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Metabolic and Endocrine Consequences of Acute Suppression of FFAs by Acipimox in Polycystic Ovary Syndrome

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To evaluate the effects of acute lowering of FFAs on glucose-induced insulin secretion and GH response to GHRH in polycystic ovary syndrome (PCOS), 27 PCOS subjects (11 lean and 16 obese) and 17 body mass index-matched controls (8 lean and 9 obese) were investigated.

Patients underwent an oral glucose tolerance test and a GHRH test before and after administration of the antilipolytic drug acipimox (250 mg orally 3 h and 1 h before the starting of the tests).

Blood samples were collected for 2 h after GHRH bolus and for 4 h after the oral glucose tolerance test. Serum concentrations of GH, insulin, glucose, and c-peptide were assayed in each sample, and the results were expressed as area under the curve (AUC).

No significant differences were found as to glucose, insulin, and c-peptide AUC before and after acute FFA plasma reduction in any of the investigated groups. Basally, lower GH-AUC was found in lean PCOS compared with body mass index-matched controls and in obese *vs.* lean controls; no significant differences were found as to the same variable between the two obese groups. The acipimox induced FFA suppression elicited in the four groups a sustained increase in the GH response to its trophic hormone; indeed, the GH-AUC nearly doubled with respect to basal evaluation in all the studied groups. However, the antilipolytic drug was not able to abolish the differences found between lean groups in basal conditions.

In conclusion, the presented data confirm that FFAs have a main role in regulating GH secretion at the pituitary level; however, it does not seem that they could explain the GH as well as insulin dysfunction of PCOS. (*J Clin Endocrinol Metab* 86: 5324–5329, 2001)

WOMEN WITH POLYCYSTIC ovary syndrome (PCOS) are characterized by an increased prevalence of metabolic disturbances; indeed, obesity is present in about 40–50% of PCOS patients (1). Furthermore, hyperandrogenism, which represents another classical feature of the syndrome, leads to a typical body fat distribution generally known as central or upper body obesity (2) and is closely related to the degree of peripheral insulin resistance (3). A key mechanism in this relationship is thought to be an enhanced mobilization of FFAs (4), since an increase in plasma FFA would contribute to insulin resistance and glucose intolerance (5–7). This hypothesis seems to be supported by the presence of a significant link between FFA plasma levels and insulin sensitivity index in PCOS subjects (8).

It has also been observed that the GH response to GHRH testing was markedly decreased in patients with PCOS compared with controls as well as in obese and hyperinsulinemic or normoinsulinemic patients with PCOS compared with matched controls (9).

Despite the fact that obesity and hyperinsulinemia have an additive influence on the impairment of GH secretion (10), it was suggested that other factors also may negatively affect GH secretion in patients with PCOS. GH has a direct lipolytic effect on adipose tissue, leading to the release of glycerol, FFA, and ketone bodies (11); on the other hand, FFAs may inhibit GH secretion by acting at the pituitary level, probably by direct inhibition of somatotroph function (12, 13).

Because increased plasma concentrations of FFAs have been reported in obese PCOS subjects when compared with body mass index (BMI)-matched control women (8), it could be speculated that FFAs might be at least partially responsible for both GH and insulin disturbances of PCOS.

Acipimox, an analog of nicotinic acid, is a powerful inhibitor of lipolysis that significantly decreases plasma FFA levels (14); thus, it represents a good tool in understanding the role of FFA depression on endocrino-metabolic disturbances of PCOS.

The present study was set to verify some hypotheses. First, we wanted to analyze if in our series FFA plasma levels were higher in obese PCOS rather than BMI-matched controls. Then, we wanted to verify whether acipimox was able to elicit GH response to GHRH and if there was a differential impact of FFA depression between PCOS and control subjects. The last aim was to analyze the presence of a cause-effect relationship between FFA and insulin resistance in PCOS.

Materials and Methods

We studied 27 consecutively seen patients with PCOS (age range, 19–33 yr) who were referred to our hospital. All the patients had spontaneous onset of puberty and normal sexual development, and all had oligomenorrhea with chronic anovulation since puberty. All the women were euthyroid, and none had taken medications known to affect plasma sex steroid levels for at least 3 months before the study.

PCOS was diagnosed on the basis of clinical findings (the presence of amenorrhea or oligomenorrhea and hirsutism), plasma androgen levels at the upper limit of or above the normal range (androstenedione,

Abbreviations: AUC, Area under the curve; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; VLDL-C, very low density lipoprotein cholesterol.

