Surfactant Protein D, a Marker of Lung Innate Immunity, Is Positively Associated With Insulin Sensitivity

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OBJECTIVE — Impaired lung function and innate immunity have both attracted growing interest as a potentially novel risk factor for glucose intolerance, insulin resistance, and type 2 diabetes. We aimed to evaluate whether surfactant protein D (SP-D), a lung-derived innate immune protein, was behind these associations.

RESEARCH DESIGN AND METHODS — Serum SP-D was evaluated in four different cohorts. The cross-sectional associations between SP-D and metabolic and inflammatory parameters were evaluated in two cohorts, the cross-sectional relationship with lung function in one cohort, and the longitudinal effects of weight loss on fasting and circadian rhythm of serum SP-D and cortisol concentrations in one prospective cohort.

RESULTS — In the cross-sectional studies, serum SP-D concentration was significantly decreased in subjects with obesity and type 2 diabetes ($P = 0.005$) and was negatively associated with fasting and postload serum glucose. SP-D was also associated with A1C, serum lipids, insulin sensitivity, inflammatory parameters, and plasma insulinase activity. Smoking subjects with normal glucose tolerance, but not smoking patients with type 2 diabetes, showed significantly higher serum SP-D concentration than nonsmokers. Serum SP-D concentration correlated positively with end-tidal carbon dioxide tension ($r = 0.54$, $P = 0.034$). In the longitudinal study, fasting serum SP-D concentration decreased significantly after weight loss ($P = 0.02$). Moreover, the main components of cortisol and SP-D rhythms became synchronous after weight loss.

CONCLUSIONS — These findings suggest that lung innate immunity, as inferred from circulating SP-D concentrations, is at the cross-roads of inflammation, obesity, and insulin resistance.

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I mpaired lung function has attracted growing interest in association with metabolic disorders (1–6). Decreased lung function has been proposed as a potential novel risk factor for glucose intolerance, insulin resistance, and type 2 diabetes (1–6). In prospective studies of middle-aged men and women without known lung disease, lower vital capacity predicted the subsequent development of type 2 diabetes. Lower forced vital capacity and forced expiratory volume in 1 s at baseline predicted hyperinsulinemia and estimated insulin resistance over 20 years

of follow-up, independent of age, adiposity, and smoking (1).

Possible mechanisms for the hypothesized link include direct effects of hypoxemia on glucose and insulin regulation (7), adverse early-life exposures and their effects on organ development (8), and lung-related inflammatory mediators and their effects on insulin signaling (9). In fact, nuclear factor interleukin-6, early growth response-1, and hypoxia-inducible factor-1 mediate inflammatory responses to chronic hypoxia in macrophages, pulmonary vascular endothelium, and smooth muscle (6,9). Cigarette smoking, an independent predictor of type 2 diabetes (10), provokes an inflammatory response (11) and is inversely associated with vital capacity. However, the link between lower vital capacity and diabetes risk was completely independent of cigarette exposure and was stronger in never-smokers (6).

Reduced vital capacity is a common residual effect of lower respiratory tract infections, including those in childhood and infancy (8), that might provoke an inflammatory response. A reduced ability to sense and eradicate pathogens could thus cause frequent respiratory tract infections, reduced vital capacity, and chronic inflammation resulting in insulin resistance and type 2 diabetes (12). The total incidence rate of infections needing hospitalization in diabetic patients was 41/1,000 persons-years compared with 16/1,000 person-years of follow-up in the general population. Roughly half of the infections were severe lung infections, suggesting impaired lung immunity in patients with type 2 diabetes (13).

Pulmonary surfactant is a complex mixture of lipids (90%) and proteins (5–10%) that constitutes the mobile liquid phase covering the large surface area of the alveolar epithelium. It maintains minimal surface tension within the lungs to avoid lung collapse during respiration. The innate immune system, by upregulating SP-D synthesis, can immediately respond to intrusion of foreign agents by helping to prevent further invasion (14). This recognition is important in the day-to-day physiology. Each day, we breathe

>7,000 liters of air, laden with inorganic and organic particles and an array of microbes. Secreted primarily by alveolar epithelial type II pneumocytes, plasma SP-D appears to increase early in the clinical course of lung injury, and its concentration is thought to reflect pulmonary epithelial injury (15).

