Lipid Club Letter

I N H O U D

Stichtende leden van de BELGIAN LIPID CLUB

Leden van het Bureau van de BELGIAN LIPID CLUB

Driemaandelijks - Juni 2005   **Vol. 17, N° 2**

Editoriaal

HET JAAR 2005 BEGINT VEELBELOVEND

Wij hopen van ganser harte dat er voortaan rekening zal worden gehouden met het totale cardiovasculaire risico van de Belgische bevolking bij de terugbetaling van vetverlagende geneesmiddelen. Daarmee zou men tegemoet komen aan de eisen van de BLC: volgens de BLC zouden meer mensen toegang moeten krijgen tot een effectieve behandeling van lipidenstoornissen.

Zoals beloofd beschikt de BLC voortaan over haar eigen website: **www.lipid-club.be**. Neemt u er eens een kijkje: u kunt er de nieuwe zakgids downloaden, u vindt er informatie over de wetenschappelijke vergaderingen die de BLC steunt en over de prijzen die door onze vereniging uitgereikt worden. Binnenkort zal er nog meer informatie op de site geplaatst worden. Uw opmerkingen en suggesties zijn van harte welkom.

In dit nummer van de Lipid Club Letter kunt u kennis maken met het werk van de winnaars van de MSD-BLC Lipidologieprijs en van de Orbitaprijs. Tegelijk willen we u eraan herinneren dat u zich kandidaat kunt stellen voor het Research Fellowship, waarmee we het Belgisch onderzoek in de lipidologie ondersteunen. Het dossier met de kandidaatstelling moet voor 30 juni 2005 worden ingediend. U vindt het deelnameformulier op de website (Prijs BLC).

Ook wordt u in dit nummer opgeroepen deel te nemen aan de werkzaamheden van het Belgisch consensusproject rond het omgaan met erfelijke hypercholesterolemie, met name bij kinderen.

Prof. F.R. HELLER,
Voorzitter BLC

ORBITA (Pfizer) PRIJS

Weight-loss associated induction of PPARalpha and PPARgamma correlate with reduced atherosclerosis and improved cardiovascular function in obese insulin-resistant mice

Wim Verreth[1], Dieuwke Dekeyzer[1], Michel Pelat[2], Peter Verhamme[1], Javier Ganame[3], John K. Bielicki[4],
Ann Mertens[1], Rozenn Quarck[1], Nora Benhabilès[5], Gérard Marguerie[5], Bharti Mackness[6], Mike Mackness[6],
Ewa Ninio[7], Marie-Christine Herregods[3], Jean-Luc Balligand[2],
Paul Holvoet[1]

[1]Cardiovascular Research Unit of the Center for Experimental Surgery and Anesthesiology,
Katholieke Universiteit Leuven, Belgium, [2]Department of Medicine, Unit of Pharmacology and Therapeutics,
Université catholique de Louvain, Belgium, [3]Department of Cardiology, Katholieke Universiteit Leuven, Belgium;
[4]Lawrence Berkeley National Laboratory, Berkeley, CA; [5]Clinigenetics, Nimes, France,
[6]University of Manchester Department of Medicine, Manchester Royal Infirmary, Manchester, UK, [7]INSERM U525,
Institut Fédératif CMV, Université Pierre et Marie Curie, Paris, France

Background

The metabolic syndrome is characterized by a group of metabolic risk factors that include obesity, raised blood pressure, dyslipidemia, insulin resistance or glucose intolerance, and a prothrombotic state, and its incidence in the Western world is rising to epidemic proportions. Obesity, particularly visceral adiposity, contributes to the clustering of the other metabolic syndrome components, such as insulin resistance/type-2 diabetes, dyslipidemia and hypertension. The molecular mechanisms underlying the metabolic abnormalities induced by visceral adiposity have yet to be fully elucidated.

Fat distributed in the abdominal region is associated closely with insulin resistance and is a risk factor for type 2 diabetes and cardiovascular disease (CVD) [1, 2]. Weight-loss in insulin-resistant obese persons reduces their CVD risk [3]. It is however not known to what extent changes in the intra-abdominal adipose gene expression profile are important for the reduction of the risk [4].

We, therefore, have investigated the cardioprotective mechanisms of weight-loss in mice with combined leptin and LDL-receptor deficiency (DKO). We have selected those mice because they have several metabolic syndrome components - obesity, hypertension, dyslipidemia, and diabetes – that are associated with increased oxidative stress and inflammation, resulting in accelerated atherosclerosis and loss of cardiovascular function [5]. Because weight-loss in DKO mice was associated with a reduction in markers of cardiovascular risk, our second objective was to identify the underlying mechanisms. Therefore we studied the effect of weight-loss on changes in the gene expression profiles in the visceral adipose tissue that correlate with changes in lipoprotein and lipid profile, oxidative stress, insulin sensitivity, glucose tolerance, blood pressure and heart rate regulation, and atherosclerosis. The following is an overview of results previously published in Circulation [6].

Experimental Protocol

In order to obtain the weight loss, food intake of DKO mice was restricted. Food intake of free-fed DKO mice was ~ 5.7g/day from weaning for 12, 24 or 36 weeks. Food intake of diet-restricted mice was restricted to 2.5g/day for 12 weeks between 12 and 24 weeks of age (24-week diet-restricted mice) or between 24 and 36 weeks of age (36-week diet-restricted mice). At 12 weeks, DKO have no detectable atherosclerosis, and their LV function is not different from that of lean mice. Starting the diet-restriction at 12 weeks is thus a proxy for a primary intervention study. At 24 weeks, DKO have already developed atherosclerosis, and their LV function is lower compared to that of lean mice, so starting the diet-restriction at 24 weeks is a proxy for a secondary intervention study.

As a result of the diet-restriction, diet-restricted mice showed a 45% weight loss compared to free-fed mice of the same age. By measuring body composition with PIXImus Mouse Densitometry (Lunar Corporation, Brussels) [7] we showed that the weight-loss was due to a 33% loss of fat mass.

