# Ultrasound examination of polycystic ovaries: is it worth counting the follicles?

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BACKGROUND: This study revisited the ultrasonographic diagnostic criteria of polycystic ovary syndrome (PCOS) and studied the relationship between the major hormonal and metabolic features of PCOS and the follicle number per ovary (FNPO). METHODS: This prospective study included 214 women with PCOS compared with 112 women with normal ovaries. Main clinical, biological and ultrasonographic markers of PCOS were assessed during the early follicular phase. RESULTS: The mean FNPO of follicles 2–5 mm in size was significantly higher in polycystic ovaries than in controls, while it was similar within the 6–9 mm range. Setting the threshold at 12 for the 2–9 mm FNPO offered the best compromise between specificity (99%) and sensitivity (75%). Within the 2–5 mm follicular range, we found significant positive relationships between the FNPO and androgens. The FNPO within the 6–9 mm range was significantly and negatively related to body mass index and fasting insulin serum level. CONCLUSIONS: We propose to modify the definition of polycystic ovaries by adding the presence of $\geqslant 12$ follicles measuring 2–9 mm in diameter (mean of both ovaries). Also, our findings strengthen the hypothesis that the intra-ovarian hyperandrogenism promotes excessive early follicular growth and that further progression cannot proceed normally because of hyperinsulinism and/or other metabolic influence linked to obesity.

*Key words:* follicle count/follicle size/PCOS/ultrasonography

## Introduction

Polycystic ovary syndrome (PCOS) is the most frequent circumstance of hyperandrogenism and anovulatory infertility in women. Its diagnostic definition by ultrasonography is currently ambiguous, especially when considering why and how ovarian ultrasonographic criteria should be used. Although current ultrasonographic criteria can be considered to be suboptimal for the diagnosis of classical PCOS, they are considered useful by several authors, including ourselves (Dewailly *et al.*, 1997), for the recognition of non-classic forms of PCOS.

Since the advent of ultrasound, numerous parameters have been proposed to morphologically define polycystic ovaries (PCO), but there is still no consensus as to their respective diagnostic value. Therefore, the definition proposed by Adams *et al.* (1985) still prevails and is used by the majority of authors today. This definition is the following: presence of $\geqslant 10$ cysts measuring 2–8 mm in diameter arranged peripherally around a dense core of stroma or scattered through an increased amount of stroma. It includes the two main histological features of PCO, namely the excessive number of follicles, also termed multifollicularity, and stromal hypertrophy.

The majority of authors using ultrasound agree with the specificity of the stromal hypertrophy (reviewed by Adams *et al.*, 1985), although this criterion is subjective and does not correlate with the biochemical indices when measured by three-dimensional ultrasound (Nardo *et al.*, 2002). We have previously proposed to use instead ovarian hypertrophy (i.e. an ovarian area >5.5 cm[2] unilaterally or bilaterally) as a morphological indicator of PCOS, since it can be more easily quantified and correlates closely with the stromal hypertrophy (Dewailly *et al.*, 1994). Others came to the same conclusions by using ovarian volume (Pache *et al.*, 1992; Takahashi *et al.*, 1995; VanSantbrink *et al.*, 1997). There is more controversy about the increased number of follicles as a morphological predictor of PCOS. In his morphological review of the PCO, Hughesdon found twice the number of all types of antral follicles, generally <4 mm in diameter, in comparison with control ovaries (Hughesdon, 1982). Since ultrasound can only detect follicles >2 mm in size, the multifollicular nature of PCO can be confused with the other causes of multifollicular ovaries (MFO) in which only the latest stages of follicular development (>4 mm) are involved. Indeed, MFO are observed by ultrasound in various physiological and pathological

situations, such as mid–late normal puberty, central precocious puberty, hypothalamic anovulation, hyperprolactinaemia and, most importantly, the early normal follicular phase in adult women, in only one ovary, before one follicle among the cohort becomes dominant. This raises the question of which threshold should be accepted if follicle number per ovary (FNPO) is used to diagnose PCO. The majority of authors have set this threshold at 10 (Adams *et al.*, 1985; Takahashi *et al.*, 1994) but some authors have recommended 15 (Fox *et al.*, 1991).

