No Seasonal Variation of Insulin Sensitivity and Glucose Effectiveness in Men

Claus Højbjerg Gravholt, Palle Holck, Birgit Nyholm, Erik Christiansen, Mogens Erlandsen, and Ole Schmitz

Insulin resistance is of pathogenetic importance for the development of non–insulin-dependent diabetes mellitus (NIDDM). However, not much is known about the variation in insulin sensitivity in the individual over longer periods. Consequently, we measured insulin sensitivity (Si) and glucose effectiveness (Sg) in healthy young men (N = 10) 5 times over a period of 15 months using a frequently sampled intravenous glucose tolerance test (FSIVGTT) with minimal-model analysis (study of seasonality). The maximal aerobic capacity ($\dot{V}o_2max$), fat-free mass, body mass index (BMI), and 24-hour ambulatory blood pressure (BP) were also assessed. Furthermore, we performed a study designed to evaluate the day-to-day variation in Si and Sg (study of day-to-day variation). Here, we studied Si and Sg in healthy young men (n = 8) within 2 weeks. In the study of seasonality, the coefficient of variation (CV) for Si was 24.0%, whereas the CV for Sg was 26.0%. Anticipating a seasonal variation in Si following a sine curve with a cycle length of 1 year and an unknown phase and amplitude, we tested this hypothesis with a multiple linear regression model that allows for different levels of Si between individuals, and failed to detect any impact due to this. Si (mean ± SD, $1.17 \pm 0.28 \times 10^{-4} \cdot min^{-1} \cdot pmol/L^{-1}$, $P = .38$), Sg ($0.023 \pm 0.006\ min^{-1}$, $P = .71$), fasting insulin (21.2 ± 7.3 pmol/L, $P = .98$), $\dot{V}o_2max$ (3.8 ± 0.6 L/min, $P = .13$), and fat-free mass (64.9 ± 2.5 kg, $P = .92$) were constant over time. In the study of day-to-day variation, we found a CV for Si of 17.3% and a CV for Sg of 23.3%. In conclusion, we found that the variations in Si and Sg were slightly higher than those found in studies performed to establish the day-to-day variation. However, no significant seasonal variation in Si and Sg was evident in this group of healthy young lean caucasian men. Consequently, indices of Si and Sg obtained at different times of the year appear comparable.

A LIMITED AMOUNT OF DATA are available on the variation in insulin sensitivity over time in man. A diurnal variation in insulin sensitivity is present, with relative insulin resistance during the early morning (6 to 9 AM) and afternoon (3 to 5 PM) compared with morning (9 to 11 AM).[1-4] Furthermore, insulin sensitivity has been found to change during the menstrual cycle in some studies,[5] while insulin sensitivity has been found to be unaltered by others.[6-8] Finally, puberty is a period of life associated with diminished insulin sensitivity.[9]

The diagnosis of non–insulin-dependent diabetes mellitus (NIDDM) has been suggested to be associated with seasonality,[10] showing the lowest incidence during the summer months and a more than 2-fold increase in incidence during winter and early spring, with intermediate incidence in late spring and autumn. Moreover, it is widely accepted that decreased insulin sensitivity is a prominent feature in the pathogenesis of NIDDM.[11,12] Thus, it is tempting to hypothesize that the time of diagnosis of overt NIDDM may be attributable, at least in part, to a seasonal influence in insulin action, and this may be apparent in individuals with normal glucose tolerance. Additionally, in the escalating number of human studies examining insulin sensitivity in various groups or during different conditions, it is important to clarify whether comparisons are justified without considering seasonality.

Consequently, we performed 2 studies, 1 examining the seasonality and the other the day-to-day variation in insulin sensitivity and glucose effectiveness. We repeatedly measured insulin sensitivity in a group of healthy young men to detect variations due to seasonal changes. In each subject, insulin sensitivity was examined 5 times in the course of 17 months using the minimal-model approach on data from a frequently sampled intravenous glucose tolerance test (FSIVGTT).[13,14] Variables known to influence insulin sensitivity such as maximal oxygen uptake ($\dot{V}o_2max$), habitual physical activity level, body composition, and smoking habits were recorded. Furthermore, we determined the short-term coefficient of variation (CV) of the methods.

