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Lipid Club Letter

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Contents

Editorial

Dear Colleagues,

This second volume of the Lipid Club Letter 2009 contains three interesting articles from members of the Belgian Lipid Club (BLC). I thank them for their contributions; the content of this volume illustrates once more the diversity of lipidology and atherosclerosis research.

Let me use this opportunity to announce important forthcoming activities of the BLC.

- *on April 23rd the BLC co-organizes with the Belgian Society of Clinical Chemistry a symposium on "Guidelines for cost-effective use of clinical chemistry" with particular emphasis on lipid parameters. Further information can be obtained from M.Langlois.*
- *the next meeting of the BLC (end of May-early June) will deal with Familial Hypercholesterolemia; more precise information will be send soon*
- *on Sept 26th the BLC co-organizes with J.Ducobu a symposium on Lipids and atherosclerosis in Charleroi. J. Ducobu has prepared an exciting program which You may have seen in the previous issue of this Letter.*
- *by the end of the year the BLC will organize a symposium on "hsCRP: risk marker, target of therapy : none or both?".*

Let me also remind You that the MSD-Schering Plough – BLC Award 2009 has been announced; deadline is July 1th 2009.

I wish You an interesting reading of this volume of the Lipid club Letter

Cordially

Prof. Dr. G. De Backer
President BLC

Responsible editor: Prof. G. DE BACKER (R.U. Gent) Editorial staff: Prof. J. DUCOBU (ULB, UMH)

THE "LIPIDO-GENOMIC DIALOGUE" BETWEEN THE MOTHER AND HER CHILD VIA THE PLACENTA BEFORE BIRTH

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1. Introduction

One hallmark of all genetic factors affecting atherosclerosis is their "lifetime persistence and consistency over time". A genetic factor does not change like age, lifestyle or other environmental factors. This may explain in part why the significant association between lipid-related genetic factors and CVD remains significant even after adjustment for lipid variables. In this particular "time aspect" of genetic factors, very few is known about what happens at the very beginning of life when the individual at his foetal stage is living in the environment of his mother.

2. An original question

In this situation of early life, we may wonder not only whether ***the genetic make up of the individuals can already affect his lipoprotein levels, as it occurs at adult age, but also whether the genetic make up of the mother could affect also his lipoprotein metabolism during the foetal state and thus the levels of lipids to which he is exposed in this critical period of life.***

No study has yet examined such influence of maternal genome of foetal lipid levels. There are however several reasons why it might be important : 1) Lipids play an important role in foetal development and foetal stage is also a critical period for the formation of organs like vessels but also for the development of lesions resembling histologically to early stages of atherosclerosis (Barker 2005, Napoli 2001); 2). The placenta expresses proteins involved in lipoprotein metabolism like LDL receptor, VLDL/apoE receptor, lipoprotein lipase at its surface and apolipoprotein-B, -E, -A1 in the placental cells (Overbergh 1995). Although never demonstrated in humans, the presence of these proteins suggests that placenta may play a role in lipoprotein metabolism in the maternal and in the foetal circulation. 3) these placental proteins which are susceptible to interact biochemically with maternal lipoproteins (encoded by the maternal genome) are encoded from the foetal genome.

Table 1. Some features of the examined polymorphisms in the general population

Genes	**APOE**		**LPL**
Polymorphism	E4	E2	X447
Prevalence	10-15% (South EU) 40-50% (North EU)	14%	20% in the Caucasian population
	(Compared to allele E3)		
TC	+ 14-22%		
LDL-C	+ 10-20 mg/dl	- 10-20 mg/dl	Variable
HDL-C	Lower		+4%
TG	Higher	Higher	-8%
OR for CHD 95%CI:	1.42 1.26 -1.61	0.98 0.66-1.46	0.83 0.7-1.0

In our two papers (Descamps 2004 & 2005), we explored the association between genetic factors and blood lipids in mothers and their foetuses at term (newborns). To do so we examined the lipoprotein and apolipoproteins levels and the polymorphisms of the genes of apolipoprotein E (APOE) and lipoprotein lipase (LPL) in 525 mothers and their newborns. APOE (E4, E3, E2) polymorphism and LPL polymorphism (X447) are common in the population and well known and understood for their effects on lipids and on CVD in the general population (Table 1). ApoE and LPL play important roles in the degradation of TG–rich lipoproteins (VLDL, chylomicrons). TG–rich lipoproteins have very high concentrations in pregnant women and may be important sources of lipid supply for the foetus. These 2 proteins are expressed abundantly in extracts of the placenta (Overbergh 1995).

The genetic test and statistical analysis are presented in details in the publications (Descamps 2004 & 2005).

3. Results and discussion

The data are summarized in Table 2. Lipoprotein concentrations in mothers were associated with the genetics of the mothers and, similarly, lipoprotein concentrations in newborns were associated with the genetics of the newborns. For example, APOE*E2 in newborns is associated with lower foetal LDL-C and apoB and the same association occurs between APOE*E2 in mothers and maternal LDL-C and apoB. LPL*X447 in mothers is associated with lower maternal triglycerides and the same association occurs in newborns (although less significant) between LPL*X447 and their triglycerides levels. These associations, refereed as "auto"-associations, are not very surprising and mimics the association usually found in most studies of adults in the general population (table 3).

Most surprising is the fact that lipoprotein concentrations of mothers are associated with DNA variations of their newborns' genes and reciprocally that lipoprotein concentrations of the newborns are associated with DNA variations of their mother's genes (refereed as "allo"-associations). For example (figure 1), APOE*E2 in mothers reduced foetal LDL-C and apoB, (even causing an additive effect on the reduction of LDL-C and apoB when APOE*E2 is also present in newborns : ***additive "allo + auto" effects***). In contrast, foetal APOE*E2 increased maternal LDL-C and apoB (as opposed to an reduction of these levels caused by the presence of APOE*E2 in mothers or in newborns : ***subtractive "auto – allo" effects***).

