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Reproductive experience promotes permanent body growth independently of growth hormone

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eLife Assessment

This **important** study reports a novel phenomenon of maternal growth during pregnancy that is independent of growth hormone (GH), adding a new dimension to maternal biology of reproduction. The evidence is **convincing** and supported by state-of-the-art methodologies conducted in mice and persuasive observations in humans with hereditary isolated GH deficiency. Revised discussion should focus on possible mechanisms, including the role of IGF2, and on directions for future research.

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Abstract

Pregnancy leads to many adaptations in the maternal body, most of which are reversible. However, reproductive experience can also result in permanent effects. Here, we investigated how pregnancy influences the somatotrophic system and the lasting effects of reproductive experience on the maternal organism. Reproductive experience induced a pronounced increase in lean body mass and longitudinal growth in both wild-type and growth hormone (GH)-deficient mice compared with age-matched virgins. Body growth was primarily observed during the first pregnancy, whereas a second gestation was mostly associated with increased adiposity. Data from a cohort of women with isolated GH deficiency (IGHD) caused by a loss-of-function mutation in the GHRHR gene revealed that nulliparous women were 7 cm shorter than those with one or more pregnancies. Increased GH secretion was observed in pregnant wild-type mice but not in pregnant GHRHR-deficient mice. Pregnancy-induced body growth is preserved despite disruption of GH-, ghrelin-, and estrogen-related signaling pathways. In conclusion, reproductive experience induces permanent changes in the maternal organism, promoting body growth in models that allow this response. Pregnancy-induced body growth appears to be independent of GH action. These findings underscore the need for further studies to investigate the long-lasting consequences of reproductive experience in females.

Introduction

Pregnancy is a unique state in which numerous physiological adaptations are necessary in the maternal organism to allow proper fetal growth and to prepare for the lactation period (Augustine et al., 2008). Among the diverse maternal adaptations, metabolic changes are particularly prominent, including alterations in appetite and glucose homeostasis, as well as increased

adiposity (Augustine et al., 2008; Ladyman et al., 2010; Zampieri et al., 2015; Teixeira et al., 2019a). Pregnancy also affects neuroplasticity and causes behavioral and emotional alterations (Larsen and Grattan, 2012; Georgescu et al., 2021). Most of these changes are reversible. However, pregnancy may lead to long-term or permanent consequences. For example, prior pregnancy experience reduces the latency to display maternal behavior in rats (Bridges, 1978), indicating long-lasting behavioral adaptations strongly shaped by prior hormonal exposure. Primiparous mice show increased body weight and reduced ambulatory activity compared with age-matched virgin animals, even several months after last contact with the pups (Ladyman et al., 2018; Teixeira et al., 2019b; Ladyman et al., 2021). Body weight retention is frequently observed after pregnancy, especially for women who experienced excessive gestational weight gain (Scholl et al., 1995; Rooney et al., 2005). Thus, pregnancy may represent a risk factor for obesity in women (Amorim et al., 2007).

Pregnancy-induced adaptations are thought to be largely driven by the marked rise in circulating hormones during gestation (Augustine et al., 2008), including progesterone and estrogens, as well as placentally derived analogues of pituitary hormones, such as chorionic gonadotropin and placental lactogens (also referred to as chorionic somatomammotropins). However, important differences are observed between humans and rodents. Humans, but not rodents, possess the *GH2* gene, which encodes the placental growth hormone (GH) variant, whose production progressively replaces pituitary GH (derived from the *GH1* gene) secretion during pregnancy (Liao et al., 2018). In contrast, rodents maintain pituitary GH secretion throughout gestation (Gatford et al., 2017; Liao et al., 2018). Additionally, human GH, both pituitary and placental variants, as well as chorionic somatomammotropins, can activate both the GH receptor (GHR) and the prolactin receptor (PrlR), explaining their lactogenic effects. In contrast, murine GH activates only the GHR (Bartke and Kopchick, 2015).

Despite species-specific differences, it is evident that hormonal exposure during pregnancy not only transiently shapes maternal physiology but may also exert lasting effects on health across the lifespan. However, the chronic consequences of reproductive experience remain poorly understood. Given that most women will experience at least one pregnancy during their lifetime, elucidating how reproductive experience influences long-term physiology and health is of substantial biological and clinical importance. Furthermore, historically, females have been underrepresented in studies (Clayton and Collins, 2014; Morselli et al., 2016; Lee, 2018). Therefore, the goal of this study is to explore how pregnancy impacts the somatotrophic system and to assess the lasting effects on the maternal organism.

Results

Pregnancy promotes permanent body growth in wild-type and GH-deficient mice

To investigate the consequences of reproductive experience, body weight and composition of C57BL/6J wild-type (WT) mice were initially measured before mating. Pregnant mice gave birth in individual cages and were maintained with their litters for 3 weeks. Two weeks after weaning (the recovery period from lactation), the females were reevaluated to determine changes relative to baseline. The control group consisted of age-matched virgin females. These assessments were also carried out in *Ghrhr*$^{lit/lit}$ mice, which carry a null mutation in the gene encoding the GHRH receptor (GHRHR) and are therefore GH-deficient and dwarf. Reproductive experience caused a robust increase in body mass (Figure 1A) and lean mass (Figure 1B) in both control and *Ghrhr*$^{lit/lit}$ mice compared with virgin groups. No changes in fat mass were observed between groups (Figure 1C). A new evaluation was performed 2 weeks after the second pregnancy and lactation cycle. Body length measurements revealed a significant increase in multiparous WT and *Ghrhr*$^{lit/lit}$ mice compared with age-matched virgin animals (Figure 1D-E). In addition, multiparous mice showed higher body and lean mass than virgin animals (Figure 1F-G). Despite pregnancy-induced growth, *Ghrhr*$^{lit/lit}$ mice remained smaller than WT females (Figure 1D-G). Fat mass increased after 2 pregnancies in both WT and *Ghrhr*$^{lit/lit}$mice compared with their

respective age-matched virgin groups (Figure 1H). Multiparous WT and *Ghrhr*$^{lit/lit}$ mice also had increased liver (Figure 1I) and kidney (data not shown) masses compared with virgin groups. Heart and brain masses increased only in multiparous *Ghrhr*$^{lit/lit}$ mice compared with virgin *Ghrhr*$^{lit/lit}$ females, whereas these tissues did not change in WT mice (data not shown). Thus, pregnancy promotes body growth in WT and GH-deficient females.

Growth is primarily induced during the first pregnancy

The following experiment tested whether a second pregnancy could also increase parameters indicative of body growth. Unlike the body weight and lean mass gains observed after the first pregnancy, WT mice subjected to a second pregnancy cycle showed no increases in body or lean mass compared with age-matched WT females (Figure 2A-B). Because females undergoing a second pregnancy are inherently older than during their first gestation, we assessed whether a first pregnancy occurring at a comparable age to the second would influence body weight. WT females that had a late first pregnancy (age-matched first pregnancy group) showed increased body and lean mass gain compared with age-matched virgin and second pregnancy groups (Figure 2A-B). Notably, the gains in body and lean mass induced by the first pregnancy were similar among the youngest and oldest females compared with their respective age-matched groups of virgin females (Figure 2A-B). No differences in fat mass gain were observed between the groups (data not shown). In contrast to WT mice, the second pregnancy resulted in a significant increase in body and lean mass in *Ghrhr*$^{lit/lit}$ females compared with age-matched virgin animals (Figure 2C-D). However, the magnitude of the increase in the second pregnancy was significantly lower than in the first (Figure 2C-D), highlighting that growth occurs primarily during the first pregnancy.

Case reports of Itabaianinha women with isolated GH deficiency suggest post-pregnancy growth

Growth plate physiology differs between humans and rodents: growth plates remain open after sexual maturation in rodents, whereas they fuse at the end of puberty in humans, thereby terminating longitudinal bone growth (Emons et al., 2011). Although pregnancy-induced longitudinal growth is not observed in normal adult women, we speculate that a slight pregnancy-induced growth may happen in certain special cases. To provide proof-of-concept evidence that gestational factors permanently influence the maternal organism in humans, we studied a cohort of individuals with severe isolated GH deficiency (IGHD) caused by a homozygous loss-of-function mutation (c.57+1G→A) in the GHRHR gene. These individuals are from the Brazilian city of Itabaianinha (Salvatori et al., 1999; Aguiar-Oliveira and Salvatori, 2021), and their mutation replicates effects seen in *Ghrhr*$^{lit/lit}$ mice (Aguiar-Oliveira and Bartke, 2019).