Subtle deficiencies in proteins of the sensing arm of the innate immune system have been found to be associated with alterations of glucose metabolism. These deficiencies run in parallel with inflammation and impaired insulin action (16).

We hypothesized that SP-D could be behind the association of lung function with impaired insulin action. For that reason, we aimed to evaluate SP-D according to metabolic and inflammatory parameters. As SP-D was associated with obesity status and impaired glucose metabolism, we evaluated the influence of weight loss on both fasting and circadian serum SP-D concentration. As glucocorticoids seem to regulate SP-D production in in vitro studies (17), we investigated the influence of circadian cortisol rhythm on serum SP-D concentration. Finally, we also studied the association of SP-D with lung function tests.

RESEARCH DESIGN AND METHODS

Cohort 1: study of circulating SP-D across categories of glucose tolerance

A total of 388 Caucasian subjects were recruited and studied. A total of 308 of them were recruited in an ongoing study dealing on nonclassical cardiovascular risk factors in Northern Spain. Subjects were randomly localized from a census and were invited to participate. The participation rate was 71%. A 75-g oral glucose tolerance test according to the American Diabetes Association criteria was performed in all subjects. All subjects with normal glucose tolerance ($n = 204$) had fasting plasma glucose <7.0 mmol/l and 2-h postload plasma glucose <7.8 mmol/l after a 75-g oral glucose tolerance test. Glucose intolerance was diagnosed in 64 subjects according to the American Diabetes Association criteria (postload glucose between 7.8 and 11.1 mmol/l). Previously unknown type 2 diabetes was diagnosed in 40 of these 308 subjects (postload glucose >11.1 mmol/l). Inclusion criteria were *1*) BMI <40 kg/m^2, *2*) absence of systemic disease, and *3*) absence of infection within the previous

month. None of the control subjects were under medication or had evidence of metabolic disease other than obesity. Alcohol and caffeine were withheld within 12 h of performing the insulin sensitivity test. Smokers were defined as any person consuming at least one cigarette a day in the previous 6 months. Resting blood pressure was measured as previously reported. Liver disease and thyroid dysfunction were specifically excluded by biochemical workup.

To increase the statistical power of the group of patients with type 2 diabetes, 80 patients were prospectively recruited from diabetes outpatient clinics on the basis of stable metabolic control in the previous 6 months, as defined by stable A1C values. Data from these patients were merged with those from the recently diagnosed type 2 diabetic patients.

Study of insulin sensitivity

In those subjects who agreed ($n = 230$), insulin sensitivity and glucose effectiveness were measured using the frequently sampled intravenous glucose tolerance test with minimal model analysis. In brief, the experimental protocol started between 8:00 and 8:30 A.M. after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10, and −5 min, after which glucose (300 mg/kg body wt) was injected over 1 min starting at time 0, and insulin (Actrapid, Novo, Denmark; 0.03 units/kg) was administered at time 20. Additional samples were obtained from a contra-lateral antecubital vein up to 180 min.

Cohort 2

Findings in the cross-sectional study were evaluated again in an independent prospective population-based survey of diabetes and cardiovascular risk factors (18). The baseline examination was carried out during 1998–1999. Randomly population-based selected subjects were evaluated to determine the prevalence of type 2 diabetes and pre-diabetes in northwestern Spain. In 2004–2005, these same subjects were invited for a follow-up examination. Seven hundred subjects participated. Circulating SP-D concentration was evaluated in randomly selected subjects ($n = 333$, 137 men and 196 women). Oral glucose tolerance test was performed as previously described (18). Measurements of SP-D were centralized in a single laboratory, as described below.

