Erythrocyte Membrane Phospholipid Composition Is Related to Hyperinsulinemia in Obese Nondiabetic Women: Effects of Weight Loss

Mohamed Younsi, Didier Quilliot, Nehmann Al-Makdissy, Irène Delbachian, Pierre Drouin, Mireille Donner, and Olivier Ziegler

The complex mechanisms by which obesity predisposes to insulin resistance are not clearly understood. According to a cell membrane hypothesis of insulin resistance, the defects in insulin action could be related to changes in membrane properties. The purpose of this work was to examine the relationship between 2 markers of insulin resistance (fasting plasma insulin [FPI] and homeostasis model assessment [HOMA IR]) and erythrocyte membrane lipid composition. In the first cross-sectional study, 24 premenopausal nondiabetic overweight women (body mass index [BMI], 32.5 ± 0.9 kg/m^2; age, 35.7 ± 2.2 years) were compared to 21 lean healthy women (BMI, 21 ± 0.4 kg/ m^2; age, 35.4 ± 2.2 years). The second study examined whether a 3-month diet-induced weight loss, which usually improves insulin resistance, could also affect the membrane phospholipid (PL) composition and fluidity in the overweight group. Overweight women had significantly higher FPI levels ($P < .0001$), HOMA IR ($P < .0001$), membrane sphingomyelin (SM) ($P < .05$), and cholesterol ($P < .05$) contents than lean women. Baseline FPI and HOMA IR were positively correlated with membrane SM ($P < .005$), phosphatidylethanolamine (PE) ($P < .005$), and phosphatidylcholine (PC) ($P < .05$) contents, and negatively with phosphatidylinositol (PI) ($P < .05$) contents in the whole population. Multivariate regression analyses showed that 2 membrane parameters, PE and SM, were among the independent predictors of FPI or HOMA IR in the whole population, but also in the lean and the obese groups separately. Intervention induced a significant reduction in body weight (-5.7% ± 0.7%), fat mass (-11.3% ± 1.4%), and FPI (-10.2% ± 5.4%). An improvement in membrane lipid composition was only observed in the insulin resistant subgroup (FPI > 9.55 mU/L). The reduction in FPI or HOMA IR was directly associated with reduction in SM and PE contents, a finding independent of the reduction in fat mass. A stepwise multiple regression analysis indicated that the changes in SM accounted for 26.6% of the variance in the changes in FPI as an independent predictor, with the changes in fat mass and PE as other determinants (27.8% and 20%, respectively, adjusted $r^2 = .704$, $P < .0001$). These results suggest that the abnormalities in the membrane PL composition could be included in the unfavorable lipid constellation of obesity which correlated with impaired insulin sensitivity.

THE RELATIONSHIP between obesity and insulin resistance has been known since about 40 years,[1] but the complex mechanisms by which obesity predisposes to insulin resistance remain poorly understood.[2] Central obesity is now considered as a cardinal feature of the metabolic syndrome, a clustering of clinical and metabolic abnormalities, including insulin resistance and hyperinsulinemia, impaired glucose tolerance or type 2 diabetes mellitus, dyslipidemia (hypertriglyceridemia and low high-density lipoprotein [HDL]-cholesterol level), and hypertension, driving cardiovascular diseases.[3-5]

According to a cell membrane hypothesis of insulin resistance, insulin action could be related to changes in cell membrane properties. Numerous studies have shown that the fatty acid composition of adipose tissue, skeletal muscle, or liver phospholipids (PLs) was closely associated with insulin action in vitro or in vivo, in animals and in humans.[6-10] Changes in membrane dynamic properties can also modulate the function of membrane proteins mediating insulin action.[11-15] Less attention has been paid to the influence of PL classes distribution in the cell membrane on insulin action. We have previously shown that changes in the PL composition of the erythrocyte membrane in obese nondiabetic normoglycaemic women were associated with 2 markers of insulin resistance. There was a positive correlation between fasting plasma insulin (FPI) and homeostasis model assessment of insulin resistance (HOMA IR) and the sphingomyelin (SM) content in the erythrocyte.[16] The same relationship has been shown for human adipocyte membranes.[17]

These results suggest that PL abnormalities could be a component of the metabolic syndrome at least in obese subjects. As insulin resistance and metabolic syndrome abnormalities are usually improved after a diet-induced weight loss in obese subjects,[18,19] we hypothetized that plasma membrane PL composition could also be influenced by weight loss. To the best of our knowledge, no information is available about how a weight loss affects the structural properties of cell membranes. This work examined the relationship between insulin resistance markers and the erythrocyte membrane PL composition both in a cross-sectional comparison of normal-weight adult women and overweight women with varying degrees of hyperinsulinemia, and in a weight loss intervention.

