UNIVERSITE DE LAUSANNE – FACULTE DE BIOLOGIE ET DE MEDECINE

Département de Médecine interne
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SURROGATE MARKERS FOR ATHEROSCLEROSIS IN OVERWEIGHT SUBJECTS WITH ATHEROGENIC DYSLIPIDEMIA

THESE

Préparée sous la direction du Professeur Gérard WAEBER
et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Myriam GENOUD

Médecin diplômée de la Confédération Suisse

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Surrogate markers for atherosclerosis in overweight subjects with atherogenic dyslipidemia

Lausanne, le 30 novembre 2011

pour Le Doyen
de la Faculté de Biologie et de Médecine

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Directrice de l'Ecole doctorale

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Rapport de synthèse

EVALUATION DE MARQUEURS PARACLINIQUES DE L'ATHEROSCLEROSE CHEZ DES SUJETS SOUFFRANT D'UN EXCES PONDERAL ET D'UNE DYSLIPIDEMIE. «Surrogate Markers for Atherosclerosis in Overweight Subjects With Atherogenic Dyslipidemia : The GEMS Project (peer-reviewed)» *Angiology 2008 Aug-Sep;59(4):484-92.*

Myriam Genoud, Vincent Wietlisbach, François Feihl, Alice Mermod, Diane Morin, Roger Darioli, Pascal Nicod, Vincent Mooser, Bernard Waeber, Daniel Hayoz, etGérard Waeber

Le syndrome métabolique (défini par les critères ATP III par la présence au minimum de 3 des facteurs suivants : taux plasmatiques d'HDL-cholestérol < 1,04 mmol/l chez l'homme et < 1.29 mmol/l chez la femme, taux plasmatiques de triglycérides > 1,69 mmol/l, tension artérielle ≥ 130/85 mmHg, glycémie ≥ 6,1 mmol/l, tour de taille > 108 cm chez l'homme et > 88 cm chez la femme) représente une constellation de facteurs de risque majeurs pour le développement de maladies cardiovasculaires. Il n'est pas encore établi actuellement quelle composante de ce syndrome contribue de manière plus marquée au risque de développer une athérosclérose. Dans le but d'éclaircir la pathogenèse de ce syndrome, une étude multicentrique intitulée GEMS (« Genetic Epidemiology of Metabolic Syndrome ») a été initiée afin de déterminer si la constellation d'une dyslipidémie avec HDL-C bas et TG élevé est un marqueur sensible de l'homogénéité génétique chez les individus atteints de syndrome métabolique.

Dans l'étude menée à Lausanne (multicentrique), la contribution de la dyslipidémie avec HDL-C bas et TG élevé dans la pathogenèse de l'athérosclérose a été évaluée par 2 examens, reconnus comme marqueurs fiables de la vasculopathie : la mesure de l'épaisseur intima média carotidienne par ultrasonographie et l'évaluation de la dysfonction endothéliale de la microcirculation cutanée. Deux groupes de sujets comparables en terme d'âge et de sexe et souffrant d'un excès pondéral (BMI > 25 kg/m2) mais normoglycémiques ont été comparés. Ces deux groupes (étude cas-témoins) étaient uniquement discordants quant à leurs profils lipidiques. Ainsi, 120 cas, définis comme ayant un HDL-cholestérol bas (≤ 25 percentile pour l'âge et le sexe dans la population générale) et des TG élevés (≥ 75 percentile) ont été comparés à 120 contrôles avec un HDL-cholestérol haut (≥ 50 percentile) et des TG bas (≤ 50 percentile). Un doppler des artères carotides et fémorales a été effectué pour déterminer l'épaisseur de l'intima média et la présence ou non de plaques d'athérome. La fonction endothéliale a été évaluée par un laser doppler sur la micro-circulation cutanée (réponse hyperémique à une occlusion transitoire de la circulation de l'avant-bras par une manchette à pression et mesure de la vasodilatation induite par un échauffement local de la peau avec de l'eau). Un enregistrement de la pression artérielle ambulatoire sur la journée (Remler) a été pratiqué chez tous les sujets.

Les résultats obtenus montrent que les cas ont une prévalence plus élevée de plaques d'athérome (médiane 1,5 ± 0,15 vs 0,8 ≥ 0,15, p<.001), une épaisseur intima média plus importante (médiane 0,66 ± 0,15 vs 0,61 ± 0,15, p<.01), ainsi qu'une réduction significative de la vasodilatation endothéliale induite par la chaleur et post-ischémique comparativement aux contrôles.

En conclusion, le profil lipidique associant un HDL-cholestérol bas et des triglycérides élevés représente un risque majeur de développer une maladie athéromateuse périphérique et est associée à une augmentation de l'épaisseur intima média et une altération de la fonction endothéliale chez les individus en surcharge pondérale. Bien qu'un HDL-cholestérol bas soit

fréquemment associé à une hypertriglycéridémie, les résultats de notre étude peuvent suggérer un rôle potentiel de la fraction HDL-cholestérol comme un puissant agent anti-athérogénique.

EVALUATION DE L'INSULINO- SECRETION INDUITE PAR LE GLUCOSE CHEZ DES SUJETS EUGLYCEMIQUES SOUFFRANT D'UNE DYSLIPIDEMIE OU NON. « Glucose-Induced Insulin Secretion in Dyslipidemic and Normolipidemic Patients With Normal Glucose Tolerance »

Diabetes Care, volume 28, number 5, pp. 1225-7, May 2005

Christophe Binnert, Myriam Genoud, Gérald Seematter, Assia Fekirini, Vincent Mooser, Gérard Waeber, Luc Tappy

Le syndrome métabolique est très prévalent dans les pays industrialisés, où il représente un enjeu majeur de santé publique en raison des risques de survenue de maladies cardiovasculaires et de diabète. La constellation d'un taux bas d'HDL-cholestérol et d'un taux élevé de triglycérides est un important constituant de ce syndrome. Le but de cette étude fut d'évaluer si l'altération du profil lipidique observée dans le syndrome métabolique est associée à une altération de la sécrétion ou de la sensibilité à l'insuline chez les sujets avec un test de tolérance au glucose normal.
L'homéostasie glucidique a été évaluée par un clamp hyperglycémique dans un groupe de sujets dyslipidémiques et un groupe de sujets normolipidémiques comparables pour l'âge et le BMI, après l'administration de dexaméthasone durant 2 jours (procédure appliquée dans le but de diminuer la sensibilité à l'insuline avec une hyperinsulinémie compensatrice). Les résultats obtenus démontrent que les sujets dyslipidémiques ont une sécrétion augmentée d'insuline induite par le glucose afin de compenser l'altération de la sensibilité à l'insuline. L'administration sur une courte période de dexaméthasone peut détecter une résistance à l'insuline sub-clinique chez les sujets dyslipidémiques. Deux hypothèses ont été émises : la première est que l'hyperinsulinémie chronique consécutive à la résistance à l'insuline pourrait contribuer à une augmentation des TG plasmatiques par une stimulation de la lipogenèse hépatique de novo et une sécrétion des lipoprotéines VLDL. La deuxième hypothèse serait que l'altération primaire du métabolisme lipidique hépatique pourrait au long court contribuer au développement de la résistance à l'insuline et de l'hyperinsulinémie. Seules des études prospectives chez des individus à risque de développer un syndrome métabolique pourraient apporter des clarifications sur ces 2 hypothèses.