PPAR Gene Expression

Several adipokines, and more specifically peroxisome proliferator activated receptors (PPAR), regulate a number of the processes that contribute to the development of atherosclerosis, including dyslipidemia, arterial hypertension, endothelial dysfunction, insulin resistance, and vascular remodeling. It has been demonstrated that PPAR deactivation (mainly obesity-related) is a key phase of metabolic syndrome initiation [8-10]. Approaches to reduce adipose tissue depots, including diet-restriction, could stimulate adipocyte differentiation and as a result positively alter PPAR levels.
PPARgamma and PPARalpha gene expression in white visceral adipose tissue was measured with quantitative RT-PCR. PPARgamma and PPARalpha gene

expression decreased in time in DKO mice (Fig. 1A), and was down-regulated compared to lean mice. Diet-restriction resulted in an upregulation of PPARalpha and PPARgamma expression in the adipose tissue of diet-restricted DKO mice compared to age-matched free-fed DKO mice (Fig. 1B). Because we observed up-regulation of PPARalpha and PPARgamma in the adipose tissue from diet-restricted compared to free-fed DKO mice, we also compared PPAR expression in extracts from the heart and aortic arch of those mice. PPARalpha and PPARgamma expression was higher in the heart and aortic arch of diet-restricted mice compared to free-fed DKO mice.
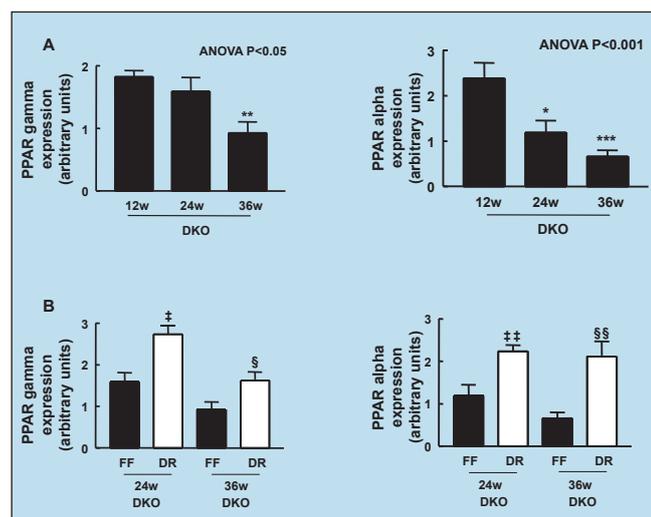
Figure 1: (A) PPARgamma and PPARalpha expression in the intra-abdominal adipose tissue from 12-week (n=8), 24-week (n=5) and 36-week (n=8) free-fed DKO mice. (B) Effect of diet-restriction on PPARgamma and PPARalpha expression at 24 (free-fed n=5; diet-restricted n=7) and 36 weeks (n=8 per group). FF= free-fed, DR= diet-restricted; *P<0.05, **P<0.01 and ***P<0.001 compared to 12-week DKO mice; ‡P<0.05 and ‡‡P<0.01 compared to 24-week free-fed DKO mice; §P<0.05 and §§P<0.01 compared to 36-week free-fed DKO mice.

Micro-array analysis of gene expression in adipose tissue

To identify genes that change according to the differences in PPAR expression we performed 3 independent experiments with Agilent Mouse cDNA micro-arrays (product number: G4104A). We compared the abundance of transcripts in two mRNA samples, one from the visceral adipose tissue from a free-fed mouse, the other from a diet-restricted mouse, both at 24 weeks. The upregulation of PPAR expression was associated with a change in the expression of genes regulating glucose transport and insulin sensitivity, lipid metabolism, oxidative stress and inflammation, resulting in a reduction of markers for cardiovascular risk.

Effect of weight loss on markers of cardiovascular risk

Weight-loss resulted in a decrease of triglyceride levels without changing total, non-HDL and HDL-cholesterol levels. Compared to free-fed DKO mice, insulin levels were lower in the diet-restricted groups but glucose levels did not change. Insulin resistance was calculated by a homeostasis model assesment (HOMA). HOMA for diet-restricted mice was 77% lower than that for free-fed mice. Diet restriction also resulted in normalization of the glucose tolerance. Weight-loss was also associated with a decrease of the titer of Ig autoantibodies against oxidized LDL indicating a decrease in oxidative stress in diet-restricted DKO mice. This decrease in oxidative stress could be attributed to an increase in the activity of two anti-oxidative enzymes, namely Paraoxonase (PON) and lecithin:cholesterol acyltransferase (LCAT).

Effect of weight loss on atherosclerosis, blood pressure, heart rate and LV function

Because weight-loss in DKO mice was associated with a reduction in markers of cardiovascular risk we then looked at the effect of weight loss on atherosclerosis, blood pressure, heart rate and LV function.

Atherosclerosis

The extent of atherosclerosis was measured by analysis of stained cross-sections from the aortic root. Lesions in 12-week free-fed DKO mice were very small fatty streaks. Plaque volumes increased from 12 to 36 weeks (Fig. 2A). Plaque volumes in 24-week diet-restricted mice were 12-times smaller than in 24-week free-fed mice. Plaque volumes in 36-week diet-restricted mice were similar to those in 24-week free-fed mice and 2.1–times smaller than in 36-week free-fed mice (Fig. 2A). Macrophage content was 52% lower (P<0.01) and Ox-LDL content was 16% lower (P<0.05) whereas smooth muscle content was 95% higher (P<0.05) in 36-week diet-restricted mice compared to 36-week free-fed mice. This indicates that the plaques in diet-restricted DKO mice are more stable compared to that of free-fed DKO mice.

There was a negative correlation between both PPARgamma and PPARalpha expression and plaque volume (Fig. 3A) and % of the plaque surface that was stained for Ox-LDL (Fig. 3B) in free-fed and diet-restricted DKO mice. This indicates that changes in PPAR gene expression in the adipose tissue could result in changes in the development of atherosclerosis.

Blood Pressure, Heart Rate and Ejection Fraction

BP signals, and HR, derived from pressure waves from the aortic arch, were measured in conscious, unrestrained animals with surgically implanted miniaturized telemetry devices (DSI model TA11-PA-C20, Datascience Corp, St Paul, MN, USA). In control (C57BL6) mice, continuous (24 hours) BP and HR recordings revealed a typical variation during the light-dark cycle, i.e. light (corresponding to a resting period for mice) values of systolic (S) BP, diastolic (D) BP and HR were significantly lower than dark (activity period for mice) values. By contrast, circadian variations of HR and BP were

totally abolished in free-fed DKO mice. These mice also had higher mean 24-hour SBP and DBP values and higher mean HR compared with controls. Diet restriction reduced all parameters in DKO mice to control values. Similar changes were observed during light and dark cycles.