Cohort 3: study of the effects of weight loss on circadian circulating SP-D

The study group included eight normotensive obese women (BMI >40 kg/m^2) evaluated before and 2 years after biliopancreatic diversion. None of the study participants had endocrine or nonendocrine diseases. They were not taking any medications, except subjects after biliopancreatic diversion, who were prescribed oral supplementation of sulfate iron (525 mg daily), calcium carbonate (1 g daily), multivitamins (Supradyn Roche, Milan, Italy) (one tablet a day), and ergocalciferol (400,000 IU intramuscular) (Ostelin fl, Teofarma, Italy) every 2 weeks. Medical histories, physical examinations, electrocardiogram, and blood screening showed that patients were in good health. Insulin sensitivity was estimated by a euglycemic-hyperinsulinemic clamp as previously described. Whole-body glucose uptake, normalized by fat-free mass (FFM) (M value in mmol · kg$_{FFM}^{-1}$ · min^{-1}), was determined during a primed constant infusion of insulin (at the rate of 6 pmol · min^{-1} · kg^{-1}).

To evaluate the intra-day SP-D and cortisol release pattern, the zero mean transformed series have been calculated:

$$v(t) = x(t) - \langle x(t) \rangle$$

where $t = 09.00, 10.00, \ldots, 08.00$. Finally the averaged patterns, before and after treatment, of these zero mean profiles were obtained and, using the Matlab (TheMathWorks, Inc.) program, were approximated by means of the finite Fourier series:

$$a_0 + \sum_{k=1}^{n} [a_k \cos k\omega t + b_k \sin k\omega t].$$

The goodness of fit was determined by means of the degree of freedom adjusted coefficient of determination: R_{adj}^2. A value of this latter parameter equal to 1 indicates a perfect agreement between the experimental data and the fitted curve.

The Ethical Committee of Catholic University approved the study, and subjects signed an informed consent document before participation.

Cohort 4: study of the association between circulating SP-D and lung function

We explored 15 obese subjects (12 women, 3 men), aged 40.4 ± 14.8 years,

with a mean BMI of 46.4 ± 8.8 kg/m². Spirometry was performed with a calibrated, dry-rolling seal spirometer (SensorMedics 2130 System; SensorMedics, Yorba Linda, CA) according to current guidelines, as previously described (19). Static lung volumes were measured by body plethysmography (SensorMedics V6200 Autobox; SensorMedics). The predicted values used for spirometric and thoracic gas volumes were those of the 1993 European update.

Patients underwent ventilatory drive assessment, including minute ventilation (V'_E), tidal volume (V_T), inspiratory time (t_I), mouth occlusion pressure at 0.1 s of inspiration ($P_{0.1}$), and end-tidal carbon dioxide tension ($P_{ET,CO2}$).

All subjects gave written informed consent after the purpose of the study was explained to them. The institutional review board of the participant institutions approved the protocol.

Analytical methods

Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. A1C was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany, and autoanalyzer Jokoh HS-10). Intra-assay and interassay coefficients of variation were <4% for all these tests.

Serum insulin levels were measured in duplicate by monoclonal immunoradiometric assay (or enzyme-amplified sensitivity immunoassay [EASIA], Medgenix Diagnostics, Fleunes, Belgium). In the replication study, insulin resistance was calculated using the HOMA value [glucose (mmol/l) × insulin (mU/l)/22.5].

Plasma sTNFR1 and sTNFR2 levels were analyzed by a commercially available solid-phase MEDGENIX sTNFR1 and sTNFR2 EASIA (BioSource Europe S.A., Zoning Industriel B-6220, Fleunes, Belgium). The intra- and interassay coefficients of variation were <7 and <9%.

Serum lipopolysaccharide-binding protein (LBP) levels were determined with a commercially available Human LBP enzyme-linked immunosorbent assay (ELISA) kit (HyCult biotechnology b.v.; PB Uden, the Netherlands). Intra- and interassay coefficients of variation were between 5 and 10%.

