Title

Metabolism and Energy Expenditure, But Not Feeding or Glucose Tolerance, Are Impaired in Young Kiss1r KO Female Mice

Journal

Endocrinology, 157(11)

ISSN

0888-8809

Authors

Tolson, Kristen P

Garcia, Christian

Delgado, Iris

et al.

Publication Date

2016-11-01

DOI

10.1210/en.2016-1501

Peer reviewed

Metabolism and energy expenditure, but not feeding or glucose tolerance, are impaired in young Kiss1r KO female mice

Kristen P. Tolson, Christian Garcia, Iris Delgado, Nuha Marooki, and Alexander S. Kauffman

Department of Reproductive Medicine, University of California, San Diego, LA Jolla, CA

Kisspeptin regulates reproduction via signaling through the receptor, Kiss1r, in GnRH neurons. However, both kisspeptin and Kiss1r are produced in several peripheral tissues, and recent studies have highlighted a role for kisspeptin signaling in metabolism and glucose homeostasis. We recently reported that *Kiss1r* KO mice display a sexually-dimorphic metabolic phenotype, with KO females displaying obesity, impaired metabolism, and glucose intolerance at 4–5 months of age. However, it remains unclear when this metabolic phenotype first emerges in development, or which aspects of the pleiotropic phenotype underlie the metabolic defects and which are secondary to the obesity. Here, we studied *Kiss1r* KO females at different ages, including several weeks before the emergence of body weight differences and later when obesity is present. We determined that at young adult ages (6 weeks old), KO females already exhibit altered adiposity, leptin levels, metabolism, and energy expenditure, despite having normal body weights at this time. In contrast, food intake, water intake, and glucose tolerance are normal at young ages, and only show impairments at older adult ages, suggesting that these impairments may be secondary to earlier alterations in metabolism and adiposity. We also demonstrate that, in addition to body weight, all other facets of the adult metabolic phenotype persist even when gonadal sex steroids are similar between genotypes. Collectively, these data highlight the developmental emergence of a metabolic phenotype induced by disrupted kisspeptin signaling, and reveal that multiple—but not all—aspects of this phenotype are already disrupted prior to detectable changes in body weight.

The neuropeptide kisspeptin (encoded by *Kiss1*) regulates reproduction by stimulating gonadotropin-releasing hormone (GnRH) neurons via its receptor, Kiss1r (formerly known as GPR54). Humans, mice, and rats with mutations in these genes show impaired puberty, hypogonadism, and infertility (1–6). In addition to being expressed in reproductive areas of the brain, kisspeptin is also expressed in multiple peripheral tissues (7–10). The receptor, *Kiss1r,* is also expressed in multiple non-GnRH brain areas and in several peripheral tissues (9–11), including metabolic tissues such as fat, liver, and pancreas. The presence of kisspeptin and its receptor outside of reproductive tissues suggests that kisspeptin signaling has additional uncharacterized roles beyond reproduction, perhaps relating to metabolism or glucose control (12, 13). However, until recently, virtually all research on kisspeptin signaling has focused on reproductive regulation.

We recently reported that, in addition to stimulating the reproductive axis, the kisspeptin system is also an important player in body weight (BW), energy balance, and glucose regulation (14). We found that, compared with WT littermates, adult *Kiss1r* knockout (KO) females maintained on a standard chow diet displayed dramatically higher BWs, but this phenotype did not emerge not until early adulthood, beginning around 10 weeks of age. By 4–5 months of age, KO females weighed a robust 30% more than WT females. In addition to becoming obese in adulthood, KO females also had increased adiposity, lep-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in USA

Received July 18, 2016. Accepted September 13, 2016.

Abbreviations:

tin levels, and impaired glucose tolerance, all measured around 4–5 months of age. This phenotype was sexually dimorphic, as male *Kiss1r* KO mice had normal BW and glucose regulation, even out to 6 months of age. Despite their obesity, adult *Kiss1r* KO females ate less than WT females. However, adult KO females displayed markedly reduced metabolic parameters, including lower respiratory rates and energy expenditure. Importantly, the BW and metabolic phenotype in *Kiss1r* KO females was not solely reflective of absent gonadal sex steroids, as chronically ovariectomized *Kiss1r* KO females still developed obesity, hyperleptinemia, reduced metabolism, and glucose intolerance compared with ovariectomized WT females.

Our previous study demonstrated that absent kisspeptin signaling can be an important factor in BW, adiposity, glucose intolerance, and metabolism in adulthood. However, other than BW, which was normal at younger adult ages, all of the metabolic parameters examined were done so exclusively in midadulthood, around 4–5 months of age. Thus, it remains unknown when the various aspects of the metabolic phenotype first emerge, or if different parameters show impairments before others. Indeed, which aspects of the KO's metabolic phenotype are secondary to the eventual obesity or which may underlie the pleiotropic phenotype remains undetermined. In this present study, we hypothesized that some aspects of the *Kiss1r* KO female phenotype, such as decreased feeding and glucose intolerance, would emerge only after (ie, as a consequence of) the onset of overt BW differences, while other facets, such as elevated adiposity and reduced metabolic rate, would be present prior to BW differences, suggesting a causal relationship between the former and the latter . To assess this issue and to begin to determine the development of the metabolic phenotype, we examined multiple metabolic parameters in *Kiss1r* KO females at several different ages, including at 6 weeks old, multiple weeks before the onset of the BW phenotype, at 10 weeks of age, around the time the BW difference first becomes detectable, and later around 20 weeks of age, when midadult females show robust metabolic impairments and marked obesity.

