Novel Expression and Direct Effects of Adiponectin in the Rat Testis


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Adiponectin is an adipocyte hormone, with relevant roles in lipid metabolism and glucose homeostasis, recently involved in the control of different endocrine organs, such as the placenta, pituitary and, likely, the ovary. However, whether as described previously for other adipokines, such as leptin and resistin, adiponectin is expressed and/or conducts biological actions in the male gonad remains unexplored. In this study, we provide compelling evidence for the expression, putative hormonal regulation, and direct effects of adiponectin in the rat testis. Testicular expression of adiponectin was demonstrated along postnatal development, with a distinctive pattern of RNA transcripts and discernible protein levels that appeared mostly located at interstitial Leydig cells. Testicular levels of adiponectin mRNA were marginally regulated by pituitary gonadotropins but overtly modulated by metabolic signals, such as glucocorticoids, thyroxine, and peroxisome proliferator-activated receptor-γ, whose effects were partially different from those on circulating levels of adiponectin.

In addition, expression of the genes encoding adiponectin receptor (AdipoR)-1 and AdipoR2 was detected in the rat testis, with developmental changes and gonadotropin regulation for AdipoR2 mRNA, and prominent levels of AdipoR1 in seminiferous tubules. Moreover, recombinant adiponectin significantly inhibited basal and human choriogonadotropin-stimulated testosterone secretion ex vivo, whereas it failed to change relative levels of several Sertoli cell-expressed mRNAs, such as stem cell factor and anti-Müllerian hormone. In summary, our data are the first to document the expression, regulation and functional role of adiponectin in the rat testis. Taken together with its recently reported expression in the ovary and its effects on LH secretion and ovarian steroidogenesis, these results further substantiate a multifaceted role of adiponectin in the control of the reproductive axis, which might operate as endocrine integrator linking metabolism and gonadal function. (Endocrinology 149: 3390–3402, 2008)

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DIPONECTIN, ALSO TERMED Acrp30 (adipocyte complement related protein), apM1 (adipose most abundant gene transcript I), GBP28 (gelatin binding protein 28), or AdipoQ, was identified in 1995–1996 as an adipocyte-specific secreted factor with structural features of the collagen superfamily (1–4). Its monomeric form is a 30-kDa protein that oligomerize to form trimers, which can further bind to generate 180-kDa hexamers or even high-molecular-weight polymers (>400 kDa), the latter being the most abundant circulating form of adiponectin in humans (5). Such a multimerization process has been documented as essential for the biological actions of adiponectin (6, 7).

Although adiponectin was identified as an adipose-secreted factor more than a decade ago, approximately coincident with the cloning of leptin (5), its full physiological relevance has emerged only in recent years, when adiponectin has been recognized as an antidiabetic adipokine, with potent insulin-sensitizing functions and thus promising therapeutic properties (5, 8–10). Of note, circulating levels of adiponectin are inversely correlated with the degree of adiposity and are overtly reduced in obesity and type 2 diabetes (4, 11, 12), whereas adiponectin administration has been shown to ameliorate insulin resistance and to induce glucose lowering in animal models of obesity (9, 13, 14). As a whole, these observations strongly suggest that decreased adiponectin levels are likely to operate as causative link between excess of adiposity and related comorbidities (15). As further proof for the relevant regulatory role of adiponectin in lipid and glucose homeostasis, its adipose expression appears tightly regulated by a wide array of metabolic cues and hormones, with either stimulatory [peroxisome proliferator-activated receptor (PPAR)-γ ligands] or inhibitory (insulin, glucocorticoids, IL-6, TNFα, GH) effects (5, 16–18).

The biological actions of adiponectin are mediated by two distinct but structurally related receptors, AdipoR1 and AdipoR2. These are seven-transmembrane spanning receptors but with differential features from the superfamily of G protein-coupled receptors, such as their inverted orientation and their predominant signaling via AMP kinase (19). Initial reports demonstrated that AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly lo-
cated in the liver (19). The physiological importance of these receptors in terms of metabolic control is now firmly established. AdipoR1−/− mice showed increased adiposity associated with decreased glucose tolerance, spontaneous locomotor activity, and energy expenditure. In contrast, AdipoR2−/− mice were lean and resistant to high-fat diet-induced obesity and showed improved glucose tolerance and reduced plasma cholesterol levels (20). Whether additional receptors can mediate these or other biological effects of adiponectin is still a matter of debate.