SUBJECTS AND METHODS

Subjects

Study of seasonality. We studied 10 young healthy men who were receiving no medication. None had any family history of type 2 diabetes and none were elite athletes. Two were smokers and 8 were nonsmokers. The age and anthropometric data are depicted in Table 1.

Study of day-to-day variation. For the study concerning the CV for the measured variables, we studied 8 young healthy men who were receiving no medication. None had a family history of type 2 diabetes, none were elite athletes, and none were smokers. Their age was 28.1 ± 4.2 years and the body mass index (BMI) was 22.8 ± 1.7 kg/m^2.

All subjects received oral and written information concerning the study prior to provision of written informed consent before the study. The protocol was approved by the local ethical scientific committee.

Experimental Procedure

Experiments during the study of seasonality of insulin sensitivity. All subjects were studied 5 times in 17 months. There was a mean of 4 months between the first and second study day, 5 months between the second and third study day, 3 months between the third and fourth study day, and 4 months between the fourth and fifth study day. Investigations were performed at the Clinical Research Center, Medical Department M, University Hospital of Aarhus, on 1 morning after an overnight fast (10 to 12 hours), which excluded caffeine consumption and cigarette

From Medical Department M (Endocrinology and Diabetes) and Medical Research Laboratories, Aarhus University Hospital, Aarhus; Steno Diabetes Center, Gentofte; and Department of Biostatistics, University of Aarhus, Aarhus, Denmark.

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Address reprint requests to Claus Højbjerg Gravholt, MD, PhD, Medical Department M (Endocrinology and Diabetes), Aarhus Kommunehospital, University Hospital of Aarhus, DK-8000 Aarhus C, Denmark.

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Table 1. Anthropometric and Body Composition Data for the 10 Normal Subjects at Each Time of Examination (mean ± SD)

Parameter	January	May	November	February	June	P*
Height (m)	184.5 ± 7.7					
Weight (kg)	77.3 ± 8.9	77.4 ± 8.6	76.8 ± 10.1	78.2 ± 9.4	77.8 ± 9.4	.68
BMI (kg/m²)	22.7 ± 1.8	22.7 ± 1.8	22.5 ± 2.2	22.9 ± 2.1	22.9 ± 2.5	.64
Fat-free mass (kg)	65.1 ± 5.3	64.9 ± 7.4	64.1 ± 6.3	65.3 ± 5.4	65.2 ± 6.6	.92

*Repeated-measures ANOVA was used to test for changes in the group between examinations.

smoking; only ingestion of tap water was allowed. At least 3 days prior to investigation, the subjects consumed a carbohydrate-rich diet with at least 300 g carbohydrate/d. Participants were asked not to perform major physical exercise for the last 3 days before examination and to refrain from alcohol intake on the day before investigation.

After a minimum initial bedrest of 45 minutes, fat-free mass was determined using bioelectrical impedance analysis (Animeter; HTS-Engineering, Odense, Denmark).[15] Bioelectrical impedance analysis was performed under the same standard conditions at every examination, with participants in the fasting state. The BMI was calculated as weight (kilograms) divided by height (meters) squared.

A FSIVGTT was performed, and the minimal model of glucose and insulin kinetics was used to analyze the data.[13,16] The insulin sensitivity index (Si) is a measure of the effect of an increment in plasma insulin to enhance the fractional disappearance of glucose from the extracellular compartment of glucose distribution and to inhibit hepatic glucose production, while the glucose effectiveness index (Sg) is a measure of the mass effect of an increment in glucose on the disappearance of glucose from the extracellular compartment and to inhibit hepatic glucose production. The minimal-model program computes fractional standard deviations or CVs on the estimated parameters (Si and Sg), providing an estimate of the precision of Si and Sg. We used exogenous insulin to accelerate glucose disappearance, as this facilitates minimal-model analysis.[17] One intravenous catheter (Viggo, Helsingborg, Sweden) was placed in an antecubital vein and another in an arterialized contralateral hand vein for blood sampling. Baseline samples of glucose and insulin were obtained at −15 and −5 minutes. Glucose (0.3 g/kg as 50% glucose) was administered as a bolus dose within 90 seconds and subsequent samples were drawn at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160, and 180 minutes. At 20 minutes, subjects received a bolus injection of insulin (0.02 U/kg; Novo Nordisk, Copenhagen, Denmark).