MATERIALS AND METHODS

Subject Characteristics

Twenty-four overweight women (age, 35.7 ± 2.2 years; body mass index [BMI], 32.5 ± 0.9 kg/m^2) and 21 normal-weight women (age, 35.4 ± 2.2 years; BMI, 21 ± 0.4 kg/m^2, all with BMI < 25) participated in the study. Overweight was defined as BMI ≥ 25 kg/ m^2, as

From the Laboratory of Nutrition and Metabolic Diseases, Medical School, Université Henri-Poincaré (UHP),Vandoeuvre les Nancy, France; and the Centre d'Investigations Cliniques INSERM-CHU, Hôpital Jeanne d'Arc, Dommartin lès Toul, France.

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Address reprint requests to Mohamed Younsi, PhD, Laboratoire de Nutrition et Maladies Métaboliques, Faculté de Médecine, BP 184, 54505, Vandoeuvre les Nancy, France.

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recommended by the World Health Organization (WHO).[18] Lean women were recruited by advertising at the Nancy University Hospital and overweight women from the outpatient clinic of the nutrition department of this hospital. No subject was taking any medication that could influence the lipid profile, glucose tolerance, or membrane fluidity,[20] and all were considered to be healthy with the exception of obesity after a comprehensive medical examination. All subjects had been weight-stable for more than 2 months before the study. All women were premenopausal. Exclusion criteria were alcoholism, diabetes mellitus, overt hyperlipidemia (cholesterol $>$ 7.8 mmol/L or triglyceride $>$ 4.6 mmol/L), binge eating disorder or bulimia nervosa, and significant depression. Written informed consent was obtained from each person before the study, and the study protocol was approved by the regional ethics committee.

Clinical Studies

Anthropometric measurement. Body weight was measured on an electronic weighing scale calibrated to 0.1 kg. Barefoot standing height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. BMI was calculated as weight (kg) divided by height squared (m^2). Circumference measurements were obtained with the subject in the standing position. The waist was measured at the midpoint between the lower rib margin and the iliac crests. The hip was defined as the widest circumference over the great trochanters. Waist circumference and the waist-to-hip circumference ratio (WHR) were used as a measure of upper-body adiposity. Whole body fat mass and fat-free mass were determined by dual-energy x-ray absorptiometry (DEXA) (model DPX I Q, Lunar, Madison, WI, program 1.3). Obese patients participated in a 3-month weight reduction program that combines moderate caloric restriction with the setting of realistic weight goal (ie, a 5% to 10 % weight loss), behavior modification and regular physical activity. The WHO equation[21] was used to estimate each patient's basal energy expenditure and an activity factor to estimate total caloric requirement (basal energy expenditure $\times$ 1.5). Only overweight women had been concerned by the hypocaloric diet. Patients were asked to reduce this intake by 600 kcal (20% to 30%). Carbohydrate, fat, and protein represented 50%, 30%, and 20%, respectively, of the total energy intake. All participants were free-living and consumed self-selected food. DEXA and laboratory analyses were performed within 5 days of the end of the menstrual cycle.

Biochemical determinations. Blood samples were taken from an antecubital vein in the morning after a 12-hour overnight fast. Fasting blood glucose was measured by the glucose oxidase method with a Beckman BGA II Glucose Analyser (Beckman Instruments, Fullerton, CA). Plasma triglycerides and cholesterol were measured enzymatically (kits from Boehringer, Mannheim, Germany). HDL-cholesterol in the plasma was determined after precipitation of very-low-density (VLDL) and low-density lipoproteins (LDL) with phosphotungstic acid. LDL-cholesterol was estimated using Friedewald's formula. Plasma insulin was determined by immunoassay (Insulin IMX, Abbott Laboratories, Tokyo, Japan). The intra-assay coefficient of variation was 4% and the interassay coefficient was 6%. Cross-reactivity with proinsulin was .05%. Fasting plasma insulin and homeostasis model assessment of insulin resistance (HOMA IR) were chosen as insulin resistance markers. HOMA IR value, which is the result of a mathematical model of the glucose-insulin interactions, has been correlated strongly with insulin resistance.[22] The HOMA IR was assessed by the formula: (fasting insulin [mU/L] $\times$ fasting glucose [mmol/L])/22.5.[23] It was based on 2 determinations of glucose and insulin levels from 2 blood samples taken within 5 minutes.

Membrane Studies

Isolation of erythrocyte membranes. Venous blood samples were collected over EDTA (1 mg/mL) from fasting subjects. Blood samples were washed twice with 30 mL of 0.9% NaCl to eliminate plasma, leukocytes, and platelets. The erythrocyte pellets were resuspended in 10 vol of hemolysis buffer (1 mmol/L Tris-HCl, 1 mmol/L EDTA, 10 mmol/L NaCl, pH 7.2) and shaken at 4°C for 30 minutes. The suspension was then centrifuged at 20,000 $\times$ *g* for 10 minutes. The supernatant was discarded and the packed cell membranes resuspended in haemolysis buffer. The same procedure was repeated until perfectly white ghosts were obtained (4 at 5 times). Membrane proteins were determined by the method of Lowry et al.[24]