Besides its dominant metabolic roles, solid evidence points out that adiponectin is a rather pleiotropic regulator of a large set of biological functions, ranging from endothelial responses and pituitary hormone secretion to cell proliferation and tumor progression (5, 21–23). This contention is further supported by the recently recognized, widespread pattern of expression of AdipoR1 and AdipoR2, which include not only muscle, liver, and adipocytes but also hypothalamus, pituitary, pancreatic β-cells, endothelial cells, and placentia (19, 24–27). Likewise, adiponectin expression has been recently proven more scattered than originally recognized, with detectable adiponectin mRNA levels in skeletal muscle, heart, placenta, pituitary, and osteoblasts (22, 24, 28–30).

It is well known that reproductive capacity is metabolically gated, and an ever growing number of neuropeptides and hormone signals, primarily involved in the control of energy balance and metabolism, have been recently proven as putative regulators of puberty maturation, gonadotropin function, and/or fertility (31). Among those, the prominent role of the adipocyte-derived hormone, leptin, in the control of reproduction has been well characterized over the last decade (32–34). In contrast, the physiological role, if any, of other adipose-born signals in the modulation of reproductive function remains ill defined. Notwithstanding, given its functional profile, the putative reproductive functions of adiponectin have begun to be explored recently. These analyses were initially focused in the eventual implication of adiponectin in female reproductive disorders linked to obesity and insulin resistance, such as polycystic ovarian syndrome. Indeed, a decrease in circulating adiponectin levels has been reported in polycystic ovarian syndrome patients (35–37). Further evidence for a physiological link between adiponectin and reproductive function came from the observation that adiponectin concentrations are invariably higher in females than in males (38) and that androgens inhibit adiponectin secretion (39, 40). Very recently expression of adiponectin and its receptors has been documented in the rat ovary, in which adiponectin has been demonstrated to modestly stimulate progesterone and estradiol secretion in response to IGF-I (41). Moreover, the presence of adiponectin in rat oviduct has been recently documented (42). Whether adiponectin is expressed and/or able to conduct direct actions in the testis and/or male reproductive tract remains unexplored to date.

Worth noting, a number of hormonal signals with key roles in energy homeostasis and metabolism are expressed and/or conduct biological actions directly at the testicular level. These have been reported in a diversity of species (from rodents to humans) and include not only leptin (for a recent review see Ref. 33) but also ghrelin, the gut-derived orexi-
mRNA analyses, in some of the above experimental groups, plasma levels of adiponectin were assayed by specific RIA. In addition, considering the previously reported effects of PPARγ ligands on adiponectin expression of adiponectin gene (16), the ability of the selective agonist of PPARγ, rosiglitazone, to modulate testicular adiponectin mRNA expression was assessed in vitro. Slices of testicular tissue were obtained from adult rats and incubated for 180 min in the presence of increasing concentrations (10^{-10} to 10^{-4} M) of rosiglitazone, as described in detail elsewhere (44). At the end of the incubation period, testis samples were processed for RNA analysis.

Our second set of experiments aimed to evaluate the expression of the genes encoding the putative adiponectin receptors, AdipoR1 and AdipoR2, in some of the experimental conditions tested above. Expression analyses were conducted in testicular samples from rats along postnatal development, after HIFX, with or without gonadotropin supplementation, and after food deprivation. In addition, expression of AdipoR1 and AdipoR2 mRNAs (and the ligand) was assessed in microdissected seminiferous tubule fragments, isolated following previously described procedures (47). Specific stages of the seminiferous epithelial cycle were identified and pooled in four major groups corresponding to stages II–VI, stages VII–VIII, stages IX–XII, and stages XIII–I of the cycle. After exhaustive washing, tubular tissue was processed for RNA analysis as described below. In addition, staged tubule preparations (20 μm segments per well) were cultured in the presence of medium alone as controls.

