0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(3):1108–1111

doi: 10.1210/jc.2005-1865

The Effects of Gonadotropin Suppression and Selective Replacement on Insulin-Like Factor 3 Secretion in Normal Adult Men

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Context: Gonadotropic regulation of the testicular Leydig cell hormone insulin-like factor 3 (INSL3) is incompletely characterized.

Objective: The objective of this study was to assess the effects of gonadotropin suppression and induced or spontaneous recovery on serum INSL3.

Design and Participants: Serum samples from 15 men enrolled in a short-term study of gonadotropin stimulation, suppression, and recovery and 11 men in a long-term study of gonadotropin suppression and spontaneous recovery were analyzed for INSL3.

Intervention: Gonadotropins were suppressed by exogenous testosterone and progestin. Recovery was spontaneous or induced with exogenous gonadotropins.

Outcome Measure: The outcome measure was serum INSL3 in relation to other reproductive hormones.

Results: Serum INSL3 was not acutely sensitive to gonadotropins. In both studies, INSL3 declined markedly with gonadotropin suppression (6–13.5% of baseline; $P < 0.05$). In the short-term study, human chorionic gonadotropin partially restored suppressed serum INSL3 within 4 d of administration (from 7.5 to 38.3% baseline; $P < 0.05$); the increase correlated with the corresponding increase in serum pro-αC ($r = 0.82$; $P < 0.01$). FSH did not stimulate the suppressed INSL3. In the long-term study, serum testosterone recovered significantly better (80% baseline) compared with serum INSL3 (38.9% baseline; $P < 0.01$) in the presence of fully recovered serum LH.

Conclusions: INSL3 is not sensitive to gonadotropin stimulation in normal men, but declines markedly in response to gonadotropin deprivation. After suppression, INSL3 was responsive to hCG 4 d after administration. After long-term suppression, INSL3 did not recover to the same degree as testosterone, suggesting that INSL3 is more sensitive to Leydig cell impairment than testosterone. **(*J Clin Endocrinol Metab* 91: 1108–1111, 2006)**

THE TESTICULAR LEYDIG cell hormone insulin-like factor 3 (INSL3) is essential for testicular descent in prenatal life (1), but is also abundantly expressed in mature postnatal Leydig cells (2), as reflected by high circulating INSL3 concentrations in adult men (3, 4). An endocrine function for INSL3 in adulthood is suggested by the expression of the gene encoding the INSL3 receptor, LGR8, in human pituitary gland, thyroid, and testis, among other tissues (3, 5). The prevention of testicular germ cell apoptosis *in vitro* by INSL3 also suggests a postnatal paracrine function (6).

Postnatally, INSL3 secretion appears to be dependent on the state of differentiation of Leydig cells, which, in turn, is dependent on LH (4). To investigate INSL3 regulation, we report the response of serum INSL3 to gonadotropin withdrawal and selective replacement or spontaneous recovery in normal adult men made experimentally hypogonadotropic using androgen-based hormonal contraceptive treatment. The INSL3 response is also compared with the corresponding response of inhibin B and pro-αC, the partially processed form of inhibin B. Pro-αC is produced in Leydig, Sertoli, and germ cells (7, 8) and is stimulated by FSH and LH (9).

First Published Online January 4, 2006

Abbreviations: DMPA, Depot medroxyprogesterone acetate; hCG, human chorionic gonadotropin; INSL3, insulin-like factor 3; T, testosterone.

Subjects and Methods

Study 1 (short-term suppression and selective replacement)

Serum samples were obtained from 15 healthy men (aged 22–45 yr) involved in a previous study examining gonadotropic regulation of inhibin-related proteins and spermatogenesis (9). The study had three phases: 1) acute gonadotropic stimulation, 2) gonadotropin suppression, and 3) selective gonadotropin replacement (9).

The 15 men (three groups of five men) received a single injection of either recombinant human FSH (1200 IU, sc), human chorionic gonadotropin (hCG; as an LH substitute; 5000 IU, sc), or FSH and hCG. These doses were used to provide acute supraphysiological levels of gonadotropin bioactivity to assess the reserve secretory capacity of Sertoli and Leydig cells. Blood was drawn daily for 8 d beginning the day before injection. After 6 wk or more, all men received 800 mg testosterone (T) implant and 300 mg depot medroxyprogesterone acetate (DMPA). After a 12-wk suppression period, men were rerandomized into three new groups to receive selective replacement with gonadotropin doses as might be used to restore spermatogenesis in hypogonadotropic men: recombinant human FSH (300 IU twice weekly), hCG (5000 IU weekly), or a combination of both. Simultaneously, all men received a second DMPA dose (150 mg) to ensure continued suppression of endogenous gonadotropins, and those receiving only FSH also received an 800-mg T implant to maintain virilization. Blood samples were obtained at wk 0, 6, and 12 of suppression and once or twice weekly throughout the 12-wk recovery period.

