDL. Starting with the detection of oxidatively modified LDL in atherosclerotic lesions [34, 35] and the evidence for an in vivo process for LDL oxidative modification [36], a number of studies linking circulating levels of oxidized LDL to cardiovascular disease emerged [37–39]. However, there is also increasing evidence that oxidative modification of lipoproteins is not limited to LDL but can affect other lipoproteins [40] with the susceptibility to oxidative modification being dependent on their fatty acid composition [41, 42]. We subsequently reported that the oxidative susceptibility of plasma LDL could be affected acutely following meal consumption [43].

3. Lipoprotein-associated oxidative stress

3.1 Autoantibodies against malondialdehyde-modified lipoproteins

While the presence of malondialdehyde-modified LDL (MDA-LDL) [38, 39, 44, 45] and autoantibodies against oxidatively modified LDL (AAb-oxLDL) [46–49] has been well accepted, the relationship between these biomarkers and atherosclerosis remains unclear. High levels of oxLDL have been reported to be associated with the presence and severity of CAD [17, 35, 38, 39, 44]. However, in spite of angiographically documented improvement in atherosclerosis with aggressive lipid-lowering intervention, plasma levels of oxLDL were actually increased [50]. Similarly, the association between AAb-oxLDL and atherosclerosis is not clear. In LDL-receptor deficient mice, levels of AAb-oxLDL positively correlated with atherosclerosis progression [51]. In the Watanabe rabbit lacking LDL receptor, these same investigators reported protection from atherosclerosis when levels AAb-oxLDL were increased by immunization with MDA-LDL [52]. Contradictory results have also been reported in humans with levels of AAb-oxLDL being associated with disease in some studies [46, 53, 54] but not in others [48, 49].

According to the oxidation hypothesis of atherosclerosis, only the smaller LDL particles could go through the endothelium, be trapped in the sub-endothelium, and undergo oxidative modification [30]. oxLDL would be in equilibrium between the endothelium and vascular space. An alternate scenario would allow plasma lipoproteins rich in highly oxidizable polyunsaturated fatty acids to attach to the arterial wall and be exposed to ROS generated in the sub-endothelium. Depending on the metabolic state preceding blood collection, levels of lipoprotein-associated biomarkers may be transiently affected resulting in different relationships between biomarkers and disease status. We have designed four different studies to examine this hypothesis.

In the first study, we administered a standardized mixed meal in the form of a 600 kcal shake to a group of patients with documented CAD and a small group of young healthy volunteers with no known risk factor for CAD [55]. Following the consumption of the shake, we observed a significant and transient reduction in plasma levels of autoantibodies (AAb) specific to MDA-LDL. This acute change was demonstrable only in individuals with documented CAD and not in healthy volunteers [55]. We have interpreted this reduction in AAb as suggestive of acute meal-induced increase in the levels of oxidatively modified lipoproteins. Thus, the time of the last meal prior to blood collection and the clearance of the intestinal lipoproteins would affect the levels of AAb-oxLDL.
In the second study, we examine the effect of different fatty acid compositions in the shake. In a small group of patients with metabolic syndrome, we examined the postprandial response with four different oral challenges administered in random order. The oral challenges were either enriched in different fatty acids, including polyunsaturated fatty acids (PSF), monounsaturated fatty acids (MSF), saturated fatty acids (SF), whereas one contained no fat (glucose). Details on the preparation of the shake have been presented elsewhere [56]. As expected we reported that this meal-induced reduction in AAb against MDA-LDL was specific for test meals rich in highly oxidizable polyunsaturated fatty acids (PSF) and could not be observed with challenges containing saturated fatty acids (SF), monounsaturated fatty acids (MSF), or only glucose (Figure 2) [56]. Thus, the composition of the last meal prior to blood collection could have an impact on plasma levels of AAb-oxLDL.

