Leukotrienes as Biomarkers of Cardiovascular Disease

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Abstract

Myocardial infarction and stroke are major causes of morbidity and mortality and result from an underlying atherosclerosis of the coronary and cerebrovascular vasculature. Atherosclerotic plaques are a site of lipid accumulation and chronic inflammation. There is a need for novel biomarkers to predict an individual's cardiovascular risk, and several inflammatory biomarkers have been explored for their prognostic value. Leukotrienes are lipid mediators of inflammation, which are formed in atherosclerotic lesions and participate in the atherosclerosis process. The local production of leukotrienes leads to high levels in atherosclerotic plaques, whereas circulating levels are negligible and difficult to measure. Ex vivo stimulation of leukocytes reflects the leukotriene synthesizing capacity and the leukotriene B_4 levels released from granulocytes in response to calcium inophore are associated with echographic measures of carotid artery vascular remodeling. Urinary leukotriene E_4 is a validated biomarker of asthma, and is increased in coronary artery disease. Salivary levels of leukotriene B_4 were recently associated with vascular stiffness and subclinical atherosclerosis. Leukotriene measures have in addition been associated with several cardiovascular risk factors, such as smoking, diabetes, obesity, and obstructive sleep apnea. The present chapter reviews the available literature using these different approaches for evaluating leukotrienes as biomarkers for cardiovascular disease.

Keywords

Atherosclerosis • Ageing • Inflammation • Leukotriene • Lipoxygenase • Obstructive sleep apnea • Pulse Wave Velocity • Saliva • Urinary biomarkers • Vascular stiffness

Abbreviations

5-LO	5-lipoxygenase
BAL	Broncho-alveolar lavage fluid
BMI	Body mass index
CABG	Coronary artery by-pass grafting
COPD	Chronic obstructive pulmonary disease

$cPLA_2$	Cytosolic phospholipase A_2
EBC	Exhaled breath condensates
eGFR	Estimated glomerular filtration rate
EIA	Enzyme immunoassay
FLAP	5-LO activating protein
GCF	Gingival crevicular fluid
GSH	Glutathione
hsCRP	High sensitivity C-reactive protein
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDL	Low density lipoprotein
LT	Leukotrienes
PCI	Percutaneous coronary intervention
γ-GT	γ-glutamyl transpeptidase

Introduction

Biomarkers of Cardiovascular Diseases

Cardiovascular disease remains the leading cause of death despite major advances in diagnostics and treatment. The major underlying pathology is atherosclerosis (Table 1), which is triggered by accumulation of cholesterol-containing low-density lipoprotein (LDL) particles in the arterial wall leading to immune activation and the recruitment of inflammatory cells (Hansson 2005; Libby et al. 2011; Bäck and Hansson 2015). The resulting atherosclerotic plaques may remain silent for a long time before plaque destabilization occurs, leading to plaque rupture and occlusion o,f for example, cerebrovascular and coronary arteries, causing myocardial infarction and stroke, respectively.

Traditional cardiovascular risk factors exhibit a high predictive value on a population level, but fail to fully predict individual risk (Hoefer et al. 2015). The identification of subjects at increased risk for plaque rupture and resulting cardiovascular events is of particular interest to select patients who would benefit from preventive actions and medical treatments. In this context, a role for inflammatory biomarkers such as high sensitivity C-reactive protein (hsCRP) has emerged as independent risk factors for acute coronary events (Libby et al. 2002), further underlining the role of inflammation in the atherosclerosis process. As stated in a

Table 1 Key facts of atherosclerosis

Atherosclerosis is characterized by lipid retention and immune activation within the vascular wall, and release of inflammatory mediators from the atherosclerotic plaques
Destabilization of atherosclerotic plaques leads to plaque rupture, thrombosis and vessel occlusion resulting in cardiovascular events, such as myocardial infarction and stroke
Biomarkers for the prediction of cardiovascular events are of substantial interest for identifying subjects who would benefit from preventive treatment and/or interventions

This table lists the key facts of atherosclerosis and the context of biomarkers

recent *Position Paper* from the European Society of Cardiology, biomarkers with causal involvement may be more valuable for risk stratification (Hoefer et al. 2015) and may also be useful for identifying novel therapeutic targets and used in drug efficacy evaluation (Bäck and Hansson 2015).