Study 2 (long-term suppression and spontaneous recovery)

Serum samples were available from 11 healthy men (aged 25–42 yr) involved in a previous study of male hormone contraceptive efficacy (10) using combined androgen (800-mg T implants every 4–6 months) and progestin (300 mg DMPA, im, every 3 months). The mean interval between the first and last depot hormone treatment was 8.5 months, after which the men were followed for spontaneous recovery of gonadal function. For the present study, blood samples drawn before and 3, 5.5, 11, and 17.5 months after the initial hormone administration. Both studies were approved by the relevant local ethics committees, and informed consent was obtained from all participants.

Assays

Serum INSL3 was measured by an immunoassay (detection limit, 0.05 ng/ml; intra- and interassay coefficients of variation, 8.0% and 11.3%) (see Ref. 4; for other hormone assays used in studies 1 and 2, see Refs. 9 and 10, respectively).

Statistics

Serum INSL3, T, and LH are presented as the mean ± SD or the mean percentage of baseline (±SD). Serum T and INSL3 at one time point and serum INSL3 at different time points were compared in Friedman and Wilcoxon tests. Serum T in response to different stimulations was compared by Kruskal-Wallis test. Trends for INSL3 and T in response to acute stimulation were analyzed in a general linear model. SPSS for Windows version 13.0 (SPSS, Inc., Chicago, IL) was used for calculations and statistical analyses.

Results

Study 1

Acute stimulation. Serum INSL3 did not increase in response to FSH, hCG, or FSH plus hCG; however, hCG resulted in a small but statistically significant ($P < 0.001$) decrease in INSL3 of 1.4% over the 8-d period. Serum T increased significantly in response to hCG and FSH plus hCG, reaching, respectively, 163% and 284% of baseline after 3 d ($P < 0.001$ for both groups). Serum T was significantly higher in the group stimulated with FSH and hCG compared with the group stimulated with hCG on d 2–7 after stimulation ($P < 0.05$ for all time points). FSH alone did not change serum T.

Suppression phase (Fig. 1 and Table 1). Gonadotropin suppression resulted in a decrease in serum INSL3 to 6.4% and 10.0% of baseline after 6 and 12 wk, respectively (both $P < 0.01$).

Selective replacement therapy (Fig. 1 and Table 1). FSH did not increase serum INSL3 at any time point, whereas hCG treatment produced a statistically significant increase in INSL3 within 4 d of administration (7.5% to 38.3% baseline; $P < 0.05$). FSH and hCG resulted in borderline significant increases in serum INSL3 within 4 d of administration (13.5% to 52.8% of baseline; $P = 0.068$). No significant additional increase was observed throughout the 12-wk recovery period in any of the groups. By the end of the recovery period, serum INSL3 was significantly lower than baseline for the group treated with FSH (9.7% baseline; $P < 0.05$). Serum INSL3 and T tended to remain below baseline values for both the hCG-treated group (44.8% and 48.1% of baseline; both $P = 0.068$) and the FSH- plus hCG-treated group (58.8% and 82.8% baseline; both $P = 0.068$).

A significant positive correlation was found between pro-αC and INSL3 in men treated with hCG or hCG plus FSH in response to the first recovery treatment dose ($r = 0.82$; $P < 0.01$ for both groups). No correlations were found between inhibin B and INSL3.

Study 2 (Fig. 2 and Table 2)

Three months after the first hormone administration, serum INSL3 had decreased to 12% of baseline and stayed suppressed to 5–13% of baseline after 5.5 and 11 months. At the final blood sampling, serum INSL3 (38.9% of baseline) and serum T (80.2% of baseline) were both significantly lower than baseline ($P < 0.01$), and final INSL3 was significantly lower than final T ($P < 0.01$). LH levels (109.4% baseline) were not significantly different from baseline.

Discussion

The lack of increase in serum INSL3 in response to acute exposure to supraphysiological levels of LH bioactivity might suggest that adult Leydig cells express maximum INSL3 in the presence of normal serum LH. This is in contrast to the capacity of Leydig cells to increase T secretion in response to exogenous hCG as well as the augmented rise in serum T in response to combined hCG and FSH. These data are in accordance with the previous proposal that T and INSL3 are differentially regulated (4).

