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Postprandial thermogenesis is reduced in polycystic ovary syndrome and is associated with increased insulin resistance

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Summary

OBJECTIVE In order to investigate the possible causes and effects of obesity in polycystic ovary syndrome resting energy expenditure, postprandial thermogenesis and insulin resistance were measured in 14 polycystic ovary syndrome subjects and in 14 controls.

DESIGN A cross-sectional study of a selected group of patients was performed.

PATIENTS Seven of the PCOS subjects were obese and seven lean. Controls were individually matched for age, race, weight, body mass index (BMI) lean body mass and percentage fat. The obese, but not lean, polycystic ovary syndrome subjects had a greater waist:hip ratio than controls (median (range) obese PCOS 0·865 (0·823–0·960) *vs* obese control 0·804 (0·823–0·940), $P < 0·025$).

MEASUREMENTS Metabolic rate was measured by continuous indirect calorimetry and insulin sensitivity was assessed by a short insulin tolerance test.

RESULTS The resting energy expenditure (REE) was similar in PCOS subjects and controls (median (range), 6796 (5489–7774) *vs* 6833 (4893–8492) kJ/day). REE correlated with LBM in the PCOS group ($r = 0·83$, $P < 0·00$) and the control group ($r = 0·82$, $P < 0·001$). Postprandial thermogenesis was reduced in polycystic ovary syndrome (obese: median 45·4 (range 33·6–100·0) *vs* 86·5 (67·2–109·2) kJ ($P < 0·05$); lean: 79·4 (73·5–108·4) *vs* 89·9 (76·0–109·2) kJ ($P < 0·05$)). Fasting insulin (9·7 ± 3·6 *vs* 4·4 ± 0·8 mU/l, $P < 0·05$) and postprandial incremental insulin rise (163 ± 31 *vs* 116 ± 15 mU/l, $P < 0·025$) were higher in polycystic ovary syndrome. Insulin sensitivity was reduced in polycystic ovary syndrome (obese: median 136 (range 92–169) *vs* 173 (109–225) μmol/l/min ($P < 0·05$); lean: 161 (138–225) *vs* 194 (161–253) μmol/l/min ($P < 0·05$)). The reduction in insulin sensitivity correlated with the reduced postprandial thermogenesis in the polycystic ovary syndrome group ($r = 0·75$, $P < 0·01$).

CONCLUSION These results confirm previous reports of hyperinsulinaemia and insulin resistance in polycystic ovary syndrome. Furthermore, polycystic ovary syndrome subjects have a reduced postprandial thermogenesis which is related statistically to the reduced insulin sensitivity. The decreased postprandial thermogenesis may predispose women with polycystic ovary syndrome to weight gain.

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Polycystic ovary syndrome (PCOS) is characterized by anovulation, hirsutism and a high prevalence of obesity (Franks, 1989). These hyperandrogenaemic women have a greater degree of hyperinsulinaemia (Burghen *et al.*, 1980; Pasquali *et al.*, 1982; Shoupe *et al.*, 1983; Chang *et al.*, 1983; Jialal *et al.*, 1987; Dunaif *et al.*, 1987; Mahabeer *et al.*, 1989) and more insulin resistance (Dunaif *et al.*, 1989) than would be predicted from obesity alone. Women with PCOS are insulin resistant even when lean (Dunaif *et al.*, 1989).

Hyperandrogenaemia in women is associated with an increased waist : hip ratio (WHR) (Hauner *et al.*, 1988) which in turn has been linked to insulin resistance (Evans *et al.*, 1984).

Obesity is associated with an elevated resting energy expenditure but the thermogenic responses to glucose (Schutz *et al.*, 1984a) and to a mixed meal (Shetty *et al.*, 1981) are reduced. It is not known whether the reduced postprandial chemogenesis (PPT) is present before the obesity develops, but glucose induced thermogenesis (GIT) is reduced when patients are re-studied after weight loss (Bessard *et al.*, 1983; Schutz *et al.*, 1984b). Furthermore, a decrease in GIT is associated with insulin resistance in obesity and in NIDDM (Ravussin *et al.*, 1985; Ravussin & Zawadzki, 1987; Golay *et al.*, 1982). It is uncertain whether the same relationship exists between insulin sensitivity and PPT in PCOS, although, in the one published study, no such relationship was observed (Segal & Dunaif, 1990).