Mesure de la pression artérielle ambulatoire: une approche pour quantifier le risqué cardiovasculaire « Ambulatory blood pressure monitoring : a mean to stratify cardiovascular risk »

Blood Press Monit. 2007 Aug ;12(4) :243-4

Waeber B, Genoud M, Feihl F, Hayoz D, Waeber G

Les recommandations actuelles pour la prise en charge de l'hypertension artérielle insistent sur l'importance d'une évaluation complète des facteurs de risque cardiovasculaires mais n'incluent pas la contribution de l'enregistrement de la pression artérielle en ambulatoire (Remler) dans la stratification du risque cardio-vasculaire.
Dans cette étude, nous avons calculé le risque cardiovasculaire global selon les recommandations 2003 de la Société Européenne d'hypertension et la Société Européenne de

cardiologie chez 127 sujets chez lesquels un enregistrement de la pression artérielle sur 12 heures (le jour) a été effectué, ainsi que la réalisation d'un doppler artériel carotidien et fémoral.
Les résultats démontrent que l'enregistrement d'une pression artérielle $\geq$ 135/85 mmHg déplace le risque cardio-vasculaire à un plus haut niveau, de même que la présence d'une hypercholestérolémie et encore plus la détection de plaques d'athérome au doppler.
D'autres études sont encore requises pour déterminer définitivement le rôle de l'enregistrement de la pression artérielle en ambulatoire (Remler) dans l'évaluation du risque cardio-vasculaire.

·ogate Markers for Atherosclerosis in Overweight Subjects With Atherogenic Dyslipidemia: The GEMS Project

n Genoud, Vincent Wietlisbach, François Feihl, Alice Mermod, Diane Morin, Roger Darioli, Pascal Nicod, Vincen Mooser, Bernard Waeber, Daniel Hayoz and Gérard Waeber

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Surrogate Markers for Atherosclerosis in Overweight Subjects With Atherogenic Dyslipidemia: The GEMS Project

Myriam Genoud, Vincent Wietlisbach, François Feihl, Alice Mermod, Diane Morin, Roger Darioli, Pascal Nicod, Vincent Mooser, Bernard Waeber, Daniel Hayoz, and Gérard Waeber

Metabolic syndrome is a constellation of major risk factors for cardiovascular disease. In affected individuals with this syndrome, the independent contribution of low high-density lipoprotein–cholesterol and increased triglyceride levels to the development of atherosclerosis remains to be clarified. We assessed the relationship between these 2 parameters and several surrogate markers for atherosclerosis. One hundred and twenty overweight cases, defined as having high-density lipoprotein–cholesterol (≤25 age- and gender-specific percentile in general population) and high triglyceride values (≥75 percentile) were compared with 120 discordant overweight controls defined on lipid values (high-density lipoprotein–cholesterol ≥50 percentile and triglycerides ≤50 percentile). Case-control pairs were matched for age and gender. Carotid and femoral arteries were examined to determine carotid intima-media thickness and the presence of atherosclerotic plaque(s). Endothelial function was assessed by laser Doppler flowmetry in the skin microvasculature. Daytime ambulatory blood pressure monitoring was performed for each subject. Cases had higher prevalence of atherosclerotic plaques (mean 1.50 ± 0.15 vs 0.80 ± 0.15, $P < .001$), increased carotid intima-media thickness (mean 0.66 ± 0.15 vs 0.61 ± 0.15, $P < .01$), and a significantly reduced temperature-induced and postischemic endothelial vasodilation compared with controls. In conclusion, low high-density lipoprotein–cholesterol and high triglycerides levels are major contributors to peripheral atherosclerosis and are associated with an increase in intima-media thickness and impaired microvascular endothelial function in overweight individuals.

The age-ajusted prevalence of metabolic syndrome as defined by Adult Treatment Program-III (ATPIII) in the United States is approximately 24%.[1] The ATPIII definition includes at least 3 of the following abnormalities: waist circumference greater than 102 cm in men and 88 cm in women; serum triglyceride levels of at least 1.69 mmol/L; high-density lipoprotein (HDL)–cholesterol of less than 1.04 mmol/L in men and 1.29 mmol/L in women; blood pressure of at least 130/85 mm Hg; and serum glucose of at least 6.1 mmol/L.[2] High-normal levels of C-reactive protein are also common in individuals with metabolic syndrome, thereby leading one to consider the presence of a low-grade chronic inflammation as one component of the metabolic syndrome.[3,4] The clustering of these metabolic components is associated with a markedly increased risk for cardiovascular disease.[2] Coronary heart disease, peripheral artery disease, and stroke are the main clinical manifestations of generalized atherosclerosis. Independently, high blood pressure, dyslipidemia, and diabetes are established risk factors for developing atherosclerosis.[1,5-8] However, in the context of multiple metabolic abnormalities, it is not yet established which component(s) of the metabolic syndrome is(are) the major contributor(s) to atherogenesis.

From the Department of Internal Medicine (MG, AM, PN, GW), Institute of Social Medicine and Epidemiology (VW), Division of Clinical Pathophysiology (FF, BW), Medical Outpatient Clinic (RD), and Division of Angiology (DH), CHUV-University Hospital, Lausanne, Switzerland; and Medical Genetics, Genetic Research, GlaxoSmithKline, Collegeville Pennsylvania (VM).

One or more authors declare a potential conflict of interest. Author Vincent Mooser is an employee of GlaxoSmithKline, which helped fund this research.

Address correspondence to: Gérard Waeber, MD, Department of Internal Medicine, CHUV, BH 10-628, 1011 Lausanne, Switzerland; e-mail: Gerard.Waeber@chuv.ch.

Since the clinical manifestations of atherosclerosis may be apparent only decades after the initiation of the disease process, there has been an intensive search for surrogate markers of atherosclerosis. In order to be useful, these markers have to be easily measurable and should correlate with the clinical manifestations of atherosclerosis. The determination of the carotid intima-media thickness (IMT) has been established and validated as a surrogate marker of cardiosvascular risk. This parameter can be measured noninvasively by ultrasonography and provides a quantitative evaluation of morphological changes in large arteries.[9,10] Endothelial dysfunction has also been shown to occur early during the development of atherosclerosis and might precede the structural changes observed in the arterial wall.[11] In most studies, endothelial function has been assessed invasively by determing the vasodilatory response to a cholinergic agent, mainly acetylcholine.[12] Noninvasive assessment of endothelial function is now possible by evaluating, for instance, the hyperemic response to a transient occlusion of the forearm of circulation, by applying transcutanously acetylcholine by iontophoresis, or by exploring the skin vasodilatation induced by local warming.[11,13]

The present investigation was undertaken in an attempt to define the contributing role of dylipidemia to the pathogenesis of atherosclerosis in metabolic syndrome. Two surrogate markers of vasculopathy (skin microcirculatory endothelial dysfunction and carotid IMT) were measured in 2 groups of overweight and normoglycemic subjects who were discordant only with respect to their lipid values. Both groups were matched for age and gender. The dyslipidemia of the 120 cases consisted of low HDL-cholesterol (HDL-C) (≤25 age- and gender-specific percentile in general population) and high triglyceride values (≥75 percentile). These cases were compared with 120 discordant controls exhibiting HDL-C ≥50 percentile and triglycerides ≤50 percentile.

Methods

Definition of Cases and Controls

Individuals age 18 to 70 years were considered as cases if they simultaneously had high serum triglycerides (≥75th age- and gender-specific percentile in general population) and low serum HDL-C (≤25th percentile). Control participants were identified if their triglyceride values were ≤50 and HDL-C ≥50th percentile. As inclusion criteria for both groups, the body mass index (BMI) had to be > 25 kg/m^2 and fasting blood glucose < 6 mmol/L. Antidiabetic treatment and/or known diabetes were considered an exclusion criteria. Subjects were also excluded if they had a BMI ≥ 35 kg/m^2, were HIV positive, a recipient of an organ transplant, or affected with familial hypercholesterolemia, if they were heavy alcohol drinkers (more than 8 units of alcohol per day), or had been under hypolipidemic drug therapy within the 4 weeks preceding the lipid evaluation. The participants were recruited at the outpatient lipid clinic of the university hospital or in the frame of health plan survey conducted in several enterprises. The institutional ethics committee approved the protocol, and informed written consent was obtained from each participant.