Using data from this rare cohort, we compared the height of 8 nulliparous patients and 9 patients with one or more pregnancies, both groups with IGHD without lifetime GH replacement therapy. The average number of children per woman was 1.8 children (Aguiar-Oliveira and Salvatori, 2021). Nulliparous women were 7 cm shorter than those with one or more pregnancies, 1.13 ± 0.04 m vs 1.20 ± 0.05 m (P = 0.009; Figure 3A). Moreover, the three shortest women, with heights of 1.07, 1.08, and 1.11 m, were nulliparous. Anecdotal reports from 6 IGHD cases also suggest residual growth in 4 women after pregnancy. This information was obtained through face-to-face personal interviews with one of the researchers (M.H. A.-O.). Patient 1 reported clear and consistent signs of somatic growth following two pregnancies. She is married to an IGHD individual, and both of her sons (currently 30 and 33 years old) are affected, as expected for this genetic background. According to documented measurements, her height increased from 1.16 m before pregnancy to 1.20 m after her two pregnancies. She also reported an increase in shoe size from 25 to 28 (Brazilian size) and changes in ring size, as a ring previously worn on the fourth finger no longer fit after childbirth. Together, these findings are consistent with clinically perceptible post-pregnancy somatic growth.

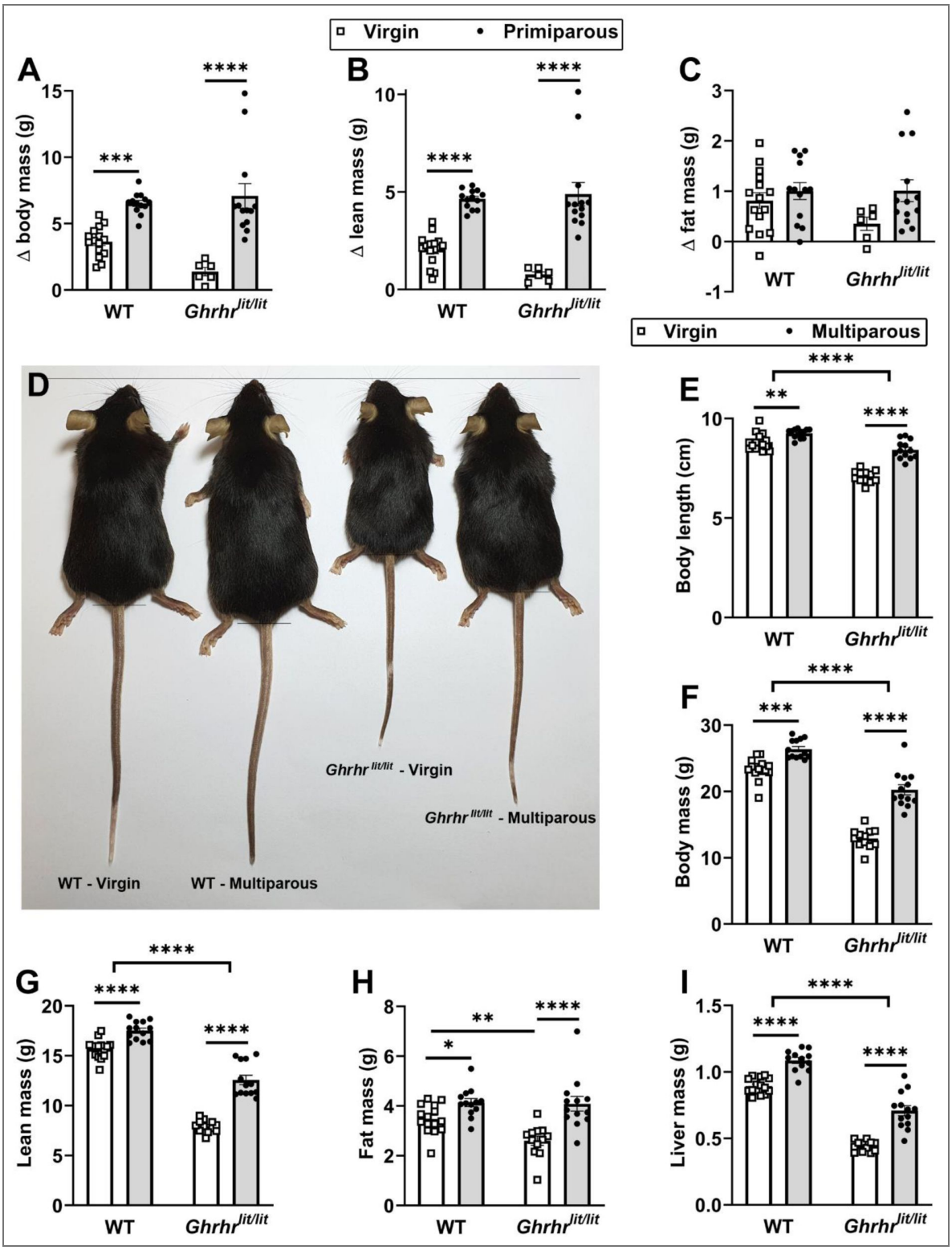

Figure 1. Pregnancy promotes permanent body growth in wild-type and GH-deficient mice.

(A-C) Differences relative to baseline in body, lean, and fat mass in virgin WT (n = 15), primiparous wild-type (WT; n = 13), virgin *Ghrhr*$^{lit/lit}$ (n = 6), and primiparous *Ghrhr*$^{lit/lit}$(n = 13). (D) Representative image showing differences in longitudinal growth between virgin and multiparous WT and *Ghrhr*$^{lit/lit}$ mice. (E-I) Body length, body weight, lean mass, fat mass, and liver mass in multiparous WT (n = 13) and *Ghrhr*$^{lit/lit}$ (n = 13) mice, relative to age-matched virgin WT (n = 15) and *Ghrhr*$^{lit/lit}$(n = 12) mice. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Statistical analysis was performed using two-way ANOVA and Holm-Sidak's multiple comparisons test.

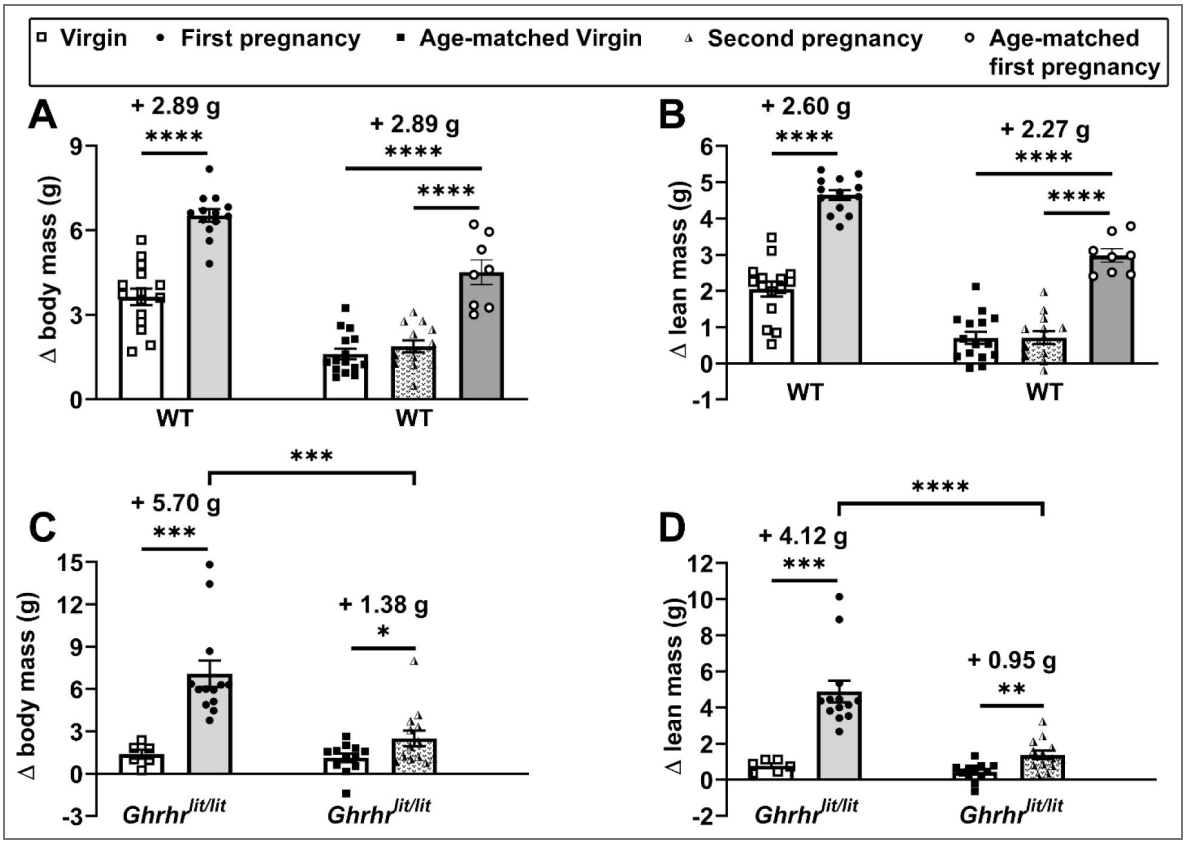

Figure 2. **Growth is primarily induced during the first pregnancy.**

(A-B) Changes in body weight and lean mass in wild-type (WT) virgins (n = 15), after the first pregnancy (n = 13), in virgins matched for age at the second pregnancy (n = 15), after the second pregnancy (n = 13), and after a late first pregnancy (n = 8). (C-D) Changes in body weight and lean mass in *Ghrhr*$^{lit/lit}$ virgins (n = 6), after the first pregnancy (n = 13), in virgins matched for age at the second pregnancy (n = 12), and after the second pregnancy (n = 13). *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Statistical analysis was performed using unpaired two-tailed Student's t-test or one-way ANOVA and Newman-Keuls multiple comparisons test.