2.0–5.6 nmol/liter; T, 0.6–2.0 nmol/liter), and the presence of bilaterally normal or enlarged ovaries containing at least 7–10 microcysts (<5 mm in diameter) on ultrasonography. In approximately 50% of cases, the diagnosis was also confirmed by laparoscopy. A normal LH/FSH ratio was not considered an exclusion criterion (15). The presence of a late-onset adrenal enzyme defect was excluded by an ACTH test (250 μg iv Synachten; Ciba-Geigy, Basel, Switzerland) according to the criteria of New *et al.* (16). Obesity was defined as a BMI of more than 25 kg/m^2 (normal range, 19–25 kg/m^2). Waist circumference was obtained as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks.

Eight lean and nine obese normo-ovulatory volunteers served as controls; the mean (±SD) length of the menstrual cycle in these subjects was 28.9 ± 2.3 d. Ovulatory cycles had been confirmed previously by midluteal plasma progesterone levels of 25 nmol/liter or greater for three consecutive cycles. No patient showed polycystic-like ovaries at the ultrasound examination.

Informed consent was obtained from each patient, and the study protocol was approved by our Institutional Review Board.

Studies were conducted in the regularly cycling control subjects during the early follicular phase of their menstrual cycles (d 2–6) and in the women with PCOS on random days. In no case had recent ovulation occurred in the women with PCOS as evidenced by retrospective measurement of serum progesterone levels on the days of the study.

The patients were hospitalized; after fasting overnight for 10–12 h, blood samples were collected for basal hormone and lipoprotein assay. Then patients underwent an oral glucose tolerance test (OGTT) (d 1). On the following day (d 2) a GHRH test was performed. On d 3 patients received acipimox (Olbetam; Pharmacia & Upjohn, Inc., Milan, Italy) at a dose of 250 mg orally at −180 min and at −60 min; at time 0 min another OGTT was performed. After 48 h (d 5), acipimox was administered in the same manner and a GHRH test was performed at time 0. In the 2 d of acipimox administration, blood samples were collected at time 0, +120, and +240 min for FFA assay. Four patients (two PCOS and two controls) had transient upper-body flushing as a side effect of acipimox administration.

Plasma levels of T, dehydroepiandrosterone sulfate, androstenedione, 17-hydroxyprogesterone, FSH, LH, SHBG, triglycerides, high-density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (vLDL-C), low-density lipoprotein cholesterol (LDL-C), cholesterol, and FFA were determined in basal conditions (d 1).

The OGTTs were performed as: at 0900 h after overnight fasting, an indwelling catheter was inserted into the antecubital vein of one arm. Blood samples were collected basally and, after ingestion of 75 g glucose in 150 ml water within 5 min, at 30, 60, 90, 120, 180, and 240 min. Insulin, glucose, and c-peptide were assayed in all samples.

The GHRH tests were performed as: at 0900 h after overnight fasting. the GHRH (Geref, Serono, Milan, Italy) was injected iv at a dose of 1 μg/kg body weight. Blood samples were collected at the time of injection (time 0) and after 15, 30, 60, 90, and 120 min from the peptide administration. GH plasma concentrations were assayed in each sample.

Samples for hormone assay were promptly centrifuged, and the plasma was stored at −20 C until assay, whereas samples for biochemical assay were assayed immediately.

All hormones were determined by commercial RIA kits (Radim, Pomezia, Italy). Gonadotropins and insulin were assayed by the dextran-charcoal separation technique. The intra-assay and interassay coefficients of variation were less than 8% and 15%, respectively, for all determinations. The kit for GH assay had a sensitivity of 0.04 μg/liter whereas intra- and interassay coefficients of variation were 2.5% and 5.8%, respectively.

For each determination all samples from the same patient were assayed simultaneously. Plasma glucose was determined by the glucose oxidase method. Total cholesterol and triglyceride concentrations were determined by an enzymatic assay (Bristol, Paris, France). HDL-C concentrations were determined after precipitation of chylomicrons, vLDL-C, and LDL-C (Boehringer, Mannheim, Germany). vLDL-C was separated (as the supernatant) from LDL-C and HDL-C by lipoprotein ultracentrifugation. A magnesium chloride/phosphotungstic acid technique was used to precipitate LDL-C from the bottom fraction after ultracentrifugation. FFAs were determined by an acyl-coenzyme A oxidase-based colorimetric method.

A normal glycemic response to the OGTT was defined according to the criteria of the American Diabetes Association (17).