Fluorescence anisotropy determinations. Isolated membranes (50 μg of proteins/mL) were labeled by adding 0.5 μL of 1,6-diphenyl-1,3,5-hexatriene (DPH) (final dilution of probe, 10^{-6} mol/L). Incubation was carried out during 30 minutes in the dark. Membrane fluidity was determined by steady-state fluorescence polarization, using a continuous excitation instrument (Fluofluidimeter, AFFIBIO, Villers-les-Nancy, France).[16] Nonlabeled membrane suspensions were measured before each series of experiments to evaluate the corrections for light scattering. The vertical IbII and the horizontal Ib$_\perp$ components for the "blank" (nonlabeled) sample were measured, followed by the corresponding fluorescence intensities IfII and If$_\perp$ of the labeled sample. Fluorescence steady-state anisotropy *r*, which is inversely related to membrane fluidity, was determined according to the relationships:

$$r = ([\text{IfII-IbII}]\text{-}[\text{If}_\perp\text{-Ib}_\perp])/([\text{IfII-IbII}] + 2[\text{If}_\perp\text{-Ib}_\perp]).$$

Membrane cholesterol and phospholipid determinations. Lipids were extracted from erythrocyte membrane with methanol and chloroform (vol/vol, 11/7) according to the method of Rose and Oklander.[25] The volume of membrane suspension corresponded to 500 μg proteins for optimal extraction. The organic phase was evaporated to dryness under a stream of nitrogen at room temperature. The lipid residue was dissolved in chloroform, and its components were separated by high-performance liquid chromatography (HPLC) on a Gold liquid chromatographic system equipped with a Model 126 pump and a Model 406 interface (Beckman, Palo Alto, CA) monitored by an IBM microcomputer (IBM, Courbevoie, France).[26] Lysophosphatidylcholine (20 μg/100 μg proteins) was added as internal standard before extraction. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and sphingomyelin (SM) were detected with an evaporative light scattering detector (SEDERE, Orleans, France). Phospholipid data were expressed as a weight concentration calculated from calibration curves established for each phospholipid. Membrane cholesterol was determined by the method of Zlatkis and Zak.[27]

Statistical Analysis

Data are presented as means $\pm$ SEM or medians with 25th and 75th percentiles. Variables were assessed for normality by the skewness and kurtosis test. Skewed data were logarithmically transformed for statistical analysis and then back-transformed to their natural units for presentation in the tables. Differences between groups were determined using 1-way analysis of variance (ANOVA). The paired and 2-sample *t* tests were used for comparison between the baseline and 3-month data and between lean and obese groups, respectively. Regression analysis was used to determine the relationships between baseline insulin resistance markers and other parameters and the relationship between the changes of these variables after weight loss. The independence and significance of variables was tested by multiple regression analysis or by stepwise multiple regression analysis based on results of the bivariate correlations. The significance level of *P* values was set at 5%. All calculations were performed using Statview software (Abacus Concepts, Berkeley, CA).

Table 1. Clinical and Metabolic Data for Lean Women, All Overweight Women, and the Overweight Women With FPI Less Than 9.55 mU/L (subgroup A), or FPI Greater Than 9.55 mU/L (subgroup B)

	Lean Women (n = 21)	All Overweight Women (n = 24)	Subgroup A (n = 9)	Subgroup B (n = 15)	ANOVA *P*
Age (yr)	35.4 ± 2.2	35.7 ± 2.2	40.9 ± 1.2	32.6 ± 3.3	NS
BMI (kg/m^2)	21.0 ± 0.4	32.5 ± 0.9[f]	31.1 ± 0.7[f]	33.3 ± 1.3[f]	<.0001
Waist circumference (cm)	71.0 ± 1.2	95.4 ± 1.4[f]	93.8 ± 1.7[f]	96.5 ± 2.1[f]	<.0001
WHR	0.76 ± 0.01	0.83 ± 0.01[f]	0.84 ± 0.03[e]	0.82 ± 0.01[e]	<.0005
Body fat (%)	24.6 ± 1.4	45.2 ± 1.0[f]	44.0 ± 1.3[f]	45.9.2 ± 1.4[f]	<.0001
Fasting plasma glucose (mmol/L)	4.77 ± 0.13	5.14 ± 0.11[a]	5.24 ± 0.16	5.08 ± 0.15	NS
Fasting plasma insulin (mU/L)	4.6 (4.1-6.0)	10.6 ± 0.7[f]	6.9 ± 0.4[a]	12.8 ± 0.6[f]†	<.0001
HOMA value	1.0 ± 0.1	2.4 ± 0.2[f]	1.6 ± 0.1[a]	2.9 ± 0.1[f]†	<.0001
Triglycerides (mmol/L)	0.73 ± 0.06	1.11 (0.72-1.52)[d]	1.27 ± 0.23[c]	1.16 (0.65-1.46)[a]	<.005
Total cholesterol (mmol/L)	4.82 ± 0.15	5.42 ± 0.20[a]	5.89 ± 0.35[c]	5.07 ± 0.18*	<.005
HDL-cholesterol (g/L)	0.67 ± 0.03	0.54 ± 0.03[b]	0.58 ± 0.03	0.52 ± 0.04[b]	<.05
LDL-cholesterol (g/L)	1.04 ± 0.05	1.30 ± 0.07[b]	1.46 ± 0.12[c]	1.21 ± 0.08	<.005

NOTE. Data are presented as mean ± SEM or medians with 25th and 75th percentiles (data that follow a log normal distribution). ANOVA: lean women *v* subgroup A *v* subgroup B.