Finally, in our third set of experiments, the direct biological effects of recombinant adiponectin on basal and stimulated testosterone (T) secretion were assessed in vitro using static incubations of adult rat tissue as described elsewhere (48, 49). Tissue samples were incubated in the presence of increasing doses of adiponectin (0.01, 0.1, and 1 μg/ml), under basal or stimulated conditions (coincubation with 10 IU/ml hCG). In addition to secretory T responses, the effects of adiponectin on the mRNA levels of several Sertoli cell-expressed genes, such as stem cell factor (SCF), anti-Müllerian hormone (AMH), inhibin-α, and inhibin-βB, were evaluated in the same tissue samples following previously published protocols (50, 51).

**RNA analysis by final-time RT-PCR**

Total RNA was isolated from testicular samples (or seminiferous tubule fragments) by the single-step, acid guanidinium thiocyanate-phenol-chloroform extraction method. Testicular expression of adiponectin, AdipoR1, and AdipoR2 mRNAs was assessed by RT-PCR, using specific primer pairs and conditions as described in detail elsewhere (24). In addition, in selected experimental designs, semiquantitative guidelines were included in all assays, yielding no amplification.

For amplification of the targets, total RNA (2 μg) was reverse transcribed using random primers and SuperScript II reverse transcriptase (Invitrogen Life Technologies, Inc., Carlsbad, CA). For PCR amplification of adiponectin, AdipoR1, AdipoR2 transcripts, the following conditions were used: denaturation at 95°C for 1 min, annealing at 60°C for 30 sec, and extension at 72°C for 1 min for 34 cycles (24). PCR amplification of SCF, AMH, and inhibin transcripts was conducted as previously reported, using conditions optimized for amplification in the exponential phase for each transcript (50).

PCR-generated DNA fragments were resolved in 3% agarose gels and visualized by ethidium bromide staining; identity of the amplicons was routinely conducted by double-strand sequencing. In all assays, liquid controls and reactions without reverse transcription resulted in negative amplification. When relevant, quantitative evaluation of RT-PCR signals was carried out by densitometric scanning using an image analysis system (GeneGnome documentation system; Bio-Rad Laboratories). For immunolabeling, testicular sections (5 μm thick) were submitted to antigen retrieval in a microwave oven and incubated overnight with the primary antibody (dilution 1:100; Sigma) and secondary antirabbit antiserum, as previously described (24). As protein control, the filters were washed and sequentially incubated with monoclonal α-tubulin antibody (dilution 1:1000; Sigma) and secondary antimouse antiserum. Detection was carried out using a chemiluminescent system (Tropix, Bedford, MA).

**Adiponectin immunohistochemistry**

Immunohistochemical detection of adiponectin peptide was carried out in 4% paraformaldehyde fixed sections of testes from adult rats. Two different primary antibodies raised against adiponectin were routinely used: rabbit anti-[Cys0]-adiponectin primary antibody (Phoenix Pharmaceuticals, Belmont, CA) and rabbit Acrp30 (N-20)-R (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). For immunolabeling, testicular sections were processed with a rabbit antiadiponectin primary antibody (ACRP 301-A; working dilution 1:100; dDiagnosic International, San Antonio, TX) and secondary antirabbit antiserum, as previously described (24). As protein control, the filters were washed and sequentially incubated with monoclonal α-tubulin antibody (dilution 1:1000; Sigma) and secondary antimouse antiserum. Detection was carried out using a chemiluminescent system (Tropix, Bedford, MA).

**Analysis of adiponectin RNA by Northern hybridization**

The pattern of expression of adiponectin mRNA was evaluated in the rat testis by means of Northern hybridization. Total RNA was isolated from adult rat testicular samples as described above. Using denaturing agarose gels, samples of 25 μg of total RNA per lane were electrophoretically resolved and transferred to nitrocellulose membranes following standard procedures (24). The filters were subsequently hybridized using a radiolabeled cDNA probe, generated using a 658-bp fragment of rat adiponectin cDNA (24). Hybridization signals were digitalized and processed by densitometric scanning using an image analysis system (Gel-2000 documentation system; Bio-Rad Laboratories).
antibody by preimmune serum. In addition, as control for antibody specificity, immunohistochemical reactions were carried out after pre-absorption of the antiserum overnight at 4 C with recombinant adiponectin.