After the FSIVGTT, a 6-minute submaximal exercise test with continuous monitoring of the heart rate was performed on a bicycle ergometer (Monark Ergometric 829 E; Monark Exercise, Varberg, Sweden) using a workload of 300 to 1,500 kpm/min, depending on the age and reported physical activity of the subject. The mean heart rate during the last 2 minutes of work (>120 bpm) was used for calculation of the maximal aerobic capacity ($\dot{V}o_2max$).[18] This indirect measure of $\dot{V}o_2max$ has been shown to correlate well with a direct measure of $\dot{V}o_2max$, with a CV less than 10%.[19,20] The level of habitual physical activity, during both work and leisure, was quantified after an interview after each examination using a questionnaire according to Saltin and Grimby.[21]

Twenty-four-hour ambulatory BP was measured by a portable lightweight monitor with an oscillometric technique (Spacelabs 90202, Redmond, WA). Readings were obtained automatically at 20-minute intervals during the day (6 AM to midnight) and once per hour from midnight to 6 AM. After a demonstration of the equipment, 5 readings were activated in the laboratory; these 5 readings were later deleted from the record before calculating 24-hour BP. The average day and night BP was calculated for each patient. The procedure has been described elsewhere.[22] The participants were then discharged and BP was measured at the participant's home. During the period of investigation, the participants ate their normal diet.

Experiments during the study of day-to-day variation in insulin sensitivity. We determined the day-to-day CV for Si and Sg, $\dot{V}o_2max$, and fat-free mass. Within 2 weeks, we performed 2 FSIVGTTs with minimal-model assessment. $\dot{V}o_2max$ and fat-free mass were measured thrice in 3 weeks. The experimental procedure was as described before.

Assays

The plasma glucose level was measured immediately in duplicate on an autoanalyzer (Beckman Instruments, Palo Alto, CA) by the glucose oxidase method. The autoanalyzer was calibrated frequently with known human plasma standards, as well as standards supplied by the manufacturer with the equipment, and the intraassay CV was less than 0.5%. The insulin level was measured by an enzyme-linked immunosorbent assay using a 2-site immunoassay that does not detect proinsulin, split(32-33)- and des(31-32)-proinsulin, whereas split(65-66)- and des(64-64)-proinsulin cross-react 30% and 63%, respectively.[23] The intraassay CV was 2.0% at a serum level of 200 pmol/L, and the interassay CV was 4%.

Calculations

The glucose disappearance constant, K_G, was calculated as the slope of the natural logarithm of glucose versus time between 8 and 19 minutes.[24] The acute serum insulin response ($AIR_{glucose}$) and plasma glucose response were calculated as the incremental area under the curve, as this provides a measure unconfounded by differences in the fasting level, using the trapezoidal rule during the first 8 minutes.

Statistical Analysis

Data were examined by Student's 2-tailed unpaired *t* test and 2-way ANOVA for repeated measures together with linear and multiple linear regression. Results are expressed as the mean ± SD. Since the time of year seems to influence the diagnosis of NIDDM, where fluctuations in diagnostic frequency per month can be described by a sine curve,[10,25] we tested the hypothesis of a seasonal variation in insulin sensitivity in normal individuals following a sine curve with a period of 1 year and an unknown phase and amplitude, anticipating a lower insulin sensitivity during parts of the year. This was achieved using multiple linear regression with random coefficients allowing for interindividual variation in the regression coefficients. Significance levels less than 5% were considered significant. All statistical calculations were performed with SPSS for Windows version 6.1.3 (SPSS, Chicago, IL) on a Pentium PC.

