potential disease mechanisms as well as for testing therapeutic strategies (15). Novel and sometimes unexpected discoveries made in animal models often become the foundation for studies using patient material, which can confirm the clinical relevance of the findings. Availability of a wide range of animal models with different advantages and varying degrees of validity in different domains directly affects the pace of advancement in translational and clinical research for diseases of the brain. The mouse models produced by Liang and collaborators are likely to play an important role in these endeavors in the dystonia research field.

Address correspondence to: Deniz Kirik, B.R.A.I.N.S. Unit, BMC D11, 221 84 Lund, Sweden. Phone: 46.46.2220564; Fax: 46.46.2223436; E-mail: deniz.kirik@med.lu.se.

1. Liang C-C, Tanabe LM, Jou S, Chi F, Dauer WT. TorsinA hypofunction causes abnormal twisting movements and sensorimotor circuit neurodegeneration. *J Clin Invest*. 2014;124(7):3080–3092.
2. Oppenheim H. Ueber eine eigenartige Krampfkrankheit des kindlichen und jugendlichen Alters (dysbasia lordotica progressiva, dystonia musculorum deformans) *Neurol Centralbr*. 1911;75:323–345.
3. Standaert DG. Update on the pathology of dystonia. *Neurobiol Dis*. 2011;42(2):148–151.
4. Albanese A, et al. Phenomenology and classification of dystonia: a consensus update. *Mov Disord*. 2013; 28(7):863–873.
5. Kramer PL, et al. Dystonia gene in Ashkenazi Jewish population is located on chromosome 9q32-34. *Ann Neurol*. 1990;27(2):114–120.
6. Ozelius LJ, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. *Nat Genet*. 1997;17(1):40–48.
7. Granata A, Schiavo G, Warner TT. TorsinA and dystonia: from nuclear envelope to synapse. *J Neurochem*. 2009;109(6):1596–1609.
8. Charlesworth G, Bhatia KP, Wood NW. The genetics of dystonia: new twists in an old tale. *Brain*. 2013; 136(pt 7):2017–2037.
9. Rostasy K, et al. TorsinA protein and neuropathology in early onset generalized dystonia with GAG deletion. *Neurobiol Dis*. 2003;12(1):11–24.
10. Argyelan M, et al. Cerebellothalamocortical connectivity regulates penetrance in dystonia. *J Neurosci*. 2009; 29(31):9740–9747.
11. Oleas J, Yokoi F, Deandrade MP, Pisani A, Li Y. Engineering animal models of dystonia. *Mov Disord*. 2013;28(7):990–1000.
12. Goodchild RE, Kim CE, Dauer WT. Loss of the dystonia-associated protein torsinA selectively disrupts the neuronal nuclear envelope. *Neuron*. 2005; 48(6):923–932.
13. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993;72(6):971–983.
14. Pouladi MA, Morton AJ, Hayden MR. Choosing an animal model for the study of Huntington's disease. *Nat Rev Neurosci*. 2013;14(10):708–721.
15. Crook ZR, Housman D. Huntington's disease: can mice lead the way to treatment? *Neuron*. 2011; 69(3):423–435.

Cardiac fibroblasts in pressure overload hypertrophy: the enemy within?

Nenad Bursac

Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA.

Cardiac fibroblasts have been long recognized as active participants in heart disease; however, their exact physiological and pathological roles remain elusive, mainly due to the lack of specific markers. In this issue of the *JCI*, Moore-Morris and colleagues used a fibroblast-specific collagen1a1-*GFP* reporter to demonstrate that fibroblast accumulation after aortic banding in murine hearts arises almost exclusively from proliferation of resident fibroblasts originating from both the epicardium and a previously unrecognized source, the endocardium. Further characterization of fibroblast origin and function in different types and stages of heart disease could lead to development of improved fibroblast-targeted cardiac therapies.

