Deficiency of the insulin-like growth factors signaling disturbs the androgen phenotype but increases aromatase activity in mouse Leydig cells

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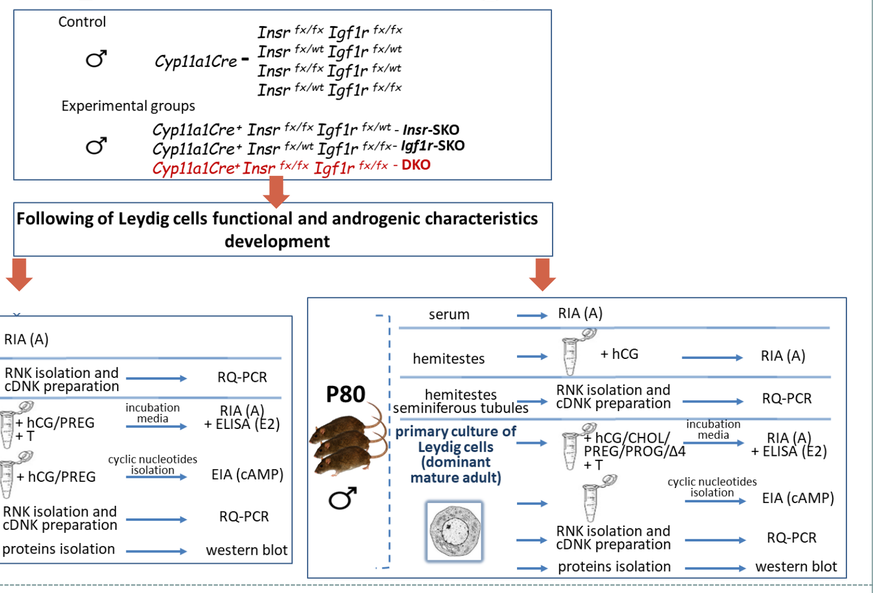
Abstract

INTRODUCTION: A growing body of evidence have pointed correlation between insulin-resistance, testosterone level and infertility, but there is scarce information about mechanisms. **OBJECTIVES:** The aim of this study was to identify the possible mechanism linking the insulin-resistance with testosterone-producing Leydig-cells functionality. **METHODS/DESIGN:** *In vivo* and *in vitro* approaches were applied . The *in vivo* model of functional genomics is represented by insulin-resistant-testosterone-producing Leydig cells obtained from the prepubertal (P21) and adult (P80) male mice with insulin and IGF1 receptors deletion in steroidogenic cells *(Insr/Igf1r-DKO).* The *in vitro* model of insulin-resistant-cell was mimicked by blockade of insulin/IGF1 receptors on primary culture of P21 and P80 Leydig cells. **RESULTS:** Leydig-cell-specific-insulin-resistance causes the loss of androgen phenotype, but induce the development of estrogenic characteristics of progenitor Leydig cells in prepubertal mice and mature Leydig cells in adult mice. Level of androgens in serum, testes and Leydig cells decrease as a consequence of the dramatic reduction of steroidogenic capacity and activity as well as all functional markers of Leydig cell. Oppositely, the markers for female-steroidogenic-cell differentiation and function increase. The physiological significance is the higher level of estradiol in double knock-out mice of both ages. Intriguingly, the transcription of pro-male sexual differentiation markers *Sry* and *Sox9* increased in P21-Leydig-cells, questioning the current view about the antagonistic genetic programs underlying gonadal sex determination. **CONCLUSIONS:** The results provide new molecular mechanisms leading to the development of the female phenotype in Leydig cells from *Insr/Igf1r-DKO* mice and could help to better understand the correlation between insulin resistance, testosterone and male (in)fertility.

KEYWORDS: estradiol, feminization, insulin/IGF1 receptors, Leydig cell, testosterone

Experimental design



Results

INSR/IGF1R deficiency alters development of the seminiferous tubules and interstitium of the testes from P21 mice