[a]$P < .05$, [b]$P < .01$, [c]$P < .005$, [d]$P < .001$, [e]$P < .0005$, [f]$P < .0001$ *v* lean women.

*$P < .05$, †$P < .0001$, overweight women with FPI >9.55 *v* overweight women with FPI <9.55.

RESULTS

Baseline

The clinical and metabolic characteristics of the 21 lean and the 24 overweight women are listed in Table 1. The BMI of the 21 lean women ranged from 17.6 to 24 kg/m^2, percent fat mass from 14.8% to 35.5%, fasting plasma insulin from 2.15 to 8.55 mU/L, and HOMA IR from 0.4 to 2.6. The BMI of the 24 overweight women ranged from 25 to 41.1 kg/m^2, percent fat mass from 38.2% to 55.2%, fasting insulin from 4.6 to 17 mU/L, and HOMA IR from 1.1 to 3.7. The 24 overweight women had significantly higher BMI ($P < .0001$), waist circumference ($P < .0001$), WHR ($P < .0001$), percent fat mass ($P < .0001$), fasting plasma glucose ($P < .05$), FPI ($P < .0001$), HOMA IR ($P < .0001$), triglycerides ($P < .001$), total cholesterol ($P < .05$), and LDL-cholesterol ($P < .01$) than the lean subjects, but they had significantly lower HDL-cholesterol concentrations ($P < .01$).

To further evaluate the effect of differences in the degree of baseline hyperinsulinemia on membrane composition, the 24 overweight subjects were divided into 2 groups on the basis of their baseline FPI, according to the cutoff of a FPI greater than the mean FPI + 2 SD measured in the control group. The 2 groups formed in this manner were arbitrarily defined as being insulin-sensitive (subgroup A, FPI < 9.55 mU/L; 9 subjects) and insulin-resistant (subgroup B, FPI > 9.55 mU/L; 15 subjects). FPI and HOMA IR in subgroup B were significantly higher than in subgroup A ($P < .0001$). Subgroup B had significantly lower total cholesterol ($P < .05$) (Table 1).

The mean values of red cell membrane cholesterol, the 5 classes of PLs, and the cholesterol/PL (Chol/PL) ratio are shown in Table 2. The entire group of overweight women had significantly higher membrane cholesterol and SM contents than the lean women ($P < .05$). They had also a lower membrane PI content ($P < .05$). The overweight women in subgroup A had significantly higher cholesterol membrane content ($P < .05$) and Chol/PL ratio ($P < .05$) than the lean women. In contrast, the hyperinsulinemic women (subgroup B) had significantly higher membrane SM ($P < .05$) and lower PI ($P < .05$) contents than the lean women. They had higher PE contents than the lean women ($P < .05$) and the women in subgroup A ($P < .01$). They had also higher total PL than the lean women ($P < .05$) and the women in subgroup A ($P < .005$).

A correlation analysis between FPI and cell membrane composition parameters was performed in the whole study population. In fact, 9 of 21 lean women had a percent fat mass above 25%. There was also a striking overlap for FPI between the lean and the overweight women. There were significant correlations between FPI and BMI ($r = .719$, $P < .0001$), waist circumference ($r = .728$, $P < .0001$), WHR ($r = .367$, $P < .05$), fat mass ($r = .675$, $P < .0001$), triglycerides ($r = .514$, $P < .0005$), and HDL-cholesterol ($r = -.543$, $P < .0005$). FPI was also positively correlated with total PL ($r = .503$, $P < .0005$), PE ($r = .458$, $P < .005$), SM ($r = .466$, P .005), and PC ($r = .364$, P .05), and negatively with PI ($r = -.332$, $P < .05$). Nearly similar correlations were obtained with HOMA IR. Multivariate regression analysis in the whole sample revealed that PE, SM, percent fat mass, and triglycerides were independent predictors for FPI (adjusted $r^2 = .689$, $P < .0001$). Membrane PE, SM contents, and percent fat mass explain a part of the variance in HOMA IR (adjusted $r^2 = .636$, $P < .0001$). The same analysis was performed in the 21 lean and 24 overweight women separately. In multiple regression analysis including all significant correlates, membrane PE, and SM contents, Chol/PL ratio and plasma HDL-cholesterol levels explain a significant part of the variance in FPI in the overweight women (adjusted $r^2 = .673$, $P < .0001$). The significance did not change after adjustment for percent fat mass. HOMA IR was explained by membrane PE contents, Chol/PL ratio, and HDL-cholesterol concentrations (adjusted $r^2 = .613$, $P < .0001$). In the lean women, membrane PE and SM contents were the only independent predictors for FPI (adjusted $r^2 = .372$, $P < .01$) and HOMA IR (adjusted $r^2 = .383$, $P < .005$).