Lack of specific cardiac fibroblast markers

Cardiac fibroblasts comprise 30% to 70% of all the cells in the healthy adult heart (1). The number of fibroblasts in the heart is not constant and changes dynamically with development, disease, and aging (2, 3). Traditionally, cardiac fibroblasts have been thought to play passive roles in the heart and to be solely responsible for maintaining homeostasis of extracellular matrix proteins, including type I and III collagens and fibronectin. Due to their ubiquitous presence in the heart, fibroblasts are well poised to actively regulate and modify cardiac function through their direct contacts with other cardiac cells and matrix as well as through secretion of different cytokines, matrix proteins, and proteases. Over the last decade, the pleiotropic roles of fibroblasts in cardiac biology and disease have been studied extensively (reviewed in refs. 4, 5); however, the lack of specific and comprehensive markers of fibroblast phenotypes has hampered the progress in this important research field. In particular, vimentin, the most inclusive marker of cardiac fibroblasts, also labels all other mesoderm-derived cells in the heart. Thymus cell antigen-1 (Thy-1, also known as CD90), discoidin domain receptor-2 (DDR2), prolyl-4-hydroxylase (P4H), transcription factor 21 (TCF21, also known as epicardin, Pod1, and capsulin), periostin, cadherin-11, and fibroblast-specific protein-1 (FSP1, also known as S100A4) have all been used to study cardiac fibroblasts, but all of these markers label only a subset of fibroblasts, have poor expression in the healthy adult heart, or nonselectively label endothelial, smooth muscle, or immune/inflammatory cells (6). Because the pathological tissue remodeling that is secondary to cardiac injury and inflammation involves contributions from both resident and extracardiac cells, the lack of adequate markers for cardiac fibroblasts may lead to erroneous conclusions about their origin, roles, and potential to be therapeutically targeted in fibrotic heart disease.
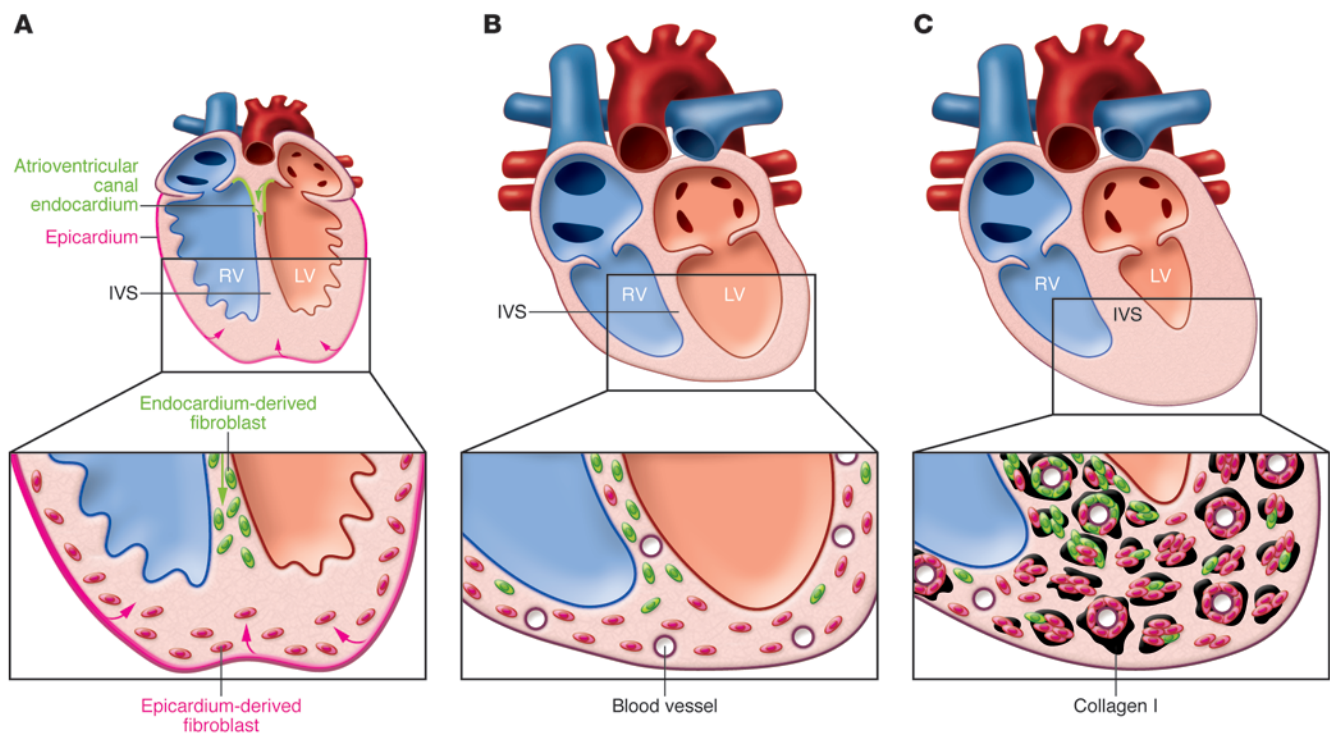
Figure 1
Cardiac fibroblasts in developing, adult, and pressure overloaded hearts. (**A**) In the embryonic heart, most fibroblasts are derived from epicardium (pink). The fibroblast lineage described by Moore-Morris et al. is generated from the atrioventricular canal endocardium (green) and migrates down the IVS. (**B**) In the adult heart, the dominant fraction of fibroblasts (~90%) in the RV and LV are of epicardial origin, while 64% of fibroblasts in the IVS derive from the endocardium. (**C**) In response to pressure overload, the heart becomes hypertrophic and undergoes fibrosis. In response to pressure overload, both epicardium-derived and endocardium-derived fibroblast lineages proliferate and accumulate in interstitial and perivascular regions along with collagen I (black). The LV undergoes more fibrosis than the RV. Endocardium-derived fibroblasts participate in perivascular fibrosis in the IVS but not in the LV or RV. These two resident lineages account for approximately 95% of fibroblasts in the healthy and pressure-overloaded ventricles.