Measurements

A questionnaire was administered to each participant to inquire about comorbid conditions such as the use of medications and tobacco and alcohol consumption. Height, weight, and waist circumference were determined in each subject. Three blood pressure measurements were obtained in the sitting position using an electronic oscillometric device (Omron/907; Omron Manufacturing of America, St. Charles, Ill). The average of the second and third systolic and diastolic blood pressure readings was used for analysis. Ambulatory blood pressure monitoring was recorded every 15 min during the day for a 12-hour period using the TM-2420, model 7 (A&D Instruments, Abington, Oxon, United Kingdom) device. A blood sample was collected after 12 hours of fasting.

Assessment of a Peripheral Atherosclerosis Score and IMT

The presence of peripheral atherosclerosis was assessed using B-mode high resolution imaging of both carotid and femoral arteries. The number of vessels containing atherosclerotic plaque(s)—defined by an encroachment of the arterial wall into the lumen ≥ 1.2 mm—was assessed as an index of peripheral atherosclerosis score (ATS). The IMT was also evaluated and standardized as proposed by Kastelein at al.[9] In brief, the average IMT was the result of the far wall across 3 segments in the left and right carotid arteries: carotid bifurcation, common carotid, and internal carotid. Validity and reproducibility of the carotid measurements using the B-mode ultrasound

protocol have been reported elsewhere.[9] The ultrasound evaluation was done by a single operator who was not aware of the lipid profile of the participants.

Endothelial Function

Microvascular blood flow was studied by laser-Doppler flowmetry as described previously.[13] Noninvasive evaluation of endothelial function in the forearm skin was evaluated in a subgroup of 99 cases and 86 controls by determining the skin blood flow response to local heating (37°C and 41°C) as well as to a transient occlusion of arterial circulation (inflation of a conventional arm cuff for 3 min at 200 mm Hg).[13]

Clinical Chemistry

Blood values for glucose, lipid, and high sensitivity C-reactive protein (hs-CRP) were assessed in the clinical chemistry laboratory of the Lausanne University Hospital using standard procedures.

Case-control Matching Procedure

Pairs of individuals of same age and gender were randomly matched from the 195 affected subjects (cases) and the 143 controls. An allowance for a limited difference in age (maximum, 3 y) was introduced in the matching criteria. In a first stage, only subjects with complete assessment of endothelial function (99 cases; 86 controls) were eligible for matching, and 74 pairs could be formed. In a second stage, all remaining cases and controls were matched, leading to the formation of 46 additional pairs. Therefore, a total of 120 pairs were available for analysis.

Statistical Analyses

The comparison of cardiovascular risk factor levels between cases and controls was based on paired *t* tests for continuous variables and on chi-square tests for categorical variables. The association between these risk factors and peripheral atherosclerosis—defined as the presence of at least 1 atherosclerotic plaque in the 4 explored arteries—was first examined in cases and controls separately using univariate logistic regression models. In order to determine whether dyslipidemia (cases, but not controls) is a determinant of peripheral atheroscelosis independently of other risk factors, a multivariate conditional logistic regression model was developed with all subjects taken together. Variables significantly associated to peripheral atherosclerosis in univariate logistic regression, in addition to dyslipidemia (ie, case vs control) were tested for inclusion in the multivariate model using a stepwise backward procedure (with statistical significance levels for entry and rejection set at $P < .10$ and $P < .20$, respectively). All statistical analyses were carried out using the STATA software (release 8.1; Stata Corp, College Station, Texas). Data are reported as mean ± SEM.

Results

Clinical Characteristics of the Participants of the Study

The clinical characteristics of the 120 cases and 120 controls are described in Table 1. The 2 groups were age and gender matched. Mean BMI was similar in both groups. Waist, waist to hip ratio, and office systolic and diastolic blood pressures were significantly higher in the affected individuals compared with the controls. The average of daytime blood pressures did not show any significant differences between cases and controls. Smokers were significantly more prevalent in the affected individuals (34% vs 21% in cases vs controls, respectively).

The laboratory values of the subjects are also described in Table 1. The lipid abnormalities were significantly more pronounced in cases. The fasting blood sugar was 5.3 ± 0.4 mmol/L and 5.4 ± 0.4 mmol/L in controls and cases, respectively. The ultrasensitive C-reactive protein values were significantly different in the affected group (2.8 ± 0.22 mg/L) compared with controls (1.6 ± 0.15 mg/L; $P < .05$). The subjects were then stratified according to their hs-CRP values.[14-16]

Evaluation of Peripheral Atherosclerosis, IMT, and Endothelial Function

We first evaluated the presence of atherosclerotic plaque(s) in both carotid and femoral artery bifurcations. A site was considered as positive if 1 or more plaque were detected in the arterial wall. Plaque was defined as an encroachement of the arterial wall into the lumen of at least 1.2 mm. The mean of positive sites in controls was 0.8 ± 0.15 compared with 1.5 ± 0.15 in cases ($P < .001$) (Figure 1A). The percentage of subjects exhibiting plaques in explored arteries was

Table 1. Clinical and Biochemical Characteristics of the Participants[a]

	Controls (n=120)	Cases (n=120)	*P* Value
Gender	83 M/37 F	83 M/37 F	1
Age, y	51 (47/55)	51 (47/55)	0.5
BMI, kg/m^2	28 (26/30)	28 (26/31)	.5
Obesity (³30)	25%	35%	.09
Waist, cm	93 (89/102)	97 (91/105)	.02
Hip, cm	107 (102/112)	106 (102/111)	.8
WHR	0.89 (0.85/0.92)	0.92 (0.88/0.95)	<.001
Abdominal obesity (>102 cm, >88 cm)	35%	50%	.02
Office SBP, mm Hg	124 (115/131)	127 (120/135)	.007
Office DBP, mm Hg	80 (70/83)	80 (75/86)	.02
Ambulatory SBP, mm Hg	130 (125/136)	133 (125/145)	.13
Ambulatory DBP, mm Hg	80 (77/87)	83 (78/88)	.07
Hypertension treated	12 (10%)	34 (28%)	<.001
BP ≥ 130/85 mm Hg	37%	53%	<.001
BP ≥ 140/90 mm Hg	9%	22%	<.001
BP ≥ 160/95 mm Hg	2%	4%	<.001
Smokers	21%	34%	.04
HDL-C, mM	1.7 (1.5/1.9)	1 (0.9/1.1)	<.001
TG, mM	0.9 (0.8/1.1)	2.4 (1.9/3.3)	<.001
TC, mM	5.3 (4.7/5.9)	6 (5.3/6.6)	<.001
TC/HDL-C	3.2 (2.6/3.6)	5.3 (4.6/6.2)	<.001
LDL-C, mM	3.1 (2.6/3.7)	3.7 (3.2/4.5)	<.001

NOTES: M = male; F = female; BMI = body mass index; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; HDL-C = high-density lipoprotein– cholesterol; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein–cholesterol.
a. Median data with interquartile range (25 and 75) in parentheses.