Model Considerations

We applied a model of seasonal variation hypothesized to follow a sine curve. We anticipated that the variation would follow a formula,

$$S_I = a + b \sin (t + \theta), \quad (1)$$

which can be transformed to

$$Si = a + c1 \times \sin (t) + c2 \times \cos (t), \quad (2)$$

where Si is insulin sensitivity, a denotes the average level, b denotes the amplitude ($b = \sqrt{c1^2 + c2^2}$), $c2/c1 = \tan (\theta)$ denotes the phase constant

(a, c1, and c2 can have different values among individuals), and t is the time of year for the individual investigation, scaled such that the period of the sine curve is 1 year. An exact confidence interval for b based on Eq 1 is difficult to compute due to the nonlinearity of the equation caused by the parameter θ. An approximate confidence interval can be obtained by first fitting Eq 2 to produce an estimate of θ from the estimates of c1 and c2. Secondly, using this value of θ as a known constant in Eq 1, this equation can be handled as an ordinary linear regression to yield a confidence interval for b.

It is statistically complicated to perform exact calculations on the power of the statistical test for seasonal variation in the multiple regression model with random coefficients. But with some simplifying assumptions, it is possible to produce a crude evaluation of the design.[26,27] Our parameter of interest is the magnitude of the amplitude b. For simplicity, we assume that $\theta = 0$ (or is known). We will consider 2 situations: (1) each individual has his own seasonal variation (individual level, amplitude, and phase) and (2) the individuals have a common nonrandom seasonal variation.

1. For a single individual, we assume that we have k + 1 equispaced observation times ($t_0, t_1, \ldots t_k$) during 1 season, ie, $t_k = t_0 + 2\pi$. Let $x_j = \sin(t_j)$, and then it follows from standard regression theory that the variance of the estimator of b is

$$\text{var}(b) = \sigma^2/\Sigma(x_j - x)^2 = \sigma^2/(k + 1)\, s_x^2, \quad (3)$$

where σ^2 denotes the (intraindividual) measurement variance. Since $x_j = \sin(t_j)$, it follows that $(k + 1)s_x^2 = k/2$, implying $\text{var}(b) = 2\sigma^2/k$. Let d be the meaningful amplitude of interest, and then standard power calculations yield

$$k = 2(z_{1-\alpha/2} + z_{1-\beta})^2\sigma^2/d^2. \quad (4)$$

2. To account for the repeated-measures design, we will assign a constant serial intraindividual correlation (ρ). Let the design consist of n individuals and k + 1 equispaced observation times ($t_0, t_1, \ldots t_k$) as before. In this situation, the variance of the estimator of b is

$$\text{var}(b) = \sigma^2(1 - \rho)/n\,(k + 1)\, s_x^2. \quad (5)$$

Thus, we obtain

$$n = (z_{1-\alpha/2} + z_{1-\beta})^2\, \sigma^2(1 - \rho)/(k + 1)\, s_x^2 d^2 \quad (6)$$

and

$$n = 2(z_{1-\alpha/2} + z_{1-\beta})^2\, \sigma^2(1 - \rho)/kd^2, \quad (7)$$

where α is the level of significance and β is the risk of a type 2 error. In the power calculations, we used an α of .05 and a β of 0.8.

RESULTS

Study of Seasonality

The age of the participants at the initial examination was 24.4 ± 1.8 years (range, 21 to 27). During the study, there were no changes in any of the body composition variables (Table 1). The basal metabolic substrates and indices from minimal-model analysis are summarized in Table 2. Insulin sensitivity did not change significantly over time (ANOVA, $P = .38$). Figure 1 shows individual values for Si at the time of examination. The CV for Si was 24%, and for the fractional standard deviation it was 3.7% ± 2.51%. Sg was also unaltered (ANOVA, $P = .71$), with a CV of 26%, and for the fractional standard deviation it was 16.0% ± 6.4%. Since the ANOVA does not account for the ordering of time, this test will have low power to detect a small seasonal variation. Therefore, we also examined the data by means of the random coefficient regression model mentioned earlier. Using this regression model without prior assumption as to when in the course of 1 year insulin sensitivity might be highest or lowest, we failed to detect any seasonal variation. In the model, we allowed for different levels of insulin sensitivity between individuals and different phases, eg, individuals could have high and low insulin sensitivity at different times of the year. Under this model, we found a value for the amplitude, b, of 0.068×10^{-5} pmol/L · min^{-1} (95% confidence interval, −0.039 to 0.174; cos(t), $P = .26$; sin(t), $P = .89$), and henceforth no indication of any seasonal variation. Applying multiple regression to detect a variation in Si due to any of the other measured variables, we failed to find any statistically significant correlations (results not shown), again allowing for a different "setpoint" of Si for each individual. When substituting Sg for Si in the formula described before, likewise, no indication of seasonal variation was found. To obtain an estimate of the power of the model to detect seasonal changes, we applied power calculations under 2 different assumptions based on the values of σ^2 and ρ derived from the present study. Assuming random (intraindividual) seasonal variation in Si and Sg, one should be able to detect at least a 30% variation in Si and Sg with a sample size of 10 and 5 sampling points, while a sample size of 41 would be necessary to detect a 15% variation in Si. Assuming nonrandom seasonal variation, a sample size of 5 should be sufficient to detect a 15% variation (Table 3).