Leukotriene Biosynthesis

Arachichidonic acid is released from cell membrane phospholipids by calcium-dependent activation of the intracellular cytosolic group *IV*A phospholipase A_2 ($cPLA_2$) and is the substrate for the formation of leukotrienes (LT; Fig 1). Metabolism by means of the 5-lipoxygenase (5-LO) enzyme, in conjunction with a 5-LO activating protein (FLAP), will yield the epoxide LTA_4, which serves as precursor for LT synthesis. Whereas the enzyme LTA_4 hydrolase leads to formation of LTB_4, the conjugation of LTA_4 with glutathione (GSH) will yield LTC_4. Subsequently, LTs are transported to the extracellular space where LTC_4 shares its subsequent metabolism with GSH by means of γ-glutamyl transpeptidase (γ-GT) and dipeptidase that cleaves the peptide bonds of the LTC_4 side chain forming LTD_4 and LTE_4, respectively (Fig 1).

Importantly, 5-LO is highly expressed in myeloid cells, e.g., granoluocytes, macrophages, and mast cells, leading to local LT biosynthesis at sites of inflammation, which in some cases also involves transcellular metabolism of LTA_4 in for

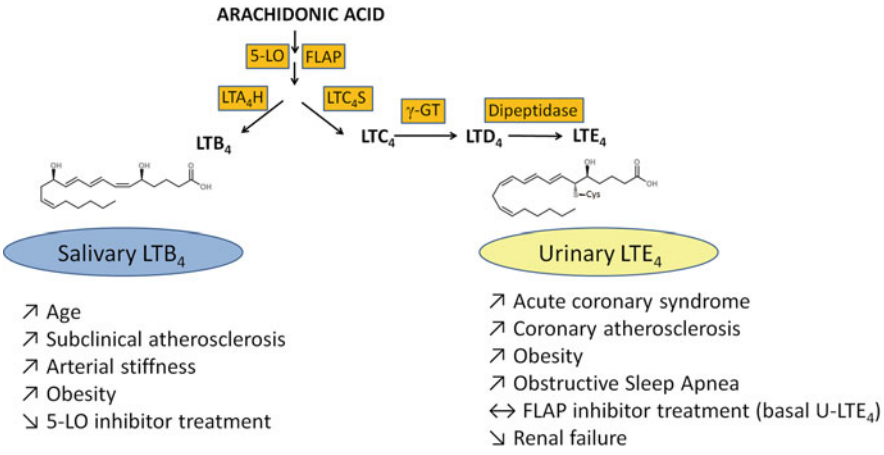

Fig. 1 Leukotriene (LT) biosynthesis through the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism, and possible biomarker applications for salivary LTB_4 (Gaber et al. 2006; Bäck et al. 2007b; Labat et al. 2013) and urinary LTE4 (Carry et al. 1992; Allen et al. 1993; Dahlen et al. 1997; Stanke-Labesque et al. 2009; Rafnsson et al. 2013; Bäck et al. 2014a) in cardiovascular disease and cardiovascular risk factors. Abbreviations: FLAP, 5-LO Activating Protein; γ-GT, γ-glutamyl transpeptidase; LTA_4H, LTA_4 hydrolase; LTC_4S, LTC_4 synthase

example endothelial cells, vascular smooth muscle cells, platelets and macrophages. In addition, 5-LO expression is regulated by promoter methylation (Katryniok et al. 2010), and LT synthesis from arachidonic acid may be induced in non-myeloid cells through epigenetic mechanisms (Nagy and Bäck 2012).

Taken together, this local leukotriene production at sites of cardiovascular inflammation can lead to high leukotriene concentrations at their sites of action, which may not necessarily be reflected in their circulating levels. The latter raises several challenges in the exploration of leukotrienes as biomarkers for cardiovascular disease, which will be the focus of the present chapter.