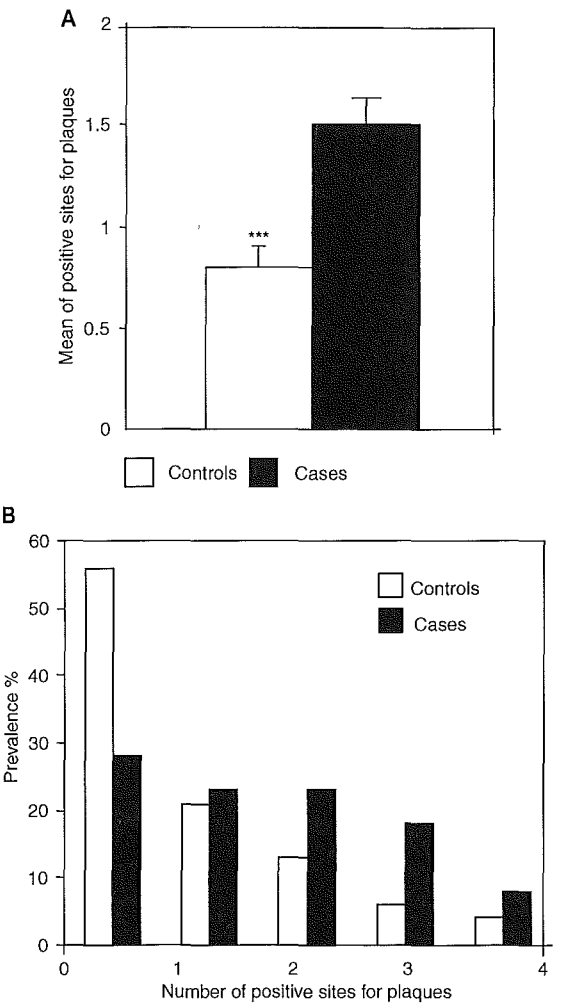

Figure 1. Detection of atherosclerotic plaques in carotid and femoral arteries. The presence of atherosclerotic plaque(s) was evaluated by B-mode ultrasound in carotid and femoral arteries. The presence of an encroachement of the arterial wall into the lumen of at least 1.2 mm was considered as a positive site. A, the mean of positive site for the presence of plaques was much higher in cases compared with controls (mean ± SEM; ***$P < .001$). B, the prevalence of subjects exhibiting 0, 1, 2, 3, or 4 positive sites for the presence of atherosclerotic plaque(s) is inversely correlated to the presence or the absence of a dyslipidemia.

much lower in the control group compared with the dyslipidemic subjects. In 55% of the controls, we did not detect the presence of any plaque in the 4 explored arteries, while only 28% of affected individulas were considered as free of any atherosclerotic plaque.

We next evaluated noninvasively by B-mode ultrasound the common carotid IMT (Figure 2). The IMT were significantly increased in cases compared with controls (0.66 ± 0.15 mm vs 0.61 ± 0.15 mm in cases vs controls, respectively; $P < .05$).

Endothelial function was also evaluated as a surrogate marker of atherosclerosis. Table 2 describes the clinical and biochemical characteristics of the 74 case-control pairs with complete assessment of microvascular endothelial function in the forearm skin. Results are shown pictorially in Figure 3. The peak increase of skin blood flow above the baseline value after the release of a 3-min arterial occlusion (reactive hyperemia) was 16% lower in cases (141 ± 6 perfusion unit [PU]) than in controls (168 ± 6, $P = .003$). A comparable difference (15%) was noted when this response was quantified as the area under curve (AUC) (cases: 21048 ± 1127 PU*sec (PU = Perfusion Unit); controls 24314 ± 1150 PU*sec; $P = .020$). Similarly, the vasodilation induced by a submaximal local thermal stimulation (skin temperature raised from 34°C to 37°C) was less marked in the cases (peak 89 ± 6 PU; AUC 19075 ± 1344 PU*sec) than in the controls (peak 11 ± 6 PU; $P = .013$; AUC

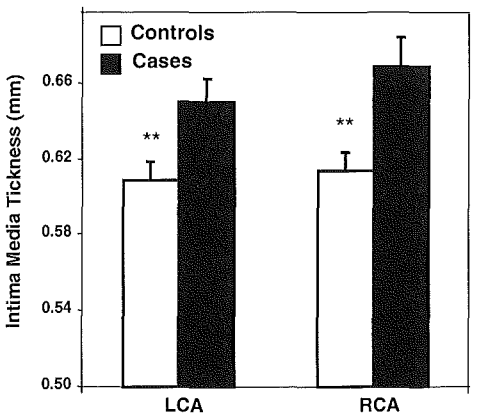

Figure 2. Intima-media thickness (IMT) assessesment in cases and controls. IMT thickness was evaluated in the left carotid arteria (LCA) and the right carotid arteria (RCA) in 120 normolipidemic controls and in 120 dyslipidemic cases. The IMTs were significantly increased in the cases compared to their discordant controls (mean ± SEM; $^{**}P < .01$).

Table 2. Clinical Characteristics of the Participants to the Endothelial Function Evaluation[a]

	Controls (n = 74)	Cases (n = 74)	*P* Value
Gender	49 M/25 F	49 M/25 F	1
Age, y	51 (47/55)	51 (47/55)	1
BMI, kg/m^2	29 (26/31)	29 (26/32)	.7
Obesity (≥30)	35%	43%	.01
Waist, cm	95 (89/103)	100 (92/106)	.2
Hip, cm	109 (103/114)	108 (104/114)	.7
WHR	0.90 (0.85/0.93)	0.91 (0.88/0.95)	.02
Abdominal obesity (>102 cm, >88 cm)	43%	59%	.02
Office SBP, mm Hg	125 (118/132)	129 (120/135)	.15
Office DBP, mm Hg	80 (73/85)	81 (75/87)	.23
Ambulatory SBP, mm Hg	130 (125/137)	133 (125/145)	.5
Ambulatory DBP, mm Hg	80 (78/87)	83 (80/88)	.3
Hypertension treated	13%	24%	<.001
BP ≥ 130/85 mm Hg	43%	57%	.01
BP ≥ 140/90 mm Hg	12 %	21%	<.001
BP ≥ 160/95 mm Hg	2.7%	4%	<.001
Smokers	24%	34%	.01
HDL-C, *mM*	1.7 (1.5/1.9)	1.1 (0.9/1.1)	<.001
TG, *mM*	0.9 (0.8/1.1)	2.4 (1.8/3.2)	<.001
TC, *mM*	5.5 (4.9/6.2)	6 (5.5/6.6)	<.001
TC/HDL-C	3.3 (2.7/3.7)	5.4 (4.6/6.2)	<.001
LDL-C, *mM*	3.2 (2.8/3.8)	3.9 (3.3/4.6)	<.001

NOTES: M = male; F = female; BMI = body mass index; WHR = waist-to-hip ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; BP = blood pressure; HDL-C = high-density lipoprotein– cholesterol; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein–cholesterol.

a. Median data with interquartile range (25 and 75) in parentheses.

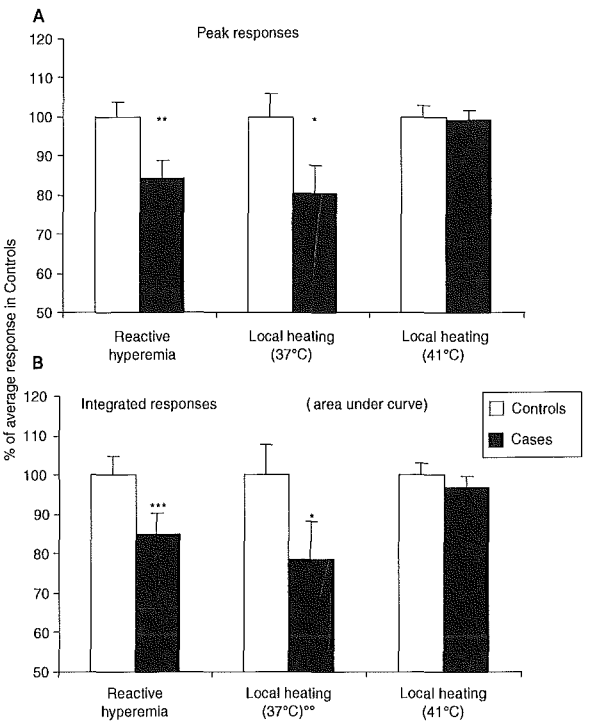

Figure 3. Endothelium-dependent vasodilatation in the skin microcirculation of case and control subjects. The time course of microvascular skin blood flow was assessed following either a 3-min occlusion of arterial inflow into the forearm (reactive hyperemia) or a brisk increase of local temperature from 34°C to either 37°C or 41°C. The reactive hyperemic response consisted in a brisk transient increase in skin blood flow, followed by a progressive return to the baseline value in 60 to 120 seconds. Local heating elicited a progressive increase in skin blood flow, reaching a steady value after approximately 10 min. All responses were quantitated as the maximal flow increase (reactive hyperemia: transient peak; local heating: steady value between 10 and 11.5 min) as well as the AUC calculated for the duration of measurement (reactive hyperemia, 120 seconds; local heating, 11.5 min). For ease of presentation, all data are normalized to the average value obtained in the control subjects and presented as mean ± SEM. $^{*}P < .05$; $^{**}P < .01$ cases compared with controls.