All other measured indices of insulin sensitivity were also

Table 2. Basal Metabolic Substrates and Minimal-Model–Analyzed FSIVGTT Data at Each Time of Examination

Parameter	January	May	November	February	June	P*
Si ($\times 10^{-5}$ pmol/L^{-1} · min^{-1})	11.0 ± 4.0	13.3 ± 2.9	11.2 ± 4.0	11.8 ± 4.5	11.2 ± 4.3	.38
Fractional SD Si	3.7 ± 4.0	3.1 ± 2.5	2.7 ± 1.8	3.4 ± 1.6	4.4 ± 2.4	.42
Sg ($\times 10^{-2}$ · min^{-1})	2.1 ± 0.5	2.6 ± 0.7	2.2 ± 0.5	2.2 ± 1.1	2.4 ± 1.1	.71
Fractional SD Sg	15.3 ± 5.7	17.8 ± 12.6	13.2 ± 3.7	17.7 ± 7.1	16.2 ± 5.9	.56
Fasting insulin (pmol/L)	2,108	2,240	1,983	2,150	2,125	.98
Acute plasma glucose response (mmol/L · 8 min)	57.6 ± 9.7	62.4 ± 7.6	62.8 ± 6.5	63.1 ± 7.6	64.3 ± 5.8	.15
Acute serum insulin response (pmol/L · 8 min)	1,965 ± 907	1,803 ± 1,087	1,812 ± 587	2,759 ± 1,937	2,323 ± 1,566	.20
Glucose disappearance ($\times 10^{-2}$ · min^{-1})	1.75 ± 0.56	2.31 ± 1.18	1.98 ± 0.46	2.47 ± 1.07	2.36 ± 1.12	.17
$\dot{V}o_2$max (L O_2/min)	334	398	403	383	388	.13
24-h systolic BP (mm Hg)	128	127	121	127	125	.13
24-h diastolic BP (mm Hg)	67	72	67	70	68	.26

*Repeated-measures ANOVA was used to test for changes in the group between examinations.