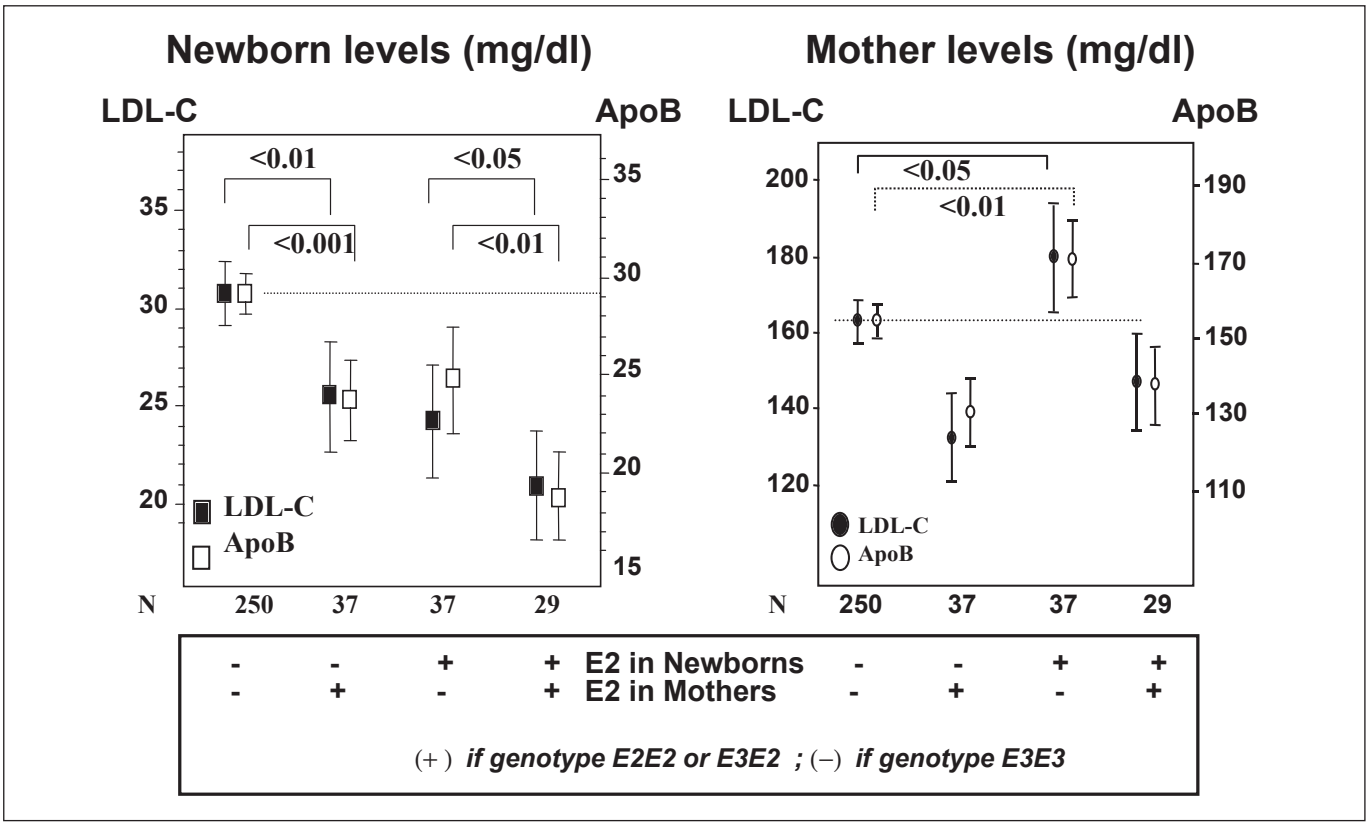

Figure 1: Legend

Concentrations (means and 95% confidence intervals) of LDL-C and apoB in various groups of mothers and newborns classified according to the carriage of APOE*E2 by the mothers and the newborns. The Y axes were scaled to set the point representing the mean values of concentrations of the referent (group A) at the same graphic level.

Table 2. Summary of our findings.

DNA variants	Effect when the DNA variation is present in mothers		Effect when the DNA variation is present in newborns	
	On maternal lipids (auto-association)	On foetal lipids (allo-association)	On maternal lipids (allo-association)	On foetal lipids (auto-association)
On LDL-C (apoB)				
E2	↓	↓	↑	↓
LPL*X447	↑	(↓)*	↓	(↓)*
On HDL-C (ApoA1)				
E2		↑		↑
LPL*X447			↑	
On Triglycerides				
LPL*X447	↓	↓	↓	(↓)

* Interaction mother-foetus.

This is the first time that such "allo"-association is demonstrated. The magnitude and the statistical contribution of the associations support the idea that these findings are important. SNPs in newborns can affect maternal lipoproteins by up to 20 mg/dL LDL-C (~12% of mean LDL-C in pregnant women) or up to 100 mg/dL TG (~38% of mean TG in pregnant women). They contribute to about 4% to the variations of maternal lipid concentrations. On the other hand, SNPs in mothers affect foetal lipoproteins by up to 6 mg/dL LDL-C (~21% of mean LDL-C in newborns) and up to 7 mg/dL TG (about 16% of mean TG in newborns) and contribute to about 10% to the variations of foetal lipid concentrations. Therefore, the magnitude and contribution of these "allo-associations" are about similar to those found with "genetic auto-associations" and to those found with non genetic factors in pregnant women and newborns. They are also in the same order of magnitude than those observed in classical genetic association studies in adults of the general population.

4. Possible mechanisms

Simple mechanisms of passive equilibrium, for example by simple diffusion or by compensatory distribution between maternal and foetal lipid pools, were excluded by the observations that some genetic variants produced opposite changes in maternal and foetal cholesterol concentrations (for example, foetal E2 reduced foetal LDL-C but elevated maternal LDL-C) and by multivariate statistical analyses adjusting for the auto SNP-lipid associations. Of course, caution must always be taken when deducing physiological mechanisms from an observed association between a genetic marker and lipid variation, as "association" does not mean "causation".

As mentioned above, maternal lipoproteins produced by the mothers are susceptible to interact (biochemically) via their apolipoproteins (encoded by the maternal genome) with the placental surface proteins (encoded from the foetal genome), like LPL and lipoprotein receptors. Foetal lipoproteins which have their own metabolism in the foetus may also interact with the placenta.