Likewise, the size range within which follicles should be counted by ultrasound is not clear. The majority of authors used a relatively wide range of diameters, i.e. from 2 to 8 mm. However, Pache *et al.* (1993) have shown that the median value of the follicle size estimated by ultrasound was significantly less in their patients with PCOS than in their control subjects (3.8 versus 5.1 mm respectively), in agreement with the pathological data from Hughesdon (1982). Therefore, it can be questioned whether counting smaller follicles would be more appropriate.

In order to elucidate these unsolved issues, we hypothesized that increasing the threshold for the number of follicles to 15 and/or narrowing the range of follicle size to 2–5 mm would improve the accuracy of the follicle count for the diagnosis of PCO and the search for functional correlates. For these purposes, we used our database including data collected prospectively in control women and in patients with PCOS.

## Materials and methods

### Patient and control populations
This study was approved by the Institutional Review Board of the Lille University Hospital. Informed consent was obtained from all patients and controls before their inclusion into the study.

### Controls
The control population consisted of 112 women with normal ovaries. These women were recruited by the Department of Assisted Reproductive Medicine in our institution. They were referred for IVF because of tubal and/or male factor infertility. Exclusion criteria included a history of menstrual disturbances (i.e. cycle length either <25 days or >35 days), hirsutism, abnormal serum level of prolactin or androgens [i.e. serum testosterone and/or androstenedione higher than our previously reported threshold, i.e. 0.7 or 2.2 ng/ml respectively (Pigny *et al.*, 1997)], PCO on ultrasound (see below) and hormonal treatment during the 3 months prior to the study.

### Patients with PCOS
A total of 214 patients with suspected PCOS had been recruited from the Gynaecology and Endocrinology clinics. The diagnosis of PCOS was based on the association of one clinical criterion [hirsutism (as assessed by a modified Ferriman and Gallwey score of >8) or menstrual disturbances (i.e. oligomenorrhoea or amenorrhoea or cycle length either <25 days or >35 days and/or ovulatory disturbances as assessed by basal body temperature chart and/or serum progesterone level <3 ng/ml in luteal phase)], with either one biological criterion (serum LH levels >6.5 UI/l, and/or testosterone levels >0.7 ng/ml, and/or androstenedione levels >2.2 ng/ml), or an ovarian area >5.5 cm$^2$ unilaterally or bilaterally at ultrasound (Pigny *et al.*, 1997).

### Serum sampling
Blood sampling was performed during the early follicular phase (EFP), i.e. between days 2 and 7 after the last menstrual period (LMP), in both PCOS patients and control women, as described previously (Pigny *et al.*, 1997). In PCOS patients, the LMP was either spontaneous or induced by the administration of didrogesterone (10 mg/day for 7 days).

### Hormonal immunoassays
Estradiol, Δ4-androstenedione, testosterone, LH and FSH were measured by immunoassays as described previously (Pigny *et al.*, 1997). Fasting serum insulin levels were measured in duplicate by a radioimmunoassay (Bi-Insulin IRMA Pasteur; Bio-Rad, Marnes la Coquette, France) that uses two monoclonal anti-insulin antibodies. Intra- and inter-assay coefficients of variation were <3.8 and 7.5% respectively. Results are expressed as mIU/l in terms of the World Health Organization 66/304 reference preparation.

Serum inhibin B was measured by a two-site enzyme immunoassay (Serotec, Oxford, UK) as described previously (Pigny *et al.*, 1997). This assay is based on the use of a specific capture monoclonal antibody directed to the β$_B$ subunit. The labelled monoclonal antibody (R1) is directed against the N-terminal portion of the mature α subunit. The results are expressed in pg/ml of partially purified forms from follicular fluid calibrated against 32 kDa recombinant inhibin B. The detection limit of the inhibin B assay was 10 pg/ml.

### Pelvic ultrasound examination
Ultrasound examination was performed between cycle days 2 and 7 with a 7 MHz transvaginal transducer (Logic 400; General Electric, Milwaukee, USA). Ultrasound measurements were taken in real time, according to a standardized protocol. The highest possible magnification was used to examine the ovaries. After the longest medial axis of the ovary had been determined, the length and thickness were measured and the area was calculated using a manual or automatic ellipse to outline the ovary as described previously (Dewailly *et al.*, 2002). Several follicles were measured in two planes of the ovary in order to estimate the size and their position. All follicles of <9 mm, but >2 mm, were counted. The diameter of several follicles was measured from the mean of two diameters (longitudinal and anteroposterior), then the number of follicles measuring >5 mm or ≤5 mm was established by scanning each ovary from the inner margin to the outer margin in longitudinal cross-section.