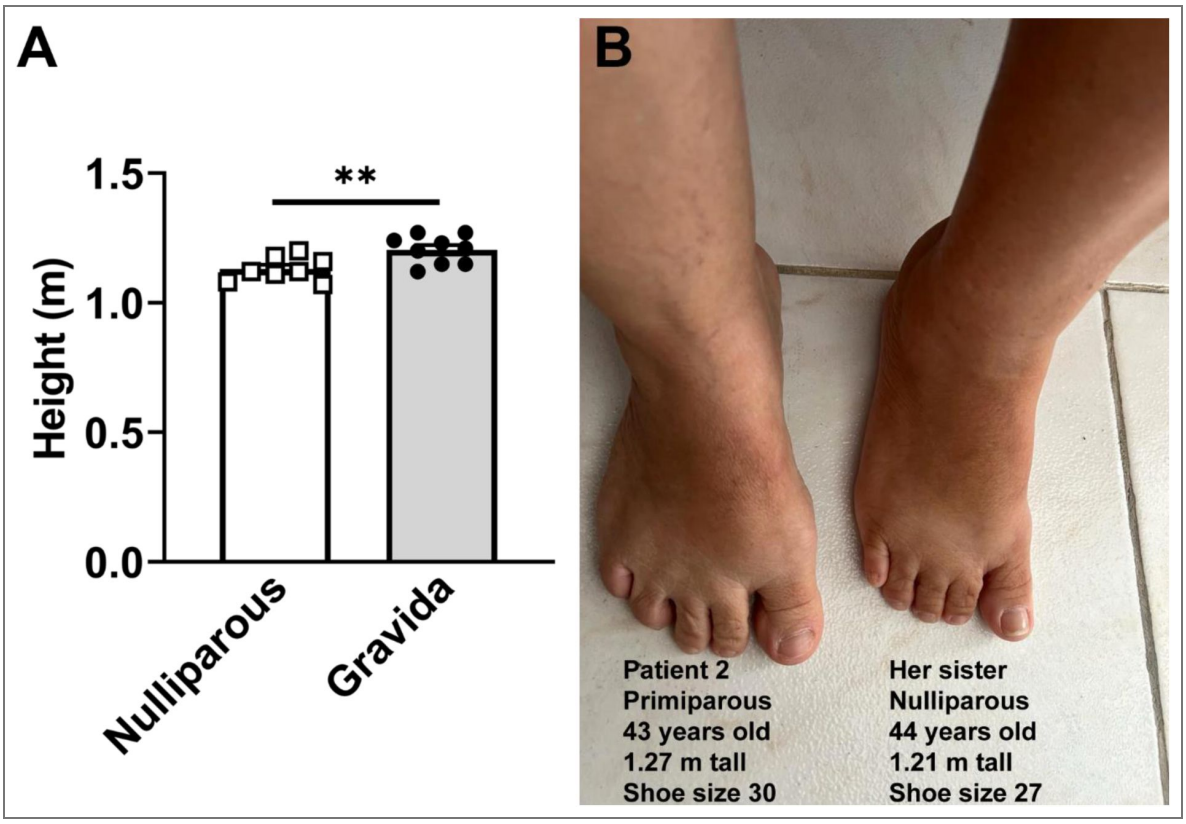

Figure 3. Evidence of post-pregnancy growth in women with isolated GH deficiency.

(A) In a cohort of women with isolated GH deficiency caused by a loss-of-function mutation in the GHRHR gene, nulliparous women (n = 8) were shorter than those with one or more pregnancies (n = 9). **, P = 0.009 (unpaired two-tailed Student's t-test). (B) Comparison of foot size between two sisters with IGHD, one nulliparous and another primiparous. Image of the feet of two sisters with severe isolated GH deficiency caused by a homozygous loss-of-function mutation in the GHRHR gene. In addition to a larger shoe size, patient 2, who had a heterozygous son 9 years ago, is also taller than her nulliparous sister.

Three additional women with IGHD also reported body changes suggestive of growth after pregnancy, though less noticeable than those of patient 1. Patient 2 (43 years old) had a documented increase in height from 1.26 m before pregnancy to 1.27 m at the present. She has one heterozygous son (8.8 years old). She reported an increase in shoe size from 29 to 30, and her graduation ring no longer fits; however, she also gained weight, which may have contributed to the ring size changes. Her feet are larger than her sister's; she is 44 years old, 1.21 m tall, shoe size 27, and nulliparous (Figure 3B). Patient 3, treated with GH in childhood and early adolescence, was 1.44 m tall before pregnancy. She had a heterozygous boy 13 years ago; now she is 36 years old and 1.47 m tall. Patient 4, currently 28 years old, was treated with GH in childhood and early adolescence. She had a heterozygous girl 3 years ago. Her current height is 1.42 m, but before pregnancy, she was 1.40 m tall. In contrast, 2 sisters with IGHD reported no signs of pregnancy-induced growth. Patient 5, 1.24 m tall, aged 56, has a daughter, aged 35, and two sons, aged 30 and 29, each heterozygous. Patient 6, 1.27 m tall, aged 68, has two heterozygous daughters, aged 46 and 44, respectively. However, no information about their heights before pregnancy is available. Despite these two cases, our data indicate a slight post-pregnancy growth in women with severe IGHD.

Increased GH secretion in pregnant WT mice but not in *Ghrhr*$^{lit/lit}$ females

Because GH is the major circulating factor that induces longitudinal growth in mammals (Zhou et al., 1997; Dehkhoda et al., 2018), we investigated whether WT and *Ghrhr*$^{lit/lit}$ mice exhibit alterations in GH secretion during pregnancy (gestational age 14-17 days). Confirming previous studies (Gatford et al., 2017; Kaur et al., 2020; Wasinski et al., 2022), pregnancy increased GH secretion in WT mice compared with virgin animals (Figure 4A, C). This increase was associated with higher GH pulse frequency and elevations in both pulsatile and basal secretion (Figure 4C-G). The contribution of basal to total GH secretion increased in pregnant WT mice (Figure 4H). As expected, non-pregnant *Ghrhr*$^{lit/lit}$ mice showed blunted GH secretion (Figure 4B). Importantly, pregnancy did not stimulate GH secretion in *Ghrhr*$^{lit/lit}$ mice (Figure 4C-H), indicating that GHRH signaling is required for pregnancy-induced increases in GH secretion in mice.

Pregnancy differentially modulates hepatic GHR signaling in WT and *Ghrhr*$^{lit/lit}$ mice

GH action in the liver plays a key role in controlling growth because GHR signaling stimulates hepatic insulin-like growth factor 1 (IGF-1) secretion, particularly by activating the signal transducer and activator of transcription 5b (STAT5b) pathway (Udy et al., 1997; Teglund et al., 1998; Grimley et al., 1999). GH action in the liver is not only controlled by the pattern of GH secretion but also by hepatic GH sensitivity (Touvier et al., 2009; Yamamoto et al., 2013; de Sousa et al., 2025b). A reduced expression of STAT5b protein was observed in the liver of pregnant WT and *Ghrhr*$^{lit/lit}$mice (Figure 5A,C). Phosphorylation of STAT5 (pSTAT5) was also reduced in the liver of pregnant WT compared to virgin animals (Figure 5B-C). Hepatic pSTAT5 was reduced in *Ghrhr*$^{lit/lit}$mice, compared to virgin WT, and did not change in pregnant animals (Figure 5B-C). Serum IGF-1 and hepatic *Igf1* mRNA levels were reduced in pregnant WT mice compared with virgin animals (Figure 5D-E). In contrast, pregnancy increased serum IGF-1 and hepatic *Igf1* mRNA levels in *Ghrhr*$^{lit/lit}$mice (Figure 5D-E).