24314 ± 1862 PU*sec; $P = .024$, approximate relative difference 20% for both variables). By contrast, the much stronger hyperemia elicited by raising skin temperature from 34°C to 41°C did not differ between the 2 groups (peak 356 ± 12 PU in cases vs 360 ± 10 PU in controls; $P = .8$; AUC 121072 ± 4380 PU*sec in cases vs 125445 ± 3787 in controls; $P = .6$).

Evaluation of the Risk for Developing Atherosclerotic Plaque(s)

We defined the presence of at least 1 positive site in any of the 4 explored by B-mode ultrasound arteries

Table 3. Independent Risk Factors for Peripheral Atherosclerosis (ie, having at least 1 atherosclerotic plaque) in the 120 Pairs of Cases and Controls Matched for Age and Gender

Variable	Odds Ratio[a] (95% CI)	*P* Value
Controls	1.00	
Cases	4.01 (1.55-10.4)	.004
SBP < 120	1.00	
SBP 120-130	4.91 (1.01-23.8)	.048
SBP > 130	7.88 (1.49-41.5)	.015
PA = 0 (nonsmoker)	1.00	
PA 1-20	2.12 (0.46-9.67)	.331
PA > 20	4.48 (0.94-23.4)	.059

NOTES: CI = confidence interval; SBP = systolic blood pressure; PA = pack-year.
a. The odds ratios are adjusted for the other variables in table (multivariate analysis).

as a manifestation of asymptomatic atherosclerosis. The odds ratios (OR) for developing atherosclerotic plaque(s) were first analyzed in an multivariate analysis of the 240 individuals. As shown in Table 3, the major contributors for developing peripheral atherosclerosis were smoking, systolic blood pressure, and the presence of a low HDL-C and high triglyceride lipid profile. The ORs for developing atherosclerotic plaque were then analyzed in an univariate model in cases and controls separately. These data are summarized in Table 4. The likelihood of developing atherosclerotic plaque in controls increased with age, with the presence of high low-density lipoprotein (LDL)–cholesterol, with high systolic blood pressure and smoking. Regarding the cases, the likelihood for developing atherosclerotic plaque was particularly important with aging and smoking.

Table 4. Odds Ratios for Having at Least 1 Plaque According to Several Risk Factors, Separately for Cases and Controls Matched for Age and Gender

	Controls					Cases				
	n[a]	Rate, %	OR	95% CI	*P* Value	n[a]	Rate, %	OR	95% CI	*P* Value
Men	77	44.2	1.00			77	74.0	1.00		
Women	36	44.4	1.02	0.46-2.24	.977	36	66.7	0.70	0.30-1.66	.420
Age 38-49 y	45	28.9	1.00			45	55.6	1.00		
Age 50-54 y	35	57.1	3.28	1.29-8.31	.012	35	74.3	2.31	0.88-6.03	.087
Age 55-65 y	33	51.5	2.61	1.02-6.69	.045	33	90.9	8.00	2.13-30.1	.002
Office SBP < 120	45	33.3	1.00			33	69.7	1.00		
Office SBP 120-130	39	46.2	1.71	0.71-4.15	.232	31	71.0	1.06	0.36-3.11	.911
Office SBP > 130	29	58.6	2.83	1.08-7.43	.034	49	73.5	1.20	0.45-3.20	.709
Office DBP < 75	46	41.3	1.00			46	66.7	1.00		
Office DBP 75-82	34	41.2	0.99	0.40-2.45	.991	34	75.0	1.50	0.51-4.41	.461
Office DBP > 82	33	51.5	1.51	0.61-3.71	.370	33	72.9	1.35	0.51-3.53	.546
TC < 5.3	51	37.3	1.00			18	61.1	1.00		
TC = 5.3-6.2	39	48.7	1.60	0.69-3.73	.276	46	69.6	1.45	0.47-4.53	.518
TC > 6.2	23	52.2	1.84	0.68-4.97	.231	49	77.6	2.20	0.69-7.02	.184
LDL-C < 3.0	41	34.1	1.00			19	57.9	1.00		
LDL-C = 3.0-3.9	34	41.2	1.35	0.53-3.46	.531	32	69.8	1.60	0.49-5.20	.434
LDL-C > 3.9	20	65.0	3.58	1.16-11.0	.026	44	75.0	2.18	0.70-6.80	.179
Glycemia < 5.0	33	45.5	1.00			20		1.00		
Glycemia = 5.0-5.6	55	41.8	0.86	0.36-2.06	.739	53	0.7	0.65	0.20-2.07	.464
Glycemia > 5.6	25	48.0	1.11	0.39-3.14	.847	40	1.1	1.15	0.33-4.03	.829
PA = 0	53	32.1	1.00			37	56.8	1.00		
PA = 1-20	41	48.8	2.02	0.87-4.47	.102	26	76.9	2.54	0.82-7.79	.103
PA > 20	18	72.2	5.51	1.69-17.9	.005	49	81.6	3.38	1.28-8.96	.014
CRP < 1	46	45.5	1.00			26	62.5	1.00		
CRP = 1-3	41	41.0	0.83	0.35-2.00	.685	38	69.4	1.36	0.46-4.05	.577
CRP > 3	20	47.4	1.08	0.37-3.17	.889	43	73.8	1.69	0.58-6.85	.338
Total	113	44.2	1.00			113	71.2	3.19	1.84-5.54	<.001

NOTES: OR = odds ratio; CI = confidence interval; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; LDL-C = low-density lipoprotein–cholesterol; PA = pack-year; CRP = C-reactive protein.
[a]The number of patients in the categories of a given variable may not add up to the total number of cases and controls because of missing values.

Discussion

High blood pressure, smoking, diabetes, and dyslipidemia are established risk factors for atherosclerosis. The metabolic syndrome includes several of these factors and represents an heterogenous disease where several cardiovascular risk factors occur in various degrees and in different combinations in a given individual. It is therefore difficult to evaluate the contribution of a single proatherogenic risk factor in individuals with metabolic syndrome. In an attempt to better characterize metabolic syndrome, a large multicentric study was recently undertaken to evaluate whether the low HDL-C and high triglyceride lipid profile observed in metabolic syndrome is a sensitive marker of genetic homogeneity in subjects with metabolic syndrome. The study was named GEMS (Genetic Epidemiology of Metabolic Syndrome) and was designed as a family-based study to compare 1436 affected subjects with 1672 unaffected individuals.[17] The inclusion criteria to identify cases and controls in GEMS were the ones used in this present study. Using this simple lipid-based criteria, 72% and 88% of the affected participants in the age group of 36 to 54 and ≥ 55 years, respectively, met the criteria of ATPIII definition for metabolic syndrome.[17] In the present study, cases and controls defined by the GEMS criteria were carefully matched for age and gender. The BMI was similar in both groups. Among inclusion criteria, a glycemia < 6 mmol/L was required. Office blood pressures were slightly different in both groups. However, the daytime average ambulatory blood pressures were not different between cases and controls. Thus, these cases and control subjects represented a quite comparable and homogenous group of individuals meeting several criteria of metabolic syndrome, except with regard to the lipid profile. This mode of selection was chosen to better explore the contribution of dyslipidemia as part of the metabolic syndrome to the development of peripheral atherosclerosis.

Since the clinical manifestations of atherosclerosis develop often late in life, we used several surrogate markers for the presence of subclinical atherosclerosis.