Patients in whom transvaginal ultrasonography was inappropriate (virgin or refusing patients) were excluded from the analysis, as well as those in whom no follicle was seen in either the right or the left ovary and/or in whom the ovarian area was below the lower normal limit, i.e. 2.5 cm$^2$. Patients with at least one follicle <9 mm in diameter at ultrasound, or a serum estradiol level >80 pg/ml, were also excluded from the study so as not to confound the data with the presence of a dominant follicle.

### Statistical methods
For the FNPO, three different size categories (2–5, 6–9 and 2–9 mm) were considered for separate analysis. Within each size range, the data used for statistical analysis was the mean of observed values for the left and right ovaries. Statistical significance between mean values was attributed to two-tailed $P < 0.05$. Significant relationships between the various parameters were evaluated by the Pearson correlation coefficient.

Receiver operating characteristic (ROC) curves (Zweig and Campbell, 1993) were constructed to examine the diagnostic test performance, i.e. the ability to discriminate between controls and patients with PCOS. Sensitivity against (1 – specificity) was plotted at

each level, and the area under the curve was computed by the non-parametric Wilcoxon statistical test (Zweig and Campbell, 1993). Area under the curve represents the probability of correctly identifying controls and patients with PCOS. A value of 0.5 means that the test result is no better than chance.

The statistical analysis was performed using Statview 4.5 (Abacus Concepts Inc., Berkeley, CA, USA).

## Results

Table I shows the main clinical, hormonal and ultrasound features of each population. The day of sampling and ultrasound examination (from 2 to 7 days after the LMP) had no significant effect on these variables as determined by analysis of variance.

### Diagnostic value of the follicle count according to follicle size

Within the 2–5 and 2–9 mm ranges, the mean FNPO was significantly higher in the PCOS group than in controls, while it was similar between the two groups within the 6–9 mm follicular range (Table I). Within all size ranges, the individual FNPO values from patients with PCO overlapped those of the control group (Figure 1).

The diagnostic value of the FNPO within each follicular size range has been assessed by the areas under the ROC curves (see Materials and methods). No difference was observed between the 2–5 mm and the 2–9 mm FNPO which both yielded area values indicating a good diagnostic performance (Table II and Figure 2), much better than the one obtained with the 6–9 mm FNPO.

The ROC curves allowed estimation of the sensitivity and specificity of a given threshold (Table II). Increasing the threshold of either the 2–5 mm or the 2–9 mm FNPO from 10 to 15 yielded a 100% specificity but substantially decreased sensitivity (Table II). Likewise, using the 2–5 mm instead of the 2–9 mm FNPO improved the specificity but decreased the sensitivity, irrespective of threshold FNPO. Setting the threshold at 12 for the 2–9 mm FNPO offered the best

compromise between specificity (99%) and sensitivity (75%) (Table II).

### Clinical and hormonal correlations with follicle count according to follicle size in patients with PCOS

Table III shows the relationships between the FNPO, within each follicular size range, and the main clinical and biological markers of PCOS.
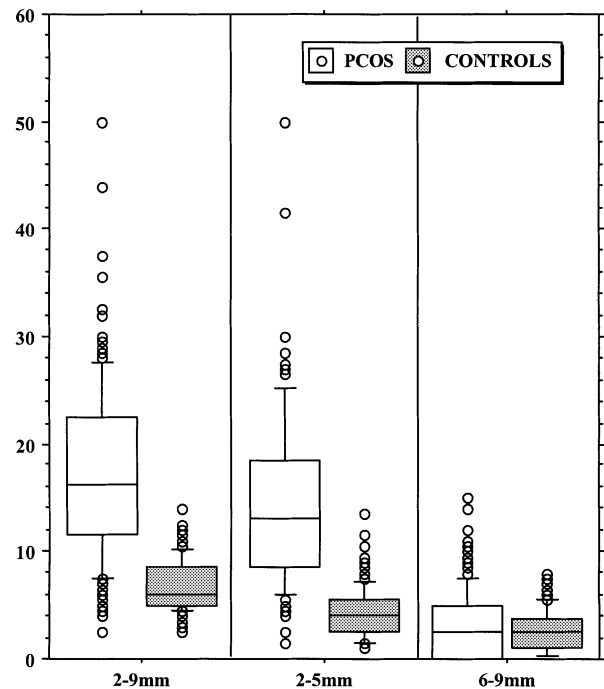


