

Low testosterone levels and unimpaired melatonin secretion in young males with metabolic syndrome

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Summary

The interrelations between testosterone, insulin and melatonin levels in males with metabolic syndrome (MS) are still not clarified, especially in young age groups. The aim of the present study was to compare the testosterone serum levels in young men with MS to those in healthy controls, and to determine the possible changes in their melatonin rhythm, as well as the relation between melatonin, insulin and lipid profile. Fasting insulin and testosterone concentrations were measured in 10 healthy nonobese and 10 MS patients. Blood samples for melatonin, insulin and luteinizing hormone (LH) were collected at 19.00, 03.00 and 11.00 hours. A significant difference was found between the testosterone levels in controls and patients. Luteinizing hormone levels in both groups were similar, however, higher night LH levels in MS patients were observed. No changes in the melatonin concentrations of the two groups were found. In conclusion, total testosterone levels were significantly lower in young men with MS compared with healthy age-matched controls. Mild hypoandrogenia in hyperinsulinaemic patients was not related with changes in their melatonin levels. No alterations in the endogenous melatonin rhythm of the MS patients were found.

Introduction

The clinical consequences of insulin resistance and compensatory hyperinsulinaemia have become a serious public health problem [insulin resistance syndrome or metabolic syndrome (MS)]. Therefore, it is important to clarify the pathogenetic factors and results of hyperinsulinaemia. Different studies have demonstrated the important role of androgens in men with MS and cardiovascular diseases. Muller *et al.* (2005) showed that low serum levels of endogenous total testosterone, bioavailable testosterone and dehydroepiandrosterone sulphate (DHEA-S) were related to the MS, lower insulin sensitivity and higher fasting serum insulin levels. They concluded that low endogenous total testosterone and sex hormone-binding globulin levels appear to increase the risk of MS in middle-aged and elderly men independently of the fasting insulin levels and body composition measurements (Muller *et al.*, 2005). Men with total testosterone levels in the lower third of studied subjects were over 3.5-fold more

likely to have the MS than men with the concentrations in the upper third and were nearly two times more likely to have the MS even after adjusting for body mass index (BMI) (Laaksonen *et al.*, 2003). Thus, hypoandrogenism is an early marker for disturbances in insulin and glucose metabolism that may progress to the MS and frank diabetes and may contribute to their pathogenesis (Laaksonen *et al.*, 2004). These studies have shown the correlations between endogenous sex hormones, insulin, MS, diabetes and cardiovascular diseases. Most of them included middle-aged or elderly men. However, little is known about whether similar relationships exist in young men.

Numerous animal studies found a correlation between insulin and the melatonin rhythm (Lima *et al.*, 1994; La Fleur *et al.*, 2001; Peschke *et al.*, 2002; Nishida *et al.*, 2003). However, other studies did not support the crucial role of melatonin on the insulin action (Bizot-Espiard *et al.*, 1998). Consequently, further studies are necessary to clarify the possible relations between melatonin and insulin resistance, especially in humans. On the other

hand, the pineal gland and melatonin influence the hypothalamo-pituitary-gonadal axis in many mammalian species (Arendt, 1998). Melatonin could downregulate the gonadotrophin-releasing hormone-induced luteinizing hormone (LH) secretion from the pituitary gland, but the hormone may also affect the androgen secretion by direct action on Leydig cells (Valenti *et al.*, 1995; Vanecek, 1999). Increased testosterone synthesis, inhibited by melatonin treatment, was found after pinealectomy in rats (Kus *et al.*, 2002). Our previous studies showed altered melatonin rhythm in hypogonadal men (Kumanov *et al.*, 2005). Therefore, the aim of the present study was to clarify the possible correlation between testosterone and insulin in males of reproductive age and to study the role of melatonin in hyperinsulinaemic patients with mild hypoandrogenia.

Materials and methods

Subjects

Twenty males volunteered for the study. Ten patients fulfilled the WHO criteria for the MS (mean age 30.00 ± 3.66 years) and 10 persons served as controls (mean age 30.70 ± 2.82 years). The patients had visited the ambulatory because of obesity and/or arterial hypertension. They underwent a standard oral glucose tolerance test with 75.0 g glucose and in three of them impaired glucose tolerance was established. None was diabetic. Hypothyroidism and Cushing syndrome were excluded as reasons for obesity. The controls were chosen among the medical staff of the hospital or their friends. The experimental protocol was explained to all of them and informed consent was obtained.