Materials and Methods

Animals and experimental ages

Kiss1r KO mice, described previously (14–17), were generated by mating heterozygous breeders and maintained on a mixed C57Bl6 × 129S1/SvImJ background. All mice were genotyped and sexed by PCR of tail DNA. In each experiment, *Kiss1r* KO mice were compared to control littermates (siblings). Weaned littermates (~3 weeks old) were housed at 2–3 per cage (mixed genotype) in a 12-hour light/12-hour dark cycle (lights off at 6:00 PM) with ad libitum water and standard rodent chow (3.5 kcal/g, 45.2% available carbohydrate, 11.4% fat, 17.2% crude protein). All experiments were approved by the University of California, San Diego Institutional Animal Care and Use Committee.

KO and control mice were studied at 3 specific ages: 6 weeks old ("young adults", several weeks prior to any detectable changes in body weight in the KOs), 10 weeks old (around the age when body weight differences first start to become apparent, with KO's weighing more than controls), and 18–20 weeks old (when body weights, feeding, metabolic rates, and glucose tolerance are all significantly impaired in the KOs). Mice at each age were subjected to body weight and body composition analyses, glucose tolerance testing, hormone analysis, feeding and water intake measures, and metabolic analyses in CLAMS cages.

Our prior study indicated that adult body weight was still dramatically higher in KO than control females even when both genotypes had been chronically without gonadal sex steroids. However, additional measures beyond body weight were not assessed in that study. Here, we also studied a cohort of adult mice at 18–20 weeks old that were first ovariectomized months

Table 1. Metabolic and body weight parameters in Kiss1r KO females at different ages or in adult OVX mice

Phenotypic Parameter	6 weeks	10 weeks	18–20 weeks	18–20 weeks (OVX)
Increased body weight	No	v (10%)	v (32%)	v (22%)
Increased adiposity	v (35%)	v (60%)	v (125%)	v (100%)
Increased leptin	v (80%)	v (140%)	v (300%)	v (65%)
Decreased VO2	v (20%)	v (22%)	v (28%)	v (22%)
Decreased CO2	v (20%)	v (22%)	v (30%)	v (25%)
Decreased energy expenditure	v (15%)	v (15%)	v (20%)	v (15%)
Decreased food intake	No	No	v (35%)	v (25%)
Decreased water intake	No	No	v (45%)	v (25%)
Glucose intolerance (increased AUC)	No	v (18%)	v (32%)	v (25%)

A "No" indicates that there was no significant difference in this measure between control and KO females at this age; a check mark indicates this measure was significantly different from controls. Percentage values in parentheses indicate approximate % increase or % decrease relative to control females at same age. Percentage values for adult OVX KOs are in comparison to adult OVX control mice similarly ovariectomized at 2.5 weeks of age. Food intake, water intake, VO2, VCO2, and energy expenditure values are for the dark period of the light-dark cycle, when mice are most active. Note that food and water intake at 10 weeks were both normal in the dark cycle though moderately reduced in the light portion.

earlier, before the normal pubertal period and any significant sex steroid secretion. In this case, both KOs and control siblings were bilaterally ovariectomized at 2.5 weeks old, thereby equalizing gonadal sex steroid secretion (ie, making it absent) in both genotypes for the remaining 16–18 weeks until time of metabolic analyses.

Body composition analyses

Body composition was determined in female *Kiss1r* KO and control mice by dual energy x-ray absorptiometry (DEXA). Mice were single housed and fasted for 4–6 hours and then anesthetized (IP injection; ketamine 100 mg/kg, xylazine 10 mg/kg). Body weight was measured and lean muscle mass and fat mass were determined by scanning with a GE Lunar Pixi Densitometer Machine (n = 7–17/group).

Leptin assays

Blood was collected retro-orbitally and the serum stored at –20°C. Serum samples were assayed for leptin using a Mouse/Rat Leptin Quantikine ELISA Kit (#MOB00, R&D Systems; n = 5–7/group), per the manufacturer's instructions. The assay sensitivity is 0.02 ng/ml, intra-assay coefficient of variant is 4%, and interassay coefficient of variation is 7%.

Metabolic and Energy Expenditure Analyses

Indirect calorimetry was performed at each age using a 12-cage equal flow CLAMS calorimeter system (Columbus Instruments) coupled with photosensors to detect movement. Females were habituated to the metabolic cages (single-housed) for 2 days prior to data acquisition (n = 6–9/genotype). O2 consumption (VO2) and CO2 production (VCO2) were measured every 12 minutes/cage. Respiratory exchange ratio (RER) was calculated as the quotient of VCO2/VO2. In addition, feeding was measured in 12 minutes intervals and calculated for hourly feeding.

Glucose tolerance tests

Glucose tolerance tests (GTT) were performed on *Kiss1r* KO and control mice on a standard chow diet. Mice were fasted for 6 hours beforehand with free access to water. Blood glucose was measured using a handheld glucometer (One Touch UltraMini, LifeScan, Inc.) just before IP glucose injection (time 0; 2g/kg BW