**Figure 1.** Box-and-whisker plots showing median follicle counts in series of patients with polycystic ovary syndrome (PCOS) (*n* = 214) and in controls (*n* = 112). Within the 2–5 and 2–9 mm follicular ranges, respectively 2.5 and 14% controls were above the threshold of 10, while within both of these follicular size ranges, none was above the threshold of 15. Conversely, within the 2–5 and 2–9 mm ranges respectively 41 and 25% patients with PCOS were below the threshold of 10, and respectively 66 and 52% were below the threshold of 15.

**Table I.** Main clinical and hormonal features in controls and in patients with polycystic ovary syndrome (PCOS)

|  | Controls (*n* = 112) | PCOS (*n* = 214) | *P*[a] |
|---|---|---|---|
| Age (years) | 30.0 (25–34.7) | 27.2 (21.4–33.6) | 0.0001 |
| Body mass index (kg/m$^2$) | 23.1 (19.4–32) | 26.7 (20–40.3) | 0.0001 |
| Testosterone (ng/ml) | 0.27 (0.09–0.50) | 0.48 (0.21–0.84) | 0.0001 |
| Androstenedione (ng/ml) | 1.41 (0.88–2.03) | 2.20 (1.35–3.30) | 0.0001 |
| Estradiol (pg/ml) | 30 (20–51) | 33 (20–52) | NS |
| LH (IU/l) | 4.1 (2.3–6.9) | 6.2 (2.4–12.4) | 0.0001 |
| FSH (IU/l) | 6.0 (4.6–7.6) | 5.1 (3.5–6.5) | 0.0001 |
| Inhibin B (pg/ml) | 84 (50–138) | 89 (43–152) | NS |
| Insulin (mIU/l) | 3.0 (1.4–7.4) | 5.3 (1.5–16.9) | 0.0001 |
| Ovarian area (cm$^2$) | 8.1 (6.1–10.3) | 12.6 (9.6–16.0) | 0.0001 |
| No. of follicles |  |  |  |
|   2–9 mm | 6.0 (4.5–10) | 15.5 (10–27.5) | 0.0001 |
|   2–5 mm | 4 (1.65–7) | 12 (6.5–24) | 0.0001 |
|   6–9 mm | 2.5 (0.5–5.5) | 2.5 (0–8) | NS |

Values are expressed as the median, with the 10th–90th percentile range in parentheses.
[a]Student's *t*-test.
NS = non-significant.

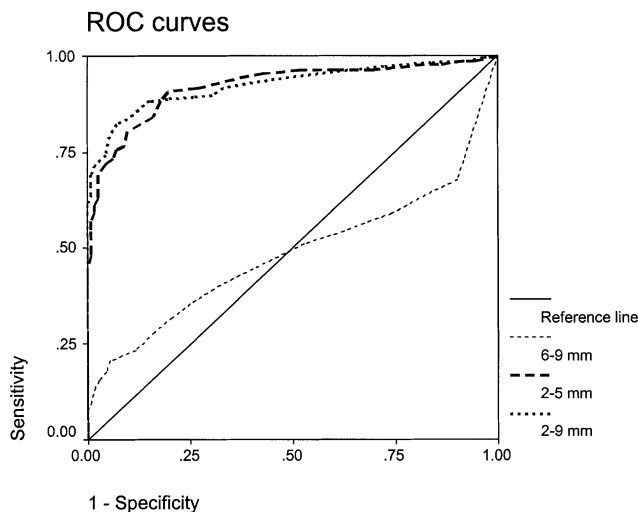**Table II.** Receiver operating characteristic (ROC) curve data

|  | Area under the ROC curve | Threshold | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| FNPO |  |  |  |  |
| 2–5 mm | 0.924 | 10 | 65 | 97 |
|  |  | 12 | 57 | 99 |
|  |  | 15 | 42 | 100 |
| 6–9 mm | 0.502 | 3 | 42 | 69 |
|  |  | 4 | 32.5 | 80 |
|  |  | 5 | 24 | 89 |
| 2–9 mm | 0.937 | 10 | 86 | 90 |
|  |  | 12 | 75 | 99 |
|  |  | 15 | 58 | 100 |

The diagnostic value of the follicle number per ovary (FNPO) within each follicular size range has been assessed by the areas under the ROC curves. Area under the curve represents the probability of correctly identifying controls and patients with PCOS. A value of 0.5 means that the test result is no better than chance. The ROC curves also allowed estimation of the sensitivity and specificity of a given FNPO threshold.