**T measurements by specific RIA**

T levels in static incubation media were measured using a commercial kit from MP Biomedicals (Costa Mesa, CA). All medium samples were measured in the same assay. The sensitivity of the assay was 1 ng/ml and intraassay coefficient of variation was 4.5%.

**Adiponectin measurements by specific RIA**

Plasma levels of adiponectin were assayed in selected experimental groups using a commercial double-antibody RIA kit from Linco Research (St. Charles, MO), as previously described (24). All samples were assayed in duplicate in the same assay. The limit of the assay sensitivity was 1 ng/ml and interassay coefficient of variation was 6.6%.

**Presentation of data and statistics**

Semi-quantitative, real-time RT-PCR analyses were carried out, at least in quadruplicate, using independent RNA samples. For presentation, in each experimental design, the expression levels in control/reference groups were assigned to a value of 100, and the others were normalized accordingly. Tissue incubations were conducted in duplicate, with a total number of 10–12 samples/determinations per group. Quantitative data are presented as mean ± SEM. Results were analyzed for statistically significant differences using ANOVA, followed by Tukey’s test. P ≤ 0.05 was considered significant.

**Results**

**Adiponectin expression in rat testis: developmental, hormonal and metabolic regulation**

Testicular expression of adiponectin, at the mRNA and protein levels, was first explored in samples from adult rats using a combination of RT-PCR/Southern blot, Northern hybridization, Western blot, and immunohistochemistry. Initial RT-PCR analyses revealed that adiponectin gene is expressed in adult testis tissue, with amplicons similar in size to those obtained from white adipose tissue; immunolabeling of tubulin is shown as loading control. In all assays, assessment of adiponectin expression, at mRNA or protein level, in WAT is presented as positive control.
ern blot and immunohistochemistry. The former detected a major peptide band of 30 kDa in testicular homogenates, similar in size to that observed in WAT (Fig. 1C). For both testicular and WAT samples, additional bands of higher molecular mass (>50 kDa) were also detected, whereas omission of primary antibody abolished immunolabeling (data not shown). In addition, the presence of adiponectin immuno-reactivity was demonstrated in adult rat testicular sections, with a predominant location in Leydig cells, identified at high magnification as cellular clusters in the interstitial space. In contrast, weak to negligible adiponectin immunolabeling was observed in the seminiferous tubules (Sertoli and germ cells) (Fig. 2, A and B). Specificity of adiponectin immunostaining was confirmed by omission and preabsorption of the primary antibody, procedures that completely blocked labeling of testis sections (Fig. 2C).

Subsequent analyses were directed toward the characterization of developmental, hormonal, and metabolic regulation of adiponectin gene expression in the rat testis. Assessment of mRNA levels of adiponectin throughout postnatal development, from the neonatal period to adulthood, demonstrated the persistent expression of the transcript in the rat testis. Yet its relative levels significantly varied along the age: moderate levels were observed before puberty (∼30 d old rats) that increased thereafter, with peak expression in early adult (60 d old) samples (Fig. 3A). In addition, the influence
on testicular adiponectin mRNA expression of the major hormonal regulators of the testis, namely pituitary gonadotropins, was assessed using HPX rats, with or without gonadotropin replacement. Long-term (4 wk) HPX failed to induce major changes in the relative levels of adiponectin mRNA, which were not further affected by replacement with hCG (10 IU/rat per 24 h for 7 d; hCG as a superagonist of LH); or FSH (7.5 IU/rat per 24 h for 7 d) (Fig. 3B). Of notice, however, long-term HPX induced dramatic changes in testis weight (HPX: 309.0 ± 17.5 mg/testis; HPX+hCG: 585.0 ± 27.0 mg/testis; HPX+FSH: 447.0 ± 15.0 mg/testis; controls: 995.0 ± 37.0 mg/testis). Thus, in addition to relative levels of the transcript (i.e. relative mRNA abundance in 2 µg of total RNA), total expression of adiponectin mRNA per testis was also calculated after correction of relative values by testis weight (arbitrary units per milligram testis weight). In contrast to relative levels, 4 wk HPX induced a significant decrease in absolute expression of adiponectin mRNA per testis; a response that was partially prevented by hCG treatment. Conversely, FSH supplementation to HPX rats evoked only a marginal increase in total adiponectin mRNA levels (Fig. 3C). Contrary to its testicular effects, 4 wk HPX caused a significant rise in circulating levels of adiponectin that was not prevented by protocols of hCG or FSH replacement (Fig. 4A).