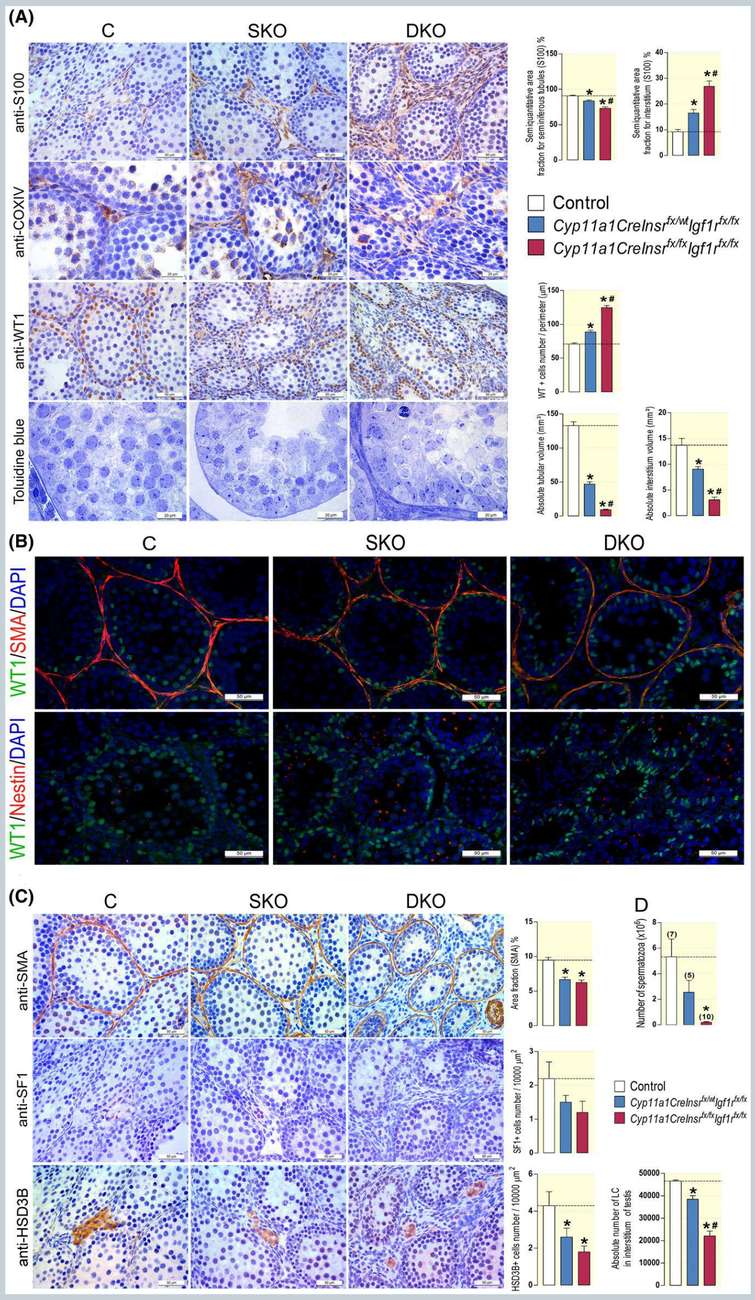


FIGURE 1. Deletions of *Insr* and *Igf1r* in steroidogenic cells alter the development of seminiferous tubules and interstitium of testes from prepubertal/P21 mice, leading to the decrease in number of Leydig cells in prepubertal/P21 as well as in the number of spermatozoa in adult/P80 mice.

(A)Histological analysis showed decreased seminiferous tubules fraction, increased interstitial fraction and number of WT1+ cells in both single knock-out (SKO) and double knock-out (DKO) mice. Besides, absolute tubular and interstitium volumes significantly decreased in both SKO and DKO mice and these effects were more prominent in DKO mice.
(B) Immunostaining for SMA and nestin.
(C)Histological analysis showed decreased SMA and HSD3B staining in both SKO and DKO mice, as well as decreased absolute number of Leydig cells in interstitium of the testes.
(D)The number of spermatozoa significantly decreased in DKO adult/P80 mice, probably as a consequence of apoptosis of spermatogonia seeing in the testes of P21 mice.
Bars represent group mean ± SEM values from three independent experiments. p<0.05: *vs. control, # vs.

INSR/IGF1R deficiency impairs testicular steroidogenesis and affects functional characteristics of Leydig cells from P21 and P80 mice