The influence of maternal SNPs on foetal lipoprotein metabolism may occur through the compositional change of maternal lipoproteins due, in the case of APOE SNP, to the alteration of their apolipoproteins or, in the case of LPL SNP, to an alteration of their lipid composition produced by the change of lipolytic activity. These compositional changes may secondarily affect the capture of the maternal lipoproteins at the placental surface and their metabolism into lipids. As the placental cells express apoB and apoA1, they are well equipped to repack these lipid components into new lipoproteins or shipped them directly into the foetal circulation via the umbilical veins. A change in the entry of these lipids associated with maternal SNP may thus affect the levels of foetal lipoproteins derived from the placenta.

The influence of foetal SNPs of the lipolytic enzyme, LPL, on maternal lipoprotein metabolism may occur because the foetal SNPs modify the expression of lipolytic proteins (LPL) at the surface of the placenta (foetal genome encodes placental proteins) and can thus affect the lipolysis of maternal lipoproteins which come in contact with placental surface enzymes. How do qualitative changes in foetal apolipoprotein affect mater-

nal lipid levels is more mysterious. It is possible that placenta excretes apolipoproteins E in the microenvironment of the intervillous space and that these placental apolipoproteins E can bind to the maternal lipoproteins that could be then more rapidly processed through placental apoE receptors (LDLR or LRP) (Descamps 1993). Another possibility is that intracellular apolipoproteins E in syncitiotrophoblasts control the lipid transfer into placental lipoproteins destined to foetal circulation.

Our observations reinforce the idea that lipoprotein receptors and lipolytic enzymes lying in the placental trophoblast cells, at the interface with maternal blood, capture or hydrolyze maternal lipoproteins.

5. Are these observations important for cardiovascular (CVD) epidemiology ?

It is tempting of course to derivate practical applications of these findings in the field of CVD epidemiology. As maternal genes affect lipid levels in their newborns, does it mean that the genotypes of mothers influence later CV risk in their offsprings? Conversely, as foetal genes affect the lipid levels in mothers, does it mean that the genotypes of individuals influence later CV risk in their mothers? If confirmed, such theory of a role of early "allogenomic" exposure on the development of future atherosclerosis would add a further layer of complexity to the already complicated relation between CVD and genetics.

To disentangle the contribution of maternal genetics from the own genetic influence of an individual on atherosclerosis, would require to design a study that compares, in a adult population, the incidence of clinical manifestations of atherosclerosis amongst those who were born from mothers carrying a risk genotype compared to those who were born from mothers not carrying this genotype, taking of course into account the confounding effect of the carriage of the risk genotype by the individuals themselves. Such project will necessitate to overcome several problems like the recruitment of a large number of patients, a long period of follow-up, the sampling of DNA from the mothers and the control of many confounders.

Meanwhile, let us just mention an epidemiological observation that is coherent with such theory. Parental history of CHD has long been recognised as a risk factor for CHD but the relative importance of the mother's role seems more important than the father's role to CHD risk. Some studies have however demonstrated that the relative risk is higher if the parent is the mother (Sesso 2001, Brown 2002 for MI, Welin 1987 for stroke). In the most recent follow-up study in the Malmö Preventive Project (22 444 men and 10 902 women), the age-adjusted relative risk (RR; 95%CI, after full adjustment for risk factors) for a son to experience a CVD event was increased in relation to a maternal positive family history of CVD mortality before 75 years when compared with no maternal history, RR 1.51 (1.23–1.84; $P < 0.001$). The corresponding adjusted RRs for father–son heritage was RR 1.22 (1.02–1.47; $P < 0.05$), mother–daughter RR 0.87 (0.54–1.41), and father–daughter RR 1.20 (0.83–1.73) (Nilsson 2004). We should also note that other diseases like type II diabetes have also demonstrated greater maternal–offspring transmission (Cox 1994, Dabelea 2000).

An explanation for the relative importance of maternal versus paternal transmission of CVD may be that the offsprings are exposed to the lipid effects of lipid-related genotypes of their mothers. If some genes carried by women are associated with greater CV risk for their own life and favour during their

Table 3. Comparison of genetic associations with lipids (and CVD) in the classical studies of adult general populations and in our studies in newborns and mothers.

Allele	CV Risk	Affected Lipids	Lipids Variation in General population	Lipid variation in Newborns with Maternal or foetal genotype		Lipid variation in Mothers with Maternal or foetal genotype	
E2	↓	LDL-C	↓	↓	↓	↓	
E4	↑	LDL-C	↑	=	=	=	=
S2	↑	HDL-C LDL-C		↓	↑ ↑		
S447X	↓	HDL-C TG LDL-C	↑ ↓ ???	= ↓ ↓	= ↓ ↓	↑ ↓	= ↓

pregnancy the development of atherogenic conditions in their foetus, CVD in individuals will be more strongly associated with CVD in their mothers than with CVD in their fathers.

6. Other implications

Beyond the demonstration of a statistical association and its potential use as new risk factors, yet to confirm, our study brings new arguments about a physiological concept, so difficult to prove in humans, that placenta plays an active role in the lipoprotein metabolism of the mothers and that the lipoproteins of the mothers provide effectively lipid components to the foetuses

There are many other situations like (foetal, placental or maternal) hormonal metabolism or preeclampsia with which lipid-related genotypes have been correlated and in which our findings can apply. Interestingly, preeclampsia that has been associated with lipid-related genotypes is also associated with an increased CVD risk in young and elderly women (Irgens 2001), as well as with increased risk of hypertension and of death from stroke in later life (Wilson 2003).

A more recent work (Witsch-Baumgartner 2004) applied elegantly the concept of allo-association between maternal genes and foetal lipid phenotypes in a disease related to cholesterol synthesis. This works examined how maternal APOE genotypes can be a modifier of the Smith-Lemli-Opitz syndrome (SLOS). SLOS is an autosomal recessive malformation with mental retardation syndrome and is caused by mutations in the delta-7-sterol-reductase gene (DHCR7), which impair endogenous cholesterol biosynthesis and make the growing embryo dependent on exogenous (maternal) sources of cholesterol.
The authors demonstrated that the SLOS patients' clinical severity scores were significantly correlated with the maternal apoE genotypes but not with the paternal apoE genotypes. This study, like ours, suggests that the efficiency of cholesterol transport from the mother to the embryo is affected by the maternal APOE genotype.