High-sensitivity CRP is a recognized biomarker of chronic inflammation.[15] In a large prospective study, it was shown that CRP was a better predictor of the risk of cardiovascular events compared with the LDL-C.[14] The predictive values of 11 atherothrombotic biomarkers for the occurrence of atherosclerosis in the same cohort of 14916 physicians was also reported.[16] Out of the 11 biomarkers, the total cholesterol HDL-C ratio and the hs-CRP were the strongest independent predictors of development of peripheral arterial disease.[16] This has been recently challenged as hs-CRP was found to be only a moderate predictor of coronary heart disease.[18] Hs-CRP levels were shown, however, to correlate with fasting triglycerides and glucose concentrations, obesity, and blood pressure. It seems therefore appropriate to consider hs-CRP as a biomarker of metabolic syndrome. This view is supported by the results of the present study: the mean average of hs-CRP levels were indeed significantly higher in cases compared with controls.

In the present study, we found that subjects with the low-HDL and high triglyceride lipid profile had significantly increased carotid IMT compared with normolipidemic controls. The number of atherosclerotic plaques was also significantly greater in cases than controls. The variable used to define subclinical atherosclerosis was the presence of at least 1 positive site in any of the 4 explored arteries. In a multivariate analysis, the likelihood for developing atherosclerotic plaques was strongly enhanced in cases compared with controls (OR: 4.01; confidence interval [CI]: 1.55-10.4; $P = .004$). In cases, age above 55 years was the strongest risk factor for developing atherosclerotic plaques (OR: 8,0; CI: 2.13-30.1; $P < .002$). In controls, the prevalence of atherosclerotic plaque was much lower and, if present, the plaque was associated with higher levels of LDL-C, smoking, or high systolic blood pressure. Thus, among several criteria of metabolic syndrome, the low HDL-C and high triglyceride lipid profile appeared to be a strong and independent contributor to peripheral atherosclerosis.

The use of B-mode IMT measurements to investigate the determinants of atherosclerosis disease has been validated in many studies, including the Atherosclerosis Risk in Communities Study (ARIC) and the Rotterdam Study.[10,19-23] Solid evidence documented the association between carotid IMT and coronary heart disease, stroke, claudication, and high blood pressure. In the ARIC study, a 0.2-mm increase in carotid IMT was shown to be associated with a 28% increase in relative risk for stroke and 33% increase in relative risk for myocardial infarction. Eric de Groot and co-authors studied the characteristics of arterial walls of subjects with familial hypercholesterolemia (FH) and compared them with

those of age-matched nondyslipidemic subjects.[9] In children at age 10, the IMTs in FH subjects or their controls were similar. However, the progression of IMTs varied considerably with age so that, in average, the IMT increase observed with age was at least twice as large in FH subjects than in controls. As an example, a mean common carotid artery IMT of 0.78 mm was measured in healthy 76-year-old subjects while the same value was already observed at the age of 40 y in subjects with FH. Considering our data, the average IMT of 0.61 mm in the controls matched approximately an average age-adjusted IMT for healthy 45-year-old individuals.[9] On the other hand, the average IMT of 0.66 mm measured in the cases corresponds approximately to the IMT measured in 55-year-old normolipidemic individuals.[9]

As a surrogate marker of subclinical atherosclerosis, we also evaluated endothelial function in a subset of individuals with or without atherogenic dyslipidemia. We used a laser Doppler imaging system to measure skin blood flow in the forearm. The device was established as a highly reproducible tool to evaluate in the forearm the postischemic hyperemia as well as the vasodilation induced by local warming of the skin, responses that are thought to be mediated mainly by the endothelium.[13] We found that the cases have a blunted thermal-induced vasodilatation as well as a reduced reactive hyperemia compared with age-gender-BMI–matched individuals with a favorable lipid profile. These data are in accordance with the accumulating evidence suggesting that endothelial dysfunction might be an early marker for subclinical atherosclerosis.[11] Our subjects were overweight, and previously published data demonstrated that obesity, with or without insulin resistance, has no effect per se on endothelial function measured in the forearm.[24] So the impaired-endothelial vasodilatation observed in our cases compared with their discordant controls is most likely the result of the low HDL and high triglyceride lipid profile. These results are in agreement with a recent observation of R J Biosendial and coauthors.[25] They studied endothelial function in 9 subjects with isolated low HDL-C linked to heterozygote carries of the ABCA1 mutations. In these individuals, they measured an impaired vasodilatory effect of the endothelium, which was corrected after a single, rapid infusion of apolipoprotein A-I/phosphatidylcholine discs (apoA-I/PC).

Taken together, these data support the conclusion that the low-HDL-C and high triglyceride lipid profile is a strong risk factor for developing peripheral atherosclerosis. This lipid profile is associated with an abnormal endothelial function and an increase in IMT compared to individuals with high HDL-C and low triglycerides levels. Although the low HDL-C is frequently associated with hypertriglyceridemia, our data extend the potential role of HDL particles as potent antiatherogenic agents.[26-28]

Acknowledgment

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Glucose-Induced Insulin Secretion in Dyslipidemic and Normolipidemic Patients With Normal Glucose Tolerance

CHRISTOPHE BINNERT, PHD[1]
MYRIAM GENOUD, MD[2]
GÉRALD SEEMATTER, MD[1]
ASSIA FEKIRINI, MD[1]
VINCENT MOOSER, MD, PHD[3]
GÉRARD WAEBER, MD, PHD[2]
LUC TAPPY, MD[1,4]

The metabolic syndrome is highly prevalent in industrialized countries, where it represents a major public health burden due to the associated high risk of diabetes and cardiovascular disorders (1,2). High plasma triglyceride and low HDL cholesterol are prominent features of this syndrome (2,3). Large epidemiological studies have shown associations with both insulin resistance and low insulin secretion (4). The aim of this study was to evaluate whether the alterations of blood lipid, observed in the metabolic syndrome, are associated with alterations of insulin sensitivity or secretion in subjects with normal glucose tolerance. Glucose homeostasis was evaluated during a two-step hyperglycemic clamp in a group of dyslipidemic patients and in a group of normolipemic subjects of similar age and body weight. Subjects were studied after short-term administration of dexamethasone to evaluate reciprocal changes in insulin secretion and insulin sensitivity (5–8).

RESEARCH DESIGN AND METHODS — Twenty dyslipidemic subjects (13 men and 7 women) with BMI >27 kg/m^2 but having normal glucose tolerance, as documented by a standard oral glucose tolerance test (fasting glycemia 94 ± 7 mg/dl, 2-h glycemia 95 ± 17 mg/dl) (9), were selected for the study. They had a mean (±SD) age of 46.6 ± 7.6 years, body weight of 85.4 ± 10.3 kg, height of 173.2 ± 9.2 cm, BMI of 28.6 ± 4.1 kg/m^2, waist circumference of 98.9 ± 11.0 cm, waist-to-hip ratio of 0.91 ± 0.08, fasting plasma triglyceride concentration of 2.25 ± 0.84 mmol/l, fasting total plasma cholesterol concentration of 5.66 ± 1.04 mmol/l, fasting HDL cholesterol concentration of 1.06 ± 0.11 mmol/l, and blood pressure of 123 ± 13/78 ± 8 mmHg. They were compared with a group of 20 normolipemic subjects (18 men and 2 women) with normal glucose tolerance (fasting glycemia 96 ± 7 mg/dl, 2-h glycemia 95 ± 22 mg/dl) and similar age (52.6 ± 6.3 years), body weight (91.8 ± 17.2 kg), height (176 ± 10 cm), BMI (29.6 ± 3.9), waist circumference (99.6 ± 12.2 cm), waist-to-hip ratio (0.91 ± 0.07), and blood pressure (126 ± 11/80 ± 7 mmHg) but had normal triglyceride (0.80 ± 0.21 mmol/l), total cholesterol (5.03 ± 0.91 mmol/l), and HDL cholesterol (1.71 ± 0.27 mmol/l) concentrations. All subjects were recruited from the GEMS (Genetic Epidemiology of Metabolic Syndrome) project study population (10). Fourteen dyslipidemic and no normolipemic patients fulfilled the criteria for the metabolic syndrome (2). Six dyslipidemic and three normolipemic subjects were receiving treatment for high blood pressure, and two dyslipidemic patients were being treated with statins.