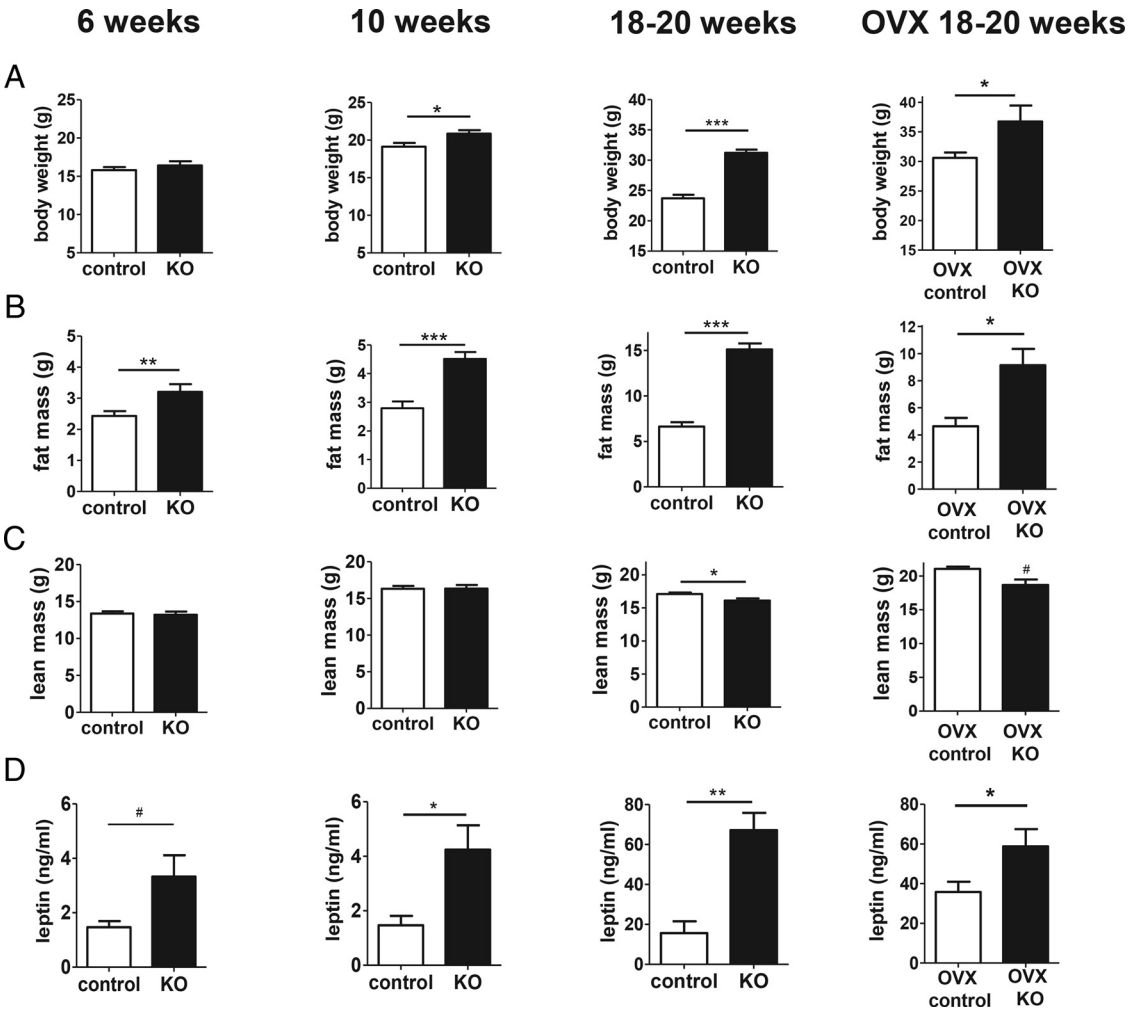

Figure 1. Body weight, body composition, and leptin levels in *Kiss1r* KO females fed a standard diet. Body weight (A), fat mass (B), lean mass (C), serum leptin (D) of KO and control females at 6, 10, or 18–20 weeks of age. n = 5–17 per group. For the cohort of 18–20 week OVX mice, both genotypes were ovariectomized at 2.5 weeks of age and remained without gonadal sex steroids for the rest of the study. Note the differing scales of the y-axes for younger vs. older animals. $*P < .05$, $**P < .01$, $***P < .001$; #, nonsignificant trend for genotype difference ($P < .06$).

in saline) and subsequently at 15, 30, 45, 60, 90 and 120 minutes postadministration (n = 8–13/group).

Statistical Analyses

All data are expressed as the mean ± SEM for each group. For data at single points (non–repeated measures), comparisons were made using unpaired 2-tailed t tests. For repeated measures (GTT), 2-way repeated-measures ANOVA was performed, with Bonferroni post hoc tests directly comparing genotypes at specific points. Statistical significance was set at $P < .05$.

Results

Kiss1r KO females already have elevated adiposity and leptin levels at young adult ages, prior to detectable differences in body weight

Female *Kiss1r* KO mice weighed the same as control littermate females at 6 weeks (Figure 1A), with a small (10%) but statistically significant difference in body weight at 10 weeks ($P < .05$; Figure 1A), compared to a more robust 32% difference in body weight in adults (18–20 weeks, $P < .001$; Figure 1A). However, even though there was no difference in body weight at 6 weeks of age, adiposity was already significantly increased in KOs by over 30% at this age ($P < .01$; Figure 1B). By 10 weeks of age, adiposity was increased by over 60% ($P < .001$; Figure 1B), which then rose to over 125% in adulthood ($P < .001$; Figure 1B). In contrast to fat mass, lean mass was not significantly different between KOs and controls at 6 or 10 weeks of age, and showed only a minor decrease (6%) in KOs in adulthood, as previously reported (Figure 1C). Similar to fat mass, serum leptin levels (Figure 1D) were also significantly elevated in KOs in adulthood ($P < .01$; 300%) and at 10 weeks ($P < .05$; 140%), with a nonsignificant trend ($P = .058$) at 6 weeks (80%), despite their body weights being normal at 6 weeks of age and only slightly higher at 10 weeks of age.