Corroborating the results of the protein expression, pregnancy reduced *Stat5b* mRNA levels in both WT and *Ghrhr*$^{lit/lit}$ mice (Figure 5F). Conversely, hepatic *Ghr* and *Prlr* mRNA levels increased during pregnancy in WT and *Ghrhr*$^{lit/lit}$ mice (Figure 5G-H). *Esr1* mRNA expression was reduced in *Ghrhr*$^{lit/lit}$ mice and was not affected by pregnancy (Figure 5I). Hepatic *Socs2* and *Cish* RNA levels, transcripts that are indicative of GH action and regulate GH sensitivity

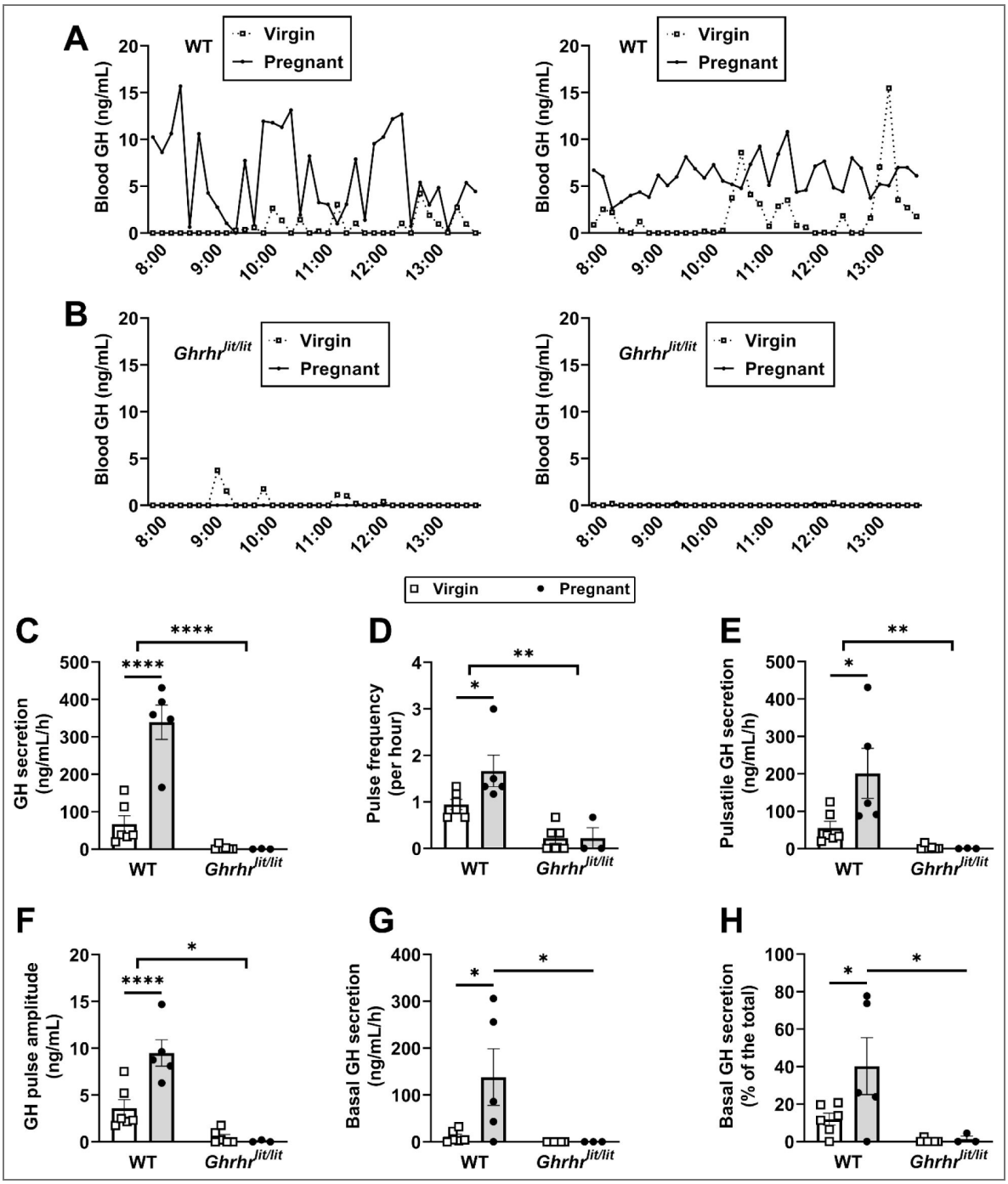

Figure 4. Increased GH secretion in pregnant WT mice but not in pregnant *Ghrhr*$^{lit/lit}$ mice.

(A-B) Representative examples of the pattern of GH secretion in two pregnant and virgin WT mice and in two pregnant and virgin *Ghrhr*$^{lit/lit}$ mice. (C-H). Total GH secretion, GH pulse frequency, pulsatile GH secretion, GH pulse amplitude, basal (non-pulsatile) GH secretion, and contribution of basal secretion to total GH secretion in virgin WT (n = 6), pregnant WT (n = 5), virgin *Ghrhr*$^{lit/lit}$ (n = 6), and pregnant *Ghrhr*$^{lit/lit}$ (n = 3) mice. The pregnant mice were at gestational ages 14-17 days. *, P < 0.05; **, P < 0.01; ****, P < 0.0001. Statistical analysis was performed using two-way ANOVA and Holm-Sidak's multiple comparisons test.

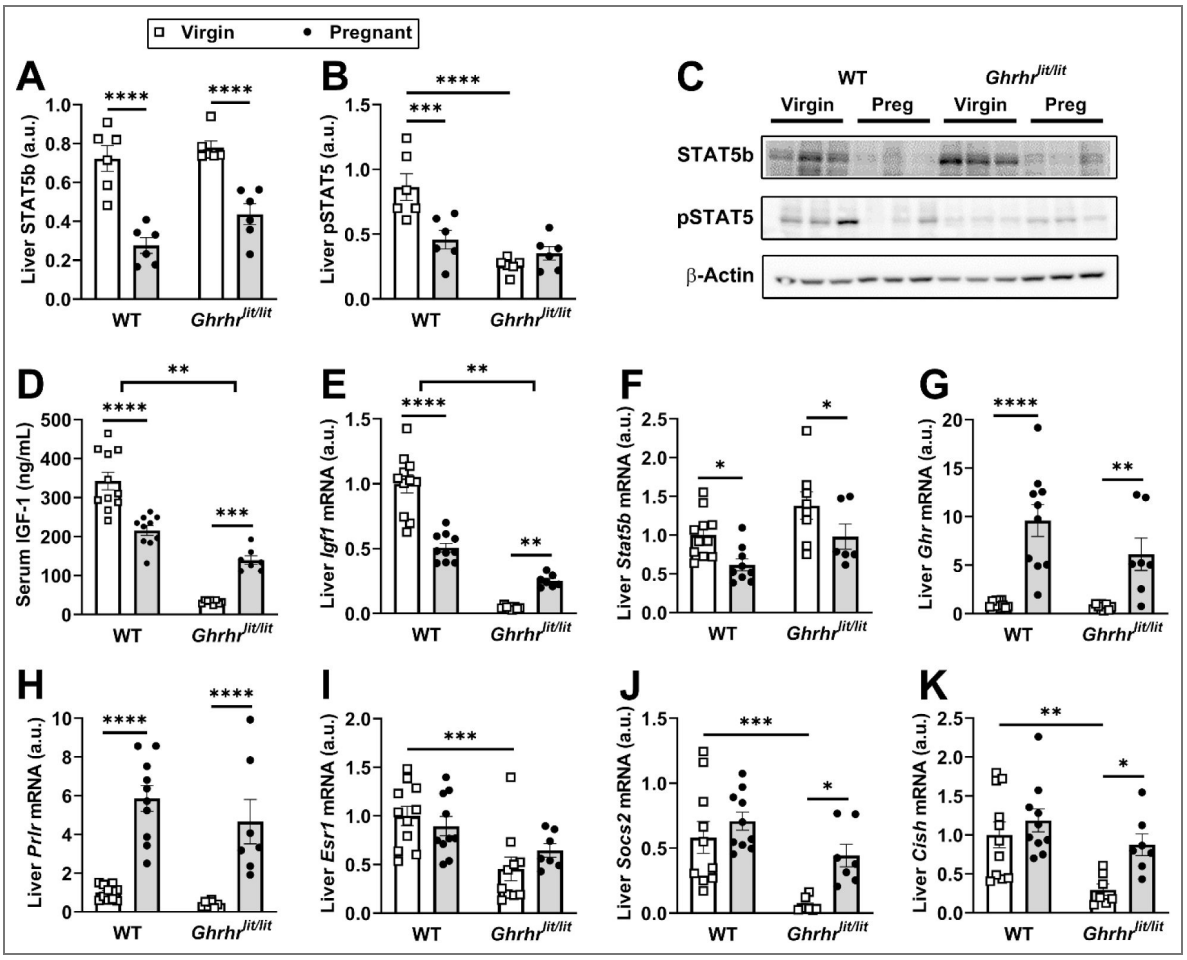

Figure 5. Pregnancy differentially modulates hepatic GHR signaling in WT and *Ghrhr*$^{lit/lit}$mice.