To determine LV function, transthoracic echocardiography of C57BL6, 24-week free-fed and 24-week diet-restricted mice was performed using a Philips SONOS 5500 with a 5-12 MHz S12 neonatal ultraband cardiac phased probe. At 24 weeks, ejection fraction of DKO mice was significantly lower (38±7.1 % vs. 51±6.2 %; P<0.01) compared to control C57BL6 mice. Diet restriction restored ejection fraction to 50±5.5 (P<0.001) (Fig. 2B).
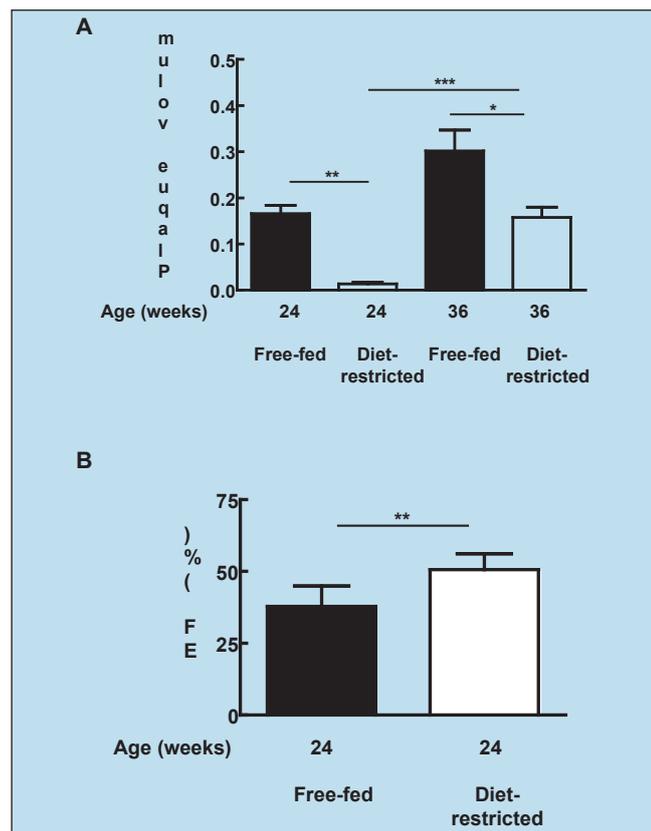

Figure 2: (A) Plaque volumes in the aortic arch from 24- and 36-week free-fed (n=10 and n=8 respectively) and diet-restricted (n=10 for both) DKO mice. (B) Ejection fraction (EF) in free-fed and diet-restricted DKO mice at 24 weeks. *P<0.05; **P<0.01; ***P<0.001.

Conclusions

Inhibition of atherosclerosis and improvement of cardiovascular function following weight-loss in leptin-deficient, obese and insulin-resistant mice can be explained by expressional changes in the adipose tissue of key genes regulating insulin sensitivity, oxidative stress, lipid metabolism, inflammation and endothelial function, most of which are under the transcriptional control of PPARs. Our observation of restored/increased expression of PPARs in the same condition and the correlation between PPAR expression and plaque volume in DKO mice, and the accumulation of Ox-LDL in particular, points to the critical role of these transcription factors

both in the pathogenesis and the potential treatment of these features of the metabolic syndrome. Because PPARalpha and PPARgamma are down-regulated in double knockout mice and because they feature most of the metabolic syndrome components, they could prove to be a useful model to test the effects of PPAR agonists and statins.
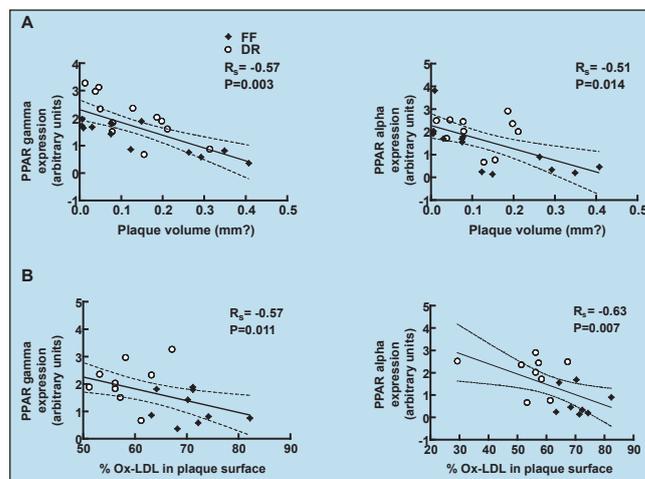
Figure 3: (A) Correlation of PPARgamma (n=25), respectively PPARalpha (n=23), expression with aortic plaque volume in free-fed (FF) and diet-restricted (DR) DKO mice. (B) Correlation between both PPARgamma (n=19) and PPARalpha (n=17) expression and Ox-LDL in aortic plaques in free-fed and diet-restricted DKO mice. Rs= Spearman correlation coefficient.

REFERENCE LIST

(1) Haffner SM, Mykkanen L, Festa A, Burke JP, Stern MP. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. Circulation 2000;101(9):975-80.

(2) Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest 1995;96(1):88-98.

(3) McLaughlin T, Abbasi F, Kim HS, Lamendola C, Schaaf P, Reaven G. Relationship between insulin resistance, weight loss, and coronary heart disease risk in healthy, obese women. Metabolism 2001;50(7):795-800.

(4) Henkin L, Bergman RN, Bowden DW et al. Genetic epidemiology of insulin resistance and visceral adiposity. The IRAS Family Study design and methods. Ann Epidemiol 2003;13(4):211-7.

(5) Mertens A, Verhamme P, Bielicki JK et al. Increased low-density lipoprotein oxidation and impaired high-density lipoprotein antioxidant defense are associated with increased macrophage homing and atherosclerosis in dyslipidemic obese mice: LCAT gene transfer decreases atherosclerosis. Circulation 2003;107(12):1640-6.

(6) Verreth W, De KD, Pelat M et al. Weight loss-associated induction of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma correlate with reduced atherosclerosis and improved cardiovascular function in obese insulin-resistant mice. Circulation 2004;110(20):3259-69.