Metabolic regulation of testicular expression of adiponectin mRNA was explored in a number of experimental settings. Food deprivation for 48 or 72 h did not induce detectable changes in the relative mRNA levels of adiponectin (Fig. 5A), even after central supplementation of fasted rats with leptin (data not shown). Notably, 48 h fasting evoked the expected rise in plasma levels of adiponectin (Fig. 4B). Chronic treatment with dexamethasone in vivo decreased testicular expression of adiponectin mRNA; hypothyroidism was without any effect. However,
the circulating levels of adiponectin increased in hypothyroid rats, remained unaltered after l-thyroxine administration and were decreased by dexamethasone (Fig. 4, C and D). Finally, in vitro exposure to increasing doses (10⁻¹⁰ to 10⁻⁴ M) of the agonist of PPARγ, rosiglitazone, evoked a significant reduction in the testicular levels of adiponectin mRNA, which was statistically significant at all doses tested (Fig. 6).

Expression of AdipoR1 and AdipoR2 genes in rat testis and direct testicular effects of adiponectin

Expression of AdipoR1 and AdipoR2 mRNAs was initially demonstrated in adult rat testes by means of RT-PCR, with amplicons similar in size to those obtained from skeletal muscle (AdipoR1) and liver (AdipoR2), and whose identity was confirmed by direct sequencing (Fig. 7A). Considering the proven expression of AdipoR1 mRNA in blood cells (24) and given the difficulty to discriminate whether amplification of this transcript in homogenates from whole testicular tissue might be due to blood contamination, RT-PCR analyses were also performed in homogenates of seminiferous tubule fragments, isolated under the stereomicroscope and thoroughly washed prior RNA isolation (i.e. to eliminate the presence of blood cells). These assays showed that AdipoR1 mRNA, but not AdipoR2, is expressed in the seminiferous epithelium (Fig. 7B), thus suggesting a compartmentalized distribution of AdipoRs within testicular tissue: AdipoR2 is not expressed in the seminiferous epithelium (but rather in the interstitium), whereas AdipoR1 is expressed in the tubular compartment. On this basis, and considering the methodological limitations indicated above, samples of whole testicular tissue were used to specifically evaluate the developmental and hormonal regulation of AdipoR2 gene expression, whereas analyses on AdipoR1 mRNA were selectively conducted in staged seminiferous tubule fragments.

As was the case for the ligand, persistent expression of AdipoR2 mRNA was detected in the rat testis along postnatal development. However, in contrast to adiponectin, relative levels of this transcript were high at the neonatal period, declined thereafter during the infantile and prepubertal stages, and partially increased in adult animals (Fig. 8A). In addition, testicular expression of AdipoR2 appeared sensitive to gonadotropin regulation because its relative mRNA levels were significantly increased by hCG treatment in long-term HPX rats (Fig. 8B). Moreover, when total expression of

![Fig. 6](https://academic.oup.com/endo/article/149/7/3390/2454969)

**Fig. 6.** Regulation of adiponectin mRNA expression in rat testis by the selective ligand of PPARγ, rosiglitazone. Relative levels of adiponectin mRNA were assayed by RT-PCR in slices of testicular tissue after in vitro exposure for 180 min to rosiglitazone maleate (TZD) at 10⁻¹⁰ to 10⁻⁴ M doses. Semiquantitative values are the mean ± SEM of at least five independent determinations. Groups with different superscript letters are statistically different (P < 0.01; ANOVA followed by Tukey’s test).
AdipoR2 mRNA per testis was calculated (see above), it was noticed that 4 wk HPX induced a significant decrease in absolute expression of AdipoR2 mRNA per testis, whereas hCG, and to a lower extent FSH, treatment induced an increase in total AdipoR2 mRNA content (Fig. 8C).

