

Fasting Insulin Pulsatile Secretion in Lean Women with Polycystic Ovary Syndrome

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Summary

The aim of our study was to evaluate rapid insulin pulses and insulin secretion regularity in fasting state in lean women with polycystic ovary syndrome (PCOS) in comparison to lean healthy women. PCOS (n=8) and controls (n=7) underwent every minute blood sampling for 60 min. Insulin pulsatility was assessed by deconvolution and insulin secretion regularity by approximate entropy methodology. PCOS had higher testosterone ($p<0.02$), prolactin ($p<0.05$) and lower sex hormone binding globulin (SHBG) ($p<0.0006$) levels than controls. Approximate entropy, insulin pulse frequency, mass, amplitude and interpulse interval did not differ between PCOS and controls. PCOS had broader insulin peaks determined by a common half-duration ($p<0.07$). Burst mass correlated positively with testosterone ($p<0.05$) and negatively with SHBG ($p<0.0004$) and common half-duration correlated positively with prolactin ($p<0.008$) and cortisol levels ($p<0.03$). Approximate entropy positively correlated with BMI ($p<0.04$) and prolactin ($p<0.03$). Lean PCOS patients tended to have broader insulin peaks in comparison to healthy controls. Prolactin, androgens and cortisol might participate in alteration of insulin secretion in PCOS-affected women. Body weight and prolactin levels could influence insulin secretion regularity.

Key words

Insulin • Pulse • Polycystic ovary syndrome • Diabetes mellitus

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Introduction

Insulin is physiologically secreted in a pulsatile manner with a variable basal insulin release. The

contribution of pulsatile insulin secretion to total insulin secretion has been estimated as being at least 70 %, both in fasting and in postabsorptive states (Porksen *et al.* 1995, Porksen 2002).

Insulin pulsatility is composed of rapid regular pulses of 5 to 15 min in frequency, superimposed on ultradian (slow) oscillations with a period of 80-150 min. It has been assumed that rapid insulin pulses suppress hepatic gluconeogenesis and slow insulin pulses contribute to glucose utilization in the peripheral tissues (Porksen 2002). Glucose is the most important regulator of insulin secretion, with a feedback loop between slow pulses and blood glucose levels. Rapid insulin pulses, in addition to being driven by glycaemic oscillations, are controlled by the hypothetical intrapancreatic neuronal pacemaker arising from rich pancreatic innervation. Many other metabolic substrates, hormones or neurotransmitters also influence insulin secretion (Stagner *et al.* 1980, Ahren 2000, Porksen *et al.* 2000).

Polycystic ovary syndrome is one of the most common endocrinopathies of women in their fertile years. Together with the reproductive abnormalities of chronic anovulation and hyperandrogenism, hyperinsulinaemia and insulin resistance (IR) are present in a relevant number of women affected with PCOS. The incidence of impaired glucose tolerance (IGT) and diabetes mellitus type 2 (DM 2) is significantly increased in PCOS in comparison with healthy control subjects (Ehrmann *et al.* 1999, Legro *et al.* 1999).

Besides insulin resistance, insulin secretory dysfunction is another risk factor for DM 2. The alterations in insulin pulsatile secretion and insulin

release orderliness might be an early marker of beta-cell dysfunction. Abnormal insulin pulsatile patterns were detected even in first degree relatives of patients with DM 2, who still had normal first phase secretory response to glucose (O'Rahilly *et al.* 1988). Insulin secretion in PCOS was studied in a limited number of studies (Dunaif *et al.* 1996, Ehrmann *et al.* 1995, Holte *et al.* 1994, Vrbíková *et al.* 2007) and the data about insulin pulsatile release in PCOS is especially sparse (Armstrong *et al.* 2001). Concerning slow insulin pulses, others described significantly higher basal insulin secretory rates and attenuated secretory responses to meals in obese women with PCOS (O'Meara *et al.* 1993).

The aim of our study was to evaluate the rapid insulin pulses and the regularity of insulin secretion in lean women affected with PCOS in comparison to healthy lean women.