ELISA of SP-D

The measurements of SP-D concentrations were centralized in a single laboratory and analyzed by a sandwich enzyme immunoassay (Human SP-D ELISA RD194059100; BioVendor Laboratory Medicine, Brno, Czech Republic) according to the manufacturer's instructions. The assay has a sensitivity of 2.2 ng/ml. Intra-assay coefficient of variation was <5%, and interassay coefficient of variation was <10%.

Plasma insulinase activity

In brief, 150 μl serum was incubated with 750 μl Tris buffer 100 mmol/l, 1% BSA. After 30′ at 30°C, ¹²⁵I-insulin (iodinated at 14B position, ~10,000 cpm) is added and then incubated at 37°C for 30, 60, and 90 min. Trichloroacetic acid (10%), which precipitates intact insulin, is finally added to this mixture. After centrifugation at room temperature, the supernatant is separated. Radioactivity units are measured in the supernatant and in the precipitated fraction. As trichloroacetic acid precipitates only intact insulin, degraded insulin remains only in the supernatant. Results are expressed as percent radioactivity found in the supernatant relative to total activity measured in the tube.

Statistical methods

Descriptive results of continuous variables are expressed as mean (SD). Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test and then variables were given a base 10 log-transformation if necessary. These parameters (insulin sensitivity [S_I], glucose effectiveness [S_G], triglycerides, LBP, and SP-D) were analyzed on a log scale and tested for significance on that scale. The anti–log-transformed values of the means (geometric mean) are reported in the tables found in the online appendix (http://care.diabetesjournals.org/cgi/content/full/dc09-0542/DC1). The relation between variables was tested using Pearson's test and stepwise multiple linear regression analysis. We used the χ^2 test for comparisons of proportions and the unpaired t tests for comparisons of quantitative variables. General linear model was also used to calculate circulating SP-D values after adjusting for age and BMI. The statistical analyses were performed using the program SPSS (version 12.0).

RESULTS

Cohort 1: circulating SP-D across categories of glucose tolerance and smoking status

Circulating SP-D was skewed to the left in the population studied. Log-transformed serum SP-D followed a normal curve and was then used in all the analyses performed. Characteristics of the subjects and the comparisons with type 2 diabetic subjects are shown in supplemental Table 1. Subjects with glucose intolerance or type 2 diabetes were significantly older, heavier, and showed lower insulin sensitivity and glucose effectiveness than subjects with normal glucose tolerance.

Circulating SP-D was significantly lower among patients with type 2 diabetes (supplemental Table 1). After adjustment for BMI, age, and smoking status, mean log-transformed serum SP-D was significantly lower in patients with type 2 diabetes (1.79 ± 0.15 [61.6 ng/ml] vs. 1.91 ± 0.17 in subjects with glucose intolerance (81.2 ng/ml) and 1.88 ± 0.13 in subjects with normal glucose tolerance (75.8 ng/ml) ($P = 0.005$).

We also observed an interaction between smoking and glucose tolerance status. Smokers with normal glucose tolerance showed significantly higher mean log–SP-D than nonsmokers (1.92 ± 0.28 vs. 1.84 ± 0.22, $P = 0.03$), and a trend was observed in subjects with impaired glucose tolerance (2.00 ± 0.22 vs. 1.88 ± 0.23, $P = 0.08$) but not in patients with type 2 diabetes (1.78 ± 0.25 vs. 1.79 ± 0.20, $P = 0.92$) (supplemental Fig. 1).

Serum SP-D concentrations did not differ between patients with type 2 diabetes treated with statins, fibrates, insulin, hypoglycemic agents, antihypertensive agents, or allopurinol versus patients who did not receive these drugs. However, those patients receiving aspirin ($n = 46$) showed a trend toward decreased serum SP-D concentration (1.74 ± 0.23 vs. 1.81 ± 0.20, $P = 0.08$) despite similar age (58.3 ± 8.7 vs. 56.5 ± 11.8, $P = 0.4$) and BMI (29.7 ± 4.1 vs. 28.9 ± 3.7, $P = 0.3$). Obese subjects showed significantly decreased serum SP-D concentrations (log SP-D 1.80 ± 0.20 vs. 1.86 ± 0.24, $P = 0.03$).