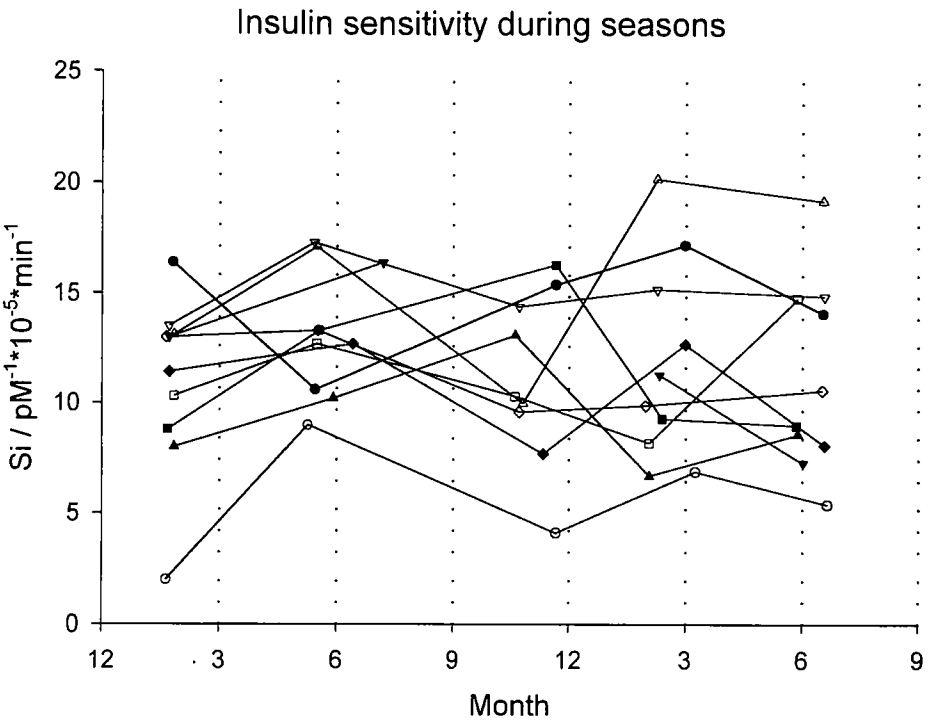

Fig 1. Individual values for Si at each examination, with individual lines drawn directly through each point. The x-axis depicts months, where 1 equals the start of January, 2 equals the start of February, and so on. Dotted lines indicate the start of a new season, ie, spring, summer, autumn, and winter. The CV for the 5 examinations was 24%.

statistically unchanged. Fasting insulin (ANOVA, $P = .98$), $\dot{V}O_2$max (ANOVA, $P = .13$), BMI (ANOVA, $P = .64$), and fat-free mass (ANOVA, $P = .92$) were constant over time within the group and individually. CVs for fasting insulin and $\dot{V}O_2$max were 35% and 16%, respectively. The acute response of insulin and glucose was unaltered, with a CV of 38% and 10%, respectively. Also, K_G was unchanged, with a CV of 33% (Table 2).

Twenty-four-hour BP (systolic ANOVA, $P = .13$; diastolic ANOVA, $P = .26$) was unchanged (Table 2).

The assessment of habitual physical activity by interview[21] did not disclose any major changes in individual activity during work and leisure. Three individuals reduced their physical activity during the study period from one group to another. The other 7 individuals did not change their level of physical activity. When introducing physical activity as an independent variable in multiple regression analysis, we could not demonstrate any significant correlation with any of the main outcome measures.

Study of Day-to-Day Variation

We found a day-to-day CV for Si of 17.3% (day 1, $15.1 \pm 4.5 \times 10^{-5}$ pmol/L · min^{-1}; day 2, $14.9 \pm 6.5 \times 10^{-5}$ pmol/L · min^{-1}), for Sg 23.3% (day 1, $2.2 \pm 0.7 \times 10^{-2}$ · min^{-1}; day 2, $2.1 \pm 0.5 \times 10^{-2}$ · min^{-1}), and for $AIR_{glucose}$ 21.4% (1,763 ± 1,596 pmol/L · 8 min). The CV was 9.4% (day 1, 3.8 ± 0.7 L O_2/min; day 2, 4.1 ± 0.6 L O_2/min; day 3, 3.9 ± 0.7 L O_2/min) and 5.8% (day 1, 82.0% ± 3.3% fat-free mass; day 2, 82.3% ± 2.9% fat-free mass; day 3, 82.1% ± 2.8% fat-free mass) for $\dot{V}O_2$max and fat-free mass, respectively.

DISCUSSION

For more than 70 years, it has been known that the onset of insulin-dependent diabetes is most common during the fall,

Table 3. Power Calculations With Determination of Sample Size Under Two Different Assumptions: (1) Assuming Random (intraindividual) Seasonal Variation in Si and Sg and (2) Assuming Nonrandom Seasonal Variation

Si	Sample Size (n)	Sg	Sample Size (n)
1. Random intraindividual seasonal variation*		1. Random intraindividual seasonal variation*	
d = 15% (1.75 pmol/L^{-1} × 10^{-4} · min^{-1})	41.0	d = 15% (0.0040 · min^{-1})	34.9
d = 30% (3.51 pmol/L^{-1} × 10^{-4} · min^{-1})	10.2	d = 30% (0.0081 · min^{-1})	8.7
d = 50% (5.84 pmol/L^{-1} × 10^{-4} · min^{-1})	3.4	d = 50% (0.0135 · min^{-1})	3.1
2. Nonrandom intraindividual seasonal variation*		2. Nonrandom intraindividual seasonal variation*	
d = 15% (1.75 pmol/L^{-1} × 10^{-4} · min^{-1})	5.2	d = 15% (0.0040 · min^{-1})	4.7
d = 30% (3.51 pmol/L^{-1} × 10^{-4} · min^{-1})	1.3	d = 30% (0.0081 · min^{-1})	1.2
d = 50% (5.84 pmol/L^{-1} × 10^{-4} · min^{-1})	0.5	d = 50% (0.0135 · min^{-1})	0.4
Estimated value for σ^2	.080	Estimated value for σ^2	.000036
Estimated value for ρ	.50	Estimated value for ρ	.46