Acknowledgement

This work is dedicated to my mother, Monique Cornez (1935–1997) and my brother, Stéphane Descamps (1963–1997). I would like also to thank the numerous mothers who consented to participate to these studies as well as the nurses and doctors of the Department of Obstetrics and Gynecology, who contributed to collect blood sample of the mothers and their babies. I am also deeply indebted to my technical colleagues who helped me to settle molecular biology used in these studies: Monique Bruniau, Jean-Claude Hondekijn, Sylvie Mabille, Eric Tarentino.

Abbréviations

ApoA-I: apolipoprotein A-I protein; ApoB: apolipoprotein B-100; APOE: gene of the apolipoprotein E protein (ApoE); CVD: cardiovascular disease; LDLR: gene of the LDL receptor; LPL: lipoprotein lipase; LRP: LDL receptor-related protein; M: men; PCR: polymerase chain reaction; SNP: single nucleotide polymorphism; TG: Triglycerides ; TRL: triglyceride rich lipoproteins; W: women

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EZETIMIBE STORY : NEW DATA FROM A NEW STUDY

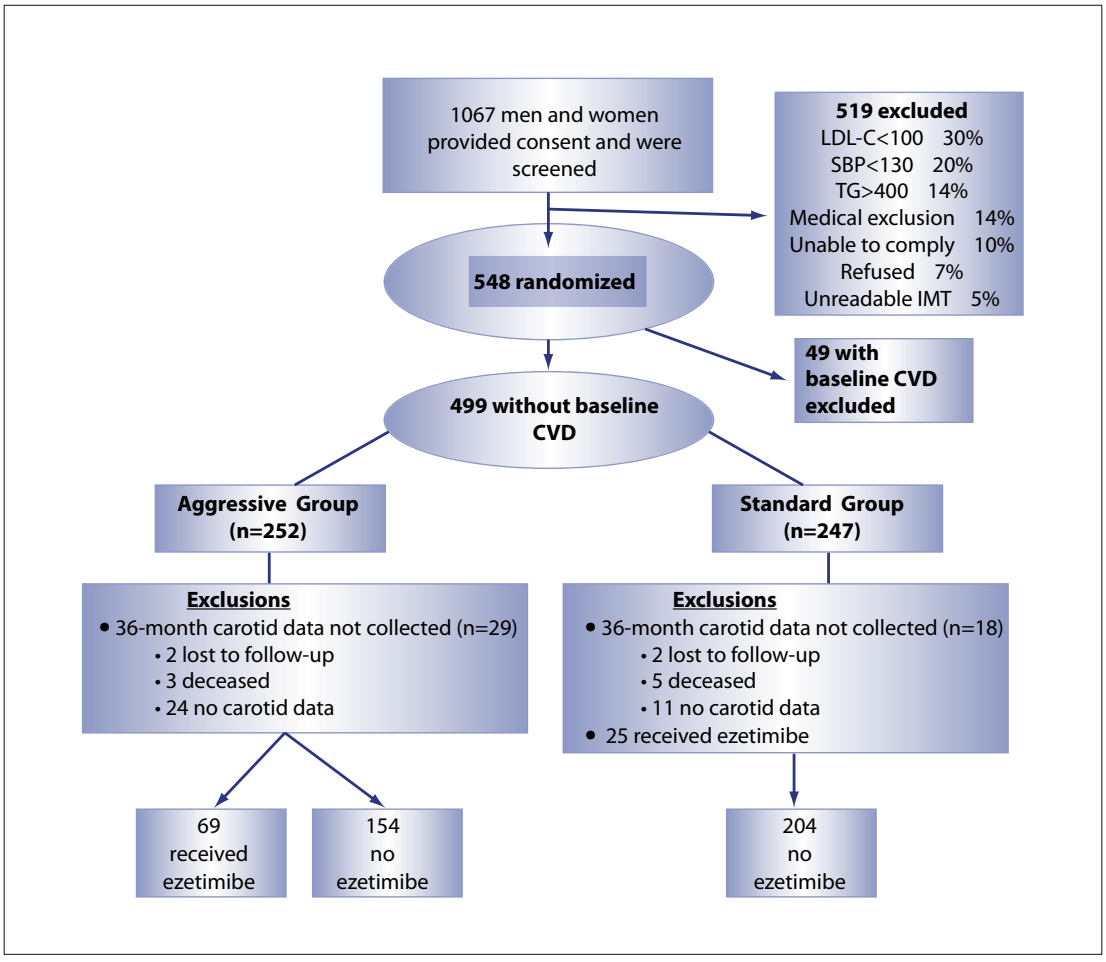
"STOP ATHEROSCLEROSIS IN NATIVE DIABETICS STUDY (SANDS)"

published in : "JAMA 2008; 299:1678-1689, by HOWARD B et al. // discussed by J. DUCOBU (UMH)

The SANDS study looked exclusively at American Indian men and women with type 2 diabetes, hypertension, and dyslipidemia. Patients were randomized to drug treatment to reach the ***standard targets*** of 100 mg/dL or lower for LDL cholesterol, 130 mg/dL or lower for non-HDL cholesterol, and 130 mm Hg or lower for systolic blood pressure (SBP). Comparatively, others were randomized to a more ***aggressive target*** of 70 mg/dL or lower for LDL cholesterol, 100 mg/dL or lower for non-HDL cholesterol, and 115 mm Hg or lower for SBP.