The Role of Leukotrienes in Cardiovascular Disease

Leukotrienes exert potent actions on inflammatory reactions, being active at nanomolar concentrations at specific G-protein coupled leukotriene receptors (Bäck et al. 2011) expressed on several target cells in atherosclerotic lesions (Bäck et al. 2014b). Leukotrienes exert diverse proinflammatory effects with implications for atherosclerosis development and cardiovascular disease, inducing leukocyte recruitment and activation, smooth muscle cell proliferation, and endothelial dysfunction (Bäck and Hansson 2006; Bäck 2009). In addition, the leukotriene pathway has been linked to cardiovascular calcification in aortic valve stenosis (Nagy et al. 2011). The degree of calcification can alter the biomechanical properties of the vascular wall (Bäck et al. 2013; Kwak et al. 2014) and is also of importance since microcalcifications in the fibrous cap might be associated with plaque rupture (Otsuka et al. 2014). Importantly, leukotriene receptor antagonists used in the treatment of asthma have been associated with a decreased risk of stroke and myocardial infarction (Ingelsson et al. 2012) and antileukotriene has been evoked as putative therapeutics in cardiovascular prevention (Bäck and Hansson 2015). Given this implication of the LT pathway as a causal factors in cardiovascular diseases (Bäck 2009), there is an increasing interest to monitor leukotrienes as biomarkers in cardiovascular disease (Hoefer et al. 2015).

Leukotriene Measurements: Methodological Considerations

The analytical challenge for leukotriene quantitation lies in the detection of small amounts of leukotrienes in different biological fluids, e.g., urine, saliva or cell supernatants. Several analytical methods have been described including enzyme immunoassays (EIA) or chromatography tandem mass spectrometry methods.

EIA is an antibody-based method, the validity of which depends on the specificity of the antibody used. Although easy to perform, the main limitation of this strategy is the cross-reactivity of the antibody with other potential interfering compounds in the matrix, despite a previous purification step. This point is a major concern when analyzing urinary-LTE_4. In addition, EIA does not allow the separation of

enantiomers or diastereoisomers, and provides, for example, an overestimation of LTB_4 concentration in supernatant of challenged cells.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a more specific method based on a chromatographic separation followed by detection of the compound of interest on its mass/charge ratio. Following an enrichment step by solid phase extraction (Hardy et al. 2005) or on-line extraction (Armstrong et al. 2009) these methods offer greater specificity, precision and accuracy and allow for example the separation of LTB_4 and its isomers 6-trans-LTB_4 and 6-trans-12-epi LTB_4 (Stanke-Labesque et al. 2012) thanks to the chromatographic step.

When compared to EIA, the concentrations of LTE_4 measured by LC-MS/MS are lower in urine from patients with asthma highlighting the greater specificity of tandem mass spectrometry (Armstrong et al. 2009). There is hence a huge need to organize international interlaboratory proficiency testing programs to standardize the quantitation of leukotrienes.

Human Vascular Leukotriene Production

Ex vivo studies of vascular specimens have confirmed that leukotriens are locally produced within the human vascular wall. This has, for example, been studied in the pulmonary vasculature (Piper et al. 1988) and perfused and ventilated human lungs (Kiss et al. 2000). Importantly, atherosclerotic lesions exhibit an increased leukotriene formation compared with healthy vessels, as demonstrated by studies of human vascular segments derived from both carotid endarterectomies (De Caterina et al. 1988), and human coronary arteries (Piomelli et al. 1987).

Recent methodological advances have opened up for selective lipidomic analysis, which has revealed that lipoxygenase metabolites are the predominant arachidonic acid products formed in carotid atherosclerotic lesions, compared with cyclooxygenase products (Liu et al. 2013). In addition, human abdominal aortic aneurysms and stenotic aortic valves are also sites of local leukotriene formation (Houard et al. 2009; Nagy et al. 2011; Kochtebane et al. 2013). Taken together, these studies provide the rationale for exploring leukotrienes as biomarkers of cardiovascular disease.