Associations with variables of glucose metabolism and inflammation

In all subjects as a whole, circulating SP-D correlated significantly with serum glucose 30′ post–oral glucose tolerance test, LBP, TNFR2, and insulin sensitivity. In subjects with altered glucose tolerance, these associations were strengthened. In this subgroup, we also observed significant associations with A1C, fasting triglycerides, HDL cholesterol, and glucose effectiveness (supplemental Table 2). The association between SP-D and insulin sensitivity was most significant in subjects with glucose intolerance ($r = 0.40$, $P = 0.002$; supplemental Fig. 2).

Circulating SP-D run in inverse proportion to serum LBP (patients with type 2 diabetes showed the highest serum LBP concentration and the lowest serum SP-D concentration in both smokers and non-smokers) (supplemental Fig. 3).

We performed a multiple linear regression analysis to predict circulating SP-D. We considered as independent variables those with significant association on univariate analysis. When all subjects were considered as a whole, only age ($P = 0.01$) and fasting glucose ($P = 0.001$) contributed independently to 11% of SP-D variance (4 and 7%, respectively), after controlling for the effects of age, BMI, smoking status, fasting triglycerides, LBP, and insulin sensitivity. When the analysis was performed in subjects with altered glucose tolerance, fasting glucose ($P < 0.0001$, $r^2 = 0.18$) and fasting triglycerides ($P = 0.001$, $r^2 = 0.15$) contributed independently to 33% of SP-D variance.

Study of possible mechanisms

To evaluate the possible mechanisms behind the association between SP-D and glucose metabolism, we studied insulinase activity in a sample of consecutive subjects whose characteristics did not differ significantly from the remaining subjects. We found that serum SP-D was significantly associated with insulinase activity ($P = 0.005$) (supplemental Fig. 4).

Cohort 2

SP-D was evaluated in 333 subjects (137 men) without previously known type 2 diabetes, mean age 50.7 ± 7.6 years, BMI 27.6 ± 4.6 kg/m^2, in whom plasma samples were available. These subjects were significantly younger than the whole co-hort ($P = 0.03$) but was otherwise similar in sex, BMI, and fasting glucose values. Among these subjects, circulating SP-D was negatively associated with BMI ($r = -0.19$, $P = 0.001$), systolic blood pressure ($r = -0.13$, $P = 0.01$), and fasting glucose ($r = -0.14$, $P = 0.009$). The findings were especially significant among nonsmoking subjects ($n = 254$) in whom SP-D was negatively associated with BMI ($r = -0.14$, $P = 0.02$), systolic blood pressure ($r = -0.13$, $P = 0.03$), and fasting and postload glucose levels ($r = -0.16$, $P = 0.009$, and $r = -0.13$, $P = 0.04$, respectively). Among non-smoking women ($n = 157$), the most significant association was between SP-D and postload glucose levels ($r = -0.21$, $P = 0.008$), and among nonsmoking men ($n = 97$), the most significant association was between SP-D and systolic blood pressure ($r = -0.33$, $P = 0.001$). Among current smokers ($n = 79$), only the association of SP-D with BMI persisted significantly ($r = -0.27$, $P = 0.015$).

Cohort 3

Both fasting and 24-h area under the curve of serum SP-D concentration decreased significantly after weight loss (from 70.7 ± 34.8 to 31.1 ± 4.3 ng/ml, $P = 0.02$, and from $1,594 \pm 831$ to 702 ± 106 ng · ml^{-1} · h^{-1}, $P = 0.03$, respectively). The change in 24-h area under the curve of serum SP-D concentration tended to be associated with the change in insulin sensitivity ($r = -0.77$, $P = 0.07$). A finite Fourier series with $n = 8$ approximated the experimental SP-D data with $R^2_{adj} \approx 0.75$ in basal condition and with $R^2_{adj} \approx 0.81$ after weight loss (supplemental Fig. 5). Frequency composition was similar before and after weight loss, but after surgery, the spectral component fluctuations had lower amplitude than before. These results suggest a decrease in protein fluctuations. The principal spectral component had a period $\approx 24 \frac{hour}{cycle}$; other components, with a lower amplitude, were found at $\approx 12 \frac{hour}{cycle}$ and $\approx 6 \frac{hour}{cycle}$.