To achieve the aggressive lipid targets, patients were treated first with a statin, and if they failed to reach the treatment goal, ezetimibe was added. In total, 154 subjects were treated with a statin alone and 69 received a statin and ezetimibe, for a total of 223 in the aggressive-target arm. In the standard target group, 204 individuals were randomized to statin treatment. (figure 1)

Figure 1



After three years, there was a regression in the carotid intima media thickness (CIMT) in the aggressively treated patients compared with an increase in CIMT in those randomized to standard treatment targets. The regression in CIMT in those randomized to aggressive targets occurred in patients who received ezetimibe/statin therapy and in those who received only a statin. Reducing LDL and non-HDL cholesterol to very low levels in patients with diabetes results in a regression of carotid intima-media thickness (CIMT) over a three-year period (table 1),

Table 1

SANDS: Follow-up carotid IMT measures						
		Aggressive target:		Group difference between:		
CIMT measure	Standard groupe	Statin/ ezetimible group	Statin alone	Ezetimibe/ statin and statin alone (p)	Ezetimible/ statin and standard therapy (p)	Statin alone and standard therapy (p)
Mean change, 36 mo	0.039	-0.025	-0.012	0.01 (0.999)	0.06 (0.001)	0.05 (0.001)

Interestingly, the regression of CIMT occurs in aggressively treated patients who reach treatment goals with ***statins alone*** and in those treated with ***statins and ezetimibe combination*** therapy. It's a unique study design ***because it compares two different targets rather than comparing two different drug-treatment regimens.*** That doesn't make a lot of difference how you get there, it's getting to the low LDL and non-HDL levels that counts.

Compared with ***ENHANCE*** study, where baseline IMT was low (some believe too low to detect to any meaningful change as a result of treatment), baseline IMT in ***SANDS*** was 0.81 mm. In addition this trial was three years, not two years, like in ***ENHANCE***, an important distinction, as the benefit of treatment emerged within the last 18 months.

Moreover,the patient population is quite different from the population of ***ENHANCE***, which was constituted of a group of familial hypercholesterolemia patients who were well-treated with statin therapy since childhood.

Getting patients to goal without an add-on therapy is difficult. Treating these patients to LDL cholesterol of less than 100 mg/dL still leaves a high amount of residual risk, . Ezetimibe is proven to be the easiest and most effective adjunct to get them to goal."

There have been concerns raised with ezetimibe and cancer, notably in the ***Simvastatin and Ezetimibe in Aortic Stenosis*** (SEAS) trial, although later analyses suggest the finding is an outlier . ***Sands study*** observed no cancer signal, although it was underpowered to detect such events.

78th European Atherosclerosis Society Congress - EAS 2010

Begin : June 20, 2010
End : June 23, 2010

City : Hamburg - **Country :** Germany

Website : http://www2.kenes.com/eas/Pages/Home.aspx

E-mail : eas@kenes.com

Description : EAS 2010, the 78th European Atherosclerosis Society Congress, Hamburg, Germany, 20-23 June, 2010, will feature a comprehensive lineup of lectures, workshops, presentations, and educational symposia on the latest treatments of atheosclerosis and cardiovascular disease

5th Symposium LIPIDS AND ATHEROSCLEROSIS

Saturday 26th of september 2009

Auditoire Point Centre Gosselies
CHU CHARLEROI, ULB

9.00- 9.30 : Yingmei Feng (KUL)
Protective roles of ApoA-I by incorporation of endothelial progenitor cells

9.30-10.00 : Damien Calay, Martine Raes (FUNDP)
Régulations cellulaires induites par les LdL oxydées

10.00-10.30 : Tim Vande Parre , Arnold Herman (U.Antwerpen)
Role of amyloid precursor protein in atherosclerosis and in Alzheimer disease

10.30-11.00 : Break

11.00-11.30 : Karim.Zouaoui , Pierre Van Antwerpen (CHU Charleroi, ULB)
Troubles du sommeil et maladies CV Et/ou Importance de la carbamylation dans l'athérosclérose

11.30-12.00 : Michel Langlois (AZ Bruges)
Leçons de l'étude ASKLEPIOS

12.00-12.30 : Philippe Lesnik, John Chapman (Inserm Paris)
Rôle de l'apoptose des macrophages dans la progression de la plaque

With the help of the Belgian Lipid Club and of Fonds pour la Recherche Médicale dans le Hainaut (FRMH)

CHANGES IN LIPOPROTEIN COMPOSITION AND FUNCTION INDUCED BY DIFFERENT REVASCULARITATION PROCEDURES

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The present article summarizes the work that has been supported by a Belgian Lipid Club Research Fellowship in Lipidology 2006-2008. Results of the first clinical study of this work have been published as an original article: "Changes in Plasma LDL and HDL Composition in Patients Undergoing Cardiac Surgery. Hacquebard M., Ducart A., Schmartz D., Malaisse WJ., Carpentier Y.A. Lipids. 2007; 42 (12): 1143-53". Results of the second clinical study of this work are part of a manuscript submitted for publication: "Comparison of oxidative stress produced by on-pump and off-pump coronary surgery. Fontaine D., Pradier O., Hacquebard M., Stefanidis C., Carpentier Y.A., de Cannière D., Fontaine J., Berkenboom G.".

Introduction

Various pathological conditions, such as severe sepsis, major surgery, burn injury, accidental trauma, and myocardial infarct, are associated with a systemic inflammatory reaction called acute phase response. This host response not only increases the production of acute phase reactant proteins but also induces major changes in the metabolism of plasma lipids and lipoproteins[1].

In contrast to changes in plasma lipids which have been measured in various pathological conditions, the composition of plasma lipoproteins has been mainly studied in conditions of sepsis with severe inflammation, and substantial changes have been documented in LDL and HDL[2, 3]. Major surgical injury also induces a severe acute phase response with postoperative decreases of plasma (as well as LDL and HDL) cholesterol concentration as reported after cardiac surgery. In contrast to sepsis which is a very heterogeneous condition, cardiac surgery with cardiopulmonary bypass (=on-pump surgery) follows strict routine procedures and represents a fairly standardized insult. In addition to surgical trauma, contact of blood cells and plasma components with artificial membranes of the extracorporeal circuit, and ischemia-reperfusion are responsible for the severe systemic inflammation observed after on-pump surgery[4]. In a first clinical study we determined the compositional changes occurring in LDL and HDL at the peak of the inflammatory response after on-pump surgery.