Each participant took part in a two-step hyperglycemic clamp (target glycemia of 135 mg/dl during 60 min and of 180 mg/dl during the next 60 min) to simultaneously evaluate glucose-induced insulin secretion and glucose/insulin-mediated glucose disposal. Participants received 2 mg/day dexamethasone during the 2 days preceding the clamp procedure and 0.5 mg on the morning of the procedure. [6,6-$^2H_2$]glucose (bolus 2 mg/kg, continuous infusion 20 μg/kg/min for 60 min before the clamp, then exogenous glucose labeled with 1.25% [6,6-$^2H_2$] glucose [hot infusate approach] [11]) was used to calculate whole-body glucose kinetics. The results obtained were compared by means of unpaired *t* tests.

RESULTS — At their inclusion in the study, dyslipidemic and normolipemic subjects had similar fasting plasma glucose (94 ± 7 vs. 97 ± 7 mg/dl) and insulin (31.5 ± 4.9 vs. 31.7 ± 7.4 mU/l). After administration of dexamethasone for 2 days (Table 1), dyslipidemic subjects had fasting glucose and insulin concentrations that were not significantly different from those of normolipemic subjects. During the clamp procedure, similar rates of exogenous glucose infusion were necessary to maintain target glycemia (Table 1). However, the rates of exogenous infusion divided by steady-state plasma insulin concentration were lower in dyslipidemic than in normolipemic subjects, indicating impaired glucose/insulin-induced glucose metabolism. Compared with normolipemic subjects, dyslipidemic subjects had 41 and 74% higher plasma insulin concentrations during the low and high plateaus of glycemia, respectively. Basal endogenous glucose production and its suppression at both plateaus of glycemia were similar in dyslipidemic and normolipemic subjects.

CONCLUSIONS — This study focused on dyslipidemic patients free of any disorder of glucose homeostasis. Such pa-

From the [1]Department of Physiology, Medical School, University of Lausanne, Lausanne, Switzerland; the [2]Department of Internal Medicine, Lausanne University Hospital, Lausanne, Switzerland; [3]GlaxoSmithKline, Collegeville, Pennsylvania; and the [4]Division of Endocrinology, Diabetes and Metabolism, Lausanne University Hospital, Lausanne, Switzerland.

Address correspondence and reprint requests to Prof. L. Tappy, Département de physiologie, 7 rue du Bugnon, 1005 Lausanne, Switzerland. E-mail: luc.tappy@unil.ch.

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Parameters of glucose metabolism during the two-step hyperglycemic clamp procedure in dyslipidemic and normolipemic overweight subjects with normal glucose tolerance*

	Dyslipidemic	Normolipemic	*P*
n	20	20	
Plasma glucose (mg/dl)			
Basal	108.9 ± 17.6	105.6 ± 8.3	0.49
Clamp first plateau	133.6 ± 3.9	136 ± 3.4	0.06
Clamp second plateau	179.1 ± 5.5	182 ± 6.7	0.10
Plasma insulin (mU/l)			
Basal	28.3 ± 13.9	20.9 ± 12.3	0.09
Clamp first plateau	60.1 ± 28.8	42.6 ± 25.4	0.05
Clamp second plateau	157.2 ± 70.7	90.3 ± 45.5	0.002
Glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)			
Clamp first plateau	1.08 ± 0.61	1.25 ± 0.70	0.43
Clamp second plateau	3.23 ± 1.30	2.83 ± 0.99	0.33
Glucose infusion rate: insulin ($mg \cdot l \cdot kg^{-1} \cdot min^{-1} \cdot mU^{-1}$)			
Clamp first plateau*	0.023 ± 0.016	0.065 ± 0.095	0.05
Clamp second plateau†	0.027 ± 0.018	0.049 ± 0.041	0.03
Endogenous glucose production ($mg \cdot kg^{-1} \cdot min^{-1}$)			
Basal	1.9 ± 0.4	1.9 ± 0.6	0.84
Clamp first plateau	0.7 ± 0.3	0.5 ± 0.3	0.08
Clamp second plateau	0.5 ± 0.2	0.4 ± 0.3	0.22

Data are means ± 1 SD. *Values at the end of each plateau; †values averaged during the last 30 min of the clamp at each plateau of glycemia.

tients offer the possibility to investigate the contribution of insulin sensitivity and/or secretion in the pathogenesis of dyslipidemia. Glucose homeostasis was evaluated by a two-step hyperglycemic clamp after administration of dexamethasone, a procedure aimed at decreasing insulin sensitivity with compensatory hyperinsulinemia. The rate of exogenous glucose infusion divided by plasma insulin concentration was lower in dyslipidemic than in normolipemic subjects, indicating a lower insulin- and glucose-induced glucose disposal. Although this protocol was not performed without dexamethasone administration, these results are consistent with some degree of insulin resistance in dyslipidemic subjects. Dyslipidemic subjects also had significantly higher plasma insulin concentration at each plateau of glycemia, consistent with impaired insulin sensitivity, but this also indicates that their glucose-induced insulin secretion was not decreased. This hyperinsulinemia cannot be attributed to differences in BMI or body fat distribution, since dyslipidemic and normolipemic patients had similar characteristics. Since both groups had similar plasma glucose and insulin concentrations before dexamethasone, we propose that short-term dexamethasone administration offers a way to detect subclinical insulin resistance in dyslipidemic patients.

In conclusion, our results indicate that dyslipidemic, glucose-tolerant patients have increased glucose-induced insulin secretion to compensate for impaired insulin sensitivity. We propose that these alterations are indicative of insulin resistance in dyslipidemic patients. The chronic hyperinsulinemia consecutive to insulin resistance may possibly contribute to increase plasma triglyceride concentrations by stimulation of hepatic de novo lipogenesis and secretion of VLDL lipoproteins (12,13). Conversely, it is also possible that primary alterations of hepatic lipid metabolism, leading to hypertriglyceridemia and low HDL levels may in the long-term contribute to the development of insulin resistance and hyperinsulinemia (14,15). Only prospective studies in individuals at risk for the metabolic syndrome will provide clarification between these two hypotheses.

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Ambulatory blood pressure monitoring: a mean to stratify cardiovascular risk

Bernard Waeber[a], Myriam Genoud[b], François Feihl[a], Daniel Hayoz[c] and Gérard Waeber[b]

Objective **Current hypertension guidelines stress the importance to assess total cardiovascular risk but do not describe precisely how to use ambulatory blood pressures in the cardiovascular risk stratification.**

Method **We calculated here global cardiovascular risk according to 2003 European Society of Hypertension/ European Society of Cardiology guidelines in 127 patients in whom daytime ambulatory blood pressures were recorded and carotid/femoral ultrasonography performed.**

Results **The presence of ambulatory blood pressures ≥ 135/85 mmHg shifted cardiovascular risk to higher categories, as did the presence of hypercholesterolemia and, even more so, the presence of atherosclerotic plaques.**

Conclusion **Further studies are, however, needed to define the position of ambulatory blood pressures in the assessment of cardiovascular risk.** ***Blood Press Monit*** **12:263–265 © 2007 Lippincott Williams & Wilkins.**

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Keywords: ambulatory blood pressure monitoring, cardiovascular risk, carotid ultrasonography, hypercholesterolemia, hypertension

[a]Division of Clinical Pathophysiology, [b]Department of Medicine and [c]Division of Angiology, University Hospital, Lausanne, Switzerland

Correspondence to Professor Bernard Waeber, MD, Division of Clinical Pathophysiology, CHUV, University Hospital, MP14/214, CH-1011 Lausanne, Switzerland
Tel: +41 21 314 07 60; fax: +41 21 314 25 18;
e-mail: Bernard.Waeber@chuv.ch

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Introduction

The usefulness of ambulatory blood pressure monitoring (ABPM) in the diagnosis and the management of hypertension is now well recognized [1–3]. Blood pressures recorded during everyday activities provide a better estimate of cardiovascular (CV) risk than blood pressure readings obtained conventionally in a medical setting [4]. Current hypertension guidelines recommend operational thresholds for ambulatory blood pressure readings (ABPs). For instance, The Working Group on Blood Pressure Monitoring of the European Society of Hypertension proposes to consider as normal ABPs < 135/85 mmHg during daytime and < 120/70 mmHg during night-time [3]. Similar limits were set by experts in the USA [2].