Plasma and Serum Leukotriene Measurements

Analyses of blood samples withdrawn at the site of obstructive atherosclerotic coronary lesions have revealed undetectable LT levels (Brezinski et al. 1992). In contrast, LTC_4 and LTD_4 (but not LTE_4) were detectable in samples taken at the site of the coronary lesion immediately after the balloon inflation during percutaneous coronary intervention (PCI) (Brezinski et al. 1992). Although the latter study provides compelling evidence that plaque rupture may be the stimulus triggering appearance of these mediators in plasma, no peripheral blood samples were

withdrawn at the time of the angioplasty and the extrapolation to systemic plasma measures of leukotrienes remains unknown.

Another study reported increased LT plasma levels (in samples withdrawn from the femoral artery) in 19 patients with myocardial infarction compared with 12 healthy controls. These elevated levels persisted for more than 1 week and had returned to values comparable to those observed for control subjects at 1 month from the myocardial infarction (Takase et al. 1996). This observation may indicate that leukocyte activation in myocardial infarction persists, but it remains unclear whether the results either reflect the atherosclerotic plaque rupture or the myocardial damage following the ischemia.

Several initial studies measured plasma levels of leukotrienes during coronary artery bypass grafting (CABG), and generated somewhat variable results. Despite pronounced coronary atherosclerosis, plasma levels of LTB_4 and LTC_4 are negligible in patients undergoing CABG (Gimpel et al. 1995). However, the perioperative extracorporeal circulation during CABG may increase plasma LTs to detectable levels, especially after release of the aortic cross-clamp (Jansen et al. 1991; Gimpel et al. 1995; Denizot et al. 1999). In contrast, one study reported unchanged LTC_4 concentrations during CABG in both the radial artery and the coronary sinus (Bengtson et al. 1989).

These reported either low or undetectable LT levels in plasma samples are contrasted by high levels in serum sample (Houard et al. 2009), raising the notion that serum LT levels may be due to ex vivo LT production from, for example, neutrophil granulocytes during the coagulation process in the tube. In support of the latter, serum LTB_4 levels are associated with the time from blood sampling to centrifugation (Houard et al. 2009), hence emphasizing the limits of measuring systemic circulation LT levels as biomarkers.

LT Release from Ex Vivo Stimulated Leukocytes

Since plasma LT levels do not necessarily reflect the local production at the site of the vascular inflammation, another approach is to isolate leukocytes from human blood followed by ex vivo stimulation with calcium ionophore, which activates 5-LO by means of increased intracellular calcium. Given that leukocytes are the predominant source of LTs biosynthesis, ex vivo stimulation of circulating leukocytes may reflect the leukocyte capacity of LT formation.

Using this approach, an increased LTB_4 formation in subjects with a history of myocardial infarction has been described (Helgadottir et al. 2004). In the latter study, myocardial infarction patients exhibiting the highest stimulated leukocyte LTB_4 production were carriers of the described 5-LO haplotype, which was associated with increased cardiovascular risk (Helgadottir et al. 2004), supporting stimulated LT release as an appropriate measure of LT-producing capacity. Furthermore, the levels of LTB_4 released from calcium ionophore-stimulated granulocytes, derived from subjects with obstructive sleep apnea, exhibited a significant association with

carotid artery measures of vascular remodelling (Lefebvre et al. 2008), supporting the use of stimulated LT release as biomarker for subclinical atherosclerosis (Stanke-Labesque et al. 2014). Finally, LTB_4 formation is increased in granulocytes collected after the extracorporeal circulation during CABG, with the elevation being persistent during the first 24 postoperative hours, and was suggested to have a significant role in the postoperative outcome (Gadaleta et al. 1994).

Taken together, evaluating LT-synthesizing capacity in ex vivo stimulated leukocytes has been explored as biomarkers of genetic variations in LT-producing enzymes (Helgadottir et al. 2004), subclinical atherosclerosis (Lefebvre et al. 2008) and in thoracic surgery (Gadaleta et al. 1994). However, the extensive experimental work involved in the sample preparation may limit the use of ex vivo stimulated leukocytes for the evaluation of LTs as biomarkers for cardiovascular disease.