Regarding circadian rhythm of cortisol, a five terms Fourier series approximate the experimental data with $R^2_{adj} \approx 0.98$ in the basal condition as well as after weight loss (supplemental Fig. 5). The spectral components with higher ampli-tudes were found at $\approx 24 \frac{hour}{cycle}$ and at $\approx 12 \frac{hour}{cycle}$. The higher-order harmonics were negligible. However, the component at $\approx 6 \frac{hour}{cycle}$ was more evident than others (supplemental Fig. 5).

Taking into consideration the relationships between diurnal variability of cortisol and SP-D, we have considered the first, second, and fourth harmonic of the estimated Fourier series. In fact, these components are the most representative for both parameters.

Before weight loss, we observed that the first harmonic had a similar pattern in both parameters, with a small time lag for SP-D (supplemental Fig. 5). In fact, the first component of SP-D had its maximum value at the middle of the day. The second and fourth harmonics showed opposite phases: cortisol assumed peak values when SP-D reached a minimum value.

After weight loss, the examined harmonics of SP-D changed their phases (Fig. 1). Apart from a small time lag, the second and fourth harmonics were in phase; on the contrary, the first harmonic of SP-D showed a phase near opposite that of cortisol. In other words, weight loss induced a synchronous variability of SP-D and cortisol components with a period of $\approx 12 \frac{hour}{cycle}$ and $\approx 6 \frac{hour}{cycle}$.

Cohort 4

We observed no associations between serum SP-D concentration and any of the parameters of lung function tested, both before and after weight loss. Serum SP-D concentration was not significantly associated with forced vital capacity, forced expiratory volume in 1 s, or expiratory reserve volume. However, serum SP-D concentration correlated positively with end-tidal carbon dioxide tension (P_{ET,CO_2}) ($r = 0.54$, $P = 0.034$) in all subjects as a whole.