On-pump surgery has for a long time been considered as the standard procedure for coronary revascularization. However, the inflammatory response and oxidative stress induced by surgical trauma and extracorporeal circulation may result in severe functional consequences such as endothelial injury and/or cardiac, renal, hepatic or pulmonary dysfunction. In an effort to reduce such postoperative complications, alternative techniques to conventional on-pump revascularization have been developed, such as coronary artery bypass performed on a beating heart without the use of cardiopulmonary bypass (=off-pump) and percutaneous transluminal coronary angioplasty (PTCA). In a second clinical study we compared the impact of different coronary revascularization techniques on changes in lipid-related parameters and on oxidative stress.

Material and Study Protocol

Study 1: Changes in plasma LDL and HDL composition in patients undergoing on-pump surgery.

The study group included 21 patients undergoing elective on-pump cardiac surgery (n=21, 19 male and 2 female; mean age: 69 ± 3 years). Major exclusion criteria were: unstable conditions, patients on steroids or with body mass index >30 kg/m^2, plasma creatinine >1.5 mg/dl, altered liver function tests, or diabetes mellitus. None of the patients received corticosteroids in the perioperative period.

Blood samples were drawn immediately before operation and on day 2 post-surgery. Measurements of inflammatory proteins, selected apolipoproteins (apo), plasma lipid parameters and antioxidants were performed before surgery and on postoperative day 2 in the first 6 patients. In the 15 following patients, plasma lipoprotein fractions were isolated and analysed for their content in lipid and apo components, alpha-tocopherol as well as hydroperoxides. The study was approved by the Ethical Committee of Erasme Hospital (Université Libre de Bruxelles, Brussels, Belgium) and all participating patients received complete information and signed a written consent form.

Study 2: Lipoprotein profile and oxidative stress after different coronary revascularization procedures.

The study included 30 patients undergoing either on-pump revascularization (n=10; 8 male and 2 female; mean age: 71 ± 2 years), off-pump revascularization (n=10, 8 male and 2 female; mean age: 66 ± 4 years), or PTCA (n=10, 9 male and 1 female; mean age: 63 ± 5 years). Major exclusion criteria were: unstable conditions, left ventricular ejection fraction<45%, evidence of infection, plasma creatinine>1.5 mg/dl, altered liver function

tests. It should be noted that, at the time of this study, the guidelines of the Department of Anesthesia imposed systemic administration of glucocorticoids at anesthesia induction to patients undergoing on-pump surgery.

Blood samples were drawn at the time of anaesthesia induction (baseline, T0), immediately after skin closure (T1) and again 24 h after surgery (T2). Samples were assayed for selected plasma apo and lipids. Malondialdehyde (MDA), total plasma antioxidant status, alpha-tocopherol and paraoxonase activity were measured as parameters of oxidant and antioxidant status. In addition, rat aorta was incubated with plasma samples drawn at different time points and production of superoxide anion was measured.

The study was approved by the Ethical Committee of Erasme Hospital (Université Libre de Bruxelles, Brussels, Belgium) and all participating patients received complete information and signed a written consent form.

Results

Changes in plasma LDL and HDL composition in patients undergoing on-pump surgery

CRP concentration increased from 0.41 ± 0.18 mg/dl (preoperative) to 13.90 ± 3.90 mg/dl (p<0.005) and SAA concentration from 0.22 ± 0.06 to 25.40 ± 5.77 mg/dl (p<0.005) on postoperative day 2. The inflammatory response was associated with decreased levels of apo B and AI, total cholesterol, triglycerides and alpha-tocopherol (Table 1). Beyond decreases in plasma lipid concentrations, cardiac surgery induced substantial modifications in plasma lipoproteins. Apo B in LDL fraction decreased from 71.5 ± 9.5 mg/dl to 43.2 ± 8.7 mg/dl (p<0.001) on postoperative day 2, reflecting a marked reduction (-46%; p<0.0001) in the number of circulating LDL particles. LDL cholesteryl ester content relative to apo B concentration remained unchanged post-surgery while triglyceride (+113%;p<0.001), free cholesterol (+22%; p<0.05) and phospholipid (+23%; p<0.025) were raised relative to apo B, indicating an increased particle size (Fig. 1). In HDL, an abrupt rise in apo SAA (p<0.05) was observed together with a decrease in apo AI (-22%; p<0.005). Cholesteryl ester content in HDL fraction decreased in parallel to apo AI concentration while triglycerides (+34%;

Table 1. Plasma concentration of selected proteins, apolipoproteins, lipids and antioxidant measurements

Biological variables	Preoperative (n=6)	Postoperative (n=6)
CRP (mg/dl)	0.41 ± 0.18	13.90 ± 3.90 [c]
SAA (mg/dl)	0.22 ± 0.06	25.40 ± 5.77 [c]
ApoB (mg/dl)	94.8 ± 17.3	54.5 ± 10.1 [a]
ApoAI (mg/dl)	183.8 ± 14.8	126.4 ± 10.0 [d]
TC (mmol/l)	5.17 ± 0.43	3.31 ± 0.31 [b]
TG (mmol/l)	1.42 ± 0.34	0.97 ± 0.17
alpha-tocopherol (µmol/l)	33.76 ± 3.43	22.30 ± 3.44 [b]

Values are expressed as means ± SEM. P values were calculated by paired Student t test. [a]P<0.05; [b]P<0.01; [c]P<0.005; [d]P<0.001; [e]P<0.0001 versus preoperative value. CRP, C-reactive protein; SAA, Serum amyloid A; apoB, apolipoprotein B; apoA1, apolipoprotein A1; TC, total cholesterol; TG, triglycerides; PON, paraoxonase.

Figure 1.