(A-C) Western blot analysis to determine the hepatic expression of STAT5b and pSTAT5 proteins in virgin WT (n = 6), pregnant WT (n = 6), virgin *Ghrhr*$^{lit/lit}$ (n = 6), and pregnant *Ghrhr*$^{lit/lit}$ (n = 6) mice. (D-K) Serum IGF-1 levels and hepatic mRNA expression in virgin WT (n = 11), pregnant WT (n = 10), virgin *Ghrhr*$^{lit/lit}$ (n = 8), and pregnant *Ghrhr*$^{lit/lit}$ (n = 7) mice. The pregnant mice were at gestational ages 14-17 days. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Statistical analysis was performed using two-way ANOVA and Holm-Sidak's multiple comparisons test.

(Greenhalgh and Alexander, 2004), were also reduced in *Ghrhr*$^{lit/lit}$ mice compared to WT animals (Figure 5J-K). Moreover, pregnancy increased *Socs2* and *Cish* RNA expression only in *Ghrhr*$^{lit/lit}$mice (Figure 5J-K).

Altogether, although WT mice exhibit increased GH secretion and hepatic *Ghr* expression, molecular analyses do not indicate higher GH action in the liver. In contrast, *Ghrhr*$^{lit/lit}$mice showed evidence of greater GH action in the liver, including increased circulating and hepatic IGF-1 levels, as well as greater *Socs2* and *Cish* expression.

Pregnancy-induced body growth is preserved despite disruption of GH-, ghrelin-, and estrogen-related signaling pathways

To gain further insights into the mechanisms driving post-pregnancy body growth, the effects of pregnancy were investigated across different mouse models. Mice carrying a GHR deletion in hepatocytes (Alb$^{\Delta GHR}$) show blunted circulating IGF-1 levels and growth deficits despite higher GH secretion (List et al., 2014; Wasinski et al., 2023; de Sousa et al., 2025b). We observed that a first pregnancy caused a robust increase in body weight and lean mass in Alb$^{\Delta GHR}$ mice, which was comparable to that seen in control animals (Figure 6A-B). Alb$^{\Delta GHR}$ females also showed increased body weight after the second pregnancy, but this was explained by pregnancy-induced increases in fat mass (Figure 6C). Thus, GHR signaling in hepatocytes is not necessary to induce pregnancy-induced growth. Ghrelin is a GH secretagogue, and impaired ghrelin signaling may affect body growth (Peris-Sampedro et al., 2021; Punt et al., 2025).

However, ghrelin receptor-deficient mice (GHSR KO) showed increases in body weight and lean mass similar to those of control animals (Figure 6D-E). Sex hormones also regulate growth, and estrogen receptor α (ERα) signaling activates the STAT5 pathway in the liver and IGF-1 synthesis (Venken et al., 2005). In addition, estradiol treatment stimulates growth in GHR-deficient mice (Venken et al., 2005), and estrogen response elements are found in the mouse *Stat5b* gene (Furigo et al., 2014). Nonetheless, hepatocyte-specific ERα ablation did not prevent pregnancy-induced increases in body weight, lean mass, and adiposity (Figure 6F-H). We also evaluated mice carrying brain GHR inactivation, as this is a model of gigantism caused by loss of GH negative feedback (Furigo et al., 2019; Teixeira et al., 2019a; Wasinski et al., 2020; de Sousa et al., 2025a). First pregnancy increased body, lean, and fat mass in Nestin$^{\Delta GHR}$ females, as observed in control animals (Figure 6I-K).

Discussion

Pregnancy causes profound endocrine and metabolic changes that are conserved across mammals. The maternal organism undergoes significant hormonal shifts that not only temporarily alter the body but may also lead to lasting effects. Here, we demonstrated that reproductive experience resulted in increased body growth in WT mice, independent of GH. This growth was mainly observed after the first pregnancy, while subsequent reproductive experiences primarily increased body fat. In humans, puberty triggers the fusion of growth plates, leading to the end of longitudinal bone growth (Emons et al., 2011). Height gain is typically limited after menarche, averaging 6.6-8.94 cm depending on the studied cohort (Spear, 2002; Gardstedt-Berghog et al., 2024; Gaete et al., 2025; Xu et al., 2026). Previous studies indicate that pregnant women may experience a slight but significant decrease in height due to weight gain, vertebral compression, and increased lordosis (Scholl et al., 1993; Xu et al., 2026). Therefore, using height as a measure of post-pregnancy growth in humans may be unreliable, which may explain why normal gravidas do not show longitudinal growth. However, increased foot size has been reported after pregnancy (Wetz et al., 2006; Dunn et al., 2012). Despite the lack of evidence for post-pregnancy longitudinal growth in healthy women, the current results in mice suggest that pregnancy activates GH-independent growth pathways. In species with open growth plates, like rodents, this could result in actual longitudinal growth, whereas in humans, it may instead lead to tissue remodeling or physical changes that do not increase height but may have

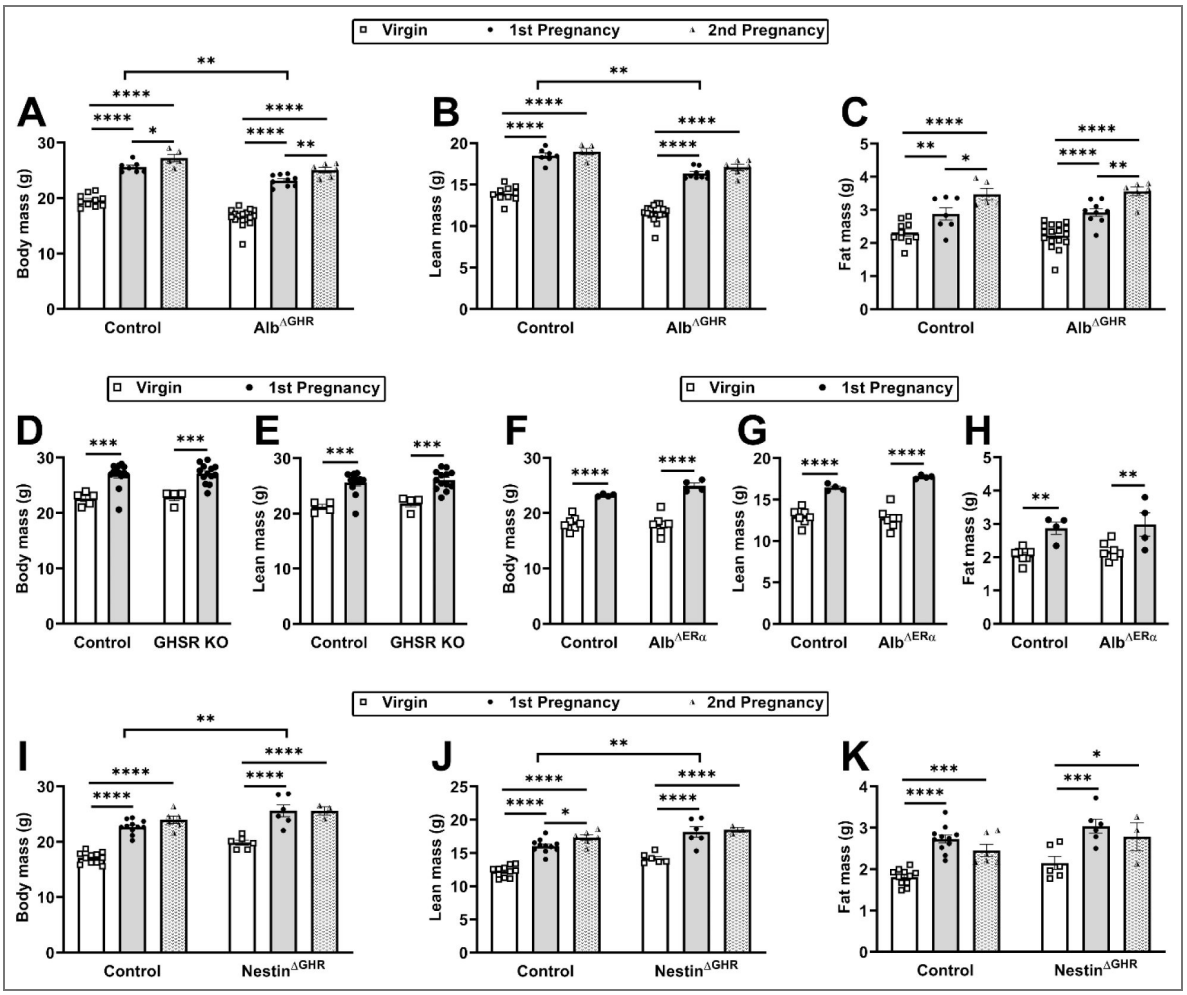

Figure 6. Pregnancy-induced body growth is preserved despite disruption of GH-, ghrelin-, and estrogen-related signaling pathways.