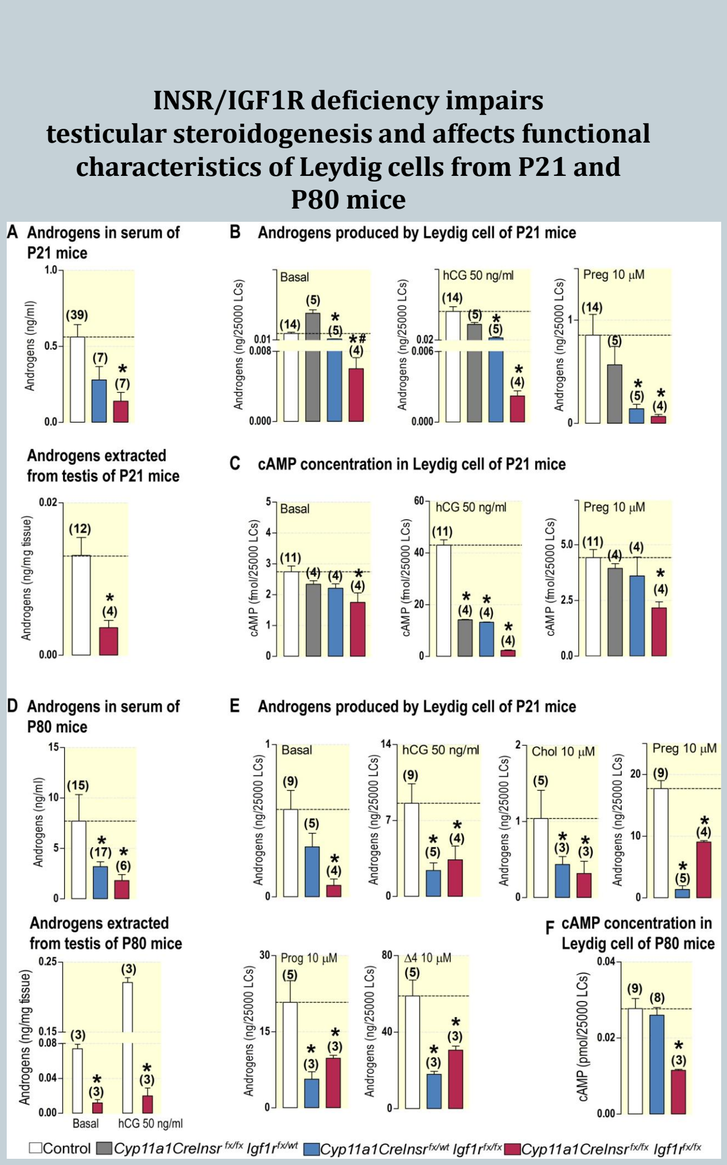


FIGURE 2. Deletion of *Insr* and *Igf1r* decreases serum and testicular androgens, steroidogenic capacity and activity, as well as cAMP content in Leydig cells of prepubertal and adult mice.

(A) Androgens level in serum and testes of P21 and (D) P80 mice. (B) Steroidogenic capacity (hCG-response) and HSD3B-activity (pregnenolone-response) of Leydig cells from P21 mice. (C) cAMP concentration in Leydig cells of P21 and (F) P80 mice. (E) Steroidogenic capacity and activity of Leydig cells from P80 mice.
Bars represent mean ± SEM (n = number of mice). P < .05: *vs. control; #vs. *Igf1r*-SKO

INSR/IGF1R deficiency increases transcripts of the main steroidogenic inhibitors (*Arr19*, *Dax1*), specifically in Leydig cells, but not in seminiferous tubules from P21 and P80 double knock-out (DKO) mice

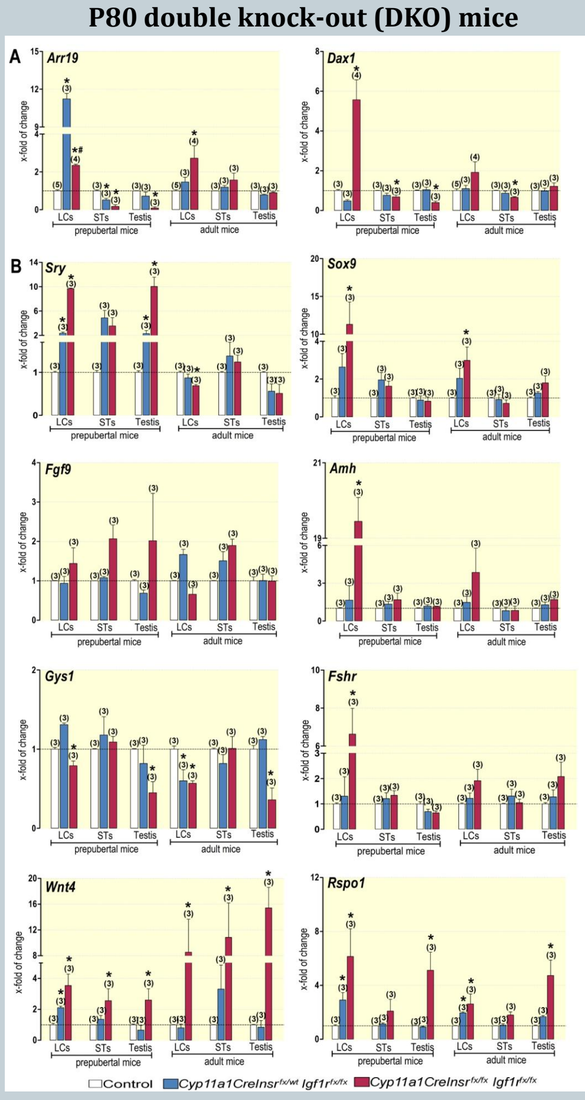


FIGURE 3. Deletion of *Insr* and *Igf1r* oppositely regulate the transcriptional pattern of the main steroidogenesis related repressors (*Arr19 and Dax1)* (A), but increases both pro-male and pro-female markers in Leydig cells and seminiferous tubules (B) of prepubertal and adult mice.
Bars represent mean ± SEM (n = number of mice). P < .05: *vs. control