Study protocol

All subjects underwent a complete general assessment, including height, weight, general status and blood pressure. The fasting glucose, lipid profile, high-density lipoprotein (HDL) cholesterol levels, triglycerides (TG), total cholesterol, insulin and total testosterone were determined in samples taken in the morning between 08.00 and 09.00 hours after an overnight fast. BMI and homeostatic model assessment for insulin resistance (HOMA-IR) were calculated [$BMI = \text{weight}/\text{height}^2$, $HOMA-IR = \text{fasting insulin (mE ml}^{-1}\text{)} \times \text{fasting glucose (mmol l}^{-1}\text{)}/22.5$]. None of the subjects had jet lag and no one was a night-shift worker. None of them had taken aspirin or beta-blockers, and they had regular sleep schedules. Otherwise, subjects were unrestricted in their usual activities. During the investigation, lights were off and the patients were in bed between 22.00 and 07.00 hours. Blood samples for

melatonin, LH and insulin were taken at 03.00 hours (using red dim light) from intravenous catheter. Other two samples were taken at 11.00 and 19.00 hours. After centrifugation sera were separated and frozen at -20°C .

Metabolic syndrome

The MS was defined according to WHO modification: hyperinsulinaemia (fasting serum insulin concentrations in the top of the 25% of the nondiabetic men) or impaired glucose tolerance and at least two of the following: abdominal obesity ($BMI \geq 30$); dyslipidaemia ($TG > 1.7 \text{ mmol l}^{-1}$ or $HDL < 0.9 \text{ mmol l}^{-1}$) or hypertension (blood pressure $\geq 140/90$ or anti-hypertensive medication) (Alberti & Zimmet, 1998; Laaksonen *et al.*, 2003).

Hormone measurement

Melatonin was measured through melatonin human radioimmunoassay (RIA) kit (DRG International, Mountainside, NJ, USA). The intra-assay variation of the kit was 7.4% for the low concentrations and 4.3% for the high concentrations. The inter-assay variation was 11.7% for the low concentrations and 14.3% for the high concentrations. The cross-reactivity of the kit ranged between 0.8 and <0.01 against a variety of melatonin metabolites, analytical sensitivity was 0.5 pg ml^{-1} . The LH was measured through hLH Spec kit (DELFIA; Perkin Elmer, Turku, Finland). Analytical sensitivity was 0.005 U l^{-1} , the intra-assay variation of the kit was 2% and the inter-assay 3.1%. The cross-reactivity against other glycoprotein hormones was below 0.05%. The testosterone determination was performed using commercially available RIA kit (DELFIA, Perkin Elmer). Analytical sensitivity was 0.03 nmol l^{-1} , the intra-assay variation of the kit was 5.5% and the inter-assay was 6.8%. Cross-reactivity against 5-alpha dihydrotestosterone was 27.2% and against other steroid hormones below 0.01%. Insulin was measured through IRMA/Immunotech, Beckman Coulter Co. (Prague, Czech Republic). Analytical sensitivity was 0.5 mIU l^{-1} , intra-assay below 4.3% and inter-assay below 3.4%. No cross-reactivity was observed against proinsulin and C-peptide.

Statistics

Statistical analysis was conducted through SPSS v. 11 for Windows (SPSS, Chicago, IL, USA). After the Kolmogorov-Smirnov test for normality of the distribution, the two-tailed Pearson correlation was performed. The correlation was considered significant at the 0.05 level. Student's *t*-test was used where appropriate.

Results

As expected, the patients with MS showed higher fasting insulin levels (mIU l^{-1}) (22.03 ± 2.43 versus 7.58 ± 1.20 , $P < 0.001$), HOMA-IR (5.01 ± 0.57 versus 1.69 ± 0.28 , $P < 0.001$), BMI (kg m^{-2}) (36.30 ± 1.82 versus 24.95 ± 0.56 , $P < 0.001$), lower HDL levels (mmol l^{-1}) (0.95 ± 0.04 versus 1.21 ± 0.10 , $P = 0.029$) and no differences in the levels of total cholesterol (mmol l^{-1}) (4.83 ± 0.34 versus 4.72 ± 0.28 , $P > 0.05$) compared with the controls (Table 1). Serum total testosterone levels (nmol l^{-1}) in the MS group were significantly lower (12.09 ± 1.17 versus 21.52 ± 2.36 , $P = 0.002$) in comparison with the healthy subjects. Night LH levels (U l^{-1}) were higher in the metabolic group (5.42 ± 0.70 versus 3.52 ± 0.51 , $P = 0.043$). Moreover, the quotient between night LH levels and morning testosterone concentrations was significantly higher in the patients with MS (0.50 ± 0.09 versus 0.18 ± 0.03 , $P = 0.005$). There was no significant difference between melatonin levels (pg ml^{-1}) at 11.00 hours (16.25 ± 3.84 versus 11.16 ± 1.11 , $P > 0.05$), 19.00 hours (11.62 ± 1.22 versus 11.53 ± 1.04 , $P > 0.05$) and 03.00 hours (50.99 ± 9.41 versus 48.00 ± 8.31 , $P > 0.05$) or melatonin night/day difference (36.55 ± 9.20 versus 36.66 ± 7.86 , $P > 0.05$) in the two groups. The results were similar when the subjects were divided into groups according to their BMI, HOMA-IR or fasting insulin.