Methods

After an overnight fast, the participants were brought to the Clinical Research Unit in Institute of Endocrinology. The protocol was approved by the Institutional Ethical Committee. Before entering the study, written informed consent was obtained after written and oral information. The study group consisted of 8 women affected with PCOS fulfilling the diagnostic criteria by using definition ESHRE/ASRM (2004). The diagnosis of PCOS is accepted, when 2 out of 3 from: oligo/amenorrhoea, hyperandrogenaemia and or clinical manifestation of either acne or hirsutismus and ultrasonographic morphology of polycystic ovary are present. The control group (C) consisted of 7 healthy women with regular menstrual cycles and no clinical or biochemical signs of hyperandrogenemia. Pelvic ultrasonography was not performed in healthy controls. Both groups were without medication affecting carbohydrate metabolism including oral contraceptives at least for the preceding 3 months and without family history of diabetes mellitus. Impaired glucose tolerance, diabetes mellitus, thyroid dysfunction, hyperprolactinemia, hypercortisolism and the late onset congenital adrenal hyperplasia were all excluded using the appropriate biochemical parameters or tests.

Basal blood samples for the determination of testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone, estradiol, 17-hydroxyprogesterone (17-OHP) were taken in the fasting state in the early follicular

phase of the menstrual cycle (between 1st and 5th day at 7-8.30 a.m.) or in the case of secondary amenorrhoea at any time. After the cannulae were placed in an antecubital vein for sampling purposes, the women affected with PCOS and control women underwent frequent blood sampling in 1-minute intervals for 60 min. Blood (cca 2 ml) was collected from 5 s before to 5 s after each minute. After finishing each blood sampling 0.6 ml of saline solution was administered to dead space. Ten seconds before sampling, the saline solution from the dead space was withdrawn and discarded. Serum insulin concentration was estimated each minute. The samples were analyzed the same day as the study was performed. The resultant insulin concentration time series was analyzed by deconvolution analysis. Insulin secretion regularity was estimated by approximate entropy. Both analyses are described below.

Quantitative insulin sensitivity check index (QUICKI) was calculated as $1/(\log(G_0) + \log(I_0))$ where G_0 is fasting plasma glucose (milligrams per deciliter), and I_0 is fasting plasma insulin (microunits per milliliter) (Katz *et al.* 2000).

Serum glucose concentrations were measured using the glucose-hexokinase method (Integra 400 plus, Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin was determined in singlet by ELISA (Roche Diagnostics GmbH, Mannheim, Germany) using two monoclonal murine antibodies. Intrassay CV 1.5 % and interassay CV 4.9 % are for insulin detection range of $5.93 \pm 0.09 \mu\text{U/ml}$. The higher insulin detection range is $14.5 \pm 0.13 \mu\text{U/ml}$ with intrassay CV 0.9 % and interassay CV 3.7 %. Minimal detection concentration is $0.20 \mu\text{U/ml}$. The assay has no cross-reactivity with proinsulin. C-peptide was measured by the electrochemiluminescence immunoassay ECLIA (Modular E170, Roche Diagnostics GmbH, Mannheim, Germany). The cross-reactivity of antiserum with both insulin and glucagon is less than 0.05 % with intra- and inter-assay CV of 0.5 % and 1.6 %, respectively. Testosterone was determined by standard RIA using anti-testosterone-3-carboxymethyloxime: bovine serum albumin (BSA) antiserum and testosterone 3-carboxymethyloxime-tyrosylmethyl ester- ^{125}I as a tracer; the intra-assay and inter-assay coefficients of variation (CV values) were 10.2 % and 10 % respectively, and the sensitivity is 0.21 nmol/l (normal range 0.4-3.0 nmol/l). Prolactin was measured in serum by chemiluminescence immunoanalysis ECLIA (Elecsys Prolactin II, Roche, Germany) (normal range 6-29.9 ng/ml). Androstenedione was determined using a standard RIA with anti-