All results are expressed as mean ± SEM. Insulin, glucose, c-peptide, and GH plasma concentrations are also expressed as the area under the curve (AUC) after glucose ingestion or GHRH bolus, calculated by the trapezoidal rule.

Data were stored and analyzed using the Statistical Package for the Social Sciences, release 5.0 (SPSS, Inc., Chicago, IL) on an IBM-compatible computer. Not normally distributed variables were logarithmically transformed. The two-tailed *t* tests for paired data were used to compare the effects of acipimox within each group of subjects. Comparison between the groups were made by one-way ANOVA, and any significant difference identified using the Bonferroni correction for multiple comparisons. Values of $P < 0.05$ were considered significant.

The power of the study was evaluated using the Statistics for Biomedical Sciences, version 4.02 by Stanton A. Glantz (Windows version by R. Goldstein and R. Solomon, 1997; McGraw Hill, Milan, Italy). Because the main evaluation concerns the insulin variation after acipimox administration, the study was set to identify an FFA depression-mediated change of at least 20% in the insulin response to glucose load in the total group of obese PCOS and control patients (16 plus 9, 25 women); considering an $\alpha = 0.05$, the power calculation was 0.866. In the separate evaluation of PCOS and control obese subjects, with a 25% expected variation in the insulin-AUC under acipimox treatment and considering an $\alpha = 0.05$, the power calculation was 0.876 for PCOS women and 0.748 for controls.

Results

Based on their BMI, patients were divided in four groups: lean PCOS (11 patients), lean controls (8 patients), obese PCOS (16 patients), and obese controls (9 patients). No subject showed impaired glucose tolerance or noninsulin-dependent diabetes mellitus.

Table 1 shows the clinical and endocrine features of the groups studied. PCOS subjects showed higher waist to hip ratio and androgen levels when compared with BMI-matched controls. Within PCOS and control women no differences were found between lean and obese groups, except for SHBG levels, which were lower in obese groups (significantly only in PCOS).

Table 2 shows the metabolic parameters and lipidic patterns of the groups studied. Obese PCOS subjects showed lower GH plasma levels when compared with lean PCOS ($P < 0.01$) patients. Triglyceride plasma levels were higher in the obese groups when compared with lean ones. No significant difference was found for any of the other investigated variables; also, FFA levels did not significantly differ between groups, even though obese PCOS women showed slightly higher levels with respect to the other three groups.

After the acipimox administration, at time 0, 120, and 240 min FFA levels were at least 50% lower than the basal values in all groups without any significant difference among the groups (range, 0.09–0.31 mEq/liter).

Figure 1 shows glucose, insulin, and c-peptide response to oral glucose load, expressed as AUC before and after acipimox administration in the four analyzed groups. No significant differences were found in basal conditions between the lean as well as the obese groups, whereas the insulin AUC was higher in obese groups compared with lean ones. No difference was found in the evaluation of acute FFA plasma reduction within each group; the treatment was uneffective in abolishing the differences found in basal conditions. To verify the lack of any kind of acipimox-mediated effect on

TABLE 1. Clinical and endocrine features of the studied groups

	Lean PCOS	Lean controls	Obese PCOS	Obese controls
Number of patients	11	8	16	9
BMI (Kg/m^2)	23.01 ± 0.66	22.11 ± 0.79	31.60 ± 1.13[a]	29.78 ± 0.43[a]
Waist/hip ratio	0.84 ± 0.06	0.73 ± 0.02[b]	0.90 ± 0.05	0.81 ± 0.10[b]
T (nmol/liter)	2.29 ± 0.19	1.32 ± 0.84[c]	2.42 ± 0.16	1.42 ± 0.14[b]
Androstenedione (nmol/liter)	7.75 ± 0.64	4.92 ± 0.85[b]	9.52 ± 1.05	4.93 ± 0.06[c]
DHEAS (μmol/liter)	4594.37 ± 540.24	3590.92 ± 307.75	4901.81 ± 412.01	3961.24 ± 326.36
17-OHP (nmol/liter)	2.45 ± 0.24	1.54 ± 0.21[c]	2.42 ± 0.20	1.44 ± 0.22[c]
SHBG (nmol/liter)	45.92 ± 5.19	44.61 ± 5.17	28.11 ± 2.96[d]	25.97 ± 3.92[d]
LH (IU/liter)	8.84 ± 1.89	6.27 ± 0.81	8.21 ± 0.95	7.03 ± 0.51
FSH (IU/liter)	5.08 ± 0.56	6.68 ± 1.12	5.64 ± 0.53	5.41 ± 0.33

Data are expressed as mean ± SEM.
[a] $P < 0.01$ between lean and obese groups.
[b] $P < 0.01$ *vs.* PCOS counterparts.
[c] $P < 0.05$ *vs.* PCOS counterparts.
[d] $P < 0.05$ between lean and obese groups.