Table 1 Characteristics (mean \pm SEM) of the two groups: patients with metabolic syndrome (MS) and healthy controls

	Control group	Patients with MS
<i>n</i>	10	10
Age (years)	30.70 ± 2.82	30.00 ± 3.66
Insulin – 08.00 hours (mIU l^{-1})	7.58 ± 1.20	$22.03 \pm 2.43^*$
HOMA-IR	1.69 ± 0.28	$5.01 \pm 0.57^*$
BMI (kg m^{-2})	24.95 ± 0.56	$36.30 \pm 1.82^*$
Triglycerides (mmol l^{-1})	1.10 ± 0.09	$2.27 \pm 0.49^*$
HDL cholesterol (mmol l^{-1})	1.21 ± 0.10	$0.95 \pm 0.04^*$
Total cholesterol (mmol l^{-1})	4.83 ± 0.34	4.72 ± 0.28
Testosterone (nmol l^{-1})	21.52 ± 2.36	$12.09 \pm 1.17^*$
Melatonin – 11.00 hours (pg ml^{-1})	16.25 ± 3.84	11.16 ± 1.11
Melatonin – 19.00 hours (pg ml^{-1})	11.62 ± 1.22	11.53 ± 1.04
Melatonin – 03.00 hours (pg ml^{-1})	50.99 ± 9.41	48.00 ± 8.31
Mel DELTA (pg ml^{-1})	36.55 ± 9.20	36.66 ± 7.86
LH – 11.00 hours (U l^{-1})	3.76 ± 0.69	4.21 ± 0.54
LH – 19.00 hours (U l^{-1})	3.77 ± 0.48	4.90 ± 0.55
LH – 03.00 hours (U l^{-1})	3.52 ± 0.51	$5.42 \pm 0.70^*$
Insulin – 11.00 hours (mIU l^{-1})	8.14 ± 2.92	$20.91 \pm 4.47^*$
Insulin – 19.00 hours (mIU l^{-1})	13.87 ± 4.50	23.43 ± 9.80
Insulin – 03.00 hours (mIU l^{-1})	5.86 ± 1.49	8.94 ± 0.84
LH/testosterone quotient	0.18 ± 0.03	$0.50 \pm 0.09^*$

* $P < 0.05$.

HOMA-IR, homeostatic model assessment for insulin resistance; BMI, body mass index; HDL, high-density lipoprotein; DELTA, melatonin night/day difference; LH, luteinizing hormone.

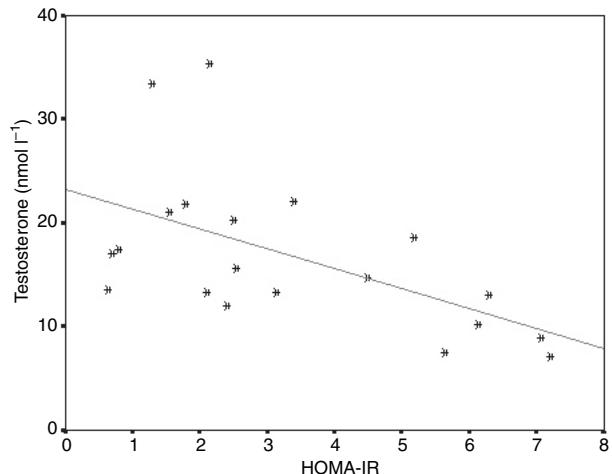


Fig. 1 Correlation between testosterone levels and homeostatic model assessment for insulin resistance index ($r = -0.557$; $P = 0.011$).