Table 2. Membrane Parameters for Lean Women, All Overweight Women, and Subgroups A and B at Baseline or After Weight Loss

	Lean Women (n = 21)	All Overweight Women (n = 24)	Subgroup A (n = 9)	Subgroup B (n = 15)	ANOVA *P*
Baseline					
Membrane cholesterol	239 ± 13	284 ± 14[a]	297 ± 20[a]	277 ± 16	.06
Phosphatidylethanolamine (PE)	265 ± 9	277 ± 8	250 ± 11	293 ± 8[a]†	<.05
Phosphatidylinositol (PI)	66 ± 7	48 ± 4[a]	55 ± 7	44 ± 4[a]	<.05
Phosphatidylserine (PS)	125 ± 6	123 ± 5	113 (105-119)	127 ± 7	NS
Phosphatidylcholine (PC)	239 ± 11	248 ± 8	229 ± 13	260 ± 10	NS
Sphingomyelin (SM)	92 ± 4	103 ± 4[a]	96 ± 4	107 ± 5[a]	.05
Total phospholipids	762 ± 21	796 ± 14	748 ± 19	824 ± 14[a]‡	<.05
Cholesterol/phospholipids	0.32 ± 0.02	0.36 ± 0.02	0.40 ± 0.03[a]	0.34 ± 0.02	.0744
DPH anisotropy	0.248 ± 0.001	0.246 ± 0.001	0.245 ± 0.002	0.246 ± 0.001	NS
After weight loss					
Membrane cholesterol	239 ± 13	243 ± 12#	258 ± 17	233 ± 16¶	NS
Phosphatidylethanolamine (PE)	265 ± 9	264 ± 7	236 ± 11	280 ± 6§	<.05
Phosphatidylinositol (PI)	66 ± 7	40 (36-54)[a]	43 ± 4¶	50 ± 6¶	<.05
Phosphatidylserine (PS)	125 ± 6	116 ± 4	117 ± 5	115 ± 6	NS
Phosphatidylcholine (PC)	239 ± 11	256 ± 8	250 ± 10	259 ± 11	NS
Sphingomyelin (SM)	92 ± 4	99 ± 4	97 ± 6	100 ± 5	NS
Total phospholipids	762 ± 21	782 ± 15	743 ± 20	805 ± 18	NS
Cholesterol/phospholipids	0.32 ± 0.02	0.31 ± 0.02‖	0.34 ± 0.02	0.29 ± 0.02¶	NS
DPH anisotropy	0.248 ± 0.001	0.244 ± 0.001[a]	0.247 ± 0.001	0.242 ± 0.001[b]*#	<.01

NOTE. Data in μg/mg proteins for PE, PI, PS, PC, SM, and cholesterol are presented as means ± SEM or medians with 25th and 75th percentiles (data that follow a log normal distribution). ANOVA: lean women *v* subgroup A *v* subgroup B.

[a]$P < 0.05$, [b]$P < 0.01$ *v* lean women.

*$P < .05$, †$P < .01$, ‡$P < .005$, §$P < .001$, subgroup B *v* subgroup A.

¶$P < .05$, ‖$P < .01$, #$P < .005$, post weight loss *v* baseline.

Effects of Weight Loss

Following the 3-month hypocaloric diet, a weight loss was noted in 23 of 24 overweight women. The weight of 1 subject remained stable. Mean weight loss was 4.7 ± 0.6 kg, (-5.7 ± 0.7 %, $P < .001$), or expressed as BMI a reduction from 32.5 ± 0.9 to 30.6 ± 0.9 kg/m^2 ($P < .001$). Waist circumference decreased by 4.5% ± 0.8% in response to weight loss ($P < .0001$), and WHR by 1.7% ± 0.5% ($P < .01$). The loss of fat mass was 4.1 ± 0.5 kg (-11.3% ± 1.4%, $P < .0001$). There was only a 0.4 ± 0.3 kg decrease in the fat-free mass.

The weight loss significantly reduced FPI levels (-10.2% ± 5.4%, $P < .05$) in the whole obese group, but insulin remained higher than in the lean subjects. The decrease in the HOMA IR (-8.4% ± 5.8%) did not reach the significance ($P = .08$). Fasting plasma glucose, triglyceride, and HDL–cholesterol concentrations did not change after weight loss. However, total cholesterol and LDL-cholesterol significantly decreased by –5.6% and –6.9%, respectively ($P < .05$).

The mean changes in the membrane parameters are shown in Table 2. Membrane cholesterol was reduced by 12.4% ± 4.4% ($P < .005$) and Chol/PL ratio by 11.1% ± 4.5% ($P < .01$). Other changes were not statistically significant.