TABLE 2. Metabolic parameters and lipidic patterns of the studied groups

	Lean PCOS	Lean controls	Obese PCOS	Obese controls
Number of patients	11	8	16	9
Fasting glucose (mmol/liter)	4.22 ± 0.14	4.73 ± 0.20	4.43 ± 0.08	4.29 ± 0.12
Fasting insulin (pmol/liter)	61.84 ± 7.46	51.95 ± 6.26	86.67 ± 5.14	71.46 ± 9.30
Fasting c-peptide (pmol/liter)	470.02 ± 61.88	486.57 ± 70.22	665.31 ± 62.06	556.08 ± 72.82
GH (μg/liter)	2.11 ± 0.29	2.27 ± 0.35	0.81 ± 0.14[a]	1.42 ± 0.35
Cholesterol (mmol/liter)	4.14 ± 0.09	3.97 ± 0.20	4.61 ± 0.26	4.80 ± 0.37
LDL (mmol/liter)	2.27 ± 0.06	2.04 ± 0.11	2.99 ± 0.25	3.25 ± 0.34
HDL (mmol/liter)	1.44 ± 0.07	1.74 ± 0.21	1.20 ± 0.09	1.22 ± 0.06
vLDL (mmol/liter)	0.32 ± 0.04	0.31 ± 0.06	0.28 ± 0.02	0.34 ± 0.05
Triglycerides (mmol/liter)	0.69 ± 0.09	1.08 ± 0.13[b]	0.65 ± 0.04	1.15 ± 0.08
FFA (mEq/liter)	0.53 ± 0.14	0.52 ± 0.08	0.62 ± 0.10	0.52 ± 0.06

Data are expressed as mean ± SEM.
[a] $P < 0.01$ between lean and obese groups.
[b] $P < 0.01$ between lean and obese groups.

insulin-AUC in obesity we also evaluated as an entire group the obese PCOS and controls; we found superimposable results before and after FFA reduction (131,410 ± 9,521 *vs.* 128,999 ± 9,399 pmol/liter × 240 min, respectively).

Figure 2 shows the GH response to the GHRH test in lean as well as obese PCOS and control women. Under basal conditions, lower GH-AUC was found in lean PCOS compared with BMI-matched controls and in lean *vs.* obese controls; no significant difference was found as to the same variable between the two obese groups. The acipimox-induced FFA reduction elicited in the four groups a sustained increase in the GH response to its trophic-releasing hormone; indeed, the GH-AUC nearly doubled with respect to basal evaluation in all the studied subjects. Lean PCOS subjects showed, under acipimox treatment, GH-AUC similar to that of the lean controls evaluated in basal conditions; on the other hand, it remained significantly lower when compared with the GHRH-stimulated GH secretion in acipimox pretreated lean controls. No significant differences were found in a similar comparison between the obese groups, in which the GH response was always blunted with respect to lean ones.

Discussion

In 1963 Randle *et al.* (18) postulated the operation of a glucose/fatty acid cycle, based on experiments on perfused rat heart and hemi-diaphragm. They found that by increasing the availability and thereby the oxidation of FFA, the oxidation of glucose was decreased, thus suggesting the presence of a competition between lipids and glucose as a source of energy in the muscle cells. In human *in vivo* experiments FFAs have been shown to impair glucose uptake by both cardiac and skeletal muscle (19).

Furthermore, FFAs are capable of altering the gene expression of some hypothalamic hormones (20); indeed, it has been shown by *in vivo* (21) and *in vitro* (12) data that an increase in FFA blocks somatotroph function in a dose-related manner.

Holte *et al.* (8) reported that obese women with PCOS had markedly increased plasma FFA concentrations. This may be due to the increased truncal-abdominal fat mass, with a high lipolytic activity (22), and a generally impaired insulin suppression of FFA release from adipose tissue in the insulin-resistant state (23). Furthermore, the observed effect of T in facilitating FFA release from abdominal fat tissue (24) could be of importance in determining the metabolic alterations found in PCOS.