A strong correlation was observed between insulin and BMI ($r = +0.837$, $P < 0.001$) as well as between HOMA index and BMI ($r = +0.788$, $P < 0.001$). Total testosterone was negatively correlated with insulin ($r = -0.559$, $P = 0.010$), BMI ($r = -0.555$, $P = 0.011$) and HOMA index ($r = -0.557$, $P = 0.011$) (Fig. 1). No relationships between testosterone as well as LH and melatonin levels were observed.

Discussion

Our results showed that testosterone, fasting insulin levels and BMI were strongly correlated in men at reproductive age. We also found that young males with MS have significantly lower levels of total testosterone compared with nonobese age-matched subjects. It is hypothesised that the low normal levels of testosterone in young men could have a pathogenetic role in the development of MS. Our results are similar to other studies which have found an association between both total and free testosterone and insulin resistance. Tsai *et al.* (2004) reported a significant correlation between testosterone, body fat and insulin resistance in a cross-sectional cohort analysis of middle-aged nondiabetic men. They also established that a greater number of MS components and waist >102 cm predicted lower serum testosterone levels at follow-up (Tsai *et al.*, 2004, 2005). Some studies have shown the predictive role of the baseline total testosterone to the onset of type 2 diabetes after 8 years of follow-up (Oh *et al.*, 2002). Low total testosterone levels also independently predict the development of MS and diabetes in middle-aged men (Laaksonen *et al.*, 2004). Furthermore, Phillips (2004) suggested the conception of glucose-insulin-lipid-hyper-

tension-testosterone-oestrogen (GILHT-E) syndrome, accentuating on the testosterone-oestrogen ratio and the role of sex hormones as risk factors for myocardial infarction.

Despite the growing evidence about the relationships between male sex hormones, insulin and cardiovascular complications, little is known about the underlying physiological mechanisms. Some authors found lower LH levels and attenuated LH pulse amplitude in patients with severe obesity (Giagulli *et al.*, 1994). However, our results are in accordance with recently published data of men aged 25–64 years. The authors hypothesised that in insulin resistant states, Leydig cells steroidogenesis is impaired because of target organ resistance to insulin action and/or production of cytokines (or hormones) by adipose tissue (Pitteloud *et al.*, 2005). Probably not only the Leydig cells, but also the germ cells could be affected, based on the observed reduction in semen quality associated with high BMI (Jensen *et al.*, 2004). In our study among a small group of young hyperinsulinaemic patients a tendency to higher night LH levels and increased LH/testosterone quotient was observed. These data confirmed indirectly the hypothesis of mild steroidogenesis impairment and enhanced LH response. Extensive longitudinal studies are necessary to clarify the pathogenetic mechanisms of mild hypoandrogenia in men with MS and the possible role of testosterone treatment. They could also accept or reject the hypothesis for the MS in young males as an expression of the 'subclinical hypogonadism'. Nevertheless, the measurement of total testosterone in young patients with MS seems to be justified in clinical practice.

Our previous studies showed altered melatonin secretion in hypogonadal patients. Darkness-dependent release of melatonin in males with hypogonadotropic hypogonadism was significantly higher in comparison with the healthy men, while it was significantly reduced in patients with hypergonadotropic hypogonadism (Kumanov *et al.*, 2005). However, the mild hypoandrogenic state in hyperinsulinaemic patients is not related with changes in melatonin rhythm, suggesting that factors other than testosterone are responsible for melatonin fluctuations by hypo- and hypergonadotropic hypogonadism. This is in line with our previous preliminary data (Robeva *et al.*, 2006).

Another aim of our study was to investigate the relationships between melatonin and components of the MS and especially hyperinsulinaemia. Different studies have shown contradictory results about relationships between melatonin and insulin. For example, supplementation with melatonin in middle-aged male rats mimics some youthful energy regulatory responses, decreasing body weight, intra-abdominal adiposity, and plasma insulin and leptin concentrations (Wolden-Hanson *et al.*, 2000).

In humans, melatonin treatment did not change glucose or serum lipid profiles (Pawlowski *et al.*, 2002), but lower endogenous nocturnal melatonin levels were observed in patients with diabetes type 2 (Tutuncu *et al.*, 2005). To the best of our knowledge, this is the first study of the melatonin rhythm in nondiabetic patients with MS. Our results did not show any differences between melatonin rhythms in hyperinsulinaemic and normoinsulinaemic males. No alteration of the endogenous melatonin rhythm in our small group of young hyperinsulinaemic patients was observed. However, an indirect interaction between melatonin and insulin could not be excluded. Only large cross-sectional and longitudinal studies could clarify the role of the age-related melatonin decline to the metabolism, because of the strong variation in melatonin levels among different individuals and in different age groups.

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