![Figure 2.](image)

**Figure 2.** Meal-induced changes in levels of AAb against MDA-LDL: Effect of meal composition. Following an oral challenge, there was a transient reduction in the circulating levels of autoantibodies (AAb) to malondialdehyde-modified lipoproteins. The acute change was observed only with a challenge enriched in the highly oxidizable polyunsaturated fatty acids (PSF) and not with monounsaturated fatty acids (MSF), saturated fatty acids (SF), or glucose.

In the third study, we examine the effect of therapy with a lipid-lowering drug known to have antioxidant properties on this postprandial response. For this we chose simvastatin, a well-known cholesterol-lowering drug that has previously been reported to have antioxidant properties [57], In a group of hypercholesterolemic patients we examined the postprandial response at baseline and after four months of treatment with simvastatin (40 mg/day). The composition of the test meal and laboratory analyses have been previously described [55]. We observed no difference in fasting levels of AAb against MDA-LDL (Figure 3A) despite a 30% reduction in LDL. However, the meal-induced reduction in AAb against MDA-LDL was completely blunted (Figure 3B) indicative of a reduction in oxidative stress in the arterial wall with simvastatin therapy. This result would suggest that the oxidative state of the arterial wall could have an impact on plasma levels of AAb-oxLDL.
In the fourth study, we used an intravenous bolus of heparin (60 IU/kg body weight) to dislodge metabolites that would be attached to heparan sulfate proteoglycans lining the arterial wall (unpublished data, pre- and post-heparin plasma samples were made available courtesy of Dr. RB Goldberg and colleagues at the University of Miami).

In addition to the measurements of AAb against MDA-LDL and LDL-IgG immune complexes as previously described [55] we also measured levels of oxLDL and myeloperoxidase in pre- and post-heparin samples by immunoassay (Mercodia, Winston-Salem, NC, USA). MPO is an enzyme released by leukocytes with the specific task of producing reactive oxidants for the destruction of ingested microorganisms within phagosomes [58, 59] and has been linked to atherosclerosis [60]. MPO is also recognized as a key catalyst for lipoprotein oxidation [61]. Concentrations of apoB and apoA-I, the key structural protein in TRL + LDL and HDL, respectively, were also measured by immunoturbidometric method (Sekisui Diagnostics, Burlington, MA, USA) on the AU480 automatic chemistry analyzer (Beckman Diagnostics, Brea, CA, USA).

Figure 4A illustrates acute reductions in plasma TG by 59.1% and 55.8% for individuals with normal glucose tolerance test (NGT) as compared to individuals with impaired oral glucose tolerance test (IGT + DM), respectively. Concomitant with this reduction in plasma TG, circulating levels of oxidatively modified LDL (oxLDL) were significantly increased, 27% and 23% (p < 0.001) in NGT and IGT + DM, respectively. Plasma levels of AAb against MDA-LDL (15.6% and 21.8%) and LDL-IgG immune complexes (21.9% and 16.9%) were also increased, independent of diabetes status (p < 0.001). We also note a significant increase (9.4% and 9.3%, p < 0.001) in plasma levels of myeloperoxidase (MPO) by immunoassay. There was no difference in levels of CHOL, apoB, or apoA-I between the pre- and post-heparin plasma samples (Figure 4B). Thus, lipoprotein-associated markers released into the circulation by the bolus of heparin were preferentially oxidatively modified. This is direct evidence that levels of oxidatively modified biomarkers in plasma may be underestimated due to
retention along the arterial wall of these metabolites. Depending on the propensity of oxidatively modified lipoproteins to adhere to the arterial wall, plasma levels of both oxLDL and AAb-oxLDL may not reflect the true levels of these biomarkers.