CONCLUSIONS — The main findings of this study are as follows: *1*) serum SP-D concentration was significantly decreased in patients with type 2 diabetes (age-, BMI- and smoking status-adjusted). *2*) The findings were replicated in an independent cohort of subjects in whom circulating SP-D correlated with several metabolic variables (namely, BMI, fasting, and postload glucose levels and
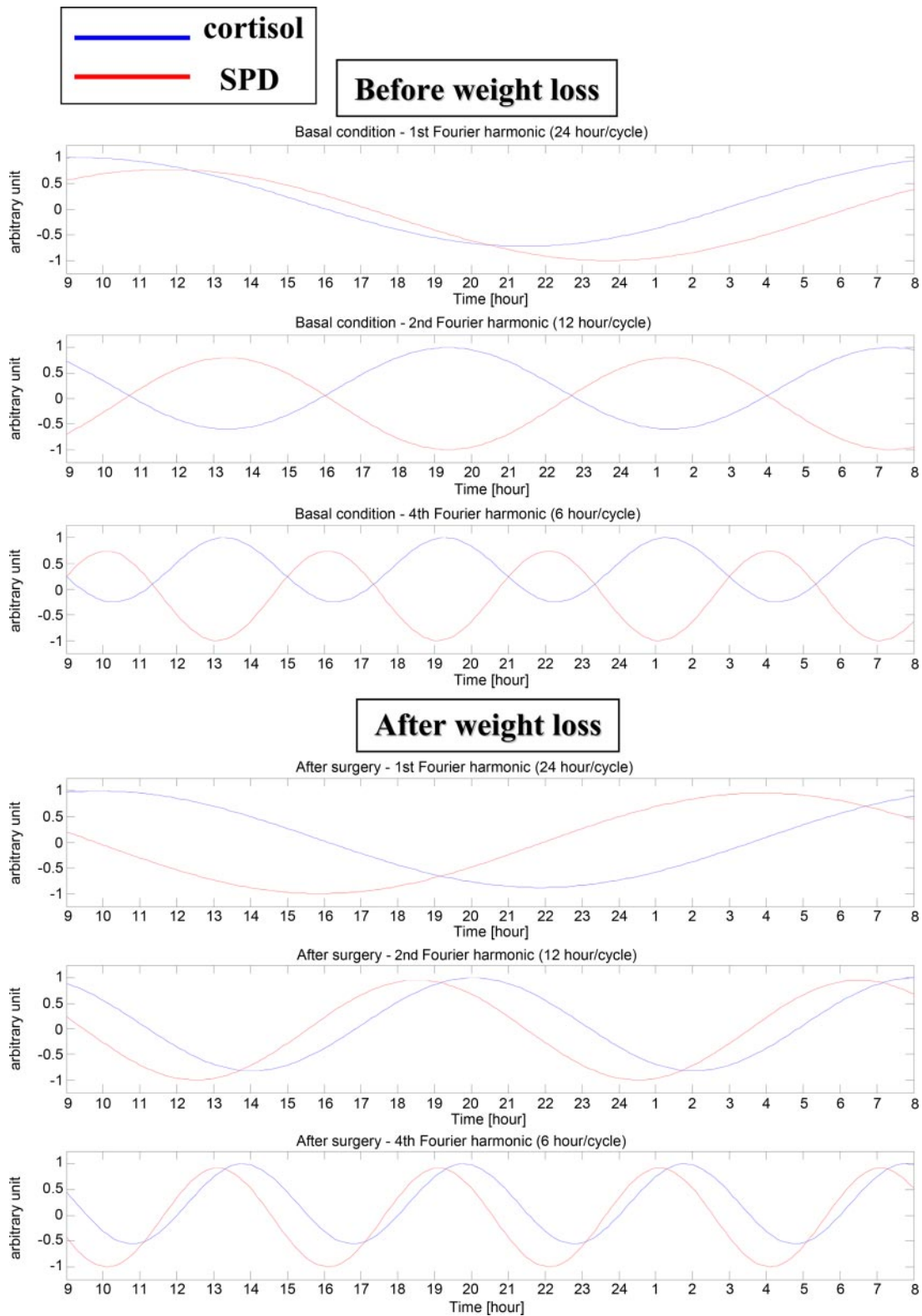
Figure 1—*Comparison of the circadian rhythm (harmonics) of serum SP-D and cortisol concentrations before and after weight loss.*

blood pressure). *3*) Weight loss led to significantly decreased serum SP-D concentrations and to important changes in their circadian rhythm. *4*) Serum SP-D concentration was not significantly associated with parameters of lung function.

Weight loss had no effects on ultradian cortisol secretion, whereas the pattern of the spontaneous 24-h secretion of SP-D was modified: those hours of the day in which SP-D had a peak before weight loss were those hours in which a valley of

SP-D concentration after weight loss was observed (supplemental Fig. 5). Moreover, after weight loss, the components with a period of $\approx 12 \frac{hour}{cycle}$ and $\approx 6 \frac{hour}{cycle}$ of cortisol and SP-D became synchronous (Fig. 1).

The effects of glucocorticoids on SP-D production have been previously described to be regulated at the level of transcription in in vitro studies (17). Cortisol levels are reduced in morbid obesity, a clear anabolic condition, and cortisol rhythm is disrupted. As previously described, free cortisol levels increase and its rhythm returns to be regular after massive weight loss secondary to malabsorptive bariatric surgery (20). It is likely that this normalized cortisol rhythm conveys the normalization of SP-D rhythm through both a quantitative and a temporal control of the surfactant protein synthesis. This would also explain why SP-D levels decreased after weight loss. Further research is needed in subjects without morbid obesity.