The syndrome is also characterized by a blunted GH response to GHRH administration (9, 25), which is heterogeneously represented in relation to both obesity and hyperinsulinism. The impairment of GH secretion found in PCOS women seems to be present at the pituitary level, even if the physiopathological events underlying the impaired somatotroph function remained unclear (8).

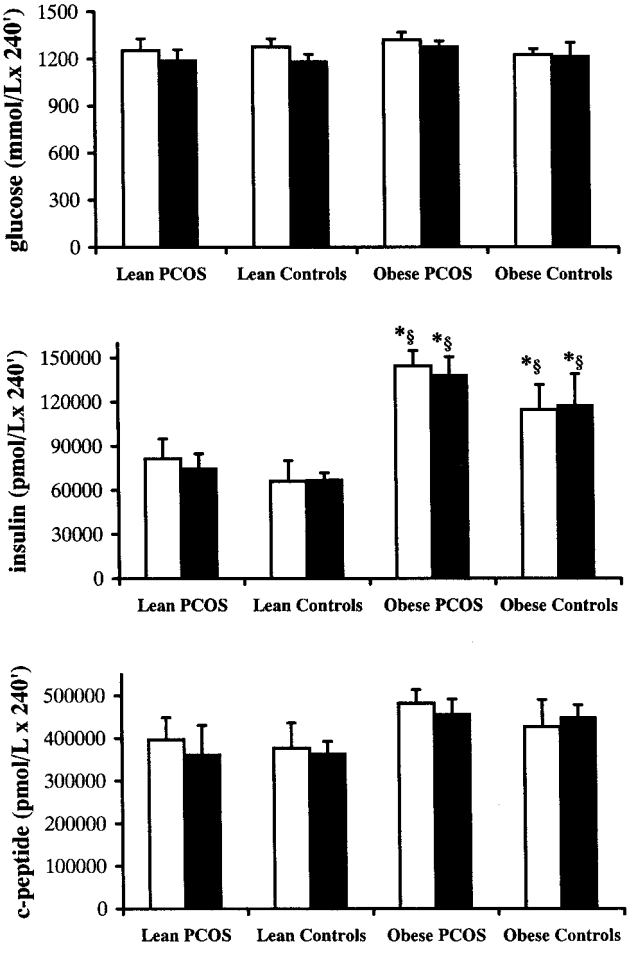

FIG. 1. Glucose, insulin, and c-peptide response to oral glucose load, expressed as AUC in the four groups studied before (□) and after (■) acipimox administration. All data are expressed as mean ± SD. Significance: *, $P < 0.01$ *vs.* lean PCOS; §, $P < 0.05$ *vs.* lean controls.

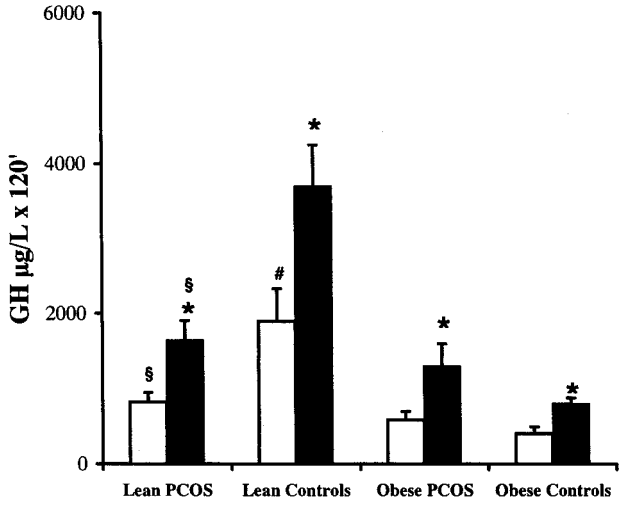

FIG. 2. GH response to GHRH, expressed as AUC in the four groups studied before (□) and after (■) acipimox administration. All data are expressed as mean ± SD. Significance: *, $P < 0.05$ before *vs.* after treatment; §, $P < 0.05$ PCOS *vs.* controls; #, $P < 0.05$ lean *vs.* obese.

In light of these data from the literature, the hypothesis that FFAs might be at least partly responsible for the insulin and GH dysfunction of PCOS seems rather intriguing.

In our series we did not find significant differences among the four investigated groups as to the FFA plasma levels; only obese PCOS women showed a slight, but not significant, increase in plasma FFA concentrations. These data are not in keeping with those reported by Holte *et al.* (8) about higher FFA levels in PCOS; it is possible that factors other than PCOS status might influence the FFA plasma levels, such as environmental or dietary factors. On the other hand, a recent report found a tendency toward higher FFA concentrations and defective suppression of rate of lipid oxidation in obese PCOS subjects during the hyperinsulinemic clamp (26). It could be argued also that small variations in the FFA levels as well as different sensitivity to circulating FFA concentrations might be able to influence insulin metabolism or GH secretion.