Table 1. Subject characteristics

		PCOS	Controls
Age (years)		27 (20–42)	29 (23–40)
Weight (kg)	lean	59·0 (53·5–71·1)	57·5 (49·5–73·9)
	obese	81·5 (74·3–118·2)	85·0 (77·2–114·5)
BMI	lean	21·3 (19·2–24·4)	22·6 (18·6–24·0)
	obese	32·8 (27·0–48·7)	33·1 (26·7–41·3)
Lean body mass (kg)	lean	45·6 (44·1–54·0)	44·7 (36·8–56·9)
	obese	54·7 (46·0–60·1)	56·5 (48·7–63·3)
Percentage fat	lean	20·6 (11·3–23·0)	23·6 (14·2–25·6)
	obese	39·3 (29·5–49·3)	39·5 (27·2–45·7)
Waist:hip ratio	lean	0·758 (0·725–0·844)	0·763 (0·700–0·848)
	obese	0·865 (0·823–0·960)	0·804* (0·823–0·940)
Testosterone (nmol/l)		3·4 ± 0·8	2·0 ± 0·5**

Values are given as median (range) except for plasma testosterone, levels of which are expressed as mean ± SD. BMI represents body mass index.
* $P < 0.025$, ** $P < 0.01$.

The purpose of this study, therefore, was to investigate PPT in women with PCOS and individually matched controls and to relate these findings to insulin sensitivity.

Subjects and methods

Subjects

Fourteen PCOS subjects were compared with 14 controls who were individually matched for age, weight, race, body mass index (BMI, weight/height^2), lean body mass and percentage fat mass (Table 1). Subjects were divided into obese ($n = 7$) and lean ($n = 7$) using a BMI > 25 and a fat mass > 26% as the criteria for obesity. For the purpose of this study PCOS was defined by the clinical features: amenorrhoea or oligomenorrhoea (menstrual cycle longer than 35 days) and/or hirsutism (score greater than 8 (Ferriman & Gallwey 1961)) with polycystic ovaries on ultrasound scanning (Adams *et al.*, 1986). Thirteen of the 14 patients were oligomenorrhoeic. Subjects were studied in the follicular phase of the menstrual cycle or, if oligo or ameno-rrhoeic, randomly. Seven were hirsute, 11 had raised testosterone, and one had neither. All subjects were healthy apart from the clinical features of PCOS and none of the controls had any known illness. No subject had taken medication for 3 months prior to the investigation. Informed consent was given by all subjects before the study, which was approved by the Parkside District Health Authority.

Experimental methods

Subjects were studied on two occasions; on one, lean body mass was estimated and energy expenditure measured; on the other, insulin resistance was assessed. Waist:hip ratio was defined from the minimal waist measurement and the maximal hip measurement, a method shown to have significant associations with indices of lipid and carbohydrate metabolism (Houmard *et al.*, 1991). Lean body mass was measured by bioelectric impedance. In the post absorptive state a 800 μA alternating current at 50 kHz was passed from the right hand to the ipsilateral foot. The impedance obtained was used to calculate total body water (TBW = height^2/(impedance × 0·585 + 1·825)) and lean body mass (LBM = TBW/0·73). Fat mass percentage was defined as 100 (total weight − lean body weight)/total weight (Kushner *et al.*, 1990).

Insulin resistance was assessed using a short insulin tolerance test (Bonora *et al.*, 1989). The linear slope of glucose decline in the first 15 minutes after insulin was used as the index of insulin-mediated glucose disposal. Subjects were fasted 10–12 hours overnight, following which a cannula was inserted in a forearm vein. The arm was heated to 45–50°C to arterialize the blood sample. Intravenous insulin (0·05 U/kg body weight) was given and samples taken at −15, 0, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 minutes for glucose estimation. The linear slope of arterialized plasma glucose decline, 3–15 minutes after insulin, was measured and expressed as μmol/l glucose fall per minute.