(7) Nagy TR, Clair AL. Precision and accuracy of dual-energy X-ray absorptiometry for determining in vivo body composition of mice. Obes Res 2000;8(5):392-8.

(8) Tenenbaum A, Fisman EZ, Motro M. Metabolic syndrome and type 2 diabetes mellitus: focus on peroxisome proliferator activated receptors (PPAR). Cardiovasc Diabetol 2003;2(1):4.

(9) Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004;10(4):355-61.

(10) Ferre P. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes 2004;53 Suppl 1:S43-S50.

GENETIC TEST MAY HAVE ADDITIONAL PREDICTIVE POWER OVER AND ABOVE THE ACCEPTED RISK FACTORS IN PATIENTS WITH SEVERE HYPERCHOLESTEROLEMIA.[1]

Olivier S. Descamps

Centre de recherche médicale de Jolimont et département de médecine interne,
Centre Hospitalier Jolimont-Lobbes,
Rue Ferrer 159, 7100 Haine-Saint-Paul
PH : 064 23 31 67 – E-mail descaoli@skynet.be

The present article summarizes two works recently published in European Journal of Clinical Investigation [1 & 2].

Abstract

Among 273 patients with severe hypercholesterolemia and a family history of early cardiovascular disease, we demonstrated that, those carrying LDL-R or ApoB genes mutations causing familial hypercholesterolemia (63 men and 59 women) had significantly greater carotid (delta = +0.27 mm in men and 0.10 mm in women) and femoral (delta = +0.22 mm in men and +0.20 mm in women) intima media thickness and were more likely to have positive exercise test (OR=6.15 in men OR=4.76 in women) and coronary calcification on CT scan (OR=3.90 in men OR=2.34 in women) than those without familial hypercholesterolemia (87 men and 64 women). Given the fact that such findings are associated with greater occurrence of CHD events, we suggest that molecular-genetic identification of familial hypercholesterolemia may better the awareness of the high cardiovascular risk of these patients and should motivate more intensive preventive hygieno- et chemo-therapy.

INTRODUCTION

Currently, patients with severe hypercholesterolemia and family history of early cardiovascular disease (CVD) are considered at high risk of CVD, whatever the underlying cause of this hypercholesterolemia and the familial aggregation of early CVD. With the emerging capability to perform genetic analysis, a critical issue is whether precise identification of the underlying molecular defect by genetic analysis may be of any help to improve assessment of CVD risk and prevention management.

Some hereditary defects of lipid metabolism such as familial hypercholesterolemia (FH), have been well characterized at the molecular level. FH is a autosomal dominant disorder caused by the presence at the heterozygous state of one mutant allele of the LDL-receptor (LDL-R) gene [3] or of the apolipoprotein B (APOB) gene [4]. Its prevalence is approximately 1 in 500. There is no doubt that cardiovascular events and atherosclerosis [3] develop more frequently and earlier in patients with FH in comparison to normolipemic patients. But in clinical practice, the question is rather whether FH individuals are at greater CHD risk compared to non-FH individuals who are clinically undistinguishable from FH because they have the same phenotype (that is : severe hypercholesterolemia and the same familial history of early CVD).

In our work [1, 2], we assessed this question by evaluating non invasively carotid [1], femoral [1] and coronary [2] atherosclerosis in series of patients free of any CVD symptoms (primary prevention) but displaying the same CVD risk phenotype with severe hypercholesterolemia and familial history of early CVD).

MATERIALS AND METHODS

In our lipid clinics, we recruited 273 patients (25-65 years) with severe hypercholesterolemia (cholesterol level above the 95th percentile for age and sex [5]) and a family history of early cardiovascular disease (at least one first-degree relative with early cardiovascular disease before the age of 55 years in men or 65 years in women). Persons with any known history of CVD or abnormal resting electrocardiogram were excluded from this study. The local ethical committee approved the study and the patients gave informed consent to participate to the study.

The patients were classified as having FH or not FH on the basis of the molecular screening for LDLR and APOB mutations performed in our laboratory as previously described [1, 2, 6]. Carotid and femoral atherosclerosis [2] was evaluated by the ultrasonographic measurement of intima-media thickness (IMT) in the carotid and femoral arteries [7]. Atherosclerosis in coronary arteries [2] was estimated non invasively by exercise stress testing (EST) [8] that allows for the determination of physiological variables in relation to blood flow limitation and by helical computed tomography [9] that provides anatomic information by detecting calcific deposits in the coronary arteries (see paper [1,2] for more detailed methodology). Although intima-media thickness was assessed in all 273 patients, exercise stress testing and helical computed tomography were performed in a large subset of the selected patients (N=235, 95 FH and 140 non FH). For ethical reason, helical computed tomography was only performed in women above 35 years. Amongst our patients, exercise stress testing (EST) was interpretable in 42 FH men, 66 non-FH men, 32 FH women and 36 non-FH women. The EST was equivocal in the other patients due to inadequate level (heart rate<85% of predicted maximum) of exercise (mostly, prematurely stopped due to limb cramps).
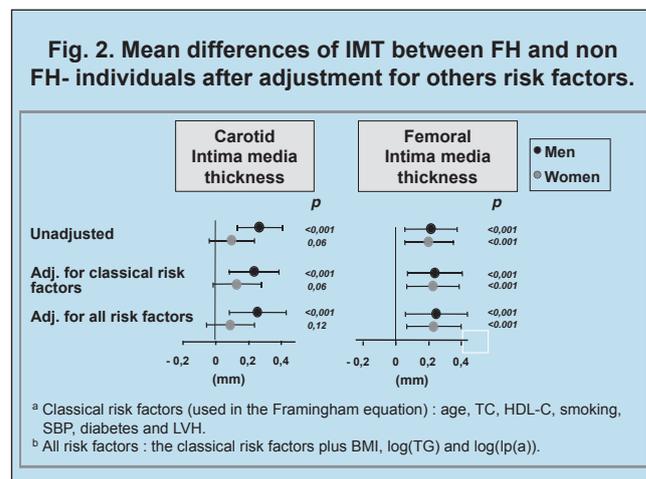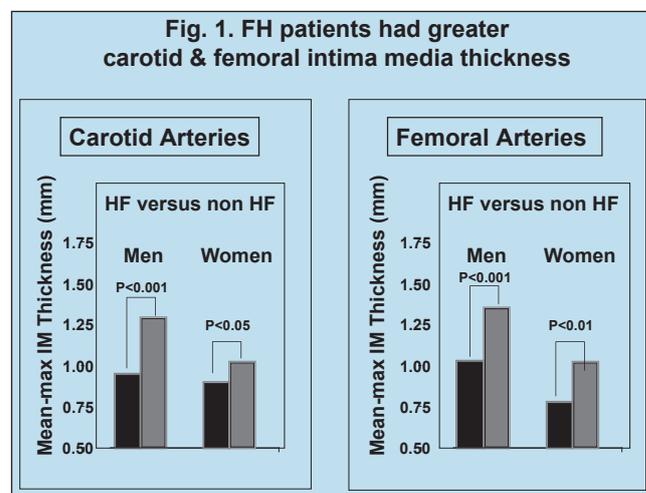
RESULTS