(A-C) The effects of first and second pregnancies on body weight, lean mass, and fat mass in control virgin (n = 10), control 1st pregnancy (n = 7), control second pregnancy (n = 5), $Alb^{\Delta GHR}$ virgin (n = 17), $Alb^{\Delta GHR}$ 1st pregnancy (n = 9), and $Alb^{\Delta GHR}$ second pregnancy (n = 6). (D-E) Body weight and lean mass in control virgin (n = 5), control 1st pregnancy (n = 14), GHSR KO virgin (n = 4), and GHSR KO 1st pregnancy (n = 13). (F-H) Body weight, lean mass, and fat mass in control virgin (n = 7), control 1st pregnancy (n = 4), $Alb^{\Delta ER\alpha}$ virgin (n = 7), and $Alb^{\Delta ER\alpha}$ 1st pregnancy (n = 4). (I-K) The effects of first and second pregnancies on body weight, lean mass, and fat mass in control virgin (n = 11), control 1st pregnancy (n = 11), control second pregnancy (n = 6), $Nestin^{\Delta GHR}$ virgin (n = 6), $Nestin^{\Delta GHR}$ 1st pregnancy (n = 6), and $Nestin^{\Delta GHR}$ second pregnancy (n = 3). *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Statistical analysis was performed using two-way ANOVA and Holm-Sidak's multiple comparisons test.

lasting effects that are still not well understood. Thus, the mouse model offers mechanistic insight into conserved pregnancy-related anabolic processes that may operate independently of the somatotropic axis.

Multiparous *Ghrhr*$^{lit/lit}$ mice showed significant gains in weight (+57%) and lean mass (+59%) after pregnancy compared with age-matched *Ghrhr*$^{lit/lit}$ virgins. In fact, their relative weight and lean mass gains were much higher than those observed in WT mice, which showed ~13% more body weight and lean mass than WT virgins. Thus, GH-deficient mice are considerably more sensitive to pregnancy-induced growth than WT mice. The profound growth deficit in *Ghrhr*$^{lit/lit}$mice likely enhances their sensitivity to pregnancy-induced growth factors. Human data are in accordance with these findings as children with severe GH deficiency (GHD) show a higher response to GH replacement than patients with less severe or questionable GHD (Savage and Bang, 2012; Wit et al., 2019). Accordingly, patients exhibiting the lowest response during GH provocation tests showed the highest responses to GH replacement treatment (Donbaloglu et al., 2023). Regarding the capacity of women with IGHD to present post-pregnancy growth, residual spinal growth is frequently observed after skeletal maturity, particularly in the 1–2 years following the peak of puberty, often averaging just a few millimeters to 1 cm in height (Ghanem and Rizkallah, 2020). The increased responsiveness to growth factors, as well as the late menarche (±17 years) and, therefore, delayed bone maturation (Aguiar-Oliveira and Salvatori, 2021), can also explain why adult women with severe IGHD retained some growth capacity after pregnancy. Importantly, identifying adult women with untreated IGHD who subsequently experienced pregnancy is extremely challenging. GH replacement therapy is typically initiated early in life, and untreated individuals are rare. Therefore, although a limited number of cases were available for study, this sample represents a highly valuable and unique clinical resource.

Unlike WT females, *Ghrhr*$^{lit/lit}$ mice show no increase in GH secretion during pregnancy, maintaining suppressed levels similar to those of nulliparous *Ghrhr*$^{lit/lit}$ mice. Thus, these findings revealed that GHRH signaling is required for pregnancy-induced increases in GH secretion in mice. Remarkably, this result indicates that pregnancy-induced body growth is independent of GH levels. However, the expression of genes typically induced by GHR signaling, such as *Igf1*, *Socs2*, and *Cish*, was upregulated in the liver of pregnant *Ghrhr*$^{lit/lit}$mice compared with virgin animals. The increased circulating IGF-1 levels in *Ghrhr*$^{lit/lit}$ mice may have contributed to their greater relative growth during gestation. In contrast, pregnant WT mice showed decreased circulating IGF-1 levels and hepatic *Igf1* mRNA expression compared to virgin animals. These results are consistent with another study that also observed decreased IGF-1 secretion and hepatic *Igf1* mRNA expression in mid-pregnant mice (Travers et al., 1990). In humans, IGF-1 levels decrease during the first trimester of gestation (Caron et al., 2010; Persechini et al., 2015), but increase during mid- and late-pregnancy, when they correlate with placental GH levels (Caufriez et al., 1993). Altogether, pregnancy differentially modulates the hepatic expression of genes commonly induced by GHR signaling in WT and *Ghrhr*$^{lit/lit}$ mice.

In an attempt to identify specific hormonal factors involved in pregnancy-induced body growth, mice with hepatocyte-specific deletion of GHR or ERα were generated. Confirming previous findings that pregnancy-induced body growth is independent of GH, hepatic GHR deletion did not prevent increases in body weight and lean mass in primiparous and multiparous mice. Although estradiol treatment can stimulate growth in GHR-deficient mice and activate the STAT5 pathway and IGF-1 synthesis in the liver (Venken et al., 2005), hepatocyte-specific ERα KO mice also exhibited normal pregnancy-induced body growth. Similar findings were observed in GHSR-deficient mice, demonstrating that pregnancy-induced body growth is ghrelin-independent. Finally, a gigantism model (Nestin$^{\Delta GHR}$ mice) was tested to determine whether pregnancy-induced growth can occur in mice that already have increased baseline lean body mass (+16%) compared with control animals. However, pregnancy also increased body and lean mass in Nestin$^{\Delta GHR}$ mice, in a manner comparable to control animals, suggesting that this growth potential persists even in animals that begin gestation with greater somatic length.

Several questions arose from our findings. For example, why does body growth occur primarily after the first pregnancy, regardless of the age at which it occurs? We speculate that pregnancy hormones, particularly sex hormones, may modify the growth plate of long bones, limiting further growth in subsequent gestations. Future studies should investigate the impact of pregnancy on growth plate structure and function. Another interesting finding is that multiple gestations are associated with increased body adiposity in mice. Leptin resistance is a hallmark of pregnancy, favoring physiological increases in food intake and fat mass (Mounzih et al., 1998; Trujillo et al., 2011; Ladyman et al., 2012; Zampieri et al., 2015; Andreoli et al., 2019; Gustafson et al., 2019). If leptin resistance persists in the postpartum period, it may favor fat retention. However, previous studies indicate normal leptin sensitivity in female mice following pregnancy (Ladyman et al., 2018; Andreoli et al., 2019; Teixeira et al., 2019b).

It is also possible that each pregnancy may cause some degree of fat retention, so multiple pregnancies lead to a significant increase in body adiposity simply through an additive effect. Regardless of the mechanism that causes pregnancy-induced fat gain, it is evident that pregnancy may represent a risk factor for obesity (Scholl et al., 1995; Rooney et al., 2005; Amorim et al., 2007). Finally, our study was not intended to distinguish the effects of pregnancy from those of lactation. Lactation stimulates the secretion of prolactin and oxytocin (Augustine et al., 2008; Ladyman et al., 2010). In mice, lactation is a period of high metabolic demand due to the cost of milk production, which is compensated for by hyperphagia (Woodside et al., 2012; Zampieri et al., 2015; Teixeira et al., 2019a). Thus, lactation may have contributed to the growth of the females and should be considered alongside gestation when interpreting our results. Moreover, female mice housed with sterile males exhibit increased body weight and reduced lifespan (Garratt et al., 2020). Therefore, mating per se, even in the absence of fertilization, is sufficient to influence body weight and longevity, representing an additional factor—beyond pregnancy and lactation—that may have contributed to the parameters analyzed.