For many years, management strategies have been based on the presence or absence of individual risk factors. Typically, this was the case for hypertension. More recently, the rationale for a multifactorial CV risk assessment approach became apparent [5,6]. The aim is, by determining the individual's absolute CV risk, to better target the therapeutic interventions and, thereby, to render them more beneficial and more cost-effective. The latest hypertension guidelines made available by the European Society of Hypertension and the European Society of Cardiology have adopted the global risk assessment approach [1]. According to these recommendations, the global CV risk of a given individual can be calculated by taking into account various parameters, including the level of blood pressure *per se*, the presence or absence of additional risk factors, target organ damage or associated clinical conditions. This study was undertaken to assess how global CV risk would be influenced by using ABPs in its calculation. The study was performed by giving to elevated daytime ABPs the same potential adverse impact as the presence of either a CV risk factor or a target organ damage.

Participants and methods

A total of 127 participants [91 men, aged 49.7 ± 6.1 years (mean ± SD), and 36 women, aged 50.4 ± 5.7 years] were included. They were all participants to an international study designed to explore the genetic basis of the metabolic syndrome and its constituent phenotypes [7]. They were selected for this analysis as each of them underwent a daytime ABPM and an ultrasonographic examination of carotid and femoral arteries. Blood samples for measurement of fasting blood glucose and lipid concentrations were also obtained from all these participants. The study protocol was approved by our institutional ethics committee and an informed written consent was obtained from each participant.

1359-5237

Clinic BPs were measured in the sitting position by an experienced nurse using an electronic oscillometric device (OMRON 907, OMRON Healthcare Europe, Hoofddrop, The Netherlands). Three readings were taken at 2-min intervals and the average of the last two was used for analysis. ABPs was recorded every 15 min during daytime over a 12-h period using the TM-2420 Boso (Bosch and Sohn, Jungingen, Germany) device. The presence of peripheral atherosclerosis was assessed using a B-mode high-resolution imaging of both carotid and femoral arteries. The ultrasound evaluation was performed by a single operator. An atherosclerotic plaque was defined as the presence of an encroachment of the arterial wall into the lumen ≥ 1.2 mm.

The global CV risk of the 127 participants was estimated using the stratification proposed by the 2003 European Society of Hypertension/European Society of Cardiology (ESH/ESC) guidelines [1]. Beyond the levels of clinic systolic and diastolic blood pressure the following parameters were taken into account; total cholesterol as a risk factor if > 6.5 mmol/l (model 1); daytime ABPs $\geq 135/85$ mmHg considered as a risk factor (model 2); presence of atherosclerotic plaque(s) as a target organ damage (model 3); daytime ABPs $\geq 135/85$ mmHg as a determinant of CV risk, assumed to have the same importance as the presence of target organ damage (model 4). According to the 2003 ESH/ESC guidelines, there exists four risk categories indicating an approximate absolute 10-year risk of CV disease of either less than 15% (low added risk), 15–20% (moderate added risk), 20–30% (high added risk) or above 30% (very high added risk).

Results

Table 1 shows the number of patients in each category of CV risk according to the different models used for stratification.

Table 1 Number of patients in each category of global cardiovascular (CV) risk according to the different models used for stratification

	CV risk			
Stratification based on	Average	Low	Moderate	High
(a) Clinic BPs	97	28	2	0
(b) Clinic BPs + Total cholesterol >6.5 mmol/l (model 1)	80	34	13	0
Daytime ABPs ≥ 135/85 mmHg[a] (model 2)	67	33	27	0
(c) Clinic BPs + Atherosclerosis (model 3)	43	9	35	40
Daytime ABPs ≥ 135/85 mmHg[b] (model 4)	67	3	14	43

[a]In attributing to increased ABPs the same impact on CV risk as any independent CV risk factor. [b]In attributing to increased ABPs the same impact on CV risk as the presence of any target organ damage. BP, blood pressure; ABP, ambulatory blood pressure.

Taking into account only clinic BPs most participants exhibited an average or a low-added risk. A modest but clear-cut shift to higher risk categories was observed when the presence of hypercholesterolemia was included in the risk stratification (model 1).

A similar shift to higher-risk categories was observed by considering high daytime ABPs as an independent CV risk factor (model 2). The adverse impact of high ABPs appeared more important than that of hypercholesterolemia. Indeed a greater number of participants was present in the moderate added risk category when using high ABPs ($n = 27$) than high cholesterol levels for risk stratification ($n = 13$). Notably, no patient was found at high risk regardless of the model used for stratification.

Taking atherosclerosis as a marker of target organ damage (model 3) increased markedly the risk status. A large number of participants ($n = 40$) showed a high-added risk when including this parameter in the global risk calculation.

When high daytime ABPs were assumed to be a load over all CV risk for the same degree as the presence of atherosclerosis (model 4), there was also a major shift of the risk distribution to higher categories. This was manifested by the large number of participants ($n = 43$) included in the high-added risk class.

Discussion

ABPM has proven very useful in the evaluation of hypertensive patients as it better reflects CV risk than BPs measured conventionally by doctors [4]. The indication of ABPM in patients with suspected 'white-coat' hypertension (also called isolated office hypertension) is now widely accepted [1,2] and a recent analysis has confirmed the cost-effectiveness of this technique [8]. The interpretation of blood pressure profiles recorded away from the medical environment is, however, still debated. A major issue is whether it is justified or not to keep a patient untreated if he or she repeatedly exhibits high office BPs, but normal BPs during everyday activities. Thus, 'white-coat' hypertension may not be totally innocent: (i) it seems to be linked with an increased probability to develop sustained hypertension, regardless of the setting of BP measurement; and (ii) it might be associated with an heightened risk of suffering target organ damage compared with individuals having normal conventional BPs [9].

Today global risk assessment is strongly recommended to avoid overtreatment in patients who have a low CV risk and, conversely, undertreatment in patients with high CV risk [10]. It appears therefore attractive to include ABPM in the stratification of CV risk. This was performed in this study in participants to a survey in which a number of investigations were performed, including ABPM, lipid

profile determination and examination of carotid and femoral arteries by ultrasonography. This allowed the calculation of global CV risk of these participants, using the framework proposed in the 2003 ESH/ESC guidelines [1]. Different models were tested for incorporating ABPs into this framework, by assigning it the same loading on global risk assessment as either hypercholesterolemia or presence of target organ damage (atherosclerotic plaques). The confirmation of hypertension by ABPM shifted CV risk to higher categories. This was particularly true when ABPs were given the same importance as target organ damage. Notably, there was no high-risk patient by taking into account only clinic BPs and total cholesterol values. Similar observations were made by attributing to high ABPs the same deleterious impact as hypercholesterolemia. In contrast, the estimation of risk based on clinic BPs and the presence of either atherosclerotic plaques (without consideration of ABPs) or high ABPs (without consideration of atherosclerotic plaques) gave comparable results, each attributing approximately one-third of the studied participants to the high risk class.

These observations support the view that abnormally elevated daytime ABPs contribute importantly to global CV risk. The value of ABPM in stratifying CV risk, however, remains to be verified in prospective trials.

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