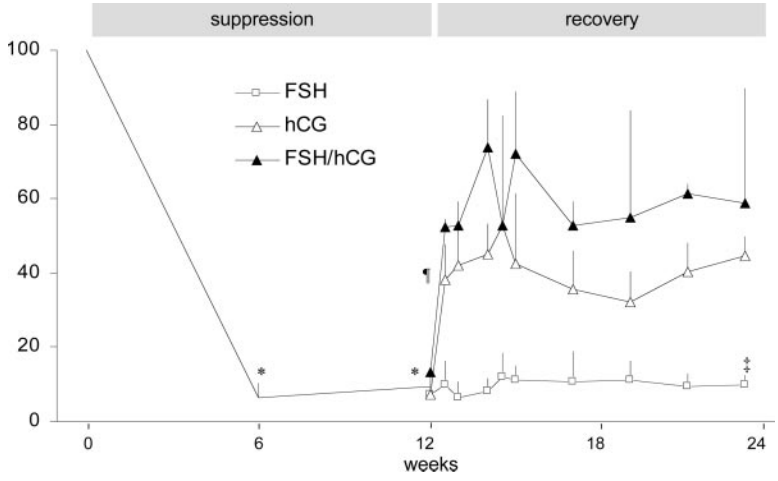

FIG. 1. Changes in serum INSL3 in response to the suppression of endogenous gonadotropins by an androgen-progestin contraceptive regimen (suppression) and subsequent 12 wk of treatment with hCG, FSH, or both FSH and hCG (recovery). Note that INSL3 was significantly lower after 6 and 12 wk of suppression compared with baseline (*, $P < 0.01$). In the group treated with hCG, INSL3 increased significantly 4 d after the first hCG administration (¶, $P < 0.05$). In the group treated with FSH, the final serum INSL3 concentration was significantly lower compared with baseline (‡, $P < 0.05$). Data are shown as the mean percentage of baseline (±SD; n = 5 men/group).

TABLE 1. Short-term study (INSL3 serum levels in response to gonadotropin suppression and selective replacement)

	INSL3 (ng/ml)	INSL3 (ng/ml)		
		FSH (n = 5)	hCG (n = 5)	FSH + hCG (n = 5)
Baseline (n = 15)	1.06 (±0.35)			
Suppression (n = 15)				
6 wk	0.08 (±0.05)[a]			
12 wk	0.11 (±0.06)[a]			
Selective replacement				
0 d		0.07 (±0.07)	0.09 (±0.03)	0.15 (±0.05)
4 d		0.10 (±0.10)	0.43 (±0.13)[b]	0.59 (±0.17)
12 wk (final)		0.09 (±0.06)	0.52 (±0.10)[b]	0.65 (±0.22)

Data are presented as mean (±SD).
[a] $P < 0.01$, INSL3 serum levels significantly lower than baseline.
[b] $P < 0.05$, INSL3 serum levels significantly higher than d 0.

A substantial decline in serum INSL3 was observed 6 wk after gonadotropic suppression. The decrease may have occurred sooner, as suggested by the marked suppression of serum INSL3 in two infertile men 30 d after GnRH analog treatment (3). The extent of INSL3 decline is in line with the decrease observed previously for serum T (11) and intratesticular T (12, 13) in response to gonadotropin suppression, suggesting that T and INSL3 are similarly susceptible to gonadotropin deprivation.

INSL3 has been suggested to be a marker of Leydig cell differentiation (2, 4, 14). Because no histological data are available, we cannot determine whether degeneration of Leydig cells and/or simply the absence of LH are responsible for the decrease in INSL3. However, rat studies report that deprivation of LH results in Leydig cell atrophy/hypotrophy within the first week of gonadotropin deprivation (15).

The initial steep increase in INSL3 in response to hCG after suppression illustrates that when Leydig cells are deprived of LH, INSL3 secretion is indeed acutely sensitive to hCG. Again, the state of Leydig cell differentiation is unknown; however, the acute increase could be attributed to the restoration of Leydig cell function. Alternatively, the Leydig cells may still be fully differentiated, but a threshold might exist below which additional LH will stimulate INSL3 secretion, and above which no additional INSL3 is released. The fact that neither INSL3 nor T recovered fully within the 12-wk recovery period may also be interpreted as some degree of Leydig cell dysfunction subsequent to suppression or, alternatively, that administered hCG does not stimulate Leydig cells to the same extent as natural pulsatile LH secretion (16).

We found a strong correlation between the increases in pro-αC and INSL3 in response to hCG immediately after the 12-wk suppression period. In normal men, serum pro-αC, in contrast to INSL3, responded to acute hCG stimulation with a rapid increase (9), suggesting that Leydig cell secretion of INSL3 and pro-αC is differentially regulated by hCG, yet responds similarly to hCG after LH deprivation.