Urinary LTE_4

Validation of Urinary-LTE_4 as a Biomarker

Injection of radiolabeled LTC_4 or LTE_4 to healthy volunteers revealed that these LTs appear as LTE_4 in the urine within 1–2 h after infusion (Orning et al. 1985; Sala et al. 1990; Maclouf et al. 1992). In contrast, only low levels of LTB_4 metabolites can be detected in urine samples after LTB_4 injection (Berry et al. 2003). Subsequent studies established the use of urinary LTE_4 as a biomarker for asthma and allergen-provoked bronchoconstriction (Dahlen et al. 1997; Dahlen and Kumlin 1998; Balgoma et al. 2013). There are in contrast only a limited number of studies applying urinary LTE_4 as cardiovascular biomarker.

Urinary-LTE_4 in Myocardial Infarction and CABG

In acute myocardial infarction an increase in urinary LTE_4 has been reported, decreasing to control values 3 days after ischemia (Carry et al. 1992). As discussed above, while this approach does not allow distinguishing plaque rupture from myocardial damage as the cause of increased LTs, the latter study suggests that urinary LTE_4 decreased to concentrations similar to controls at the third day after the ischemic event. This is in contrast to a study of patients undergoing CABG who had significantly elevated urinary LTE_4 compared with healthy controls (Allen et al. 1993). Whether the apparent differences between those studies were due to more extended atherosclerosis in the CABG candidates cannot be concluded from the available data. Nevertheless, taken together those studies suggest that urinary LTE_4 may be used for both the evaluation of baseline values in atherosclerosis and reflect changes in acute coronary syndromes and myocardial ischemia. The latter notion is supported by the further increase in urinary LTE_4 observed after CABG

surgery, with a peak of urinary LTE_4 at the second postoperative day (Allen et al. 1993).

Limitations of Urinary LT Measures

In a recent study, urinary LTE_4 did not correlate with either macro- or microvascular endothelial function in a cohort of diabetes patients with microalbuminuria (Rafnsson and Bäck 2013). In contrast, urinary LTE_4 levels were significantly decreased with impaired renal function, and multivariate analysis revealed the estimated glomerular filtration rate (eGFR) as an independent predictor of urinary LTE_4 concentrations in these patients (Rafnsson and Bäck 2013). These data imply that renal function should be considered when studying urinary biomarkers, especially when evaluating cardiovascular risk, for which renal function may be a significant confounder. However, in a cohort with normal renal function, no significant associations between urinary LTE_4 and eGFR were detected (Bäck et al. 2014a), hence supporting the use of this biomarker in the absence of renal failure.

Another limitation of urinary LTE_4 has emerged from clinical studies of LT synthesis inhibitors. Whereas the allergen-induced increase in urinary LTE_4 concentrations is effectively inhibited by the FLAP antagonist BAYx1005/DG031, the basal prechallenge urinary LTE_4 concentrations were unaltered by this treatment (Dahlen et al. 1997). In addition, a study evaluating effects on cardiovascular biomarkers reported an unexpected increase in urinary LTE_4 in subjects treated with BAYx1005/DG031 (Hakonarson et al. 2005). Those studies hence question the use of urinary LTE_4 for the evaluation of pharmacological LT synthesis efficacy in cardiovascular clinical trials.

Salivary LTB_4

In the exploration of biomarkers for pulmonary disease, LT levels have been measured in exhaled breath condensates (EBC). Studies in thoracic surgery have shown that while LTB_4 levels in EBC are unaltered during CABG, significant changes were observed in pulmonary lobectomy (Moloney et al. 2004), supporting that biomarkers in EBC may more accurately predict pulmonary compared with cardiovascular diseases. However, studies of LT concentrations in EBC revealed the possibility of EBC contamination with LTs derived from the oral cavity (Gaber et al. 2006).