In conclusion, reproductive experience, including mating, pregnancy, and lactation, activates redundant, multi-hormonal anabolic programs involving growth factors and placental lactogens, among others. Our findings indicate that this coordinated endocrine-metabolic environment is sufficient to promote longitudinal skeletal growth, even in the absence of pituitary GH, in species with open growth plates. We also provided evidence that post-pregnancy growth can occur in women with severe IGHD. Thus, reproductive experience has many effects on the maternal organism.

Despite the lack of longitudinal growth in normal women, their bodies undergo significant and potentially permanent changes that need further study to understand the long-term impact on women's health.

Material and Methods

Animals

In the current study, the following mouse strains were used: C57BL/6J (The Jackson Laboratory, Bar Harbor, ME; *RRID:IMSR_JAX:000664*), *Ghrhr*$^{lit/lit}$(The Jackson Laboratory; *RRID:IMSR_JAX:000533*), Alb^{Cre} (The Jackson Laboratory; *RRID:IMSR_JAX:003574*), Ghr^{flox} (List et al., 2014), ERα^{flox} (The Jackson Laboratory; *RRID:IMSR_JAX:032173*), Nestin^{cre} (The Jackson Laboratory; *RRID:IMSR_JAX:003771*), and GHSR-deficient mice (Zigman et al., 2005). The generation, genotyping, and validation of the experimental mice were carried out as described in previous studies from our group (Frazao et al., 2013; Furigo et al., 2019; Teixeira et al., 2019a; Wasinski et al., 2020; Wasinski et al., 2022; Wasinski et al., 2023; de Sousa et al., 2025a; de Sousa et al., 2025b). Mice had ad libitum access to regular rodent chow (2.99 kcal/g, 9.4% kcal from fat, 236 g protein/kg; Nuvilab CR-1, Quimtia, Brazil) and filtered water. The experimental procedures were approved by the Ethics Committee on the Use of Animals of the Institute of Biomedical Sciences at the University of São Paulo.

Evaluation of pregnancy-induced growth in mice

The body composition (lean and fat mass) of adult (~10-week-old) female mice was determined by time-domain nuclear magnetic resonance using the LF50 body composition mice analyzer (Bruker, Germany). Subsequently, the females were mated with sexually experienced males. Upon detection of pregnancy, females were placed in individual cages and maintained with their litters for 3-4 weeks. After weaning, the dams were regrouped in cages with up to five females. Body composition was reassessed 2 weeks after the last contact with the pups (~20-week-old), which we consider the lactation recovery period. At the same time, body composition was analyzed in age-matched virgin females as a control group. In some experiments, the primiparous females were allowed to mate again, and the entire procedure was repeated to determine the effects of the second pregnancy and lactation cycle. After the last body composition assessment, WT and *Ghrhr*$^{lit/lit}$ mice were euthanized in a CO_2 chamber, and naso-anal length was measured, as were the masses of the liver, heart, kidneys, and brain. Specifically, for GHSR KO mice and their respective controls, lean mass was calculated as total body weight minus fat mass (the sum of periuterine, periovarian, retroperitoneal, inguinal, posterior subcutaneous, and anterior subcutaneous fat depots).

Evaluation of pulsatile GH secretion and IGF-1 levels

To adapt the mice to the tail-tip blood sampling procedure, females were acclimated daily for 2-3 weeks before being placed in breeding. This adaptation continued while the females were housed with males. In pregnant females (gestational age 14-17 days) and virgin controls, a total of 36 blood samples were taken from the tail tip every 10 minutes. The procedure initially involved clipping a small portion (1 mm) from the tail tip with a surgical blade. Each blood sample (5 µL for WT and 2 µL for *Ghrhr*$^{lit/lit}$) was then transferred to a tube containing PBS with 0.05% Tween-20. After each draw, gentle fingertip pressure was applied to the tail to halt bleeding. The mice continued to move freely within their home cages, with unlimited access to food and water. Immediately after collection, samples were placed on dry ice and stored at -80°C.

An enzyme-linked immunosorbent assay (ELISA) was performed to measure blood GH levels, using a 1:22 final dilution, and the following antisera: monkey anti-rat GH as the capture antibody (1:50,000; NHPP, Cat# AFP411S; *RRID:AB_2665564*) and rabbit anti-rat GH as the detection antibody (1:100,000; NHPP, Cat# AFP5672099, *RRID:AB_2721132*). GH pulses were detected using the DynPeak pulse detection algorithm's default settings (Vidal et al., 2012). More details about this ELISA and the analysis of the GH secretion pattern are found in previous publications (Wasinski et al., 2020; Wasinski et al., 2022; de Sousa et al., 2024; de Sousa et al., 2025b). Serum IGF-1 levels were measured with a commercial ELISA kit (#MG100; *RRID:AB_2827989*; R&D Systems, Minneapolis, MN, USA).

Western Blot

Liver proteins were extracted using RIPA buffer (Sigma-Aldrich, Cat# R0278), containing phosphatase (Sigma-Aldrich, Cat# P5726 and P0044) and protease (Sigma-Aldrich, Cat# P8340) inhibitors. Samples were fractionated by polyacrylamide gel electrophoresis containing SDS (SDS-PAGE) using a running buffer (25 mM Tris; 192 mM Glycine; 0.1% SDS). Then, proteins were transferred to a nitrocellulose membrane overnight at 15 V using transfer buffer (25 mM Tris; 192 mM Glycine; 20% Methanol).

Membranes were blocked at room temperature for 2 h in 5% skim milk diluted in TBS-T buffer (10 mM Tris–HCl; 150 mM NaCl; 0.05% Tween-20). Membranes were incubated overnight in primary antibodies diluted in TBS-T containing 3% albumin. Membranes were washed three times for 10 min with TBS-T and incubated at room temperature for 1 h with respective secondary antibodies, diluted in 5% skim milk in TBS-T. After three 10-minute washes with TBS-T, the membranes were treated with chemiluminescent reagents (Clarity Western ECL Substrate, Bio-Rad, Hercules, CA; Cat# 170-5060). Bands on the membranes were revealed by automatic imaging systems. The intensities of the bands were quantified by densitometry using ImageJ software

(*https://imagej.net/ij/*) and normalized to total protein expression in the gel (Ponceau staining). Primary antibodies used for blotting were anti-phospho^{Tyr694}-STAT5 antibody (Cell Signaling Technology, Beverly, MA; Cat# 9351; *RRID:AB_2315225*; 1:1,000), anti-STAT5b antibody (Santa Cruz, Dallas, TX; Cat# sc-377069; 1:1,000), and anti-β-actin (Santa Cruz; Cat# sc-47778, *RRID:AB_626632*; 1:2,500).

Gene expression

Total RNA from the liver was extracted with TRIzol (Invitrogen, Carlsbad, CA), followed by incubation in DNase I RNase-free (MilliporeSigma, St. Louis, MO, USA). Reverse transcription was performed using 2 µg of total RNA, SuperScript II Reverse Transcriptase (Invitrogen), and random primers p(dN)6 (MilliporeSigma). Real-time PCR was performed using the 7500TM Real-Time PCR System (Applied Biosystems, Warrington, UK) and SYBR Green Gene Expression PCR Master Mix (Applied Biosystems. The following primers were used: *Actb* (forward: gctccggcatgtgcaaag; reverse: catcacaccctggtgccta), *Cish* (forward: tcgggaatctgggtggtact; reverse: taggaatgtaccctccggca), *Esr1* (forward: gcagatagggagctggttca; reverse: tggagattcaagtccccaaa), *Ghr* (forward: atcaatccaagcctgggga c; reverse: acagctgaatagatcctgggg), *Igf1* (forward: ccacactgacatgcccaaga; reverse: gtacttccttccttctcct ttgc), *Prlr* (forward: cagtaaatgccaccaacgaa; reverse: gaggaggctctggttcaaca), *Ppia* (forward: tatctgcac tgccaagactgagt; reverse: cttcttgctggtcttgccattcc), *Socs2* (forward: cgcgagctcagtcaaacag; reverse: aagt tccttctggagcctctt), and *Stat5b* (forward: ggactccgtccttgataccg; reverse: tccatcgtgtcttccagatcg). Data were normalized to the *Actb* (β-actin) and *Ppia* expression. Relative quantification of mRNA was calculated by $2^{-\Delta\Delta Ct}$.

Statistical analysis

When comparing two groups, the data were analyzed using a two-tailed, unpaired Student's t-test. When comparing three groups simultaneously, one-way ANOVA followed by the Newman-Keuls multiple comparisons test was used. Two-way ANOVA and Holm–Sidak's multiple comparisons test were used to analyze two variables (pregnancy and mutation effects). Statistical analyses were conducted with Prism software (GraphPad, San Diego, CA). All results are presented as mean ± standard error of the mean.