Expression of AdipoR1 mRNA in the seminiferous epithelium was confirmed by real-time RT-PCR analyses using staged tubule fragments devoid of blood cell contamination. These analyses revealed that AdipoR1 mRNA levels are the highest at stages II–VI, significantly decrease during stages VII–VIII and IX–XII, and increase again at stages XIII–I of the epithelial cycle. Exposure to FSH \textit{in vitro} for 24 h (10 ng/ml) failed to change the expression levels of AdipoR1 at any of the stages tested (Fig. 9). Of note, adiponectin mRNA, albeit at low levels, was also detected in seminiferous epithelial preparations, yet its relative levels remained invariant regardless of the stage of the cycle and FSH treatment (data not shown).

Finally, demonstration of AdipoR1 and AdipoR2 gene expression in the rat testis prompted us to explore the potential effects of adiponectin directly at the testicular level. On the basis of previous data on the direct actions of relevant metabolic signals in rat testis (44, 48–50), two phenomena were selectively evaluated using static incubations of testicular tissue. First, T responses to increasing doses of adiponectin (0.01, 0.1, 1 \(\mu\)g/ml) were assayed, at 90 and 180 min incubation, in basal and hCG-stimulated conditions. Recombinant adiponectin, in a dose-dependent manner, was able to inhibit basal T secretion by incubated testicular tissue, with a significant lowering of \(T\) levels being detected after challenge with 0.01 and 0.1 \(\mu\)g/ml doses of adiponectin at both time points tested. Likewise, hCG-stimulated testosterone secretion was significantly suppressed by coincubation with adiponectin, at all doses studied (Fig. 10). Second, the potential actions of adiponectin on key seminiferous tubule functions were explored \textit{in vitro} by monitoring its effects on a panel of Sertoli cell-expressed genes. In detail, the effects of increasing concentrations of adiponectin (0.01, 0.1, 1 \(\mu\)g/ml) upon the relative mRNA levels of inhibin-\(\alpha\) and inhibin-\(\beta\)B subunits, SCF and AMH, because Sertoli cell-specific signals within the tubular compartment of the adult rat testis (51), were explored. In our setting, exposure to adiponectin, at the different doses tested, failed to modify the relative expression levels of any of the targets under analysis (data not shown).

**Discussion**

Among the diversity of target tissues and cellular functions (55), expression and/or direct actions of different adipokines have been reported in the gonads (41, 44). To our knowledge, our study is the first to provide a detailed characterization of the pattern of expression and hormonal regulation of adiponectin in the testis and document the presence of functional receptors and direct biological effects of this adipokine in the male gonad. Taken together with very recent reports on the presence of adiponectin and its cognate receptors in the rat ovary (41), our data unveil the putative role of adiponectin as novel regulator of gonadal function in rodents.

The expression of adiponectin in the rat testis was documented by a combination of analytic approaches at the mRNA and protein levels. Intriguingly, a distinctive pattern of mRNA transcripts, partially different to that observed in the WAT, was detected, with two prominent species of 1.2- and less than 1.0 kb (see Fig. 1). Although the sequence identity of those transcripts was not directly determined, the combined evaluation of Northern and RT-PCR analyses strongly suggests that differences in size between adipose and testicular transcripts are likely to reside in the 3’-untranslated region. Of note, a similar phenomenon has been described for other sites of extraadipose expression of adiponectin, such as the placenta (24). Nonetheless, the fact that a similar protein band, of the expected 30-kDa size, was obtained in homogenates from fat and testicular tissues evidence that the 1.2-kDa mRNA transcript is likely to encode functional adiponectin monomers, in keeping with previous data in the mouse showing that the 1.2-kb species contains the complete open reading frame of adiponectin (54). In good agreement, discernible adiponectin immunoreactivity was observed in testicular sections from adult rats, with prominent signals being detected in interstitial Leydig cells. In contrast, faint to negligible immunostaining was observed in the seminiferous tubules, regardless of the stage of the epithelial cycle. This observation strongly suggests that the major source of testicular expression of adiponectin is located...
at the interstitium, likely in steroidogenic Leydig cells, in which this adipokine might play a functional role in the local (autocrine) control of testosterone secretion (see below). Yet weak expression of adiponectin at the seminiferous epithelium is likely to occur because its mRNA was detected, albeit at low levels, in tubular fragments throughout the epithelial cycle.