This is the first study, to our knowledge, in which the pattern of secretion of a lung innate immune protein and the in vivo relation between SP-D and cortisol are studied. The implications of this change of pattern should be further explored. In this sense, of note were the parallel variations in serum LBP and SP-D in subjects from cohort 1. The same factor (lipopolysaccharide) could lead to increased serum LBP and decreased SP-D concentrations. In a recent study, long-term exposure to lipopolysaccharide enhanced the rate of stimulated exocytosis and surfactant secretion in alveolar type II cells (21). In fact, lipopolysaccharide is extraordinarily ubiquitous in nature, being present in food and water, in normal indoor environments as a constituent of house dust, and of cigarette smoke. Both smoking as inflammatory stimulus, and plasma TNFR2 concentration, were positively associated with serum SP-D concentrations.

Interestingly, smoking led to significantly increased serum SP-D concentrations among subjects with normal glucose tolerance, but this was not observed in subjects with glucose intolerance or type 2 diabetes. This finding suggests that normal insulin action is needed to increase serum SP-D after an inflammatory stimulus. In fact, insulin receptors are present in rabbit type II pneumocytes (precisely those that produce SP-D) and insulin led

to increased surfactant synthesis in in vitro studies (22). Glucagon-like peptide 1, known to stimulate insulin secretion, also stimulated surfactant secretion in human type II pneumocytes.

The associations between SP-D and BMI have been recently reported (23). We evaluated a possible mechanism of the association between SP-D and glucose metabolism. We found a negative relationship between plasma insulinase activity and serum SP-D (Fig. 4). We took advantage of a recent article showing that SP-D is inactivated by neutrophil serine proteinases (24). Insulinase activity has been shown to be increased 14.5-fold in neutrophils from diabetic patients. A number of different peptides have been described to be degraded by insulinase, including insulin, IGF-I, and IGF-II. It is unknown whether SP-D could be cleaved by insulinase. However, a third factor leading to increased insulinase activity and decreased SP-D seems more plausible. In fact, some molecules with insulinase activity (protein disulfide isomerase) seem to control simultaneously insulin degradation and the inflammatory process.

Finally, we did not find any significant relationship between serum SP-D concentration and lung function tests. Obese subjects have respiratory impairment due to the increment of total body fat, which produces diminished compliance and increased resistance and work of breathing. Most obese subjects have an increased respiratory drive and a diminished hypercapnic response (19). These alterations are explained mainly by mechanical factors, as the extra fat load provokes higher work of breathing (19). We observed that serum SP-D concentration correlated positively with end-tidal carbon dioxide tension ($P_{\text{ET,CO2}}$), but the meaning of this association remains to be explored more in-depth. In fact, the lipoprotein complex surfactant is essential for reducing surface tension at the air–liquid interface of the lung and for lung immune host defense. Relatively little is known about the in vivo role of this protein in chronic lung diseases. Because there was no correlation of SP-D with vital capacity, this finding cannot explain why subjects with lower lung capacity are at a higher risk of diabetes.

Fasting glucose and fasting triglycerides contributed significantly to the variance of circulating SP-D concentrations. Glucose is a known ligand for SP-D. Neutralization of different viruses by SP-D is

abolished in the presence of increased glucose levels in mice. A number of respiratory illnesses to which diabetic patients show particular susceptibility are known to be agglutinated by SP-D (25). We speculate that repeated viral infection in patients with reduced SP-D levels could lead to inflammation and worsening of carbohydrate metabolism. However, the reverse hypothesis cannot be excluded: SP-D might be regulated by glucose, triglycerides, and inflammation.

In summary, the findings of the present study suggest that lung innate immunity, as inferred from circulating SP-D concentrations, could be at the crossroads of inflammation, obesity, and insulin resistance. Circulating SP-D levels should be evaluated in future studies to explore whether their concentrations predict the development of type 2 diabetes or decrease after the impairment of carbohydrate metabolism. The measurement of SP-D bioactivity in vivo and SP-D levels in bronchoalveolar lavage will be necessary to evaluate the underlying mechanisms connecting obesity, insulin resistance, inflammation, and SP-D.

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