The changes in FPI and membrane parameters were also considered within the subgroups A and B. Baseline insulin resistance status (group A *v* B) did not affect significantly the reduction in weight, fat mass, waist circumference, and WHR. Mean weight loss was similar in the 2 subgroups: -5.9% ± 1.1% and –5.5% ± 0.9% for subgroups A and B, respectively. However, subjects with a high initial FPI value (subgroup B) had a significant decrease in FPI (-18.8% ± 6.1%, $P < 0.01$) and HOMA IR (-16.2% ± 7.5%, $P < .05$). Subgroup B also had a significant decrease in membrane cholesterol (-13.7% ± 5.6%, $P < .05$), Chol/PL ratio (-12.0% ± 5.4%, $P < .05$), and DPH anisotropy (-1.71% ± 0.43%, $P < .005$) (Table 2). There was also a 5.1% ± 3.0% decrease in membrane SM content but the difference was not statistically significant ($P = .09$). In contrast, no significant changes in FPI, HOMA IR, and membrane parameters were noted in subgroup A after weight loss. Only membrane PI content was significantly decreased (-16.4% ± 9.3%, $P < .05$). The comparisons between the changes in membrane parameters between the 2 groups were significant for PE and DPH anisotropy ($P < .001$ and $P < .05$, respectively).

The relationships between the changes in FPI and clinical characteristics, plasma lipids, and red cell membrane components (expressed in percent of the baseline value) were analyzed in the whole population of overweight women by univariate regression analysis. Changes in FPI were positively correlated with changes in BMI ($r = .573$ $P < .005$), waist circumference ($r = .573$ $P < .01$), and fat mass ($r = .494$ $P < 0.05$) (Fig 1A), fluorescence anisotropy of DPH ($r = .615$, $P < .005$), and membrane SM content ($r = .516$ $P < .01$) (Fig 1B), and negatively correlated with membrane PE content ($r = -.41$ $P < .05$) (Fig 1C). In addition, the changes in membrane SM contents were negatively correlated with the changes in membrane PI contents ($r = -.497$ $P < .05$) (Fig 1D) and positively with the changes in the fluorescence anisotropy of DPH ($r = .587$ $P < .005$). The analysis of the relationships between the changes in HOMA IR and the above parameters have given similar results.

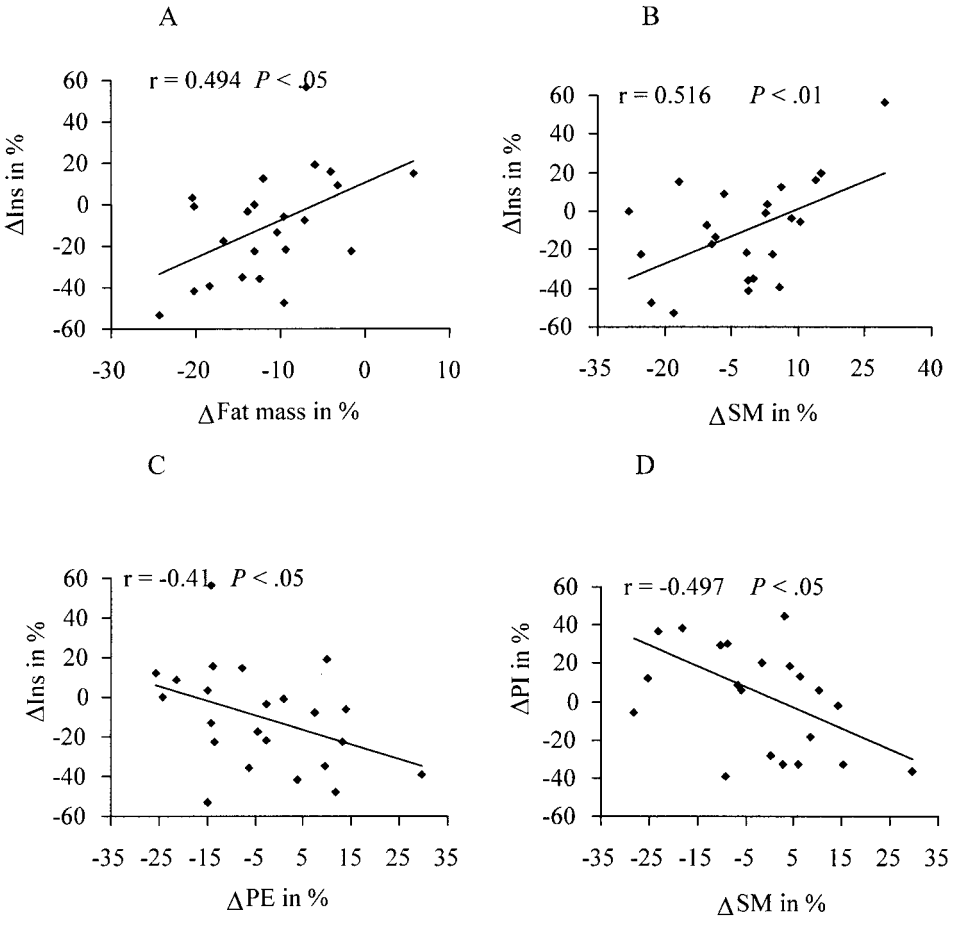

Fig 1. Relationships between the changes in (A) FPI (ΔIns in %) and percent fat mass (ΔFat mass in %), (B) membrane SM content (ΔSM in %), (C) membrane PE content (ΔPE in %), and (D) membrane SM content (ΔSM in %) and membrane PI content (ΔPI in %) in the whole population of overweight women.