**Table III.** Relationships (Pearson's correlation coefficients) between the follicle number per ovary (FNPO), body mass index (BMI) and biological parameters, within each follicular size range in polycystic ovary syndrome ($n = 214$)

|  | Estradiol (pg/ml) | Inhibin B (pg/ml) | Testosterone (ng/ml) | Androstenedione (ng/ml) | LH (IU/l) | BMI (kg/m$^2$) | Insulin (mUI/l) |
|---|---|---|---|---|---|---|---|
| FNPO |  |  |  |  |  |  |  |
| 2–5 mm | 0.123 | –0.092 | 0.266 | 0.305 | 0.166 | 0.086 | –0.116 |
|  | (NS) | (NS) | (0.0001) | (0.0001) | (0.02) | (NS) | (NS) |
| 6–9 mm | –0.114 | 0.228 | –0.154 | –0.064 | 0.021 | –0.283 | –0.252 |
|  | (NS) | (0.002) | (0.03) | (NS) | (NS) | (0.0001) | (0.0005) |

Numbers in parentheses indicate the *P*-value.
NS = non-significant.



**Figure 2.** Receiver operating characteristic (ROC) curves giving the diagnostic performance of the follicle number per ovary within each follicular size range.

Within the 2–5 mm follicular range, we found significant and positive relationships between the FNPO and testosterone, androstenedione and LH serum levels. After controlling for LH, the relationship between the FNPO and testosterone and androstenedione levels remained significant ($r = 0.228$ and $0.253$, $P = 0.001$ and $0.001$ respectively), while the relationship with LH was lost after controlling for testosterone or androstenedione ($r = 0.109$ and $0.08$ respectively).

The FNPO within the 6–9 mm range was negatively related to testosterone, body mass index (BMI) and fasting insulin serum levels, and positively related to inhibin B concentrations (Table III). After controlling for BMI or insulin, the relationship between FNPO and testosterone and inhibin B levels was no longer significant ($r = –0.105$ and $–0.098$ respectively and $r = 0.119$ and $r = 0.098$ respectively).

### Clinical and hormonal correlations with follicle count according to follicle size in controls

In this group, the 2–5 mm FNPO correlated with serum LH exclusively ($r = 0.187$, $P = 0.049$), while the 6–9 mm FNPO did not significantly correlate with any hormonal or metabolic variable.

### Correlation in both groups

No significant relationship was found between the FNPO and FSH or inhibin A serum level in any group, nor within any follicular size range (data not shown).

### Discussion

Our study was conducted to assess both the diagnostic and pathophysiological interest of the ultrasonically determined FNPO in PCOS. For diagnostic concerns, we re-evaluated

whether the threshold of 10 follicles per ovary was appropriate, as recommended by several authors (Adams *et al.*, 1985; Ardaens *et al.*, 1991; Takahashi *et al.*, 1994) for the definition of PCO. Our data with the ROC curves indicate that setting the threshold at 15 instead of 10 does not help to distinguish PCO from normal ovaries in control women. Although this brings the specificity up to 100%, it leads to a substantial loss of sensitivity. Likewise, narrowing the follicular range to 2–5 mm does not improve the diagnostic power of the FNPO, compared with data obtained by counting 2–9 mm follicles. Using a threshold value of 12 instead of 10 follicles within the 2–9 mm range seems to be a good compromise, since this kept good sensitivity (75%) while improving the specificity to 99%, thus making this parameter as valuable as the ovarian area, as previously reported (Atiomo *et al.*, 2000).