Metabolic rate was measured by continuous indirect calorimetry (Deltatrac metabolic monitor, Datex Instrumentarium, Helsinki, Finland). After an overnight fast (10–12 h), the subject acclimatized to the thermoneutral environment for at least 40 minutes. A cannula was inserted into a forearm vein on arrival. Resting metabolic rate was measured for 30 minutes after steady state was reached, before a mixed meal of Build up (Carnation, Clintec Nutrition, Surrey) was given. The energy load was 32% fat, 22% protein and 46% carbohydrate. The amount given was calculated to provide 42 kJ/kg lean body mass (10 kcal/kg LBM). Oxygen consumption and carbon dioxide production were measured for the next 2 hours and the metabolic rate calculated according to the formula of Weir (1949). The incremental area of metabolic rate above the REE was calculated and

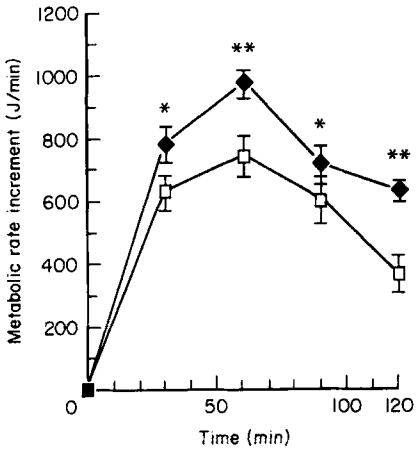

Fig. 1 The increment of energy expenditure (EE) after the mixed meal intervals in the PCOS and control subjects. Resting energy expenditure (range 4893–8492 kJ) is standardized to zero, so the increment in EE after the meal is shown averaged over 30 minutes. $*P<0.05$, $**P<0.001$. ●, Controls; □, PCOS subjects.

expressed in kilojoules. The area under the curve of the incremental rise in metabolic rate is the PPT (Fig. 1) (PPT, Post meal energy expenditure − resting energy expenditure). Blood was taken for glucose, non-esterified fatty acid (NEFA) and insulin estimation at −30, 0, 30, 60, 90 and 120 minutes. Fasting metabolite levels given in the text are the mean of −30 and 0 minute values.

Analytical methods

Metabolites were measured by an enzymatic technique using a centrifugal analyser (Cobas Bio, Roche Diagnostica), glucose with a hexokinase method (Glucose, HK, Roche Products Ltd, Herts UK) and NEFA using acyl CoA synthetase and acyl CoA oxidase (NEFA C, Wako Chemicals, Neuss, Germany). A polyethylene-glycol accelerated second-antibody system was used to measure insulin (Hampton & Marks, 1979).

Area under the curve was calculated by the trapezoidal rule. The differences between women with PCOS and their individually matched controls were compared using a Wilcoxon paired rank sum test. Correlations were sought with Pearson product moment correlation coefficient.

Results

Body composition

Despite matching PCOS subjects and controls for weight and BMI, the obese, but not lean, PCOS subjects had greater central adiposity (obese subjects waist:hip ratio PCOS *vs* controls, median (range), 0·865 (0·823–0·960) *vs* 0·804 (0·823–0·940) ($P<0.025$)).

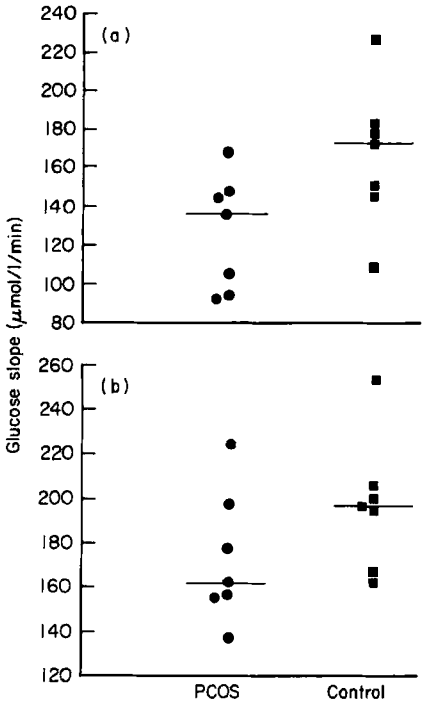

Fig. 2 Individual values for insulin sensitivity in a, the obese and b, the lean subjects with PCOS and in matched controls. The median values are indicated. Insulin sensitivity was determined as outlined in methods.