The notion of oral LTs as biomarkers is supported by studies of periodontitis, in which gingival crevicular fluid (GCF) can be sampled at the site of periodontal inflammation. LT concentrations are increased in GCF from periodontitis patients (Tsai et al. 1998; Bäck et al. 2006). Interestingly, GCF concentrations of LTs are also increased in subjects with carotid artery atherosclerotic plaques on echographic

examination (Bäck et al. 2006), providing a first piece of evidence of an association between oral LTs and cardiovascular disease.

Since GCF sampling requires dental intervention, GCF may not be a suitable matrix for cardiovascular biomarker evaluations. However, also saliva contains remarkably high levels of LTB_4 (Gaber et al. 2006; Bäck et al. 2007b). Furthermore, in contrast to urinary LTE_4 (Dahlen et al. 1997; Hakonarson et al. 2005), a reduction of basal LT production by 5-LO inhibition can be monitored by measuring salivary LT levels (Gaber et al. 2007), further reinforcing the accuracy of salivary LTB_4 as a possible biomarker.

In a cohort of 259 subjects, salivary LTB_4 exhibited a significant association with echographic measures of subclinical atherosclerosis, as defined by the carotid artery intima media thickness (Labat et al. 2013). Furthermore, the vascular stiffness was evaluated by means of pulse wave velocity. In a multivariate analysis, salivary LTB_4 was an independent predictor for increased arterial stiffness (Labat et al. 2013). Since both the intima media thickness and pulse wave velocity are prognostic markers for cardiovascular outcome, the associations with these measures provide a first indication for the potential use of salivary LTB_4 as a biomarker for cardiovascular disease. Given that sampling of unstimulated whole buccal saliva is a simple and non-invasive procedure reinforces the suitability of salivary LTB_4 for biomarker evaluation in large cardiovascular cohort studies.

LTs as Biomarkers in Relation to Cardiovascular Risk Factors

When studying leukotriene formation in cardiovascular disease it is also important to address how known cardiovascular risk factors affect LT biomarker concentrations, both in terms of confounding factors, but also as a potential causal factor and source of LTs.

Age

The normal wear and tear of aging will lead to a progressive deterioration of cardiovascular structures which may contribute to cardiovascular disease development. Moreover, aging is associated with an accumulation of cardiovascular risk factors (Thomas et al. 2001), and needs to be taken into consideration when evaluating cardiovascular biomarkers. Salivary LTB_4 is significantly increased in older subjects (Bäck et al. 2007b), whereas ex vivo stimulated LTB_4 release (Lefebvre et al. 2008; Stanke-Labesque et al. 2012) and urinary LTE_4 (Stanke-Labesque et al. 2009) appears less dependent on age. It should however be pointed out that the significant associations of salivary LTB_4 with carotid artery intima media thickness and body mass index (BMI) persisted in an age-adjusted analysis (Labat et al. 2013).

Smoking and Chronic Obstructive Pulmonary Disease (COPD)

Increased LT levels have been reported in EBC (Carpagnano et al. 2003), sputum (Keatings et al. 1996), and bronchoalveolar lavage fluid (BAL) (Zijlstra et al. 1992), derived from smokers compared with nonsmokers. LT concentrations in BAL were in addition correlated to the number of granulocytes, suggesting that increased LT levels in pulmonary samples may reflect increased neutrophilic inflammation in the lungs induced by smoking. Likewise, urinary LTE_4 is increased in smokers compared with non-smokers, and correlates with the number of cigarettes smoked (Fauler and Frolich 1997). In the latter context, also underlying pulmonary pathologies may affect the urinary LTE_4 response to smoking (Gaki et al. 2007).

In contrast, GCF and saliva LT levels do not differ between smokers and nonsmokers (Bäck et al. 2006, 2007b), suggesting that the biomarker sampling fluid may be crucial for the detection of smoking-induced LT production. Finally, with the reservation of plasma LT measures previously addressed (cf. *supra*), no difference in plama-LTB_4 concentrations between smokers and nonsmokers have been reported in a study of 61 healthy subjects (McKarns et al. 1995).