*For the sample size calculations, we used a value of 4 for k, .05 for α, and .8 for β.

winter, and spring (the cold months of the Northern Hemisphere).[25,28-30] However, recent data from the UK Prospective Diabetes Study suggest that the diagnosis of NIDDM also shows seasonal variation,[10] which also has been shown before,[25] with the incidence approximately twice as high during winter months versus summer months.[10] This seasonal pattern in presentation can be described as following a sine curve. Seasonality in glycosylated hemoglobin is present in normal children, and this could be suggestive of seasonality in insulin sensitivity.[31] It is established that insulin resistance is a cardinal factor in the pathogenesis of NIDDM, being present at least up to 10 years before the development of NIDDM.[32] Young[33] found that the heart muscle in rats is most insulin-resistant during the winter months. Furthermore, Young demonstrated that maintaining a constant day length with artificial light prevented the decrease in insulin sensitivity, which seems enigmatic since rats are primarily nocturnal animals. In various hibernators, seasonally occurring changes in insulin sensitivity have been demonstrated,[34,35] and circadian neuroendocrine rhythms of glucocorticosteroids and prolactin have been suggested to be implicated in these changes.[36] These previous studies in animal models allowed us to hypothesize the presence of seasonal variation in insulin sensitivity in healthy man.

However, we failed to detect any significant seasonal variation in insulin sensitivity, as well as glucose effectiveness, in this sample of young healthy men. A CV for Si of 24% in the course of 15 months was observed. This implies that Si varies over the course of months. By comparison, Steil et al[37] found a CV of 20% when investigating adults 3 times within 12 days, whereas Ferrari et al[38] found a CV of 14.4% when investigating 15 men twice at an interval of 3 weeks. In accordance with Steil et al and Ferrari et al, we found a CV of 17.4% for Si in the study of day-to-day variation in insulin sensitivity. Similarly, we did not demonstrate any seasonal variation in glucose effectiveness. A CV of 26% was found, as compared with a CV of 25% in the study by Steil et al[37] and a CV of 23% in the present study of day-to-day variation. These CVs point toward a very modest variation in insulin sensitivity and glucose effectiveness, since the studies by Steil et al[37] and Ferrari et al,[38] and the present study of day-to-day variation were performed to establish the variation due to the method per se. The additional variation in the study of seasonality compared with the 3 studies is only 4%, 10%, and 7%, respectively, for insulin sensitivity and 1% and 3% for glucose effectiveness. In this context, it should be noted that during an oral glucose tolerance test, intraindividual variation can be as high as 25% or even higher,[39,40] and it has been shown that among people with impaired glucose tolerance, 35% to 75% revert to normal when retested.[41]

The most simple mathematical way of describing a variable that cycles is by applying a sine function, because only 2 parameters, amplitude and phase, are needed, apart from the length of the cycle. All other models describing cyclic phenomenae depend on additional parameters; thus, an approach of increasing complexity would severely limit the statistical power of any model. When applying a multiple linear regression model to explain any type of seasonal variation in Si and Sg, no such variation could be detected. Expected to follow a sine curve, the model was without restrictions as to when insulin sensitivity might be at its highest or lowest level and allowed for different levels of insulin sensitivity between individuals and different phases; eg, individuals could have high and low insulin sensitivity at different times of the year. This regression model does not depend on equal sampling intervals, since sin(t) and cos(t) are used as regressor variables (Eq 2), eg, it is not crucial to have equidistant sampling intervals. We chose to define the length of a cycle as 1 year based on findings in studies of the time of diagnosis of type 2 diabetes. The model does not allow us to test for seasonal variation with either a shorter or longer period, eg, a cycle length of 1 month or 2 years, etc. Indeed, when studying the data, any variation with a length of cycle shorter than 1 year does not seem obvious. However, we cannot exclude a cyclic pattern following a sine function with a shorter or longer length of cycle, and likewise, we are not able to exclude the existence of other cyclic patterns. The existence of cyclic patterns other than sine functions, or more complicated sine functions, would indeed be difficult to study, due to the fact that this would increase the number of parameters involved in modeling, requiring much larger study groups and more frequent sampling. The present model also allowed us to test the impact of covariates, eg, the additional variables measured in the study. None of the measured covariates were significant predictors of either Si or Sg.