Insulin sensitivity studies

There was no significant difference in fasting plasma glucose between the PCOS and control groups (mean ± SEM, 4·7 ± 0·1 *vs* 4·8–± 0.1 mmol/l respectively). Fasting NEFA were also similar in the two groups (692 ± 83 *vs* 647 ± 82 μmol/l).

The insulin sensitivity as assessed by the slope of glucose decline, was lower in the PCOS than the control group; obese: median 136 (range 92–169) *vs* 173 (109–225) μmol/l/min ($P<0.05$); lean: 161 (138–225) *vs* 194 (161–253) ($P<0.05$) (Fig. 2). Insulin sensitivity correlated negatively with percentage fat mass (% FM) in the PCOS subjects ($r=-0.54$, $P<0.05$) but not significantly in the control group ($r=-0.42$, NS). Circulating insulin levels peaked at 4 minutes in the short insulin tolerance test and were similar in the two groups (265 ± 18 *vs* 273 ± 9 mU/l).

Mixed meal and energy expenditure

The total area under the curve of glucose during the test meal was not significantly different in the PCOS group from that in the control group (23·9 ± 0·6 *vs* 24·2 ± 0·6 mmol/l/2 h, NS). During the mixed meal the non-esterified fatty acids

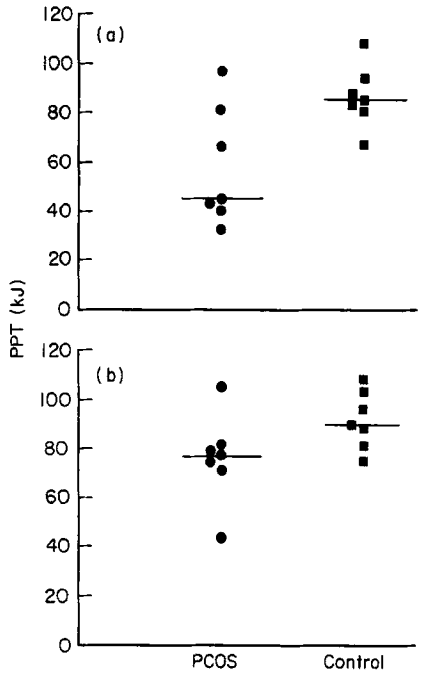

Fig. 3 Postprandial thermogenesis in a, obese and b, lean subjects with PCOS and in matched controls. Values in kJ are the sum of the increments abova basal following ingestion of a mixed meal. Median values are indicated.

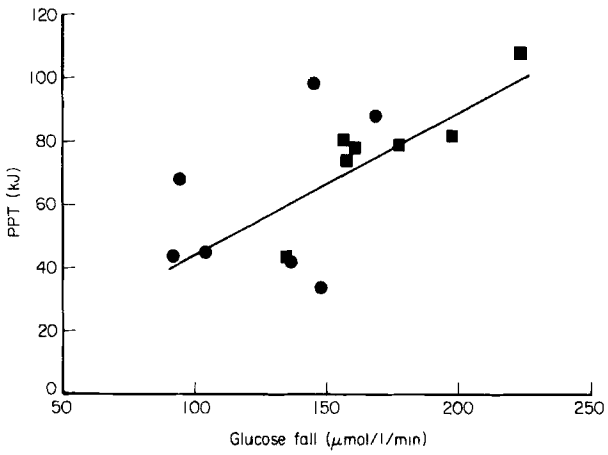

Fig. 4 The association of insulin sensitivity and postprandial thermogenesis in the subjects with polycystic ovary syndrome. Insulin sensitivity and postprandial thermogenesis (PPT) were assessed as indicated in the methods section. ●, Obese subjects; ■, lean subjects. $r=0{\cdot}75$.

were similar in PCOS and control groups. There was no significant difference in the fasting NEFA (PCOS 560 ± 60 *vs* control 593 ± 71 μmol/l), absolute NEFA fall at 60 minutes (393 ± 42 *vs* 399 ± 54 μmol/l) nor in percentage fall at 60 minutes (72 ± 5 *vs* 68 ± 4%). The fasting insulin was higher in the PCOS group ($9{\cdot}7\pm3{\cdot}6$ *vs* $4{\cdot}4\pm0{\cdot}8$ IU/l, $P<0{\cdot}05$) as was the incremental insulin rise after the meal (163 ± 31 *vs* 116 ± 15 mU/l, $P<0{\cdot}025$).