Assessed by means of ex vivo stimulated LTB_4 release, subjects with COPD exhibit an increased LT production (Mitsunobu et al. 2001; Santus et al. 2005). Importantly, in addition to sharing smoking as common risk factor, COPD has emerged as an independent risk factor for cardiovascular disease (Nishiyama et al. 2010; Yin et al. 2014). The association of COPD with an increased cardiovascular risk has been suggested to relate to both similar risk factors and to similar pathophysiological mechanisms, in which LT-induced inflammatory circuits may be involved (Bäck 2008), hence reinforcing the interest of evaluating LTs as biomarkers when assessing cardiovascular risk associated with smoking and COPD.

Diabetes

Urinary LTE_4 is increased in type 1 diabetic patients with poor metabolic control (Hardy et al. 2005) and intense glycemic control decreased urinary LTE_4 in type 1 diabetes but not in type 2 diabetes (Boizel et al. 2010). In overweight subjects, urinary LTE_4 is significantly higher in subjects with high fasting plasma glucose (Bäck et al. 2014a), supporting that diabetes and insulin resistance should be considered as possible confounders in the exploration of urinary LTE_4 as cardiovascular biomarker.

Obesity

The association between LT production and obesity was initially demonstrated in subjects with obstructive sleep apnea (Stanke-Labesque et al. 2009) and was subsequently reported in asthmatics (Giouleka et al. 2011) and in children with sleep

disordered breathing (Shen et al. 2011). Further exploration in a cohort of obese subjects revealed a significant correlation of urinary LTE_4 with the waist to hip ratio (Bäck et al. 2014a). Finally, the association of LTs with obesity and waist-to-hip ratio has also been observed for salivary LTB_4 (Labat et al. 2013).

The association of LTs not only with BMI but also waist circumference is of particular clinical interest, since abdominal obesity is a predominant cardiovascular risk factor in obesity. Adipose tissue may represent an active site of leukotriene formation in experimental studies (Bäck et al. 2007a). In addition, FLAP is expressed in human adipose tissue, with higher levels in abdominal subcutaneous fat derived from obese compared with lean subjects (Kaaman et al. 2006).

Obstructive Sleep Apnea

Obstructive sleep apnea is characterized by recurrent episodes of nocturnal upper airway obstruction leading to chronic intermittent hypoxia, which is a potent proinflammatory stimulus. Increased LT levels in urine (Stanke-Labesque et al. 2009; Shen et al. 2011), EBC (Goldbart et al. 2006) and ex vivo stimulated leukocytes (Lefebvre et al. 2008; Stanke-Labesque et al. 2012) have been reported in subjects with obstructive sleep apnea, and correlated with different measures of disease severity. The increased cardiovascular risk associated with obstructive sleep apnea has been well established (Levy et al. 2012), and leukotrienes have been implicated as possible causal factor for the accelerated atherosclerosis associated with obstructive sleep apnea (Stanke-Labesque et al. 2014).

Potential Applications to Prognosis, Other Diseases or Conditions

As outlined above, LTs are increased in different cardiovascular diseases (Table 2). Given that these potent lipid mediators of inflammation are produced locally at their site of action, systemically measured levels may not necessarily reflect increased levels at sites of inflammation, such as atherosclerotic lesions. Plasma and serum measures of LTs cannot be recommended based on the existing literature. As an alternative, assessment of leukotriene synthesis from ex vivo stimulated leukocytes provides a reliable measure of LT synthesizing capacity, but its use may be limited by the experimental preparations needed. Urinary LTE_4 has been validated as a biomarker in asthma, but only limited data are available for this biomarker in cardiovascular disease. Despite certain precautions needed, such as taking renal function into considerations, urinary LTE_4 is an interesting and feasible approach for assessing LTs as biomarkers in cardiovascular disease. However, LTB_4 concentrations cannot be measured in the urine, and for this mediator, saliva measures may represent an alternative. Finally, we draw the attention to important confounders to take into consideration when assessing LTs as biomarkers, such as age, smoking, and obesity, as well as comorbidities in terms of COPD, diabetes, and obstructive sleep apnea. In conclusion, although the limited available studies on LTs as biomarkers in