We previously reported that body weight at 18–20 weeks old was still markedly higher in KO than control females even when both genotypes had been chronically without gonadal sex steroids (14). However, additional measures besides body weight were not previously assessed. Here, we further studied a cohort of 18–20 week old adult females of both genotypes that were ovariectomized earlier at 2.5 weeks old. These OVX KOs still weighed more than OVX controls, by approximately 22%, and also still displayed higher fat mass and circulating leptin levels at 18–20 weeks of age (Figure 1A, B, D, far right column, $P < .05$ for all measures). Lean mass showed a nonsignificant trend to be slightly lower in OVX females at 18–20 weeks old (Figure 1C).

Food and water intake are not different at young adult ages prior to changes in body weight

We previously discovered that adult KO females have decreased food intake, despite their higher body weights. We measured feeding in younger animals to determine if this aspect of the KO phenotype was linked or if it was secondary (or compensatory) to the obese phenotype. Unlike in adulthood, food consumption of KO females was normal in both dark and light cycles at 6 weeks of age (Figure 2A), and was only slightly decreased in the light cycle at 10 weeks of age (Figure 2A). By comparison, in adult KO females, food intake was strongly decreased in both the dark and light portions of the light-dark cycle ($P < .001$; Figure 2A). We also examined water intake, which was previously not examined in our prior study. As with feeding, adult KOs showed significantly decreased water intake in both dark and light phases ($P < .001$; Figure 2B). However, at 6 weeks of age, there was no significant genotype difference in water intake (Figure 2B), and at 10 weeks of age, the KOs displayed a minor, though significant, decrease in water intake only during the light phase (Figure 2B).

To assess potential influence of sex steroids on these measures, feeding and drinking were also measured in a cohort of 18–20 week old mice of both genotypes that had been ovariectomized earlier at 2.5 weeks old. As in gonad-intact mice, 18–20 week old OVX KO females still consumed significantly less food and water than OVX controls during the dark phase of the light-dark cycle, when mice are most active (Figure 2, far right column, $P < .05$ for both measures).

Metabolism and energy homeostasis are already impaired at younger ages prior to detectable changes in body weight

Next, we used CLAMs metabolic cages to measure metabolic gas exchange in KOs and controls at different ages. Similar to adiposity and leptin levels, and in contrast to food intake, gas exchange was already significantly decreased at 6 weeks of age. Specifically, VO_2 was significantly decreased in both dark and light phases ($P < .001$ in dark cycle, $P < .05$ in light cycle; Figure 3A) and VCO_2 was strongly decreased during the dark phase ($P < .001$; Figure 3B). Energy expenditure was also already notably decreased at 6 weeks of age ($P < .01$; Figure 3C). By 10 weeks of age, VO_2, VCO_2, and energy expenditure were all substantially decreased in both dark ($P < .001$) and light phases ($P < .05$–0.001) of the light-dark cycle (Figure 3A-C), similar to what was observed previously in adult 18–20 week KO females (Figure 3) (14).

To determine the potential influence—or lack thereof—of gonadal steroids on these metabolic measures in

the KOs, we also studied 18–20 week old females of both genotypes that were chronically ovariectomized at 2.5 weeks old. As in gonad-intact mice, 18–20 week old OVX KOs still displayed significantly reduced VO2, VCO2, and energy expenditure compared to their OVX control siblings (Figure 3A-C, far right column, $P < .05$ to $P < .001$, depending on the measure).

Glucose tolerance in Kiss1r KO females is normal at young adult ages prior to body weight differences but disrupted at older ages

Decreased glucose tolerance can be caused by innate defects in glucose processing or secondary defects due to the inflammatory state caused by obesity. To determine whether the aberrant glucose homeostasis previously seen in adult KO females is secondary to obesity, we performed glucose tolerance tests at 6 and 10 weeks. In contrast to adult KO females, which showed elevated basal glucose ($P < .05$, Figure 4A) and severely impaired glucose tolerance (AUC $P < .01$, Figure 4B, C), at 6 weeks of age there were no genotype differences in either basal glucose levels (Figure 4A) or the response to a bolus injection of glucose (Figure 4B, C), indicating normal glucose homeostasis at this younger age. However, at 10 weeks of age, when the body weight differences are becoming significantly different, basal glucose was elevated ($P < .05$; Figure 4A), and glucose tolerance was now significantly decreased (Figure 4B, C; AUC $P < .01$). Thus, like food intake, impairments in glucose homeostasis are not present at younger ages but emerge later in adulthood when body weights are significantly elevated.

To ascertain whether absent gonadal steroids influence the glucose intolerance in the adult KOs, we also studied 18–20 week old females of both genotypes that were ovariectomized at 2.5 weeks old. As in gonad-intact mice, 18–20 week old OVX KOs still displayed significantly impaired glucose tolerance compared to their OVX control siblings (Figure 4, right column, $P < .05$).