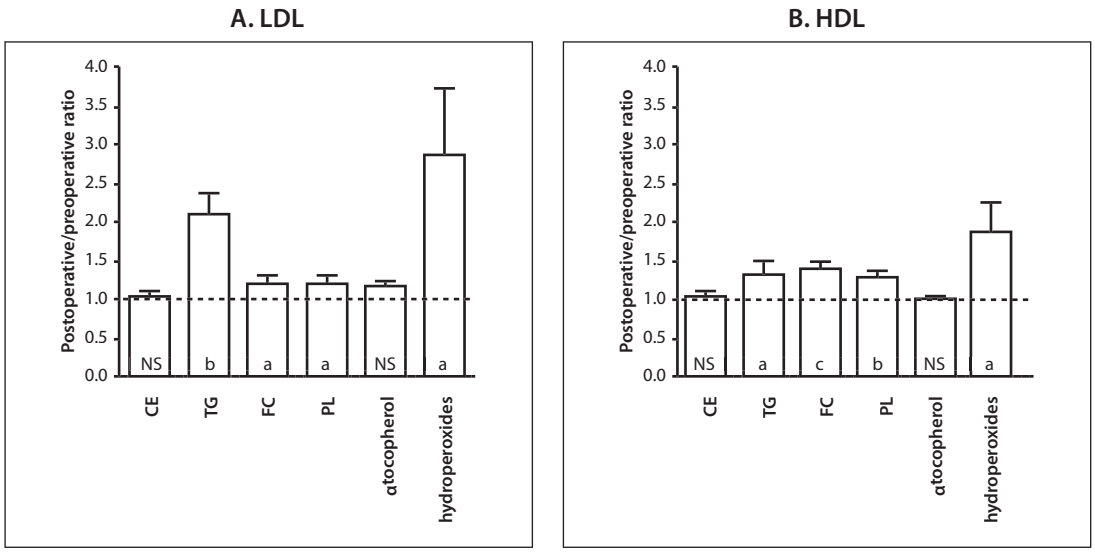

Figure 1. Postoperative changes in LDL (A) and HDL (B) composition (n=15). LDL and HDL lipid components are expressed relative to LDL-apoB and HDL-apoA1, respectively. The figure shows an unchanged cholesteryl ester (CE) content in LDL and HDL particles, an increased triglyceride (TG) content (particularly in LDL) together with an increase in surface components (free cholesterol (FC) and phospholipids (PL)); in spite of unchanged alpha-tocopherol content, LDL and HDL showed increased hydroperoxide content. Values are expressed as means ± SEM. P values were calculated by Student t test. [a]P<0.05; [b]P<0.001; [c]P<0.0001.

Table 2. Apolipoprotein (apo), total cholesterol (TC) and triglycerides (TG) concentration measured at T0, T1 and T2 in plasma from patients undergoing coronary revascularization surgery.

		ApoB/prot (mg/g)	ApoAI/prot. (mg/g)	TC/prot. (µmol/g)	TG/prot. (µmol/g)
On-pump (n=10)	T0	11.66 ± 1.57	19.46 ± 1.84	56.5 ± 7.5	23.2 ± 4.6
	T1	11.36 ± 1.43	16.94 ± 1.16[a]	53.0 ± 6.7	14.2 ± 2.4[b]
	T2	8.90 ± 1.60[c]	16.33 ± 0.99[b]	45.0 ± 6.9[c]	11.5 ± 2.1[c]
Off-pump (n=10)	T0	9.65 ± 1.29	19.96 ± 1.78	53.5 ± 3.2	20.2 ± 2.4
	T1	9.55 ± 1.15	19.02 ± 1.26	51.6 ± 2.9	13.4 ± 2.1[a]
	T2	7.52 ± 0.96[c]	17.16 ± 1.39[a]	42.6 ± 2.2[c]	13.1 ± 1.9[b]
PTCA (n=10)	T0	14.5 ± 1.8	26.7 ± 2.1	66.7 ± 7.4	17.5 ± 3.5
	T1	14.6 ± 1.9	25.7 ± 2.1	65.1 ± 8.5	16.6 ± 2.8
	T2	13.8 ± 1.6	25.0 ± 2.1	62.0 ± 7.5	18.9 ± 3.1

Results are expressed as mean values ± SEM. P values within each group were calculated by a one-way ANOVA followed by Tukey's post hoc multiple comparison test. [a]p<0.05 vs. T0, [b]p<0.01 vs. T0, [c]p<0.001 vs. T0. Concentrations are expressed relative to plasma protein (prot.) concentration to account for hemodilution.

p<0.05), free cholesterol (+43%; p<0.001) and phospholipids (+30%; p<0.001) increased relative to apo AI (Fig. 1). In contrast to unchanged alpha-tocopherol content, hydroperoxide content was increased in both LDL and HDL.

Lipoprotein profile and oxidative stress after different coronary revascularization procedures

Inflammatory markers. CRP and SAA concentrations markedly increased at 24 h post-surgery in on-pump patients, to 3.75 ± 0.50 mg/dl (p<0.001) and 7.81 ± 1.14 mg/dl (p<0.001), respectively. Inflammatory markers also increased at 24 h post-surgery in off-pump patients, to 12.33 ± 1.93 mg/dl for CRP (p<0.001) and 22.81 ± 3.97 mg/dl for SAA (p<0.001). The increase in inflammatory markers was more marked in off-pump compared to on-pump patients. PTCA did not induce any increase of CRP and SAA concentrations.

Plasma apolipoproteins and lipids. Plasma apo B concentration corrected for hemodilution was not modified at the end of the operation (T1) but was decreased by 27.5 ± 4.7% (p<0.001) in on-pump patients and by 18.1 ± 4.9% (p<0.001) in off-pump patients at 24 h post-surgery (T2) (Table 2).

In on-pump patients, apo AI concentration was decreased by 10.4 ± 4.0% (p<0.05) after the operation (T1) and by 13.4 ± 3.9% (p<0.01) at 24 h post-surgery (T2). In off-pump patients, apo AI concentration was not significantly decreased in the immediate postoperative period but was decreased by 12.6 ± 3.5% (p<0.05) at 24 h post-surgery. PTCA did not induce changes in apo B and apo AI concentrations

Plasma total cholesterol concentration was not modified in the immediate postoperative course (T1) but was significantly decreased at 24 h post-surgery, i.e. by 21.9 ± 3.2% (p<0.001) in on-pump patients and by 18.7 ± 4.9% (p<0.001) in off-pump patients (Table 2). In contrast, triglyceride concentration was already decreased in the immediate postoperative period, by 34.0 ± 6.3% (p<0.01) in on-pump and by 35.4 ± 3.9% (p<0.05) in off-pump patients. This decrease in triglyceride concentration was maintained at 24 h post-surgery in both on-pump and off-pump patients. There was no change in plasma lipid concentration after PTCA.