androstenedione-6-carboxymethyloxime: BSA antiserum and (^3H) androstenedione as a tracer. The intra-assay and inter-assay CV values were 10 % and 10.2 % respectively and the sensitivity was 0.39 nmol/l. Sex hormone binding globulin (SHBG) was estimated using IRMA method (Orion, Espoo, Finland) with an intra-assay CV of 6.1 % and an inter-assay CV of 7.9 % (normal range 43.2-94.8 nmol/l). DHEAS (normal range 2.4-14.5 $\mu\text{mol/l}$; intra-assay CV of 3.5 % and inter-assay CV of 10.2 %), 17-OHP (normal range 1.1-8.4 nmol/l; intra-assay CV of 5.2 % and inter-assay CV of 6.5 %) and estradiol (normal range 0.11-1.29 nmol/l; intra-assay CV of 4.4 % and inter-assay CV of 4.6 %) were measured by the RIA kit from Immunotech (Prague, Czech Republic). LH was determined by IRMA (Immunotech, Prague, Czech Republic) with an intra-assay CV of 3.7 % and an inter-assay CV of 4.3 %. FSH was estimated by IRMA (Immunotech, Prague, Czech Republic) with an intra-assay CV of 2.6 % and an inter-assay CV of 4.5 %. Total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were measured by photometry (Ecoline 25, Vitalab Elipse; Merck; Darmstadt; Germany) with intra-assay CVs 1.6 %, 1.7 % and 1.2 % and inter-assay CVs of 1.9 %, 2.1 % and 1.9 %, respectively.

Deconvolution analysis of the data

Deconvolution analysis was developed for endocrinological purposes as a mathematical procedure for measuring simultaneously hormone secretion and elimination. Insulin concentration time series were analyzed by deconvolution method to detect and quantify insulin secretory pulses. We employed several different deconvolution algorithms Pulse 2, Pulse 4 and AutoDecon. The principle of deconvolution software was described previously (Veldhuis and Johnson 1992, Veldhuis and Johnson 1994). Briefly, the deconvolution method is a multiparameter technique validated previously and correctly identifying more than 90 % of hormone pulses (sensitivity) as correctly recognizing their absence (Johnson *et al.* 2004). The insulin concentrations were assumed to result from five determinable and correlated parameters: 1) a finite number of separate insulin pulses occurring at specific times, 2) individual amplitudes, 3) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude), 4) basal time invariant insulin secretion and 5) monoexponential or biexponential insulin model of elimination in the systemic circulation. Biexponential model of insulin clearance was estimated with rapid (first)

2.8 min and slow (second) 5.0 min half-lives with fractional slow compartment of 0.28. We calculated monoexponential insulin half-life of 3.3 (2.8-4.1) min in PCOS and 3.2 (2.6-4.1) min in healthy controls.

We used the root mean square of residuals (RMS) and residual variance (%) to assess optimal insulin kinetics parameters (best fit curve). We derived the best fit curve with following RMS and residual variance in PCOS and healthy women (0.77 ± 0.23 vs 0.9 ± 0.26 mUI/l; 9.44 ± 4.73 vs 9.14 ± 8.79 %, respectively).

The following parameters were calculated: secretory pulse number (the number of significant secretory pulses per 60 min), interpulse interval (time in minutes separating successive pulses), pulse mass (the mass of the calculated secretory pulses) (mUI/l), amplitude (maximal secretory rate within a pulse) (mUI/l/min), AUC_{0-60} (area under the curve, overall insulin secretion per 60 min) (mUI/l), common half-duration (mUI/min) and basal secretion (mUI/l/min).

Approximate entropy (ApEn) is used as a model-independent and scale-invariant statistic. In brief, ApEn assumes the orderliness of hormonal release patterns. ApEn is computed as the sum of the negative logarithms of the conditional probabilities that subpatterns of length m in a time series recur upon next $(m + 1)$ incremental comparison within a given tolerance range r . We used $m = 1$ and $r = 20$ % of the intraseries SD, which provides a normalized (scale-independent) ApEn statistics. ApEn was in detail described previously (Pincus *et al.* 1999).

Statistics

All data are given as retransformed means with a 95 % confidence interval. Unpaired t-tests and Pearson correlation coefficients were used on transformed data to evaluate differences between groups and the mutual relationship among the variables, respectively ($p < 0.05$) was considered statistically significant. RMS and residual variance are given as means \pm SD.

Results

The clinical, basal biochemical and hormonal parameters of women affected with PCOS and healthy subjects are given in Table 1. PCOS women had significantly higher testosterone ($p < 0.02$), prolactin ($p < 0.05$), 17-OHP ($p < 0.03$) and estradiol ($p < 0.05$) concentrations and lower SHBG levels ($p < 0.0006$). We did not observe significant difference between groups in basal insulinaemia, basal glucose levels, C-peptide and QUICKI.