Discussion

Puberty and fertility in mammals are highly dependent on functionally-intact kisspeptin signaling in the brain, via kisspeptin's stimulation of GnRH neurons (1–3, 5, 6), with disrupted kisspeptin signaling causing hypogonadal hypogonadism. Because of this role, the vast majority of kisspeptin studies have focused on its actions in controlling reproduction, overlooking possible other functions of this peptide. Yet, the expression of kisspeptin and its receptor in several peripheral tissues implies other undiscovered roles for kisspeptin signaling. Indeed, some evidence suggested that kisspeptin signaling might play a role in energy balance (13, 18–20), and our recent study discovered a sexually dimorphic body weight phenotype in *Kiss1r* KO mice that was not detectable until the females reached adulthood, first notably emerging around 10 weeks of age and increasing dramatically thereafter into

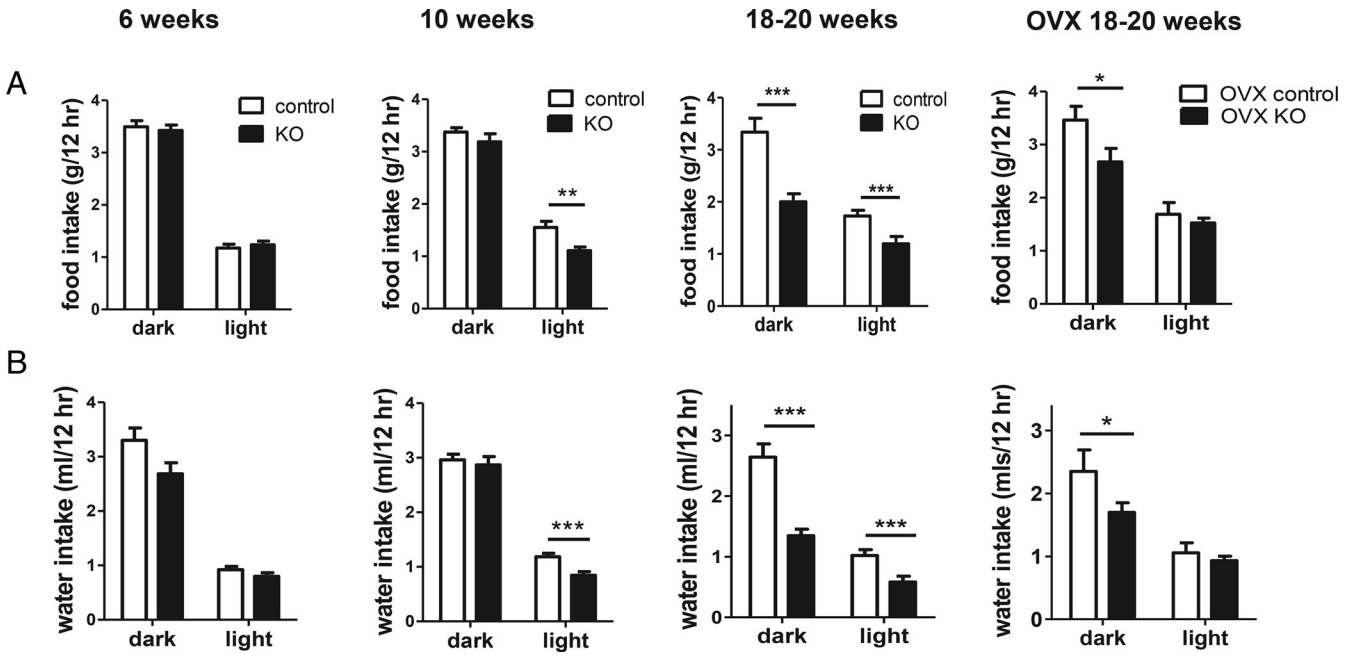

Figure 2. Food and water intake during light and dark cycles. Food intake (A) and water intake (B) in 6 week, 10 week, and 18–20 week old *Kiss1r* KO and control females. For the cohort of 18–20 week OVX mice, both genotypes were ovariectomized at 2.5 weeks of age. The gonad-intact 18–20 week old food intake graph is reused with permission from our previous publication (14) and is shown for comparison purposes to the other ages. n = 6–9 per group, $**P < .01$, $***P < .001$; #, nonsignificant trend for genotype difference ($P < .07$).

older adult ages (14). We previously found that the KO females also display greatly increased adiposity and leptin levels, decreased glucose tolerance, and markedly disrupted metabolism and energy expenditure. However, other than body weights, all these measures were taken only in midadulthood, around 4–5 months of age. Thus, the developmental timing of the various aspects of the metabolic phenotype, as well as which of these aspects might be secondary to the obesity vs which might causally underlie the development of obesity, remained unknown. Here, in comparison to older adults (18–20 weeks old), we examined multiple metabolic and glucose parameters in *Kiss1r* KO mice at 6 and 10 weeks of age, the former being young adulthood, multiple weeks before the onset of the body weight phenotype, and the latter being concurrent with the emergence of the body weight phenotype. Our results demonstrate that adiposity, elevated leptin, and decreased metabolism are already altered at younger ages, well before overt changes in body weight, whereas impaired glucose tolerance and feeding develop later in adulthood, after significant increases in body weight have been established

This study confirms our previous report of a dramatic body weight phenotype in adult *Kiss1r* KO female mice, which does not become first apparent until around 10 weeks of age and which gets progressively worse as the animals age beyond that point. We found no genotype difference in body weight in younger adults, at 6 weeks of age, as previously reported. However, when body composition was examined at 6 weeks of age, we were surprised to find that adiposity was already elevated, despite normal body weights. The degree of increased adiposity was smaller at 6 weeks than 10 weeks, which was also smaller than in adulthood, suggesting a gradual increase in adiposity that begins at least right after puberty and which likely causes the elevated body weight that emerges later around 10 weeks of age. Due to the technical limitations of our available metabolic equipment, we were unable to examine ages younger than 6 weeks old, so it remains unknown how early the increased adiposity begins to manifest. Consistent with the increased fat mass at all ages, circulating leptin was elevated as well. The small difference in lean mass that is observed in adult females was not present at 6 weeks or 10 weeks, so this difference may be due to the chronic lack of sex steroids.