Data availability

All data generated or analyzed during this study are included in the manuscript, figures and supporting files.

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Additional information

Author Contributions

Gabriel O. de Souza: Formal analysis; Investigation. **Willian O. dos Santos:** Formal analysis; Investigation. **Frederick Wasinski:** Formal analysis; Investigation. **Ligia M. M. de Sousa:** Investigation. **Andressa G. Amaral:** Investigation. **Daniela O. Gusmao:** Investigation. **Edward O. List:** Resources. **John J. Kopchick:** Resources. **Gimena Fernandez:** Investigation. **Mario Perelló:** Investigation. **Carla R. P. Oliveira:** Investigation. **Manuel H. Aguiar-Oliveira:** Investigation. **Jose Donato Jr:** Conceptualization, Data Curation, Writing - Original Draft, Project administration, Funding acquisition.

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Peer reviews

Reviewer #1 (Public review):

This work evaluates the impact of reproductive history on growth, body weight and body composition in mammals. In mice, somatic growth is stimulated by the first pregnancy while the second pregnancy increases body weight mainly by increasing adiposity. To probe the role of pituitary growth hormone (GH), the key regulator of somatic growth in these processes, was addressed by comparing the impact of reproduction on growth in normal ("wild type") and genetically GH-deficient females and by detailed characterization of the profile of fluctuations in circulating GH levels in both types of animals. Additional studies addressed the possible role of other endocrine pathways (ghrelin and estrogen) in the pregnancy-related growth. Surprisingly, reproduction-related growth was independent of GH,

ghrelin and estrogen. To determine whether these results may apply ("translate") to human physiology, data on various parameters of somatic growth were collected from women with hereditary GH deficiency. The findings indicate that GH-independent stimulation of growth by reproductive events also occurs in women.

Use of multiple animal models, rigorous characterization of GH levels in normal and GH-deficient females, and inclusion of data derived from a unique and well-characterised population of people with hereditary isolated GH deficiency and no GH replacement therapy are important strengths of these elegant and innovative studies. The results address a broader and clinically significant issue of permanent changes in body size, composition and function that result from pregnancy and lactation. This work also provides important background for further studies aimed at the identification of the mechanism involved and the role of specific reproductive events in the regulation of growth.

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Reviewer #2 (Public review):

This manuscript describes the fascinating phenomenon of growth hormone (GH)-independent growth occurring in the mother during pregnancy. This growth was most pronounced in dwarf mice that are lacking the receptor for growth hormone-releasing hormone (GHRH) and therefore showing isolated GH deficiency. However, the pregnancy-induced growth could also be observed in wild-type mice, suggesting that it is a normal part of the maternal adaptation to pregnancy. The study falls short of identifying the mechanism(s) driving this pregnancy-induced growth response, but it certainly reveals a novel insight into maternal physiology. The authors have completed a range of experiments in mice to prove that, as well as being GH independent, the pregnancy-induced growth also did not require GH signaling in the liver (i.e. not another pregnancy-specific ligand operating through the GHR to promote IGF). They also provided complementary data from a population of humans with untreated isolated GH deficiency that are broadly consistent with the hypothesis. While it is important to consider the significant species differences between rodents and humans, both in terms of growth physiology and also in terms of evolution of placental somato-mammotrophic hormones, this unique population are a valuable resource and adds credence to the study. Overall, I find this a compelling research story, but disappointingly unfinished. There are some areas where additional information could improve the ability to interpret the data, and some additional concepts that could be considered in the discussion. There are also areas where additional experiments might provide important insights. However, I think that such suggestions can be considered as appropriate for future research, rather than delaying consideration of the current manuscript.

Main comments:

(1) Data in Figure 1 are remarkable - not so much the growth in pregnancy in the wildtype mice, because while elevated GH is well known in pregnancy, but growth in the dwarf mice is indicative of GH-independent growth. From these data, it seems that there is good evidence that growth in pregnancy is an adaptive function. However, it is possible that growth is achieved in dwarf mice and that in wildtype mice may have been mediated through different mechanisms. The dwarf mice showed an increase in liver and plasma IGF1, suggestive of an additional ligand driving IGF in pregnancy. One could hypothesize that such an effect could be mediated by an additional pregnancy-specific ligand activating the GH receptor. In humans, placental growth hormone could be such a ligand, but as far as we know, there is no placental GH in mice. In contrast, the wildtype animals showed suppression of liver and circulating IGF1, and low levels of pSTAT5 in the liver during pregnancy. These data (in Figure 5) are very surprising. Given the high circulating GH in pregnancy, as well as high

placental lactogen (which would be expected to activate STAT5 in the liver through the Prlr), the low levels of pSTAT5 are unexpected and would seem to indicate some sort of acquired insensitivity to GH. Is this entirely driven by down-regulation of STAT5b protein, or could there be activation of other, negative regulators of STAT signalling, such as SOCS? What is causing such a profound suppression of STAT5? Regardless of the mechanism, this suggests that pregnancy-induced growth in wildtype mice is independent of circulating IGF1 (potentially a different mechanism or in addition to that seen in IGHD mice).

The data shown in Figure 6 are a major strength of the study, showing that the pregnancy-induced changes are not specific to one particular transgenic model, but still occur in a variety of models affecting GH through different approaches. Given the pregnancy-specific nature of the changes, however, it seems an oversight not to have evaluated the role of placental lactogens. Prlr is highly expressed in the liver, but the function of this hormone in the liver is not well established. Could the extremely high levels of PL be mediating this growth response? Given the low expression of STAT5 in the liver and the fact that plasma IGF1 is not markedly elevated, it seems more likely that this growth response may be mediated by locally produced IGF1 in target tissues.

I think these possibilities could be addressed by an expanded discussion of species variation in placental hormones, to highlight that humans have expansion of the GH locus, but rodents have expansion of the prolactin axis (see Soares, M. J. The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reprod Biol Endocrinol 2, 51, 2004). Importantly, placental GH and chorionic somatomammotropins (CSM) in humans are all variants of the GH gene, but CSM have preferential activity at Prlr. This seems to be a fundamental species difference in pregnancy biology, but has been interpreted as an example of convergent evolution, with conservation of prolactin and GH-like functions at the maternal-fetal interface, mediated by different mechanisms, likely contributing to the metabolic adaptations of the mother (see Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. Curr Opin Endocrinol Diabetes Obes. 2011; 18: 409-416). While the preceding function has focused on explaining the evolution of placental lactogens (either prolactin or GH variants), the present data suggest that there are also mechanisms to maintain growth in pregnancy, independent of GH (even in the absence of a placental GH).

(2) The human data are very interesting, and my initial impression was that it seemed unlikely to be the same phenomenon. Was there any real evidence for "growth" in pregnancy? Pubertal maturation of long bone growth might be expected to prevent further growth in adulthood. However, these issues were appropriately discussed, and it seems well justified to evaluate this unique population of women with IGHD who underwent pregnancy. It would be very interesting to know if these women experienced elevated IGF1 during pregnancy, indicative of placental GH contributing to growth. Mechanistically, this might be more like the dwarf mouse situation of IGHD, that the situation in wildtype mice (associated with liver insensitivity to GH and low IGF1).

(3) It would be useful to include investigations that isolate the effects of pregnancy and the placental hormones. Such studies could include evaluating growth in pseudopregnant mice with IGHD (pregnancy-like changes in hormones but lacking the placental contribution) and in IGHD animals that experience pregnancy but not lactation (pups removed at birth). I accept that this might be too large an additional study to add for the present manuscript.

(4) It is an important and translationally relevant observation that pregnancy increased the risk of long-term weight gain, and that after the first pregnancy, the pregnancy-induced growth response was more directed to promoting fat deposition. Does this provide any mechanistic insight? Could a metabolic adaptation result in growth?

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Reviewer #3 (Public review):

Summary:

The study describes an increase in body growth and body composition in both mice and women. In mice, the impact on growth is mainly seen during the first pregnancy, and the changes postpartum on body composition are also different during the first and second pregnancies. The study has used various knock-out models in the growth hormone axis to understand these changes as well as some gene expression analysis related to GH, IGF-1 and estrogen signalling pathways.

Strengths:

(1) The inclusion of various knock-out mouse models that allow for exploration of mechanisms related to the above-mentioned changes.

(2) The investigation of gene expression of GHR, IGF-1R and ER pathways.

Weaknesses:

The human findings are dependent on the patient's recollection of bodily changes after their pregnancies.

Conclusion:

The authors have partly achieved their aim of describing changes in growth and body composition that remain after pregnancy and the mechanisms behind these changes. This study may have importance for a wide variety of research areas as well as in the clinical setting. The study is also unique in its attempt to bridge findings in mice to a unique human model of congenital GH deficiency.

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