There was no significant difference in resting energy expenditure between the two groups, 6796 (range 5489–7774) *vs* 6833 (4893–8492) kJ/day. REE correlated with LBM in the PCOS group ($r=0.83$, $P<0{\cdot}001$) and the control group ($r=0{\cdot}82$, $P<0{\cdot}001$).

Postprandial thermogenesis was decreased in the PCOS group compared to their matched controls; obese: median 45·4 (range 33·6–100·0) *vs* 86·5 (67·2–109·2) kJ ($P<0{\cdot}05$); lean: 79·4 (73·5–108·4) *vs* 89·9 (76·0–109·2) kJ ($P<0{\cdot}05$) (Fig. 3). The increment in metabolic rate over REE for the PCOS and control groups is summarized in Fig. 1. The reduction in PPT was significant in the PCOS group at each of the 30-minute time intervals compared with their matched controls. PPT was reduced in both the lean and obese PCOS subjects (Fig. 3). PPT correlated negatively with percentage fat mass in the PCOS subjects ($r=-0{\cdot}68$, $P<0{\cdot}01$) and control subjects ($r=-0{\cdot}56$, $P<0{\cdot}05$). Postprandial thermogenesis correlated significantly with insulin resistance in the PCOS subjects ($r=-0{\cdot}75$, $P<0{\cdot}01$) (Fig. 4) but not in the control subjects.

Discussion

Insulin sensitivity was measured by means of an intravenous insulin tolerance test. Using this technique, the slope of the decline of blood glucose with time has been demonstrated previously to correlate closely with the *M* values obtained using hyperinsulinaemic euglycaemic clamps (Bonora *et al.*, 1989). Fasting blood glucose concentrations were normal in the PCOS patients and controls, of relevance in view of the fact that the insulin tolerance test has been validated only in normoglycaemic subjects.

Bioelectrical impedance used to assess lean body mass correlates well with other techniques such as densitometry, deuterated water or total body potassium (Kushner *et al.*, 1986, 1990; Lukaski *et al.*, 1985). PPT was derived following the ingestion of a mixed meal, the energy content of which was related to lean body mass (Illingworth *et al.*, 1987). This is preferable to ingestion of a fixed energy intake, as lean body mass is the major determinant of resting energy expenditure. The meal induced stimulus to energy expenditure is thus proportionately greater if resting energy expenditure is high and vice versa. For comparison between PCOS

and controls, it should be noted that each PCOS subject ingested an energy load similar to the matched control. Despite receiving a greater caloric intake, the obese women had a relatively reduced PPT. The similar glucose concentrations during the test meal allow for easier comparison of the metabolic rate in the PCOS and control subjects. The PPT was studied for 2 hours, by which time the thermogenic response was 80% complete. We have studied control and PCOS women for 3 hours with similar conclusions (unpublished observations).

Hyperinsulinaemia both fasting and in response to a mixed meal has been demonstrated in both obese and lean subjects with PCOS, suggesting resistance to endogenous insulin. This finding is in agreement with some previous studies (Burghen *et al.*, 1980; Pasquali *et al.*, 1982; Shoupe *et al.*, 1983; Chang *et al.*, 1983; Jialal *et al.*, 1987; Dunaif *et al.*, 1987; Mahabeer *et al.*, 1989). As in these studies, the patients in the present series were hyperandrogenaemic and with one exception, oligomenorrhoeic. In contrast, in a recent study of an unselected group of women with PCOS neither fasting insulin nor glucose-stimulated insulin levels were significantly different from controls. Significantly, however, relative hyperinsulinaemia was demonstrated in those women who had menstrual disturbances, compared with normal women with normal cycles (Sharp *et al.*, 1991). We have also shown resistance to exogenous insulin, thereby confirming the results of previous studies using the hyperinsulinaemic euglycaemic clamp technique (Dunaif *et al.*, 1989). The insulin resistance was observed in both lean and obese women, when compared with matched controls. Since the fasting and postprandial glucose levels were similar in the PCOS and control groups, this insulin resistance is probably not a consequence of hyperglycaemia by itself, in agreement with other studies (Dunaif *et al.*, 1989). Despite being matched for BMI and having a similar percentage of body fat, obese women with PCOS have an increased waist:hip ratio compared with controls. In hyperandrogenaemic women, without sonographic evidence of PCO, an increased WHR has also been observed (Hauner *et al.*, 1988) and a direct relationship between WHR and insulin resistance has been observed in other clinical situations (Evans *et al.*, 1984). An increase in WHR and decreased SHBG has been associated with low numbers of red, slow twitch, type 1 muscle fibres and increased white, fast twitch, type 2 fibres. This has been postulated as a mechanism for the insulin resistance (Krotekiewski & Bjorntorp, 1986). Certainly insulin resistance in non-obese hyperandrogenic women has been attributed to peripheral as opposed to hepatic insulin insensitivity, again perhaps reflecting alterations in muscle fibre type (Peiris *et al.*, 1989). The present study was not designed to assess the relative importance of WHR, SHBG and hyperandrogenaemia in the mechanism of the insulin resistance.