We previously showed that multiple measures of metabolic rate are significantly decreased in adult KO females (14), suggesting the possibility that the eventual emergence of obesity is caused by underlying decreases in metabolism and energy expenditure. However, in our original report, we only measured metabolic rats in older adults (~4–5 months old). Here, we demonstrate that metabolic

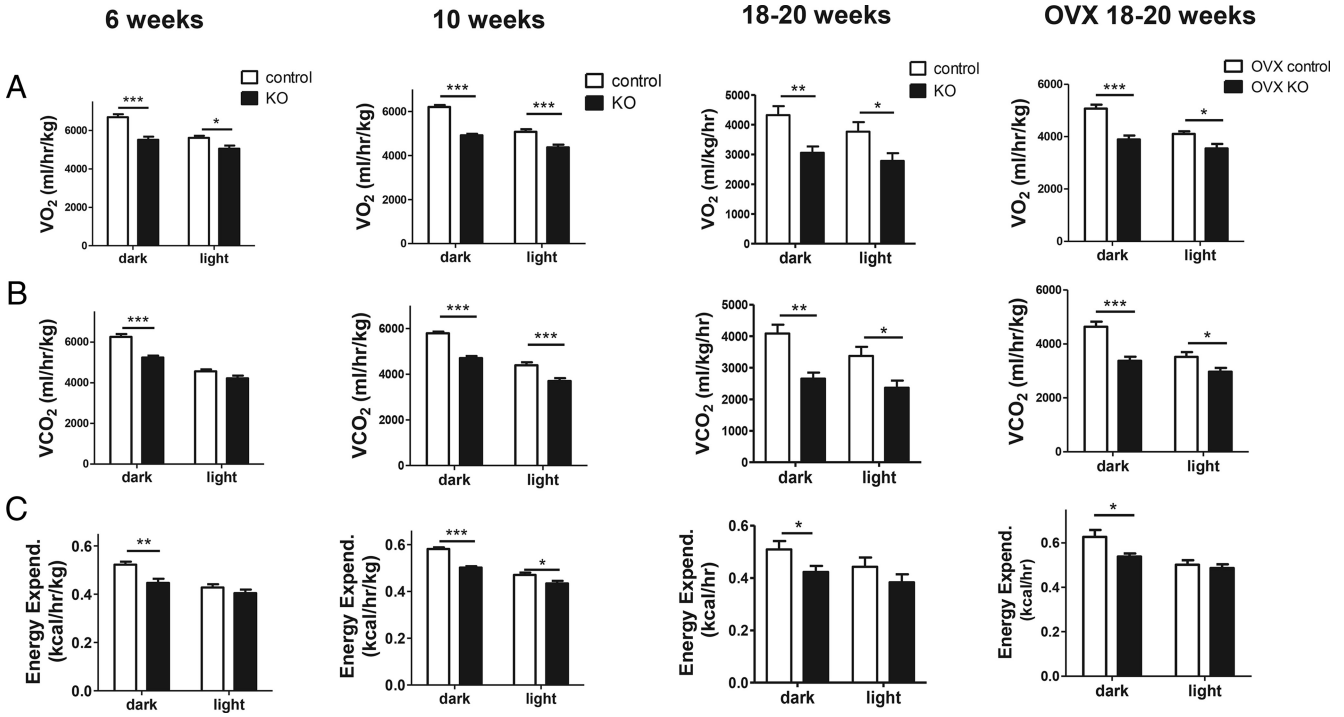

Figure 3. CLAMS cage assessments of gas exchange and energy expenditure in light and dark cycles. VO_2 (A), VCO_2 (B), and energy expenditure (C) in 6 week, 10 week, and 18–20 week old *Kiss1r* KO and control females. n = 7–9 per group. For the cohort of 18–20 week OVX mice, both genotypes were ovariectomized at 2.5 weeks of age and remained without gonadal sex steroids for the rest of the study. The gonad-intact 18–20 week old CLAMS data (third column of graphs) is reused with permission from our previous publication (14) and is shown for comparison purposes to the other ages. $*P < .05$, $**P < .01$, $***P < .001$.

rates and energy expenditure are also significantly reduced to a substantial degree at both 6 and 10 weeks of age. These data support the likelihood that permanent or early life decreases in metabolism and energy expenditure underlie the eventual emergence of the obese phenotype of adult *Kiss1r* KO females.

Unexpectedly, even though they weigh much more, adult *Kiss1r* KO females display decreased food intake rather than the expected hyperphagia, and we report here for the first time that adult KO females also have significantly decreased water intake as well. Interestingly, although adiposity, leptin levels, metabolic rates, and energy expenditure are all already showing significant elevations or decreases at 6 weeks of age, we found that the decreases in feeding and water intake are not yet present in young adults at that age, and only minor changes are present (primarily during the light phase only) for 10 week old females. These findings suggest that these feeding changes may be secondary side effects to the onset of obesity, perhaps as compensatory attempt to the higher body weight and adiposity. In fact, we hypothesize that the elevated leptin levels in KO's serve to act in the brain to lower food intake, as is well established, but this does not emerge in the KOs until older ages when the adiposity and leptin levels are high enough to provide robust leptin feedback on feeding circuits.

Like feeding and water intake, we found that defects in glucose metabolism were not present yet in KOs at 6 weeks of age, and only very minor impairments were seen at 10 weeks of age. Like the food intake data, these findings similarly imply that the impairments in glucose homeostasis present in older adult KOs are secondary (ie, a side effect) to the higher body weight phenotype, and may be due to the increased inflammation commonly seen in obese states. Indeed, increasing evidence now suggests that chronic low-grade inflammation caused by increased macrophage infiltration of adipose tissue may directly cause insulin resistance, leading to glucose intolerance and type 2 diabetes (21–24).