In the long-term study, the failure of INSL3 to recover to the same degree as T in the presence of normalized pulsatile LH illustrates that after long-term suppression of Leydig cells, T and INSL3 indeed responded differently to LH and suggest that INSL3 is more sensitive than T to impaired Leydig cell function. However, it cannot be excluded that some other (testicular) factor, also affected by gonadotropin deprivation, is involved in INSL3 regulation.

Our data raise the question of whether FSH plays a minor role in promoting baseline INSL3 secretion. The subtle decrease in serum INSL3 observed when hCG alone was administered, not when FSH was coadministered, might suggest that the 70% suppression of FSH associated with the hCG-induced rise in serum T (data not shown) may have led to a fall in INSL3 secretion by mature Leydig cells. Furthermore, although not significant, a stronger increase was observed for INSL3 in response to recovery treatment with both FSH and hCG compared with hCG alone. FSH alone, before or after gonadotropin suppression, was, however, not capable of stimulating INSL3, suggesting that if FSH has any effect on INSL3, this effect is minor, probably indirect, and observed only in combination with LH.

Notably, FSH replacement was, to some degree, capable of restoring spermatogenesis (9) in the absence of rising serum INSL3. This is in line with findings in mouse INSL3 knockout models; although fertility may be affected, spermatogenesis

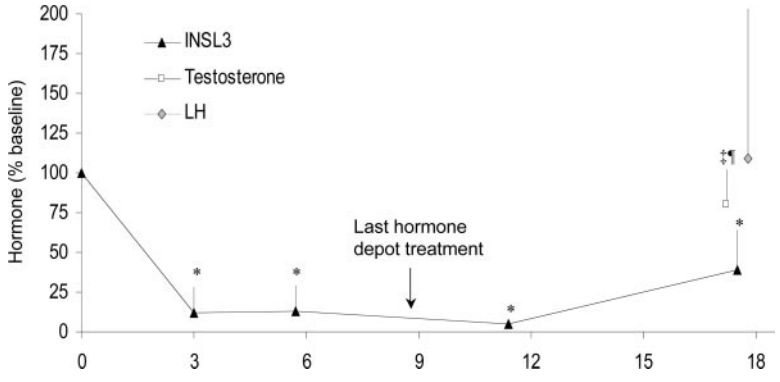

FIG. 2. Changes in serum INSL3 in response to long-term suppression of gonadotropins (T implants every 4–6 months and DMPA every 3 months) and subsequent spontaneous recovery, expressed as a percentage of baseline. Note that serum INSL3 was significantly reduced at all time points compared with baseline (*, $P < 0.01$). Serum T and LH, expressed as a percentage of baseline, are shown after 17.5 months (9 months since the last hormone depot administration). Note that serum T was significantly lower compared with baseline (‡, $P < 0.01$), but was significantly higher compared with the percentage of baseline of serum INSL3 at the same time point (¶, $P < 0.01$). LH levels were not significantly different from baseline levels. Data are shown as the mean percentage of baseline (±SD; n = 11 men).

TABLE 2. Long-term study (serum levels of INSL3, T, and LH at baseline and after recovery after long-term suppression of gonadotropins)

	INSL3 (ng/ml)	T (nmol/liter)	LH (U/liter)
Baseline	1.75 (±0.59)	17.9 (±8.1)	3.5 (±1.5)
Final (17.5 months)	0.70 (±0.49)[a]	14.3 (±7.0)[a]	3.7 (±2.7)

Hormone values are presented as mean (±SD). Last DMPA/T administration 8.5 months after onset of suppression.

[a] $P < 0.01$. After a total of 17.5 months (9 months after the last DMPA/T administration), both INSL3 and T were significantly lower as compared to baseline, whereas LH was not significantly different from baseline LH.

can occur in the absence of INSL3 (1, 17). However, the reporting of LGR8 gene expression in adult rat germ cells and a role for INSL3 in germ cell survival (6) indicates that INSL3 may indeed serve a function in spermatogenesis, although this may not be essential.

In conclusion, we have confirmed that serum INSL3 in normal men is not acutely sensitive to exogenous FSH or LH bioactivity (hCG). Suppression of endogenous gonadotropins resulted in a marked decline in serum INSL3 within 6 wk, after which INSL3 is acutely sensitive to LH action. Subsequent to long-term gonadotropin suppression, INSL3 does not recover to the same degree as T, suggesting that INSL3 may be more sensitive than T to impaired Leydig cell function.

Acknowledgments

Received August 17, 2005. Accepted December 27, 2005.

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This work was supported by the European Union (Contract QLK4-CT-2002-00603, EDEN project), the Danish Research Council (Grant 2107-04-0006), the Australian National Health and Medical Research Council (Program Grant 241000), and Postgraduate Scholarship (Grant ID-241031; to K.L.M.).

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