Testicular expression of adiponectin gene appears to be under the regulation of developmental cues and hormonal factors. On the former, moderate mRNA levels of adiponectin were detected in the rat testis before puberty, which significantly increased after pubertal maturation. The basis for such observation is yet to be defined but may involve the expansion of the adult-type Leydig cell population that takes place along the puberty transition (56). In addition, the contribution of pituitary gonadotropins, whose circulating levels increase at puberty, to this phenomenon cannot be excluded. Yet it is noticeable that relative levels of adiponectin mRNA were not overtly increased by treatment of HPX rats with either hCG (as superagonist of LH) or FSH. Nonetheless, the total content of adiponectin mRNA per testis was significantly decreased after HPX (a procedure that markedly reduced testis weight) and partially rescued by gonadotropin replacement, especially by hCG treatment. This is likely to reflect the trophic effect of hCG administration on the testis (57), and it is fully compatible with the preferential expression of adiponectin in Leydig cells within the testis because these cells represent the primary site of testicular expression of LH/CG receptors (58). Of note, circulating levels of adiponectin increased after HPX, which might be due (at least partially) to reduced secretion of androgen, which is a putative inhibitor of adiponectin expression in the
adipose (39). Yet our protocols of gonadotropin replacement were not able to normalize adiponectin plasma concentrations, suggesting the involvement of other pituitary hormones in this phenomenon.

Contrary to the modest regulatory effects of gonadotropins, testicular expression of adiponectin gene was clearly modulated by a number of metabolic hormones and factors. Among those, administration of the synthetic glucocorticoid, dexamethasone, significantly decreased, whereas repeated injection of thyroxine markedly increased, adiponectin mRNA levels in the testis. In addition, in vitro exposure to the ligand of the transcription factor PPARγ, rosiglitazone, significantly suppressed testicular expression of adiponectin mRNA; an effect detected for doses as low as 100 pm, the lowest dose tested in our experiments. In contrast, food deprivation, for up to 72 h, failed to induce overt changes in adiponectin mRNA levels in the testis, which were not affected by leptin administration either. Of note, whereas some of the above regulators (e.g. glucocorticoids) have been reported to induce similar effects in terms of adipose expression of adiponectin gene (5), significant differences become apparent when comparing the actions of proven modulators of adiponectin expression in fat tissue, such as ligands of PPARγ and fasting, which increase adiponectin expression in the adipose (5), but either decrease (PPARγ) or do not affect (fasting) it in the testis. Likewise, measurement of circulating adiponectin levels in our models suggested dissimilar effects of thyroid hormones on testicular and adipose expression of adiponectin because this could not be reliably assessed in our analyses given the presence of AdipoR1 transcripts in the interstitium, whereas expression of AdipoR2 mRNA was not detected in the seminiferous epithelium, but likely confined to the testis. The latter was under the regulation of developmental cues and gonadotropins because AdipoR2 mRNA levels increased at puberty transition and were significantly enhanced by the superagonist of LH, hCG, a phenomenon that might be causative for the observed pubertal rise of AdipoR2 mRNA in the rat testis. Admittedly, the interstitial expression of AdipoR1 gene cannot be excluded because this could not be reliably assessed in our analyses given the presence of AdipoR1 transcripts in blood cells (24), which are likely to contaminate whole-tissus homogenates. Nonetheless, clear-cut AdipoR1 transcripts were detected in isolated tubule fragments, at the different stages of the seminiferous epithelial cycle, with varying relative levels across the cycle: peak values at stages II–VI and nadir levels at stages VII–XII. This profile is suggestive of a fine tuning of adiponectin signaling in the seminiferous epithelium. Yet it
is noticeable that FSH, as a key endocrine regulator of spermatogenesis (58), is not apparently posed with major regulatory effects on the tubular expression of AdipoR1 gene.