INSR/IGF1R deficiency increases aromatase expression and activity in Leydig cells from P21 and P80 mice

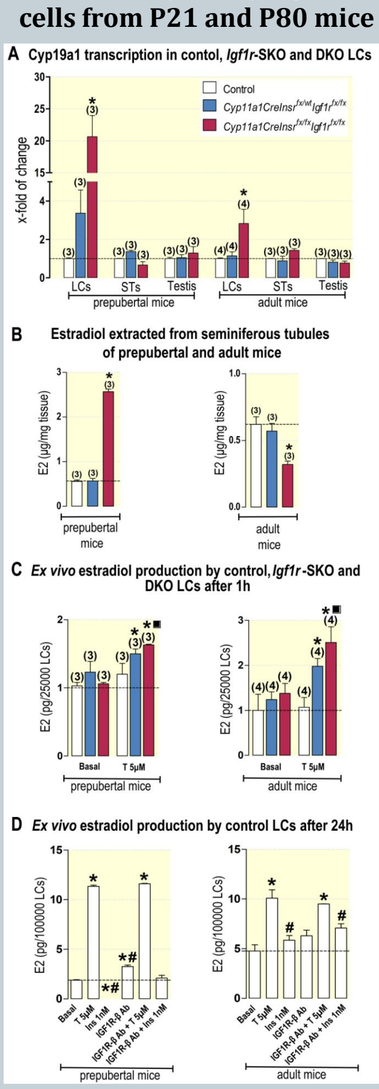


FIGURE 4. *In vivo* deletion of *Insr* and *Igf1r* increases *Cyp19a1*/aromatase transcription and activity (estradiol production) in Leydig cells of prepubertal and adult mice, while *ex vivo* manipulation with INSR/IGF1R and testosterone (T) mimicked some of the effects of *in vivo*-INSR/IGF1R deletion.

(A) Transcription pattern of *Cyp19a1* in the Leydig cells, seminiferous tubules and testes of P21 mice and P80 mice.
(B)Aromatase activity measured using estradiol (E2) produced in medium by Leydig cells from P21 and P80 mice.
(C)The level/content of estradiol (E2) extracted from seminiferous tubules of P21 and P80 mice.
(D) *Ex vivo* E2 production by Leydig cells from control mice in absence (Basal) or presence testosterone (T) or insulin (Ins) or antibody against IGF1R-β (IGF1R-β-Ab) or combinations (IGF1R-β-Ab + T, IGF1R-β-Ab + Ins).
Bars represent mean ± SEM (n = number of mice). P < .05: *vs. control mice *(*A, B – *in vivo),* or Basal-group (C); ■vs. corresponding Basal E2-production in particular group of mice (B – *in vivo*); # vs. T-treated-group (C).

Conclusions

FIGURE 5. Deletion of *Insr* and *Igf1r* changes the transcriptional pattern of the steroidogenic machinery elements and the main male/female sex markers in favor of the female-steroidogenic-cells-characteristics leading to "feminization" of Leydig cells from prepubertal and adult mice.

(A) Decreased steroidogenesis-related-activator (*Sf1)* and increased steroidogenesis-related-repressors *(Dax1 and Arr19),* together with *Esr2* could be the candidates that mediate the effect of INSR/IGF1R. Green arrows (↑) indicate increased, red arrows reduced and signs of equality (=) unchanged expression. Red rectangles (□) denote decreased and green increased transcription of the candidates regulating genes coding steroidogenic enzymes. (B) The deletion of *Insr* and *Igf1r* changes the transcription pattern of the markers of male/female sex-development. (C) The absence of INSR and IGF1R increases transcription/activity of *Cyp19a1*/aromatase, transcription of *Fshr* and the molecular markers of female sex development (*Wnt4, Rspo1),* dramatically increases steroidogenesis-related-repressors-*Dax1/Arr19* and important regulators of male sex differentiation *(Sry, Sox9),* while decreases steroidogenesis-related-activator-*Sf1.* (D) Possible molecular mechanisms leading to the development of female phenotype in Leydig cells from *Insr/Igf1r*-DKO mice.

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