Furthermore, we performed power calculations based on the present model. The results show that the ability to detect meaningful (15%) nonrandom seasonal variation would require a sample size of 5 young healthy sedentary men studied 5 times. Assuming random (intraindividual) seasonal variation in Si, one should be able to detect at least a 30% variation in Si with a sample size of 10, while a sample size of 41 should be necessary to detect a 15% variation. In the study of day-to-day variation, the reported CVs consist of at least 2 components. One component could be called the "real" day-to-day variation, meaning the variation in Si, and another component could be called random measurement error. From the present data, it is not possible to separate these 2 sources of error to yield an estimate of the "real" day-to-day variation. Previously, Campbell et al[42,43] found differences in the response to an oral glucose tolerance test in humans under extreme weather conditions in Antarctica, with a relative insulin resistance during the winter. However, these changes could readily be explained by large differences in energy intake, physical exercise, periods of continuous light and continuous dark, and large variations in temperature.

Significant seasonal variations in $\dot{V}O_2max$ have not been demonstrated except in elite athletes.[44] The intraindividual CV is small (9.4%) for $\dot{V}O_2max$ determined by the indirect method, as found in the study of day-to-day variation, and a good correlation with direct methods of determination exists with less than 10% variability.[19,20] We found a CV of 16% for $\dot{V}O_2max$ in the study of seasonal variation, which shows that the persons involved in this study led steady lives during the period of investigation. It is of course notable that the participants were also asked not to be involved in heavy exercise for the last 3 days prior to the study, since a bout of exercise in close

proximity to the investigation of insulin sensitivity has been shown to enhance measures of insulin sensitivity.[45]

Seasonal variation in the BMI is present in rural women in Third World countries[46,47] and in healthy premenopausal women,[48] but it has not been shown to be present in males. Since the BMI is known to modulate insulin sensitivity, it would be expected that seasonal changes would affect insulin sensitivity. We could not detect any seasonal changes in the BMI in the present study.

Previously, the variability of the $AIR_{glucose}$ has been assessed. To establish the CV for the acute serum insulin response to intravenous glucose, a previous study found a mean value of 8.8% in a protocol wherein subjects were examined twice separated by at least 2 weeks and no more than 4 months.[49] Smith et al[50] found a CV of 22%, in accordance with the present study of day-to-day variation (CV for $AIR_{glucose}$, 21%). The reasons for this large difference in the report by Rayman et al[49] versus the report by Smith et al[50] and the present study are not entirely clear. We used much the same approach as Rayman et al, including heating the hand to achieve arterialization of the venous blood, an individualized glucose bolus, and meticulous timing of the sampling intervals. In the study of seasonality, we found a considerably higher CV for $AIR_{glucose}$ of 38%. Contrary to Si, it appears that the acute serum insulin response tends to vary considerably during a longer period of observation.

An association between insulin sensitivity and BP is well established.[51] Seasonal variation in arterial BP has been described in mildly hypertensive individuals, in whom the variation was significantly related to the temperature, with higher systolic and diastolic BP during the winter.[52] However, in the present study, we did not observe any variation in 24-hour BP due to seasonality in normotensive individuals.

In conclusion, we found slightly higher variations in Si and Sg versus studies performed to establish the day-to-day variation. However, no significant seasonal variation in insulin sensitivity and glucose effectiveness was evident in healthy sedentary lean young caucasian men. We furthermore showed that to exclude nonrandom seasonal variation and random (individual) seasonal variation of more than 30% in Si and Sg, the design used in the current study would suffice. Consequently, indices of insulin sensitivity and glucose effectiveness obtained at different times of the year appear comparable.

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