In our previous study, we determined that the high body weights of 18–20 weeks old KO females were still present relative to control females even when both genotypes had been chronically without gonadal sex steroids since an early juvenile age (14). This suggested that the body weight phenotype was not simply a product of the KOs hypogonadism. However, additional metabolic measures beyond body weight were not assessed in that study. In the present study, we similarly studied 18–20 week old females of both genotypes that had been chronically ovariectomized earlier at 2.5 weeks old. These adult OVX KOs still demonstrated all the metabolic impairments observed in the gonad-intact KOs, including higher fat mass, elevated leptin levels, reduced VO2, lower VCO2, decreased energy expenditure, lower food and water intake, and significantly impaired glucose tolerance. Thus, all facets of the KOs metabolic phenotype were recapitulated even when both genotypes were similarly without gonadal sex steroids since a very young juvenile age. This further emphasizes that the obesity and metabolic status of these mice is not simply due to chronic lack of gonadal sex steroids,

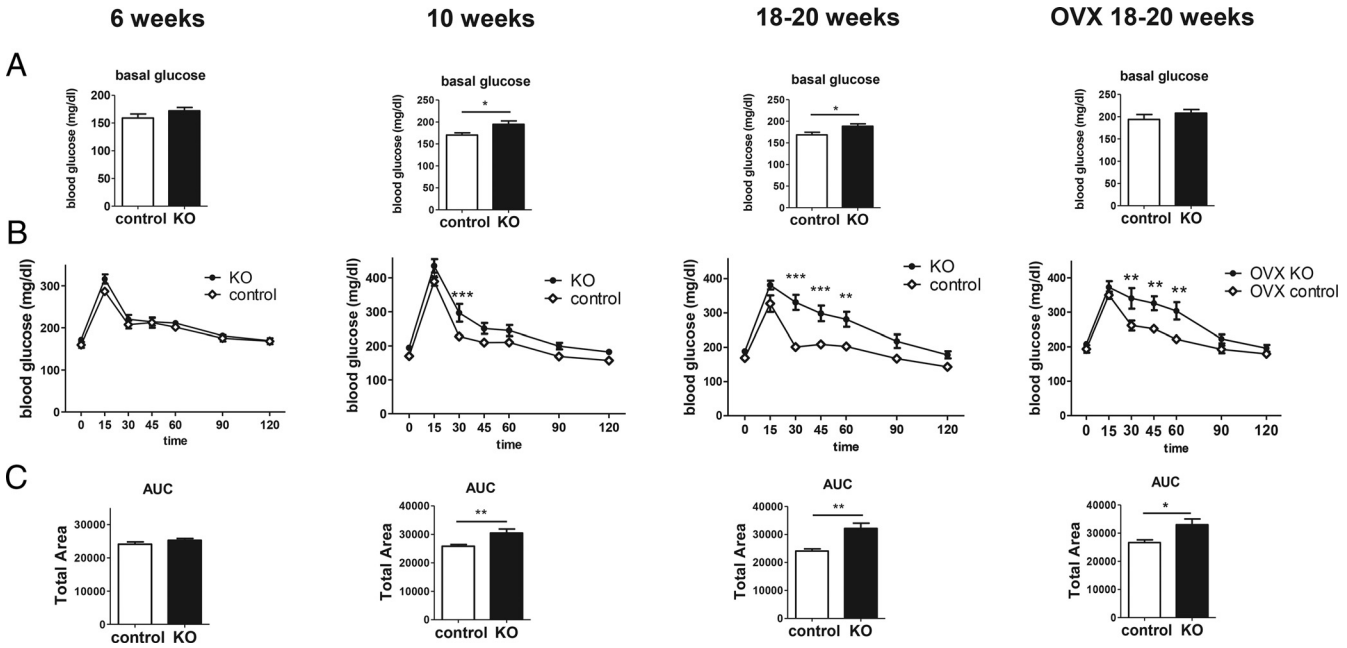

Figure 4. Determination of glucose homeostasis with i.p. glucose tolerance testing (GTT). Basal glucose (A), GTT (B), and area under the curve (AUC) (C) at 6 weeks, 10 weeks, and 18–20 weeks of age. For the cohort of 18–20 week OVX mice, both genotypes were ovariectomized at 2.5 weeks of age and remained without gonadal sex steroids for the rest of the study. n = 8–13 per group, $*P < .05$, $**P < .01$, $***P < .001$.

but rather reflects a role for kisspeptin signaling outside the reproductive axis, supported by the presence of kisspeptin and Kiss1r in several metabolic tissues.

Based on our present data on the developmental timing of different measures, we propose that impaired kisspeptin signaling inherently causes lower metabolism and energy expenditure (by mechanisms yet to be elucidated), which thereby drives increased adiposity. This increased adiposity eventually accumulates enough over development and young adulthood to later produce first an overweight and, eventually, an obese state in older adult ages. Thus, the obesity phenotype in *Kiss1r* KO females is likely due to early developmental defects in metabolism that manifest prior to detectable body weight differences. Conversely, significant alterations in feeding, water intake, and glucose homeostasis emerge at much later ages in KOs, after they have already displayed higher adiposity, body weights, and leptin levels. We therefore hypothesize that the "primary" changes in metabolism, body weight, and adiposity (and, by association, leptin levels) then likely lead to eventual "secondary" responses in food consumption and impairments in glucose homeostasis later in mid to older adulthood. This model would suggest that kisspeptin signaling itself is not a major direct regulator of food intake, water intake, or glucose processing, but rather can influence these physiological processes indirectly by altering metabolism, adiposity/leptin, and inducing an obese state. Future studies examining the role of kisspeptin signaling in metabolic health should focus on ascertaining the mechanistic role of kisspeptin in metabolism and energy expenditure, as the other parameters are likely secondary to these primary phenotypes.