According to the genetic test, we classified 122 patients (63 men and 59 women) as FH-patients and the 151 others (87 men and 64 women) as non-FH patients. In these FH patients, we found 24 different mutations in the LDL-receptor gene (108 patients) and one mutation (14 patients) in the apolipoprotein B gene. All FH patients were heterozygous. As seen in *table 1*, FH and non-FH patients had a different distribution of classical risk factors. The concentrations of LDL-C and Lp(a) were higher in FH patients whereas the prevalence of hypertension, diabetes and current smoking as well as the mean values of body-mass index and waist-to-hip ratio were greater in non-FH patients.

|	Non-FH	FH
N	151	122
Age, ans	51 ± 9	44 ± 10 *
LDL-C, mg/dl	314 ± 81	221 ± 32 *
Lp(a), mg/dl	49 ± 41	27 ± 42 **
BMI, kg/m²	28,6± 3,9	27,2 ± 4,0 *
AGR	0,94± 0,07	0,90 ± 0,07 *
Tabagisme	33%	19% *
Hypertension	34%	16% *
Diabètes	15%	3% *

* p<0,05 FH versus non-FH   **p<0,05 FH versus non-FH by log transformation

Compared to non-FH-men, FH-men had significantly greater **carotid intima-media thickness** (1.27 ± 0.47 mm versus 1.00 ± 0.40 mm) and significantly greater

femoral intima-media thickness (1.30 ± 0.53 mm versus 1.08 ± 0.46 mm). Similarly, FH-women had significantly greater femoral intima-media thickness compared to non-FH-women (1.05 ± 0.49 mm versus 0.84 ± 0.32 mm) *(figure 1)*. After adjustment for the classical risk factors *(figure 2)*, the difference of the carotid and femoral intima-media thickness between FH and non-FH patients remained statistically significant.

Fig. 1. FH patients had greater carotid & femoral intima media thickness

Fig. 2. Mean differences of IMT between FH and non FH- individuals after adjustment for others risk factors.

[a] Classical risk factors (used in the Framingham equation) : age, TC, HDL-C, smoking, SBP, diabetes and LVH.
[b] All risk factors : the classical risk factors plus BMI, log(TG) and log(lp(a)).

Coronary calcification (CC) were present in 75% of the FH-men compared with 44% of the non-FH men (odds ratio = 3.90; p<0.001) and in 53% of the FH women compared with 31% in the non-FH women (odds ratio = 2.65; p<0.01) *(figure 3)*.

Fig. 3. FH patients had greater coronary atherosclerosis
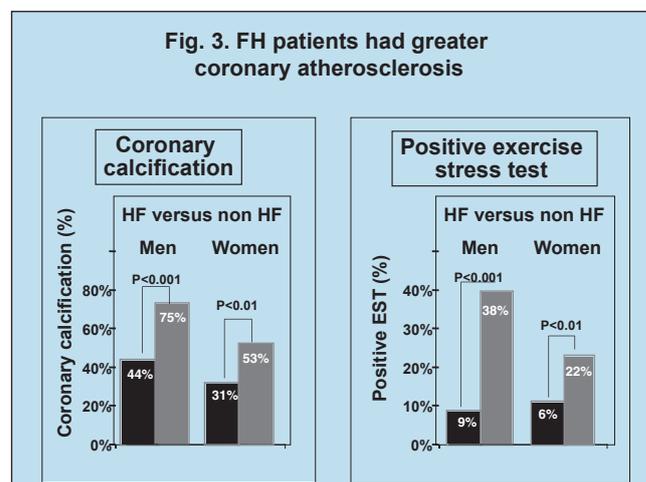
Positive exercise stress testing (EST) was present in 38% of FH men compared to 9% of non-FH men (odds ratio = 6.15; p<0.01) and in 22% of FH-women compared to 6% of non-FH women (odds ratio = 4.76 ; p=0.06). The EST was positive only on the basis of electrocardiographic criteria and none of the patient had complained of angina-like chest pain during the test. After adjustment of the odds ratios for the classical risk factors *(figure 4)*, these odds ratios for CC and for positive EST remained statistically significant.

Fig. 4. Odds ratios for coronary calcification and positive exercise stress testing in FH versus non-FH patients after adjustment for others risk factors.

	Coronary calcification Odds ratios		Positive exercise stress testing Odds ratios		
		p		p	● Men ○ Women
Unadjusted		<0,001 0,048		<0,001 0,06	
Adj. for classical risk factors		<0,01 0,07		0,03 0,21	
Adj. for all risk factors		<0,01 0,13		0,02 0,16	
	1 10 100		1 10 100		

a Classical risk factors (used in the Framingham equation) : age, TC, HDL-C, smoking, SBP, diabetes and LVH.
b All risk factors : the classical risk factors plus BMI, log(TG) and log(lp(a)).

DISCUSSION

The present work indicates that patients with genetically-ascertained FH have a higher degree of atherosclerosis than non-FH patients in the carotid, femoral and coronary arteries when compared with a cohort of non-FH patients with severe hypercholesterolemia and a family history of early cardiovascular diseases. Remarkably, the group of non-FH patients that we selected, accumulated more risk factors than the group of FH patients (higher prevalence of smoking, hypertension, diabetes and obesity) and yet, FH patients had a higher degree of atherosclerosis than non-FH patients.