Although plasma NEFA concentrations, before and after the mixed meal, were similar in the women with PCOS and controls, the co-existence of hyperinsulinaemia suggests resistance to the effects of insulin on fatty acid mobilization in addition to insensitivity to the glucose lowering effects.

No difference was observed in REE corrected for LBM between the PCOS and control women, studied where possible in the follicular phase. Other studies have found REE to be increased by 6–9% in the luteal phase of the cycle (Webb, 1986), although Westrate *et al.* (1990) demonstrated no variation throughout the menstrual cycle. The lack of a luteal phase would be a mechanism for reduced total energy expenditure in non-ovulating women, whether due to PCOS or uncomplicated obesity. In both PCOS and controls a correlation was observed between LBM and REE. These latter findings are consistent with other studies (Segal & Dunaif, 1990).

The reduction of PPT in PCOS demonstrated in this study is not unexpected given the previous reports of decreased PPT or GIT in other insulin resistant states such as obesity (Schutz *et al.*, 1984a) and non-insulin dependent diabetes (Ravussin & Zawadzki, 1987). A diminished thermogenic response to intravenous insulin and glucose during hyperinsulinaemic euglycaemic clamps has been reported in NIDDM (Ravussin *et al.*, 1987). The only other study in PCOS did not, however, demonstrate a reduction in PPT (Segal & Dunaif, 1990). This may reflect differences in the population of patients with PCOS, the different experimental protocols (specifically, differences in the energy content of the meal) or both. We used continuous ventilated hood indirect calorimetry whereas Segal and Dunaif (1990) used intermittent calorimetry with a mouthpiece which may be more disturbing for the subject. In the present study, a negative correlation was observed between percentage fat mass and PPT in the PCOS subjects and controls in keeping with previous studies (Segal & Dunaif, 1990). The PCOS subjects had a reduced PPT compared to their matched controls and despite the similar REE levels, PCOS may therefore lead to reduced 24-hour energy expenditure and predispose to obesity. In the obese subjects the difference in PPT between PCOS and controls was 42 kJ. The meal represented about one-fifth of the daily caloric intake. If this difference continued for one year it is equivalent to approximately 735 000 kJ or 1·9 kg of fat.

The biochemical mechanisms underlying the relationship between PPT and insulin resistance are unknown, both in PCOS and in the other conditions where the relationship is observed.

PCOS is a disorder with a strong genetic basis, at least in

some women (Hague *et al.*, 1988). It is also very common in Western societies, affecting up to 20% of women of reproductive age (Polson *et al.*, 1988). It is tempting to speculate that its high prevalence suggests an evolutionary advantage and the presumed decrease in 24 hour energy expenditure gives a putative basis for this. Enhanced survival in times of food deprivation with improved fertility during weight loss (Kiddy *et al.*, 1992), may have combined to provide the evolutionary gain.

We have confirmed resistance to exogenous insulin in PCOS and demonstrated a reduced PPT in PCOS which may in part explain the obesity often associated with the syndrome. The insulin resistance and reduced PPT could be a major health risk for PCOS subjects in the long term if energy intake is not restricted. The difference in insulin sensitivity between ovulatory and anovulatory women remains to be determined.

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