Table 1. Clinical, hormonal and biochemical parameters of PCOS subjects (n=8) and controls (n=7)

	Mean	PCOS		Mean	Controls		p<
		CI Lower limit	CI Upper limit		CI Lower limit	CI Upper limit	
<i>BMI (kg/m²)</i>	21.9	20.2	23.5	20.1	18.9	21.2	NS
<i>Age (years)</i>	25.1	21.8	29.5	30.2	25.1	38.1	NS
<i>Insulinaemia basal (mUI/l)</i>	5.1	3.9	6.5	4.2	3.1	5.6	NS
<i>Blood glucose basal (mmol/l)</i>	4.17	4	4.35	4.12	4	4.4	NS
<i>C-peptide (nmol/l)</i>	0.54	0.4	0.71	0.51	0.23	0.96	NS
<i>QUICKI</i>	0.39	0.37	0.4	0.4	0.38	0.43	NS
<i>Total cholesterol (mmol/l)</i>	4	3.7	4.8	4	3.6	4.7	NS
<i>HDL cholesterol (mmol/l)</i>	1.6	1.2	1.8	1.8	1.6	1.9	NS
<i>Triglycerides (mmol/l)</i>	0.75	0.6	0.9	0.7	0.4	0.9	NS
<i>Testosterone (nmol/l)</i>	3.2	2.8	3.6	2.3	0	3.3	0.02
<i>DHEAS (mmol/l)</i>	7.1	5.6	8.3	5.5	0	8.1	NS
<i>Androstenedione (nmol/l)</i>	8.6	5.9	12.9	7.4	5	11.4	NS
<i>SHBG (nmol/l)</i>	46.3	28.4	64.1	98.1	83.1	113.1	0.0006
<i>Prolactin (ng/ml)</i>	23.8	16.8	32.5	13.7	6.3	25.4	0.05
<i>Estradiol (nmol/l)</i>	0.12	0.1	0.14	0.09	0.05	0.13	0.05
<i>Progesterone (nmol/l)</i>	2.5	1.4	3.6	2.8	0	7.4	NS
<i>LH (IU/l)</i>	4.5	2.8	8.4	6.5	3.1	20.4	NS
<i>FSH (IU/l)</i>	6.3	5.25	7.8	8	6.4	10.4	NS
<i>Cortisol (nmol/l)</i>	564	442	729	448.7	306	680	NS
<i>17-hydroxyprogesterone (nmol/l)</i>	2.5	1.8	3.7	1.4	0.9	2.2	0.03

Values are given as means and 95 % confidence interval (CI); p – value-statistical significance; NS – not significant; QUICKI – Quantitative insulin sensitivity check index; LH – luteinising hormone; FSH – follicle stimulating hormone; DHEAS – dehydroepiandrosterone sulfate; BMI – body mass index; SHBG - sex hormone binding globulin

Table 2. Deconvolution analysis results of rapid insulin pulsatile secretion in PCOS (n=8) and controls (n=7)

	Mean	PCOS		Mean	Controls		p<
		CI Lower limit	CI Upper limit		CI Lower limit	CI Upper limit	
<i>Number of peaks per 1 hour</i>	9.7	7.7	11.7	9.9	7.2	12.55	NS
<i>Basal secretion (mUI/l/min)</i>	0.7	0.4	0.98	0.5	0.24	0.82	NS
<i>Half-life of insulin (min)</i>	3.3	2.8	4.1	3.2	2.6	4.1	NS
<i>AUC₀₋₆₀ (mUI/l)</i>	300.5	235.6	398.2	255.3	188.2	340.2	NS
<i>Common half-duration (mUI/min)</i>	1.97	1.1	3.37	0.89	0.51	1.49	0.07
<i>Secretory pulse amplitude (mUI/l/min)</i>	1.1	0.7	2.1	1.5	1	2.6	NS
<i>Secretory pulse mass (mUI/l)</i>	2.1	1.55	3	1.4	0.96	2.4	NS
<i>Interpulse interval (min)</i>	5.87	5	7.2	5.56	4.6	7.1	NS
<i>Approximate entropy</i>	1.14	1.09	1.19	1.08	0.94	1.16	NS