Further proof for the presence of functional adiponectin receptors in rat testis came from our in vitro analyses, testing the effects of increasing doses of the adipokine on several indices of testis function. As most salient finding, such analyses revealed that adiponectin is able to significantly inhibit basal and hCG-stimulated testosterone secretion by testicular explants. The relevance of this observation is 2-fold: 1) it further documents the ability of a number of metabolic signals, such as leptin, ghrelin, orexin-A, and resistin, to modulate testicular steroidogenesis (33, 44, 49, 59), a phenomenon that is likely to contribute to the functional coupling of metabolic status and reproduction (33); and 2) it demonstrates for the first time the ability of adiponectin to directly regulate male gonadal function. The physiological relevance and mechanisms of the latter are yet to be fully defined. Of note, our preliminary expression analyses in testicular explants did not identify any detectable effect of adiponectin on the relative mRNA levels of key factors of the steroidogenic route, such as steroidogenic acute regulatory protein, P450 side-chain cleavage enzyme, 3β-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase type III (our unpublished data), some of which are targets for the testicular effects of leptin (60). Notwithstanding, the inhibitory effects of adiponectin on basal and stimulated testosterone secretion reported here might contribute to the well-known suppression of testicular function in conditions of persistent negative energy balance (31), when systemic adiponectin levels are known to increase (5). Of note, a decrease in serum total levels of adiponectin has been reported in human males through puberty (61), a phenomenon that may play a permissive role in the pubertal increase of androgen secretion, provided that similar inhibitory effects take place in humans. Interestingly, our recent data demonstrate that adiponectin is also able to suppress basal and GnRH-stimulated LH secretion at the pituitary level (22). Thus, adiponectin could be considered an integral negative modifier of reproductive function in conditions of low adiposity linked to hyperadiponectinemia (5) via its combined actions at central (pituitary) and peripheral (testis) levels. In contrast, adiponectin is not likely to contribute to the hypoandroagenism frequently observed states of obesity and insulin resistance (62, 63) because adiponectin levels are reported to decrease in those conditions (4, 11, 12).

As reported herein for adiponectin, the expression and direct effects of resistin, another adipokine putatively involved in the regulation of glucose homeostasis and insulin sensitivity (5), have been previously documented by our group in the rat testis (44). However, the regulation and testicular actions of resistin and adiponectin appears to be markedly different because testicular expression of resistin was overtly modulated by gonadotropins and fasting in vitro, and it significantly enhanced basal and stimulated testosterone secretion in vitro (44). Of note, antagonistic actions have been reported for resistin and adiponectin in the modulation of insulin sensitivity, and their circulating levels are known to inversely change in different metabolic conditions (5). Likewise, the effects of resistin and adiponectin in terms of control of testosterone secretion appear to be opposite. Moreover, whereas a reciprocal stimulatory loop between androgen and resistin has been described (because androgens enhance adipose expression of resistin, whereas the latter increases testosterone secretion (44)), the opposite seems to be the case for adiponectin because androgens are known to decrease adiponectin expression (39), whereas adiponectin is able to suppress basal and stimulated testosterone secretion (present results). The relevance of the potential cross talk between resistin and adiponectin, not only in metabolic control but also in the regulation of testis function, is yet to be defined.

In summary, we provide herein novel evidence for the expression and potential functional role of adiponectin in the rat testis. Admittedly, some key aspects of adiponectin expression and action in the male gonad remain to be fully disclosed, including, among others: 1) its ontogenetic profile; 2) the relative importance of local vs. systemic adiponectin; 3) the mechanisms for its effects on testosterone secretion; and 4) the possibility of additional regulatory signals and functional roles (e.g. at the seminiferous epithelium). Yet our present observations are the first to disclose the potential role of adiponectin in the direct control of testicular function. From a more general perspective, our data, together with recent findings on the expression and direct actions of adiponectin at the pituitary and ovary (22, 41), substantiate the putative role of this adipokine as a metabolic modulator of the reproductive axis; a function whose physiological relevance (e.g. in coupling of energy reserves, metabolism, and fertility) and eventual pathophysiopathological implications (e.g. in conditions of disturbed adiposity, insulin resistance, and gonadal dysfunction) merit further investigation.

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