As the carotid and femoral intima-media thickening [7], the presence of coronary calcification [9] and positiveness of exercise stress test are associated to greater occurrence of CHD events [8], this suggests that FH patents are at greater risk of CVD than non-FH patients even if these last shared similar findings of severe hypercholesterolemia and a family history of early cardiovascular diseases.

Some previous studies have shown greater intima-media thickness [10], greater prevalence of coronary calcification [11-12] and greater prevalence of positive exercise stress testing [13] in FH patients compared with normolipidemic controls studies. The present study is however the first to compare FH patients with hypercholesterolemic patients likely to be confounded in routine practice.

The greatest degree of atherosclerosis in FH patients may be explained by their longer exposure to higher LDL-cholesterol concentrations [14]. It has been also suggested [15] that the higher concentrations of lp(a) found in FH patients may play a role in the higher risk of CVD of FH patients. In our study, FH patients had higher concentrations of lp(a) than non FH patients and the concentration of lp(a) was associated with greater frequencies of CC and of positive EST.

From a practical point of view, the presented situation where the group of non-FH patients accumulated even more risk factors (in term of number) than FH patients, illustrates an interesting paradox. For most physicians, it would have made no doubt that the non-FH patients had greater CVD risk on the basis of such cluster of risk factors, specially as the genetic nature of the lipid disorder is usually not known precisely in routine practice. Such impression may be misleading as the present work shows that markers of atherosclerosis appeared more frequently in FH than in non-FH patients. This reinforces the idea that the precise identification of FH, preferably by molecular DNA diagnosis, may be useful for proper CHD risk assessment, especially in primary prevention.

POST-STUDY

Although coronary angiography was not intended to be performed systematically when EST was abnormal, we decided with 7 FH-men and 2 non-FH men of the present cohort to investigate their coronary arteries. These patients cumulated severe and multiple risk factors (some had diabetes, all were current smokers, some had brothers recently died of sudden death, 2 developed typical symptoms in the following years). All of these 7 FH-men and 2 non-FH men had multiple CC, strongly positive EST as well as severe thickenings of carotid and femoral IMT. Coronary angiography demonstrated the presence of severe coronary stenosis (>75% stenosis) and, finally, PTCA was proposed in 3 patients and CABG in the 2 patients with triple vessels disease… .

This high prevalence of severe CVD was further confirmed after the present work ended (December 2000), by a survey (16) that we performed in 2001 and 2002 by recruiting all young FH-men (25-55 years old) admitted in our hospital to estimate the incidence of coronary heart diseases. In this cohort of 66 young FH men, 32% (N=21 with 8 below age 45) had earlier history of symptomatic ischaemic disease and 20% (N=13 with 7 below age 45) had significant ST/T changes during exercise stress test. Amongst the 8 patients who were further investigated by coronary angiography, 6 (with 2 below age 45) underwent coronary angiography or surgical bypass.

REFERENCES

1. Descamps OS, Gilbeau JB, Luwaert R, Heller F. Impact of Genetic Defects on coronary atherosclerosis in patients suspected of familial hypercholesterolemia. Eur J Clin Invest 2003, 33: 1-9.
2. Descamps OS, Gilbeau JP, Van Leuven F et al. Impact of genetic defects on carotid and femoral atherosclerosis in patients suspected of familial hypercholesterolemia. Eur J Clin Invest 2001;31:958-965.
3. Goldstein JL, Hobbs HH, Brown MS. Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic basis of inherited disease. 7th Ed. McGraw-Hill,Inc, 1995:1981-2030.
4. Soria LF, Ludwig EH, Clarke HRG, Vega GL, Grundy SM, McCarthy BJ. Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100. Proc Natl Acad Sc USA 1989;96:587-591.
5. Kornitzer M, Bara L, for the B.I.R.N. Study Group. Clinical and anthropomorphic data, blood chemistry, and nutritional patterns in the Belgian population according to age and sex. Acta Cardiol 1989; 44:101-144.
6. Descamps OS, Leysen X, Van Leuven F et al. The use of Achilles tendon ultrasonography for the diagnosis of familial hypercholesterolemia. Atherosclerosis 2001; 157: 514-518
7. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu C, Liu CH. The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med 1998;128:262-269.
8. Gibbons RJ, Balady GJ, Beasley JW et al.. ACC / AHA guidelines for exercise testing – a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee of exercise testing). J Am Coll Cardiol 1997;30:260-315.
9. Budoff MJ, Mao S, Zalace CP et al. Comparison of spiral and electron beam tomography in the evaluation of coronary calcification in asymptomatic persons. In J Cardiol 2001:77;181-188.
10. Wendelhag I, Wiklund O, Wikstrand J. Atherosclerotic changes in the femoral and carotid arteries in familial hypercholesterolemia. Ultrasonographic assessment of intima-media thickness and plaque occurrence. Arterioscler Thromb 1993;13:1404-1411.
11. Hoffmann U, Bodjal G, Derfler K et al. Quantification of coronary arteries calcification in patients with FH using EBCT. Eur J Clin Invest 2001;31:471- 475.
12. Jensen JM, Kruse TA, Brorholt-Petersen JU et al. Linking genotype to aorto-coronary atherosclerosis : a model using familial hypercholesterolemia and aorto-coronary calcification. Ann Hum Genet 1999;63:511-520.
13. Wojciechowski APJ, Highes LO, Raftery EB et al. Detection of asymptomatic coronary artery disease in familial hypercholesterolemia. Lancet 1989; i : 1202-1203.
14. Raal FJ, Pilcher GJ, Waisberg R et al. Low-density lipoprotein cholesterol bulk is the pivotal determinant of atherosclerosis in familial hypercholesterolemia. Am J Cardiol 1999;83:1330-1333.
15. Hobbs HH, White AL. Lipoprotein(a), intrigues and insights. Current Opinion of Lipidology 1999;10:225-236.
16. Descamps OS, de Meester A, Cheron P, Kastelein JJ, Heller FR. Clinical and preclinical Myocardial ischaemia in familial hypercholesterolemia. Atherosclerosis 2003, Supp 4, 7-8.