Values are given as means and 95 % confidence interval (CI); NS-not significant; AUC₀₋₆₀ - area under the curve, overall insulin secretion during 1 hour; p-value-statistical significance

Table 3. Correlations in PCOS subjects (n=8) and controls (n=7)

	Basal insulin secretion (mUI/l/min)		Insulinaemia basal (mUI/l)		AUC ₀₋₆₀ (mUI/l)		ApEn		Common half-duration (mUI/min)		Insulin pulse mass (mUI/l)	
	r-value	p<	r-value	p<	r-value	p<	r-value	p<	r-value	p<	r-value	p<
<i>C-peptide</i> (nmol/l)	0.78	0.008	0.75	0.008	0.75	0.008		NS		NS		NS
<i>HDL cholesterol</i> (mmol/l)	-0.85	0.004	-0.88	0.0007	-0.89	0.0006		NS		NS		NS
<i>Triglycerides</i> (mmol/l)	0.75	0.03	0.78	0.008	0.78	0.009		NS		NS		NS
<i>QUICKI</i>	-0.77	0.002	-0.99	0.0001	-0.99	0.0001		NS		NS	-0.59	0.03
<i>Prolactin</i> (ng/ml)		NS		NS		NS	0.68	0.03	0.76	0.008		NS
<i>BMI</i> (kg/m ²)		NS		NS		NS	0.57	0.04		NS		NS
<i>Cortisol</i> (nmol/l)		NS		NS		NS		NS	0.64	0.03		NS
<i>Testosterone</i> (nmol/l)		NS		NS		NS		NS		NS	0.61	0.05
<i>SHBG</i> (nmol/l)		NS		NS		NS		NS		NS	-0.88	0.0004
<i>DHEAS</i> (nmol/l)		NS	0.73	0.02	0.73	0.02		NS		NS		NS

r-value-coefficient of correlation; p – value-statistical significance; NS – not significant; QUICKI – Quantitative insulin sensitivity check index; AUC₀₋₆₀ – area under the curve, overall insulin secretion per 60 minutes; ApEn – Approximate entropy; DHEAS – dehydroepiandrosterone sulfate; SHBG – sex hormone binding globulin

Pulsatile insulin secretion results

Deconvolution analysis results are given in Table 2. Women affected with PCOS and healthy controls had similar average frequency of 10 insulin pulses per 60 min. Variation in insulin burst frequency ranges from 6 to 13 insulin pulses per hour in both groups. We did not observe differences in number of insulin pulses, basal insulin secretion, pulse amplitude, pulse mass or in interpulse interval between the two groups. PCOS-affected women had broader insulin peaks in comparison to control group, as determined by the common-half pulse duration (1.97 (1.1-3.37) vs 0.89 (0.51-1.49) mUI/min; p<0.07).

Basal insulin secretion correlated negatively with HDL-cholesterol (r = -0.85; p<0.004), QUICKI (r = -0.77; p<0.002) and positively with triglycerides (r =

0.75; p<0.03). AUC₀₋₆₀ correlated negatively with QUICKI (r = -0.99; p=0.0001), HDL-cholesterol (r = -0.89; p<0.0006) and positively with triglycerides (r = 0.78; p<0.009) (Table 3.).

Insulin pulse amplitude (r = -0.61; p<0.03) similarly as insulin pulse mass (r = -0.59; p<0.03) correlated negatively with QUICKI. Common-half pulse duration correlated positively with prolactin (r = 0.76; p<0.008) and cortisol levels (r = 0.64; p<0.03). Burst mass correlated positively with testosterone (r = 0.61; p<0.05) and negatively with SHBG levels (r = -0.88; p<0.0004) (Table 3.).

Insulin secretion regularity

Insulin burst orderliness evaluated by ApEn did not differ significantly between women affected with

PCOS and healthy subjects (1.14 (1.095-1.178) vs 1.079 (0.94-1.164), respectively; $p < 0.2$). ApEn correlated positively with prolactin levels ($r = 0.68$; $p < 0.03$) and with BMI ($r = 0.57$; $p < 0.04$).