Although making the distinction between 2–5 and 6–9 mm follicular size ranges does not improve the diagnosis of PCO, our data raise some issues of pathophysiological interest. Indeed, the PCO contains two to three times the number of all types of growing follicles (stage 1 to stage 5) in comparison with normal ovaries (Hughesdon, 1982). Recent data suggest that this increase in folliculogenesis is under the dependence of intra-ovarian androgens which promote granulosa cell proliferation and inhibit apoptosis (Vendola *et al.*, 1998), especially in small follicles which are the richest in androgen receptors (Hillier *et al.*, 1997; Weil *et al.*, 1998). This physiological effect of androgens is probably exaggerated in the PCO wherein theca cells are hyperactive, over-expressing steroidogenic enzymes. This last phenomenon might be partly independent from LH and insulin, as suggested by long-term cultures (Wickenheisser *et al.*, 2000), and could therefore operate during early follicular growth. That the significant and positive correlation between the 2–5 mm follicle number and the serum testosterone or androstenedione levels in our patients with PCOS was independent from LH fits well with this concept. Although no previous study had focused on the follicle number in the range of 2–5 mm in PCOS, our data are in keeping with others' findings. Takahashi *et al.* (1994) and Battaglia *et al.* (1999) noted a positive correlation between the number of small follicles (2–8 mm) and the serum androstenedione level or the LH/FSH ratio. Pache *et al.* (1993) also found that both testosterone and immunoreactive LH were independently correlated with the number of follicles ⩾2 mm.

To our knowledge, this is the first study that has shown that PCOS patients have a similar number of 6–9 mm follicles per ovary to controls, despite the presence of a large excess of 2–5 mm follicles in PCOS patients. This ultrasound finding substantiates the theory of the follicular arrest, which assumes that the progression of small antral follicles to selected follicles (6–9 mm in size) and to the dominant follicle cannot proceed normally in PCO (Franks, 1997). This phenomenon is important in determining the anovulation of PCOS, and it has been shown to be closely related to obesity and hyperinsulinism (Franks, 1997). Accordingly, for those patients with PCOS in our study, being overweight and hyperinsulinaemic negatively influenced the number of 6–9 mm follicles, as was the case for the serum inhibin B concentrations in our previous study (Cortet-Rudelli *et al.*, 2002). We think that both phenomena

reflect the prominent role of obesity and/or hyperinsulinism in the follicular arrest of PCOS. In agreement with this hypothesis, obese women (BMI >25 kg/m$^2$) in our series had fewer 6–9 mm follicles and a lower mean serum inhibin B level than the lean ones (2.7 ± 3.0 versus 3.8 ± 3.4 pg/ml and 82.4 ± 41.9 versus 112.6 ± 49.2 pg/ml respectively; $P < 0.02$ and $P < 0.0001$ respectively).

Like Laven *et al.* (2001), we found that the inhibin B serum level in our unstimulated patients with PCOS was positively correlated with the FNPO. However, in our study, this relationship was restricted to the 6–9 mm range and was not significant after controlling for BMI or serum fasting insulin level. In addition, no such relationship was noted in our controls. Therefore, as emphasized earlier by ourselves (Cortet-Rudelli *et al.*, 2002), the present data again stress the need always to consider the patients' weight as a confounding factor when the serum inhibin B level is significantly related to hormonal or ultrasound variables.

In conclusion, we propose to modify the ultrasound definition of PCO advocated by Adams *et al.* (1985) as follows: 'increased ovarian area (>5.5 cm$^2$) or volume (>11 ml) and/or presence of ⩾12 follicles measuring 2–9 mm in diameter (mean of both ovaries)'. This definition should help to recognize the non-classical forms of PCOS in practice and should improve the phenotypic analysis in the frame of family studies. Besides revisiting the criteria for the ultrasound diagnosis of PCOS, our ultrasound findings strengthen the hypothesis that the follicular problem in PCOS is 2-fold (Dewailly *et al.*, 2003). First, the intra-ovarian hyperandrogenism promotes excessive early follicular growth, up to the 2–5 mm follicular stage, independently from LH and insulin. Second, the entry of follicles from this increased pool to the cohort and their further progression to selected follicles (6–9 mm in size) and ultimately to the dominant follicle cannot proceed because of follicular arrest. Although little is known about the mechanism(s) of this last phenomenon, it is clear that it is exacerbated by hyperinsulinism and/or other metabolic influences linked to obesity (Franks *et al.*, 1999).

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