Acknowledgments

We thank Elena Luo, Matthew Poling, and Shannon Stephens for their technical and experimental support.

Address all correspondence and requests for reprints to: Dr. Alexander S. Kauffman, Department of Reproductive Medicine, University of California, San Diego, Leichtag Building 3A-15, 9500 Gilman Drive, #0674, LA Jolla, CA 92 093, Phone: 858–246-0219, Fax: 858–534-1438, Email: akauffman@ucsd.edu

Disclosure Summary: The authors have nothing to disclose.

This work was supported by **Grant Support:** This research was supported by NSF IOS-1 025 893, NIH R01 HD082567, and NIH P30 DK063491. Additional support provided by the Eunice Kennedy Shriver NICHD/NIH (SCCPIR) grant U54-HD012303 (U.C. San Diego). KPT was supported by NIH F32 HD076606.

References

1. **Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS, Jr., Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF, Jr., Aparicio SA, Colledge WH.** The GPR54 gene as a regulator of puberty. *N Engl J Med*. 2003;349:1614–1627.
2. **Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B.** Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N Engl J Med*. 2012;366:629–635.
3. **Uenoyama Y, Nakamura S, Hayakawa Y, Ikegami K, Watanabe Y, Deura C, Minabe S, Tomikawa J, Goto T, Ieda N, Inoue N, Sanbo M, Tamura C, Hirabayashi M, Maeda KI, Tsukamura H.** Lack of pulse and surge modes and glutamatergic stimulation of luteinising hormone release in Kiss1 knockout rats. *J Neuroendocrinol*. 2015;27:187–197.
4. **Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F, Le WW, Hoffman GE, Seminara SB.** Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. *Endocrinology*. 2007;148:4927–4936.
5. **Kirilov M, Clarkson J, Liu X, Roa J, Campos P, Porteous R, Schutz G, Herbison AE.** Dependence of fertility on kisspeptin-Gpr54 signaling at the GnRH neuron. *Nat Commun*. 2013;4:2492.
6. **Novaira HJ, Sonko ML, Hoffman G, Koo Y, Ko C, Wolfe A, Radovick S.** Disrupted kisspeptin signaling in GnRH neurons leads to hypogonadotrophic hypogonadism. *Mol Endocrinol*. 2014;28:225–238.
7. **Brown RE, Imran SA, Ur E, Wilkinson M.** KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. *Mol Cell Endocrinol*. 2008;281:64–72.
8. **Hauge-Evans AC, Richardson CC, Milne HM, Christie MR, Persaud SJ, Jones PM.** A role for kisspeptin in islet function. *Diabetologia*. 2006;49:2131–2135.
9. **Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M.** Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature*. 2001;411:613–617.
10. **Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M.** The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem*. 2001;276:34631–34636.
11. **Herbison AE, de Tassigny X, Doran J, Colledge WH.** Distribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. *Endocrinology*. 2010;151:312–321.
12. **Hussain MA, Song WJ, Wolfe A.** There is Kisspeptin - And Then There is Kisspeptin. *Trends Endocrinol Metab*. 2015;26:564–572.
13. **Song WJ, Mondal P, Wolfe A, Alonso LC, Stamateris R, Ong BW, Lim OC, Yang KS, Radovick S, Novaira HJ, Farber EA, Farber CR, Turner SD, Hussain MA.** Glucagon regulates hepatic kisspeptin to impair insulin secretion. *Cell Metab*. 2014;19:667–681.
14. **Tolson KP, Garcia C, Yen S, Simonds S, Stefanidis A, Lawrence A, Smith JT, Kauffman AS.** Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. *J Clin Invest*. 2014.
15. **Poling MC, Kauffman AS.** Sexually dimorphic testosterone secretion in prenatal and neonatal mice is independent of kisspeptin-Kiss1r and GnRH signaling. *Endocrinology*. 2012;153:782–793.
16. **Dungan HM, Gottsch ML, Zeng H, Gragerov A, Bergmann JE, Vassilatis DK, Clifton DK, Steiner RA.** The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonad-

otropin-releasing hormone/luteinizing hormone. *Journal of Neuroscience*. 2007;27:12088–12095.
17. **Dror T, Franks J, Kauffman AS.** Analysis of multiple positive feedback paradigms demonstrates a complete absence of LH surges and GnRH activation in mice lacking kisspeptin signaling. *Biol Reprod*. 2013;88:146.
18. **Fu LY, van den Pol AN.** Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. *J Neurosci*. 2010;30:10205–10219.
19. **Stengel A, Wang L, Goebel-Stengel M, Tache Y.** Centrally injected kisspeptin reduces food intake by increasing meal intervals in mice. *Neuroreport*. 2011;22:253–257.
20. **Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ.** Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. *Endocrinology*. 2010;151:2233–2243.
21. **Olefsky JM, Glass CK.** Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol*. 2010;72:219–246.
22. **Hellbronn LKaLVC.** Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des*. 2008;14:1225–1230.
23. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 2003;112:1821–1830.
24. **Shoelson SE, Lee J, Goldfine AB.** Inflammation and insulin resistance. *J Clin Invest*. 2006;116:1793–1801.