Discussion

The present study was designed to assess the rapid insulin pulses, insulin secretion regularity and the potential factors affecting insulin pulsatile secretion in lean women affected with PCOS in comparison to healthy lean women. The main findings are as follows: 1. PCOS-affected women tended to have different insulin pulsatile secretion patterns described by the broader insulin pulses in comparison to healthy controls. 2. Steroid hormones and prolactin levels were related to different aspects of insulin pulsatile secretion.

We observed the tendency toward broader insulin peaks in PCOS, but no differences between PCOS and controls in number of insulin secretory events, insulin pulse amplitude, pulse mass, interpulse interval or insulin secretion orderliness. Many studies have shown alterations in insulin pulsatility and insulin release orderliness in different states connected with insulin resistance, such as in patients with diabetes mellitus type 2 (Porksen 1999), in first degree relatives of DM 2 patients (O'Rahilly *et al.* 1988), in obesity (Peiris *et al.* 1992, Zarkovic *et al.* 2000) or in old people (Meneilly *et al.* 1999). Conversely, there are only few studies concerning insulin pulsatility in PCOS as in another states often connected with insulin resistance. In one of these studies, no significant difference in rapid fasting insulin pulse frequency, total insulin area or total pulse related area was found between PCOS and healthy controls (Armstrong *et al.* 2001).

We have found that higher prolactin levels were associated with more irregular insulin secretion as determined by higher ApEn and with different insulin secretory patterns. Prolactin could influence insulin secretion. In vitro, beta-cells of the pancreatic islets express prolactin receptors. Prolactin supports the growth of the pancreatic islets and stimulates insulin secretion (Ben-Jonathan *et al.* 2006). Prolactin could induce insulin resistance, as women with microprolactinomas were more insulin resistant than healthy women (Serri *et al.* 1986, Seki and Nagata 1991). Similarly hyperprolactinemic PCOS women were more insulin resistant than their normoprolactinemic counterparts (Bahceci *et al.* 2003). We have also observed a positive correlation between

insulin secretion regularity, as determined by ApEn values, and BMI. This finding is supported by other authors who have found an association of overweight and irregular insulin secretion with improvement after weight loss (Zarkovic *et al.* 2000).

Further, cortisol was positively correlated with the common half-duration of insulin peaks. Similarly to us, others shown that glucocorticoids could influence insulin secretion. Short-term glucocorticoid treatment impaired insulin pulsatility, probably by a disruption of the normal glucose-insulin feedback loop (Hollingdal *et al.* 2002).

Afterwards, we observed positive correlation of testosterone levels and negative correlation of SHBG and insulin pulse mass. Insulin increases the biological availability of testosterone through the suppression of SHBG synthesis. Hyperinsulinemia increases ovarian androgen production. Conversely, testosterone may indirectly contribute to insulin resistance through facilitating free fatty acids release from abdominal fat and perhaps directly from muscular tissue reducing insulin-mediated glucose uptake (Holte 1994, Holte *et al.* 1996, Cahová *et al.* 2007).

We used QUICKI to determine insulin sensitivity in PCOS-affected women and healthy controls. Both groups had the same insulin sensitivity. We observed a negative correlation between insulin sensitivity and basal insulin secretion. But we did not ascertain any correlation between insulin pulse frequency or interpulse interval and insulin sensitivity. Previously published results concerning the relationship between insulin sensitivity, measured by euglycaemic clamp, and insulin pulse frequency are, however, discrepant. One study concluded that the increased frequency of rapid insulin pulses might be related to the insulin resistance in patients with abdominal obesity. The number of rapid insulin pulses was reduced and insulin sensitivity was improved after weight loss (Peiris *et al.* 1992). On the other hand, insulin pulse frequency was not the indicator of insulin sensitivity in healthy subjects (Courtney *et al.* 2003) or women with polycystic ovary syndrome (Armstrong *et al.* 2001). These divergent results could be explained by different studied populations or by the use of varying approaches for assessment of insulin pulsatile secretion.

In conclusion, PCOS-affected women tended to have broader insulin peaks in comparison to healthy lean women. Prolactin, cortisol and androgens were related to some of the insulin pulsatile secretion characteristics,

whereas parameters describing insulin pulsatility were not significant determinants of insulin sensitivity.

Conflict of Interest

There is no conflict of interest.

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