In view of the potential interdependence of the different groups of variables that were significantly linked to the insulin resistance markers, stepwise multiple regression analyses were performed to identify significant independent determinants for the variation in fasting plasma insulin. The changes in SM accounted for 26.6% of the variance in the changes in FPI as an independent predictor, with the changes in fat mass and PE as other determinants (27.8% and 20%, respectively) (Table 3). The proportional variability accounted for by including factors selected was $r^2 = .704$ ($P < .0001$) in the model to predict the variations in FPI. The same factors explained the variations in HOMA IR.

DISCUSSION

In light of our previous observations that FPI and HOMA IR were positively correlated with membrane SM contents in obese women,[16,17] the aim of the present study was to investigate whether a diet-induced weight loss, which usually improves insulin sensitivity, could also modulate lipid composition of cell membranes. As expected from our previous studies, multivariate regression analysis showed that membrane PE and SM contents are independent predictors of FPI and/or HOMA IR in the overweight group. The same was true in the control lean group. The novel key observation was that the changes in SM and PE after weight loss were also independent predictors of the changes in FPI or HOMA IR.

Table 3. Multiple Linear Regression Model for Variation in Fasting Plasma Insulin (%) Between Baseline and Post Weight Loss

Variable	β Coefficient	Partial r^2	F	Partial P
ΔFat mass (%)	0.528	0.278	11.908	<.0005
ΔSM (%)	0.516	0.266	7.607	<.05
ΔPE (%)	−0.463	0.20	18.419	<.0001

NOTE. Multiple r^2: 0.704, F: 18.419, and $P < .0001$.

Cross-sectional studies cannot fully clarify whether membrane composition abnormalities occur as a consequence of obesity related insulin-resistance or instead might add to the risk of becoming insulin resistant. In the current study, a weight loss intervention was used to address this issue. Even a moderate weight loss in obese individuals can improve insulin sensitivity.[18,19,28] Our results indicate that a diet-induced weight loss that decreases hyperinsulinemia can correct membrane abnormalities at least partly. It is striking that changes in FPI or HOMA IR were significantly correlated with changes in SM and PE after adjustment for changes in fat mass. Interestingly, the crude classification of obese women as insulin-resistant and insulin-sensitive brought further arguments for a relationship between insulin resistance and cell membrane lipid composition. The changes in FPI and membrane parameters

differed in the insulin–resistant and insulin-sensitive groups. Changes in FPI and SM were not significant in the insulin-sensitive group. In contrast, as far as the subgroup of overweight hyperinsulinemic women was concerned, the 3-month intervention induced both a significant improvement in insulin resistance markers and a significant decrease in erythrocyte membrane cholesterol. The reduction in membrane SM content tended to reach the significance level. However, the overweight women of subgroup B for whom the decrease in fasting insulin was higher than 10% had a significant decrease in membrane SM content ($\Delta SM = -7.1\% \pm 3.2\%$, $P < .05$, $n = 11$). There was also a significant decrease in fluorescence anisotropy of DPH, indicating an increased membrane lipid fluidity. Despite the fact that changes in fluidity were weak, they may have a physiologil significance. It has been previously reported that small changes in structural and dynamic membrane properties can markedly modify D-glucose uptake,[29] the insulin binding parameters,[30] and downregulation of insulin receptors in erythrocyte membranes.[31]

To the best of our knowledge, no data on the relationships between energy restriction and membrane lipid composition are available in humans. Food restriction reduced by 50% all PLs at least in the cortical brush border membrane of rats.[32] It was not the case in the liver mitochondrial and microsomal membranes.[33] Food restriction also increased membrane fluidity of erythrocyte membranes of growing rats.[34]

To date, the precise contribution of obesity and/or insulin-resistance to the alterations in the phospholipid composition of cell membranes in obese insulin-resistant women is not understood. The altered patterns in membrane SM contents correlated with the phenotype of insulin resistance (eg, hyperinsulinemia and low HDL-cholesterol levels) in weight-stable obese individuals. Our data therefore suggest that the membrane abnormalities could belong to the cluster of metabolic abnormalities commonly found in patients with excess visceral adipose tissue.[5,35,36] It is noteworthy that the altered patterns in membrane parameters also correlated with insulin resistance markers in lean women. However, FPI and HOMA IR were not significantly correlated with percent fat mass or with index of fat distribution in this group. So, the increased membrane SM or PE contents could be considered as hallmarks of insulin resistance but not of obesity. It is of interest that plasma SM levels could have an independent predictive value for coronary heart disease after adjusting for other risk factors.[37]

Red cells could be considered as a poor surrogate of insulin sensitive cells such as adipocyte, hepatocyte, or skeletal muscle cell. However, we have previously shown that changes in erythrocyte membrane SM parallel those in adipocyte membrane in obese subjects, according to the degree of insulin resistance.[17] We therefore believe that the changes in red cell membranes can reflect those occuring in an insulin sensitive cell such as adipocyte.