REMAINING QUESTIONS ON FAMILIAL HYPERCHOLESTEROLEMIA (FH)

O.S. Descamps
Centre Hospitalier
Jolimont-Lobbes

Dr. Olivier DESCAMPS stelt de lezers van de "Lipid Club Letter" voor om samen na te denken over heterozygote familiale hypercholesterolemie, en met name over de diagnose en de behandeling bij kinderen.
Alle suggesties, opmerkingen en kritiek zijn meer dan welkom en kunnen worden verstuurd naar descaoli@skynet.be

As presented at the last meeting of the BLC, we would like to launch a new project of discussion in the Belgian Lipid Club aiming to establish guidelines on some specific questions remained unanswered on heterozygous familial hypercholesterolemia (HFH).

One of the major question that remained opened in HFH is «When should we start a treatment in HFH patients?». This implies the question «Should we treat young adults or even children ?» and, if yes, this brings the new question «how could we identify the young adults and children that need our attention ?». All these questions are not easy to answer, because there is no clear «evidence-based medicine» to support any action or abstention. There is a need however to agree on a common attitude between pediatricians, general practitioners, and internists as patients will move from one hand to the other along their life. Consistency between our attitudes may be an important determinant for long-life compliance of the patient to treatment

Historically, till the early 90ís, intervention for CVD prevention in children was not advised (except in homozygous FH) [1-3]. This attitude was based on the belief that [1] HFH children are completely asymptomatic and are not expecting to get any CVD complication before 20 years or more (why to worry so early of such relapsed problem ?), [2] that drug treatment appeared unconceivable given the unproved benefit on future CVD (at this time, it was the same debate in adults), possible hormonal disequilibrium, questionable increase of non-cardiovascular mortality and potentially associated with hormonal disequilibrium (lack of long experience with statins except with bile acid-binding resin (expected 15% reduction of LDL-C, but a very poor compliance), [3] that diet was poorly effective in children (Restriction Saturated Fat/cholesterol : -5% LDL-C), poorly complied and potentially associated with nutrition disequilibrium. Finally, some studies have also raised the issue of psychological problems (may create unnecessary and unproductive anxiety in parents and children, could lead to labeling and family conflicts) so that, it was questioned whether the psychosocial costs of labeling would outweigh the expected benefits of diets and early treatment,

We should worry about the fact that most of this «wait and see» attitude do not rely on scientific facts but mostly, on belief, lack of clear scientific evidence, lack of experience and perhaps also on the discouraging observation that implementation of CVD prevention (and cholesterol reduction to target) in adult population at risk represents already a difficult step in practice (why then, to attempt the more difficult step of children treatment ?)

Since then, some studies have brought some new scientific evidences that restimulate the debate on whether or not to care about children in the perspective of CV prevention. [4]

First, it appears that elevated cholesterol in childhood results to substantial subclinical impact in child [5] and may be a good predictor for future cardiovascular events later in life [6-8]. Early atherosclerotic lesions or fatty streaks, begin in childhood [5] and are related to cholesterol levels and other CHD risk factors that exist in adults [5]. In FH children, flow mediated dilation is impaired [9] and intima-media thickness is greater than in non FH children. [10-11]

Second, plant sterol / stanols (that allow a 10 to 15% LDL-C reduction) has entered the market [12] and trials on statins in children have shown their efficacy (with a 30 to 40% LDL-C reduction, maximum study duration: 2 years) and their good tolerance (no side effect, no growth problem, no particular hepatic of muscular toxicity) (13). FDA has approved the treatment by simvastatin in 1995, atorvastatin 10-20mg (in 2000), and pravastatin (in 2002).

Finally, treatment with statins have been showed to improve atherosclerosis in children with changes of flow-mediated diameters over 28 weeks of statins treatment [14] and of intima-media thickness over two years of statins treatment [15-16].

At the present time, some guidelines exist [17-19] in the US. Although there was no clear discussion in the last version of the European guidelines [20], some European experts have recently met and published some interesting conclusions [21].

We thus need to enter in the debate and establish an consensual attitude for the medical practice in Belgium. After collect of relevant publications on the following questions (keeping in mind the need to be in accordance with the existing consensus on these questions if they exist)

1. Do we have to screen children for cholesterol levels ? (We cannot avoid this debate if we want to develop a logical attitude)

Collect arguments in favor of cholesterol screening
1. "Tracking" of cholesterol over time.

2. The presence of atherosclerotic lesions in adults (intima-media thickness , clinical events) is related to risk factors during childhood.

3. "Tracking" of atherosclerosis over time.

4. Early atherosclerotic lesions in blood vessels of youth is correlated with childhood levels of low-density lipoprotein cholesterol and other risk factors

Collect arguments against cholesterol screening
1. Frequency of elevated cholesterol in the general population of children

2. Psychological problems specific to the knowledge of a high cholesterol in children for the child and his family.

3. Potential means for the physician to deal with these psychological problems

2. Diagnosis of FH in children.

Which criteria to suspect HFH in children : 2 situations to explore :

1. Children in a family which is known to carry FH.

2. Children where a high cholesterol level is discovered in a blood sample (see 1).

Psychological problems with identifying HFH in children.

The questions that we should address include reported concerns or worries subsequent to the notification of a diagnosis of an inherited disease in the child (Child, parents, brothers and sisters, physicians)

3. Treatment of FH in children or in young adults (that is the main question)

Who ?
Debate the absence of evidence based medicine
Target ? LDL-C
Treatment ?
1. Diet (harm ? type ? Follow-up)
2. Stanols / Plant sterols (dosage?, side effect?Ö)
3. Statins (which ?, dosage)
4. Ezetimibe (not registered, ! Do we have to introduce a demand ?)
5. Fibrates (not indicated ?)

4. Do we have specific problems with FH in Belgium ?

1. Age-specific normal values of cholesterol and LDL (HDL-C & TG) (required to set biological criteria of suspicion of FH)

2. Reimbursement of statins and ezetimibe. At the present time, only one drug is registered for children in Belgium (atorvastatin) and the current condition of reimbursement could cause a problem for many children (very few children at age 10-17 have one parent* with CHD, specially if CHD prevention has been applied in their parents).

3. Psychological aspects for diagnosis and therapy. which could be specific to our culture and education (poor education on what is inherited cholesterol in our country). Finally, there is only one drug registered for children in Belgium and the current condition of reimbursement could cause a problem for many children (very few children at age 10-17 have one parent* with CHD, specially if CHD prevention has been applied in their parents).