Parameters of oxidant and anti-oxidant status. On-pump coronary revascularization induced a 2 fold increase of MDA concentration in the immediate postoperative period (3.18 ± 0.52 vs. 1.75 ± 0.46 nmol/g protein before surgery, n=10; p<0.05); this was associated with a ~30-40% reduction of plasma total antioxidant status in the immediate postoperative period (19 ± 4 vs. 34 ± 3% before surgery, n=10; p<0.001) and at 24 h post-surgery (23 ± 2 vs. 34 ± 3% before surgery, n=10; p<0.01). In sharp contrast, off-pump coronary revascularization and PTCA did not modify these parameters at either T1 and T2 time points post-surgery.

The marked reduction in plasma alpha-tocopherol concentration following on-pump and off-pump revascularization was largely due to the reduction in circulating lipoproteins, as indicated by an unchanged alpha-tocopherol/total lipid ratio. PTCA did not affect plasma alpha-tocopherol concentration.

Paraoxonase activity was decreased immediately after coronary surgery, whether performed by on-pump (61.1 ± 7.8 vs. 80.4 ± 6.8 µmol/min.ml before surgery, n=7; p<0.001) or off-pump (65.6 ± 7.4 vs. 86.4 ± 10.1 µmol/min.ml before surgery, n=7; p<0.001) revascularization. Such a decrease in paraoxonase activity subsisted in both groups at 24 h post-surgery. This parameter was not modified after PTCA.

Finally, when plasma samples were added to rat aortic rings, postoperative plasmas (T1 and T2) from on-pump patients markedly enhanced the chemiluminescence signal (indicating an increased production of superoxide anion), by 247 ± 34% (p<0.05) after incubation with T1 plasma and by 304 ± 70% (p<0.05) with T2 plasma in comparison to the signal measured with preoperative plasma. In contrast, plasma obtained after off-pump revascularization or PTCA did not stimulate superoxide anion production in rat aortas.

Discussion

Two clinical studies were conducted to analyze changes in lipoprotein concentration and composition in relation to inflammatory reaction and oxidative stress in selected sub groups of critically ill patients, namely patients undergoing cardiac surgery with different procedures.

The first clinical study was conducted to determine compositional changes occurring in LDL and HDL particles at the peak of the inflammatory response (i.e. on postoperative day 2) in patients undergoing on-pump cardiac surgery. The study included 21 cardiac surgery patients and analyses were performed before surgery and on postoperative day 2. The marked increase in inflammatory marker SAA on postoperative day 2 paralleled and even exceeded that of CRP. The inflammatory response was accompanied by decreases in plasma levels of total cholesterol, triglycerides and alpha-tocopherol, in agreement with prior observations on the effect of cardiac surgical stress. In parallel, important changes in LDL and HDL lipid content were observed after surgery, some of which contrast with previous observations made in critically ill septic patients. By comparison with sepsis, cardiac surgery induces a comparable reduction in circulating LDL but a more limited decrease in HDL particles[5]. Furthermore, in contrast to sepsis, the increase in LDL and HDL triglyceride content was not associated to a reduced cholesteryl ester content in these particles. Lower plasma triglyceride concentrations, as observed after cardiac surgery, do not usually induce cholesteryl ester transfer protein-mediated exchanges of core lipids between triglyceride-rich and cholesteryl ester-rich lipoproteins[6]. Hence, triglyceride enrichment in LDL and HDL post-surgery may result from reduced hepatic lipase activity. The global increase in core lipids in post-surgery lipoproteins was associated with an increase in surface lipids in both LDL and HDL, strongly suggesting an increase in lipoprotein particle size. This is in contrast to sepsis, where the decrease in LDL is associated with the appearance of small size and dense (sd) LDL[7]. This data indicates that the acute phase response caused by different conditions may differently affect the composition of plasma lipoproteins, and possibly their functions. Furthermore, the reduced plasma level of alpha-tocopherol measured after cardiac surgery was entirely due to a reduced number of circulating LDL and HDL particles. Data indicates that such reduced number in alpha-tocopherol carriers after-surgery may impede the delivery of alpha-tocopherol to cells in conditions of increased requirements due to oxidative stress. An increased hydroperoxide content was measured in circulating LDL and HDL particles after cardiac surgery, suggesting that the (unchanged) alpha-tocopherol content in postoperative particles could not completely prevent the oxidative stress associated with cardiac surgery in cardiopulmonary bypass.

The second clinical study was conducted to compare the effect of different coronary revascularization procedures on lipid-related changes and oxidative stress in the immediate postoperative period. This second clinical study included 30 patients undergoing either on-pump or off-pump cardiac surgery, or PTCA. Blood samples were collected before, immediately after skin closure and at 24 h after the intervention. One limitation in this study was the systematic administration of glucocorticoids to patients undergoing on-pump surgery but not to those undergoing off-pump surgery. This difference in patient's treatment was reflected by a lower postoperative rise in inflammatory markers SAA and CRP in on-pump compared to off-pump patients. In spite of this difference in inflammatory markers, plasma concentrations of cholesterol as well as of apolipoprotein B and AI decreased to a comparable extent in on-pump and off-pump patients after correction for hemodilution. In contrast to these observations, marked differences were measured in several parameters related to oxidative stress. Data suggests that avoidance of extracorporeal circulation and myocardial ischemia-reperfusion may significantly attenuate perioperative oxidative stress. These results are in accordance with original observations by Matata et al.[8] who measured lower levels of lipid hydroperoxides, protein carbonyls and nitrotyrosine following off-pump surgery. Comparable observations were made in a more recent study in which levels of isoprostanes and plasma MDA were not substantially affected in off-pump patients[9]. Coronary revascularization performed by PTCA avoids both surgical trauma and extracorporeal circulation; using this procedure appears to blunt the inflammatory response, as well as changes in lipoprotein profile and oxidative stress.

Overall, this data on changes occurring in plasma lipoproteins after different revascularization procedures allows a distinction to be made between the contribution of extracorporeal circulation and that of surgical trauma on both lipid related parameters and oxidative stress and may help to build up strategies aimed at reducing potential deleterious changes in lipoprotein properties induced by inflammation and/or oxidative stress.

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