The pharmacological reduction of FFAs by the administration of an antilipolytic drug (acipimox), gave us the possibility to better evaluate their role, if any, on the disturbances of PCOS.

Concerning the GH response to GHRH, our lean PCOS patients showed blunted somatotroph function when compared with BMI-matched controls, whereas no significant differences were found within obese individuals, in line with previous reported data (25, 27).

The antilipolytic drug administration highlighted two different results: 1) a significant, nearly 2-fold, increase in GH response to its trophic hormone in all the studied groups; and 2) a lack of effect on glucose, insulin, and c-peptide response to oral glucose load in PCOS and control subjects.

Concerning the first result, our data confirm the hypothesis that FFA reduction makes the somatotroph cells more sensitive to GHRH stimulus. Furthermore, this effect is superimposable in all groups; indeed, acipimox administration was able to nearly double the GH secretion after GHRH bolus in all examined subjects, without differences between PCOS and controls. These data, coupled with the finding that in lean subjects the treatment was not effective in abolishing the differences between PCOS and normo-ovulatory women, seem to argue against a differential impact of FFA on somatotroph function in PCOS.

No significant changes were observed after acute FFA suppression as to the glycemic and insulinemic response to OGTT in any of the investigated groups. Although the PCOS responsiveness to acipimox has not been previously studied, the data regarding lean and obese controls are not in keeping with previous researchers reporting improvements of insulin resistance and glucose tolerance after acute lowering of FFAs in obese diabetic and nondiabetic subjects (28, 29). It could be hypothesized that this disagreement could be ascribed to different dosages or length of treatment; indeed, both the investigator groups (28, 29) chose an overnight FFA inhibition with a 3-fold administration of acipimox (250 mg orally) within a 12-h period, whereas in our study the same drug was administered twice, 3 and 1 h before the starting of GHRH or oral glucose tests.

Boden *et al.* (30) were unable to demonstrate a statistically significant reduction in insulin resistance after acutely low-

ering plasma FFA for 6 h in four healthy volunteers, thus suggesting the importance of a longer length of suppression. However, in other studies, in which FFAs were pharmacologically increased, it was suggested that inhibition of insulin-mediated glucose uptake is time dependent and may only become apparent after 3 h or more of raised FFA levels (31, 32). It is also reasonable that the same time interval could be considered adequate in showing incidental modifications of insulinemic patterns after pharmacological reduction of FFA concentrations. Piatti *et al.* (33), by orally administering placebo or 250 mg acipimox 2 h before an OGTT in 10 lean noninsulin-dependent diabetes mellitus subjects, reported a significant decrease in the incremental areas of blood glucose (−18%) and insulin (−19%) after acipimox compared with placebo. Starting from these data we performed a power calculation of our study to verify whether our negative results might be due to a bias related to a poor sample size: we evaluated together obese PCOS and controls to verify the hypothesis of a cause-effect relationship between obesity and FFA-mediated insulin resistance. Using this procedure we obtained a power of 86.6% to detect a difference of 20% in the insulin AUC, which is that reported by Piatti *et al.* (33), thus confirming the validity of our data. In the evaluation of obese PCOS subjects alone, with a 25% expected variation in the insulin-AUC under acipimox treatment, we obtained a power result of 87.6%.

At last, it does not seem that the present results could be explained by an inadequate dose in acipimox administration; indeed, this drug is effective in the suppression of lipolysis at a plasma concentration of 10^{-5} M (34), and it has been shown that oral administration of a single dose of 250 mg acipimox to humans produces a plasma concentration of acipimox of more than 10^{-5} M for 6–8 h (35).

Thus, our results were unexpected, and we are not aware of possible explanations to the lack of effect of the acute FFA suppression on glucose, insulin, and c-peptide response to oral glucose load. However, the main finding reported in our study is represented by the absence of any kind of difference between PCOS and control BMI-matched subjects as to the metabolic effects of the antilipolytic drug administration.

In conclusion, the presented data confirm that FFAs have a main role in regulating GH secretion at the pituitary level; however, it does not seem that they could explain the GH as well as insulin dysfunctions of PCOS. It remains unclear whether a long-term acipimox treatment could lead to different results; additional studies are in progress in this area.

Acknowledgments

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