We hope you will join us in this new useful and exciting mission

Dr Descamps Olivier
Hopital de Jolimont
159 , Rue >Ferrer
7100 Haine Saint Paul
Belgium
descaoli@skynet.be

REFERENCES

1. Newman TB et al. The case against childhood cholesterol screening. JAMA. 1990;264:3039-3043

2. Holtzman NA et al. The great god cholesterol. Pediatrics.1991;87:943-945

3. Hulley SB et al. Position statement: cholesterol screening in children is not indicated, even with positive family history J Am Coll Nutr. 1992;11:20S-22S.

4. Tsimikas S, Witztum JL Shifting the diagnosis and treatment of atherosclerosis to children and young adults: a new paradigm for the 21st century. J Am Coll Cardiol 2002 : 40:2122ñ2124.

5. Newman WP, Freedman DS, Voors AW, Relation of serum lipoprotein levels and systolic blood pressure in early atherosclerosis. N Engl J Med. 1986; 314:138-144

6. Olli T. Raitakari, Markus Juonala, Mika K‰h^nen, Leena Taittonen, Tomi Laitinen, Noora M‰ki-Torkko, Mikko J. J‰rvisalo, Matti Uhari, Eero Jokinen, Tapani R^nnemaa, Hans K. ≈kerblom, Jorma S. A. Viikari, Cardiovascular Risk Factors in Childhood and Carotid Artery Intima-Media Thickness in Adulthood. The Cardiovascular Risk in Young Finns Study ; JAMA. 2003;290:2277-2283.

7. Shengxu Li, Wei Chen, Sathanur R. Srinivasan, Gene Bond, Rong Tang, Elaine M. Urbina, Gerald S. Berenson, Childhood Cardiovascular Risk Factors and Carotid Vascular Changes in Adulthood. The Bogalusa Heart Study. JAMA. 2003;290:2271-2276.

8. Patricia H. Davis, Jeffrey D. Dawson, Ward A. Riley, Ronald M. Lauer. Carotid Intimal-Medial Thickness Is Related to Cardiovascular Risk Factors Measured From Childhood Through Middle Age. The Muscatine Study. Circulation. 2001;104:2815.

9. Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. J Clin Invest. 1994;93(1):50-5.

10. Lavrencic A, Kosmina B, Keber I, Videcnik V, Keber D. Carotid intima-media thickness in young patients with familial hypercholesterolaemia. Heart. 1996 Oct;76(4):321-5

11. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, Bonaa KH. Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arterioscler Thromb Vasc Biol. 1996; 16(8):984-91.

12. Williams CL, Bollella MC, Strobino BA, Boccia L, Campanaro L. Plant stanol ester and bran fiber in childhood: effects on lipids, stool weight and stool frequency in preschool children. J Am Coll Nutr. 1999;18(6):572-81.

13. Rodenburg et al. Familial hypercholesterolemia in children. Curr Opin Lipidology 2004; 15; 405-412.

14. De Jongh S et al. J Am Coll Cardiol 2002: 40(12):2117-2124

15. Wiegman A et al. Efficacy and safety of statin therapy in children. JAMA 2004;292::377-378.

16. Sijbrands EJ et al : Kyoto, Japan: 13th ISA; 2003.

17 Williams CL, Hayman LL, Daniels SR, Robinson TN, Steinberger J, Paridon S, Bazzarre T. Cardiovascular health in childhood: statement for health professionals from the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. Circulation 2002: 106:143ñ160

18. Cardiovascular Health Promotion in the Schools: A Statement for Health and Education Professionals and Child Health Advocates From the Committee on Atherosclerosis, Hypertension, and Obesity in Youth (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. Hayman et al.. Circulation 2004;110:2266-2275.

19. Expert Panel on Blood Cholesterol Levels and Adolescents. *National Cholesterol Education Program Report.* Washington, DC: US Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute; 1991. National Institutes of Health publication 91-2732

20. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njolstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM; SCORE project group. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J. 2003 Jun;24(11):987-1003.

21. Civeira F; International Panel on Management of Familial Hypercholesterolemia. Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. Atherosclerosis. 2004 Mar;173(1):55-68.

CHU t TIVOLI

Symposium

LIPIDEN EN ATHEROSCLEROSE

«State of the Art in 2005»

Zaterdag 24 september 2005 in de CHU Tivoli
Conferentiezaal, vleugel H, 1e verdieping
avenue Max Buset 34
7100 La Louvière

Met de medewerking van

Belgian Lipid Club
Fonds pour la Recherche Médicale dans le Hainaut (FRMH)

PROGRAMMA

8.30u. *Welkomstkoffie*

9.00u. Inleiding
J. DUCOBU, CHU Tivoli, La Louvière.

EERSTE SESSIE
Nieuwe gegevens over atherosclerose

9.10u. "NO en de arteriële wand"
J-L. BALLIGAND, UCL.

9.35u. "Rol van myeloperoxidase in de oxidatie van lipiden"
K. ZOUAOUI, CHU van Charleroi.

TWEEDE SESSIE
Nieuweontwikkelingenindefysiologievanlipoproteïnen

10.00u. "Nucleaire receptoren en modulatie van lipiden"
B. STAELS, Pasteurinstituut, Rijsel.

10.30u. "Van fysiologie tot nieuwe behandelingen"
M. FARNIER, Dijon.

11.00u. Discussie

11.10u. *Pauze met koffie*

DERDE SESSIE
Klinische aspecten

11.30u. "Familialehypercholesterolemie:vandiagnose tot behandeling"
O.DESCAMPS,CentreHospitalierdeJolimont.

11.50u. "Nieuwe therapeutische doelen bij het metabool syndroom"
A. SCHEEN, Universiteit van Luik.

12.10u. "Aanbevelingenvoordeklinischepraktijk–Wat is werkelijk belangrijk?
G. DE BACKER, Universiteit Gent.

12.30u. Groepsdiscussie met alle sprekers

12.50u. Opmerkingen en conclusies
M. FARNIER en J. DUCOBU.

13.00u. *Lunch*

Voor alle verdere inlichtingen:
Prof. J. Ducobu
Tel. : 064 27 66 69 - E-mail : jean.ducobu@chu-tivoli.be