3.2 Lipoprotein oxidative susceptibility

Considering the abundance of both nonenzymatic (glutathione, uric acid, bilirubin, vitamin E, vitamin C, etc.) and enzymatic antioxidants (superoxide dismutase, glutathione reductase, catalase, etc.) in the body, the mechanism(s) by which an acute increase in oxidatively modified lipoproteins would occur is unclear. The concept of oxidative susceptibility may be helpful to explain this observation. Oxidative susceptibility of isolated lipoproteins is based on the kinetics of the formation of conjugated dienes when exposed to a highly oxidative environment. By exposing the preparation of purified lipoproteins to Cu^{2+} as an artificial catalyst of oxidation, Esterbauer et al. [62] characterized the oxidative process in terms of changes in absorbance at 234 nm, corresponding to absorption by newly formed dienes. They defined the oxidative process in three phases, lag phase corresponding to the initial formation of dienes in the presence of endogenous antioxidants, propagation phase associated with the accelerated formation of dienes after antioxidant properties have been depleted, and decomposition phase. As endogenous antioxidants are consumed by initiating free radical species during the lag phase, lipoprotein particles that are more susceptible to oxidative modification would have shorter lag phase.

Increased oxidative susceptibility of plasma lipoproteins has been linked to CAD [63], stroke [64], diabetes mellitus [41] as well as kidney disease [65] among other chronic conditions. Oxidative susceptibility of LDL has also been shown to be affected by diet [66–68] and physical activity [69]. Data from our group indicate that lipoprotein susceptibility is acutely affected by meal consumption and can be altered by lipid-lowering therapy.
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Figure 5 illustrates the time-dependent formation of conjugated dienes (as assessed by optical density at 234 nm) for preparations of LDL isolated at t = 0 h (LDL₀) and at t = 4 h (LDL₄) following meal consumption. In the absence of Cu²⁺ as a catalyst, there was minimal formation of conjugated dienes with either the fasting LDL or the postprandial LDL samples. Upon incubation with Cu²⁺, there was rapid formation of conjugated dienes with the lag time of postprandial LDL (LDL₄) being significantly shorter than that of fasting LDL (LDL₀), 38.7 min versus 142.4 min [43], respectively. We hypothesize that, in individuals with metabolic syndrome, postprandial lipoproteins might be seeded with ROS during their contact with the arterial wall and therefore could be more susceptible to oxidative modification when compared to fasting lipoproteins.

Figure 6 presents changes in oxidative susceptibility of fasting and postprandial LDL following a 6-month treatment with a TG-lowering medication (ABT-335, Trilipixᵀᴹ) in a patient with metabolic syndrome. As shown (Figure 5A), in the absence of Cu²⁺, fasting LDL from this participant underwent spontaneous oxidative modification. The rate of formation of conjugated dienes was significantly slowed down after ABT-335 therapy. In the presence of Cu²⁺, the lag time of fasting LDL was increased from 28.7 min to 149.5 min after therapy. The lag time for postprandial LDL was minimally increased after therapy, 25.5 min versus 35.5 min, respectively [43].

3.3 oxLDL versus LDL

A more direct assessment of the meal-induced oxidation biomarkers is the direct measurement of oxLDL and total plasma apoB by immunoassay following meal consumption (unpublished data). Apolipoprotein B (apoB) is the primary structural
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protein in TRL and LDL. In a preliminary study, we examine the change in plasma levels of oxLDL (Mercodia, Winston-Salem, NC, USA) and apoB (Sekisui Diagnostics, Burlington, MA, USA). Figure 7 illustrates representative time-dependent changes in these metabolites following the standardized oral challenge for two participants. In both individuals, there was minimal change in total plasma apoB following the meal.
Plasma oxLDL levels were increased in both instances, reaching a maximal increase of 2.5-fold (Figure 7A) and 1.5-fold (Figure 7B) 6 h after meal consumption. While apoB and oxLDL levels have been examined previously this is the first data suggesting that they are not affected to the same degree during postprandial lipemia. Further investigation is needed to confirm this observation and to determine when oxLDL levels return back to pre-meal levels.