Table 2 Leukotrienes as biomarkers of cardiovascular disease

	Plasma	Stimulated Leukocytes	Urine	Saliva	Other
Acute Coronary Syndrome	↑[1]		↑[2]		
CABG	↑[3–5], ↔[6]	↑[7]	↑[8]		↔ in EBC[9]
PCI	↑[10]				
History of AMI		↑[11]			
Carotid Artery Echography Measures		↑[12]		↑[13]	↑ in GCF[14]
Vascular Stiffness				↑[13]	
Cardiovascular Risk factors					
Age		↔[12, 15]	↔[16, 17]	↑[18]	
Smoking	↑[19]		↑[19, 20],↔[20, 21]	↔[13]	↑ in EBC[22], sputum[23] and BAL[24], ↔ in GCF[14]
Diabetes			↑[25]		
Obesity		↔[12, 15]	↑[16, 26]	↑[13]	
Obstructive Sleep Apnea		↑[12, 15]	↑[16]		↑ in EBC[27]

This table lists the key studies of leukotrienes as biomarkers for different measures of cardiovascular disease, and of different cardiovascular risk factors. Abbreviations: *CABG* coronary artery by-pass grafting, *EBC* exhaled breath condensates, *PCI* percutaneous coronary intervention, *AMI* acute myocardial infarction, *GCF* gingival crevicular fluid, *BAL* bronco-aleveolar lavage
References: [1]Takase et al. 1996; [2]Carry et al. 1992; [3]Jansen et al. 1991; [4]Gimpel et al. 1995; [5]Denizot et al. 1999; [6]Bengtson et al. 1989; [7] Gadaleta et al. 1994; [8]Allen et al. 1993; [9]Moloney et al. 2004; [10]Brezinski et al. 1992; [11]Helgadottir et al. 2004; [12]Lefebvre et al. 2008; [13]Labat et al. 2013; [14]Bäck et al. 2006; [15]Stanke-Labesque et al. 2012; [16]Stanke-Labesque et al. 2009; [17]Rafnsson and Bäck 2013; [18]Bäck et al. 2007b; [19]Fauler and Frolich 1997; [20]Gaki et al. 2007; [21]McKarns et al. 1995; [22]Carpagnano et al. 2003; [23]Keatings et al. 1996; [24]Zijlstra et al. 1992; [25]Hardy et al. 2005; [26]Bäck et al. 2014; [27]Goldbart et al. 2006

cardiovascular disease (Table 2) are promising, there is a need for standardization of LT measurements to reflect and detect increased LT formation in cardiovascular diseases.

Summary Points

- This chapter focuses on the lipid mediators of inflammation, leukotrienes, and their role as biomarkers of cardiovascular disease, especially atherosclerosis.
- Leukotrienes are lipid mediators of inflammation, formed locally at sites of inflammation by means of 5-lipoxygenase metabolism of arachidonic acid.

- Local production of leukotrienes leads to high levels in atherosclerotic plaques, whereas circulating levels are negligible and difficult to measure.
- Ex vivo stimulation of leukocytes reflects the leukotriene synthesizing capacity and the leukotriene B_4 levels released from granulocytes in response to calcium inophore are associated with echographic measures of carotid artery vascular remodeling.
- Urinary leukotriene E_4 is a validated biomarker of asthma, and is increased in coronary artery disease, but some precautions are needed when applying urinary leukotriene E_4 as cardiovascular biomarker.
- Salivary levels of leukotriene B_4 were recently associated with vascular stiffness and sublinical atherosclerosis.
- Leukotriene measures have in addition been associated with several cardiovascular risk factors, such as smoking, diabetes, obesity, and obstructive sleep apnea.
- There is a need for standardization of LT measurements to reflect and detect increased LT formation in cardiovascular diseases.

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