The use of red cells to investigate cell membrane composition has several advantages: (1) it is an isolated plasma membrane that contains neither lipoprotein receptor nor intracellular organelles; (2) there is no de novo synthesis of cholesterol in red blood cells; (3) the red cell membrane has been extensively used to study the exchanges of cholesterol between cell and plasma[38]; (4) the erythrocyte system has the great virtue of quantity: enough membrane is available from a small blood sample to determine the plasma membrane composition.

It is not clear whether insulin resistance is a cause or a consequence of lipid disorders such as dyslipidemia[39] or alterations in the metabolism of free fatty acids,[40] and as suggested in this study, cell membrane PL composition abnormalities. Thus, we are left with 2 interpretations of the data, without clear evidence to support one over the other. The first interpretation is that hyperinsulinemia and/or insulin resistance could be responsible for changes in the membrane parameters, particularly membrane SM and PE contents. An insulin-resistant state could affect the PL composition of cell membranes through 3 mechanisms: (1) alterations in the exchanges between cells and plasma lipoproteins; (2) the presence in the plasma of molecules stimulating the cellular import of phospholipids; or (3) an effect on the synthesis of phospholipids. First, the movement of free cholesterol and PLs between cells and lipoproteins is a bidirectional process. The extent and the direction of net movement depend on the ratio of influx to efflux and are determined by both cell and acceptor properties.[41] Both can be altered by insulin-resistance. In fact, the composition of lipoproteins is abnormal in insulin-resistant subjects.[36,39,42] Changes in plasma PC or SM are rapidly transmitted to erythrocyte membranes under in vivo conditions.[43] Therefore, the PL content of erythrocyte membranes in the obese insulin-resistant women could reflect the altered composition of lipoproteins.[44,45] Second, another explanation could be related to the chemokine connective tissue-activating peptide III (CTAP-III). This peptide released by activated platelets stimulates the cellular sphingomyelin import.[46] Now, an increased platelet aggregability may be associated to insulin resistance.[47] Finally, hyperinsulinemia or insulin resistance could influence the metabolism of membrane PLs. It has been reported that insulin stimulated the synthesis of major PLs.[48] Hyperinsulinemia could therefore explain the significantly higher total PLs found in the subgroup of obese insulin-resistant women. Given the fact that the PL synthesis takes primarily place in reticulocytes,[49,50] and that the erythrocyte life is 115 days, it is likely that 75% the erythrocyte pool was renewed after the 3-month weight loss program. Furthermore, a PL biosynthesis has been reported in human mature erythrocytes.[51] We cannot therefore exclude the possibility that the decreased fasting plasma insulin after the weight loss program could play a role on the phospholipid composition of erythrocyte membranes.

The second interpretation is that insulin resistance is a consequence of membrane PL abnormalities, as previously discussed.[16,17,52] The membrane SM and PE, which explained a great part of the variance in fasting plasma insulin, could effectively have a role in the insulin action. SM is a rigidifying membrane PL that can affect the insulin signal pathway. The hydrolysis of SM by tumor necrosis factor-alpha (TNF-α) generates phosphocholine and ceramide, which serve as second messengers, activating specific kinases and phosphatases.[53] An enrichment in SM inhibits the tyrosine kinase activity in rat liver plasma membranes,[54] decreases the phosphatidylinositol phosphodiesterase activity activated by diacylglycerol,[55] and inhibits glucose transport in PL vesicles.[56] The role of changes in PE membrane content is less clear, but PE content may be an

important regulator of insulin action. A high membrane PE content leading to an increase in the head group spacing of PLs in lipid bilayers of defined composition is closely correlated with decreased protein kinase C activity, a key enzyme in insulin action.[57]

In summary, a decreased hyperinsulinemia induced by weight loss was associated with improvement of membrane lipid composition. This was observed mainly in the most hyperinsulinemic/insulin-resistant overweight women and was independent of changes in fat mass. Lipid membrane composition abnormalities could be included in the unfavorable lipid constellation of obesity, which correlated with impaired insulin sensitivity. The complex associations between hyperinsulinemia and/or insulin resistance and the membrane abnormalities, the so-called membrane hypothesis of insulin resistance, require further investigations in lean and obese subjects. Drug intervention (eg, thiazolidinediones or metformin) could provide evidence confirming this suggestion of a significant role of insulin resistance in determining PL membrane abnormalities, independent of obesity.

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