4. Metabolic flexibility

There has been a recent shift away from the traditional definition of health formulated by the WHO in 1948 as “a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity” [70]. The current concept of health is based on the definition by Huber et al. [70] in terms of flexibility, adaptability, elasticity, and robustness. In other words, health should be defined in terms of adaptation and flexibility to continuously changing external inputs [71]. This ability to adapt to a specific metabolic challenge is referred to as metabolic flexibility or phenotypic flexibility [10, 71].

The most commonly used challenge test in the clinical environment is the oral glucose tolerance test which is the primary tool for the diagnosis of diabetes [72]. Ceriello et al. [5, 6, 73–75] have published extensively on the acute effect of oral glucose administered by itself or as part of a mixed meal on biomarkers of inflammation and oxidative stress. Van Ommen and his colleagues have characterized a number of metabolic processes that could be associated with clusters of metabolites using their “PhenFlex challenge” [9, 10, 71, 76–78].

The studies by both the Ceriello team and the Van Ommen team, though very comprehensive, utilized non-physiologic dosage of fat in their challenges, 75 grams [5] and 60 grams [76], respectively. Especially, since the typical consumption of a 600-kcal meal with 30% fat composition as recommended by the AHA would correspond to only 20 grams of fat. Furthermore, the PhenFlex challenge consisted primarily of monounsaturated fatty acids (47%) and saturated fatty acids (39%). The high content of monounsaturated fatty acids may blunt any inflammatory effects of dietary fat [79, 80]. The low contents of highly oxidizable polyunsaturated fatty acids (only 14% of the fat intake) on the other hand, might not be sufficient to elicit any lipoprotein-associated oxidative stress.

To date, most studies utilizing metabolic challenges have limited themselves to looking at the transient changes in the levels of various metabolites as a function of time. Since the metabolism of many of these metabolites is inter-related, we propose to examine phenotypic flexibility in terms of trajectory analysis using two metabolites that are under coordinated control. With an oral meal challenge, the simplest pair of metabolites is glucose and insulin. We used postprandial glucose and insulin data from a group of patients with type 2 diabetes mellitus participating in a randomized double-blind, multicenter clinical trial comparing the efficacy of mid-mixture lispro versus glargine [81]. Figure 8 presents the postprandial changes in the mean levels of insulin and glucose as a function of time during glargine (Panel A) or MM-lispro (Panel B) therapy. In panel C (Figure 8C), each data point represents the normalized levels of insulin and glucose at a specific time following meal consumption. Both trajectories start out at (1,1) and return back to the starting point after 8 h. Under glargine (dashed), the maximum excursion for
glucose at $t = 2\text{ h}$ is at 1.7 (or 70% greater than fasting level) and at 1.5 for insulin. In contrast, with the administration of pre-meal lispro (solid line), the maximum excursion of glucose is 1.3 at $t = 1\text{ h}$ corresponding to an insulin level of 2.7 which reflects the combined concentrations of endogenous and exogenous insulin. This trajectory analysis can be characterized by several parameters, including the maximum excursion, slope of the maximum excursion, perimeter of the trajectory, and area enclosed within the trajectory. A large maximum excursion distance would suggest great impact of the challenge while a large perimeter and enclosed area would be indicative of poor metabolic control.

5. Conclusions

CMD are chronic conditions associated with inflammatory and oxidative stress and tend to progress over time. Abnormal fasting levels of inflammatory and oxidative biomarkers are typically indicative of presence of advanced disease conditions and may be too late for any preventive management. The ability to assess how rapidly the body can respond to a challenge and maintain metabolic homeostasis may be useful in establishing early signs of metabolic disturbances. It is crucial to note that the challenge should be similar to physiological influences and must be reflective of typical daily activities. While the use of an oral meal challenge may be used to demonstrate the beneficial effects of some nutrients, the composition of the challenge must include elements known to elicit the maximal responses in terms of inflammatory and oxidative stress. Furthermore, in addition to examining the time-dependent changes, it would be helpful to identify biomarkers that are metabolically linked as that may provide data that could elucidate potential regulatory pathways.
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