Collagen1a1-*GFP*, but not FSP1, is a robust cardiac fibroblast marker

In this issue of the *JCI*, Moore-Morris and colleagues (7) used mice in which GFP expression is driven by a collagen1a1 enhancer (8) to investigate the origin and fate of cardiac fibroblasts in healthy hearts and in pressure overload hypertrophy induced by transverse aortic constriction (TAC). In the healthy ventricles, collagen1a1-*GFP* was expressed robustly in interstitial noncardiomyocytes that were also positive for vimentin and PDGFRα, a mesenchymal marker shown recently to label cardiac fibroblasts (9). This population of GFP⁺ cells also expressed Thy-1 (in 70% of cells) but did not express markers of endothelial cells (PECAM1), pericytes (PDGRFβ), leukocytes (CD45), or smooth muscle cells (αSMA). After TAC, GFP⁺ cells maintained PDGFRα expression, did not express other cell type–specific markers, and accumulated in both interstitial and perivascular areas rich in collagen I.

On the other hand, FSP1, which is frequently used as a cardiac fibroblast marker (10), was almost exclusively expressed in CD45⁺ cells, with no expression detected in interstitial GFP⁺ fibroblasts and a limited presence in perivascular GFP⁺ fibroblasts; therefore, similar to previous studies (11, 12), FSP1 preferentially labeled immune cells. Hence, Moore-Morris et al. have identified collagen1a1-*GFP* as a robust and specific marker of cardiac fibroblasts in both healthy and pressure-overloaded hearts. It remains to be determined whether all fibroblasts in the heart are labeled with this marker.

Fibroblast origin in developing, adult, and pressure-overloaded hearts

By using the collagen1a1-*GFP* reporter along with constitutive and inducible Cre-based lineage tracing of epicardial (*Wt1* and *Tbx18*), endocardial/endothelial (*Tie2*, VE-cadherin, *Nfatc1*), and hematopoietic (*Vav*) populations, Moore-Morris

and colleagues have further explored the origin of cardiac fibroblasts in developing, adult, and pressure-overloaded hearts. Traditionally, all nonvalvular fibroblasts in the healthy heart have been thought to derive from epicardium (2). Thus, it was surprising that epicardial-specific Cre drivers labeled only 30% of GFP⁺ cells in the interventricular septum (IVS), while 92% of GFP⁺ cells in the LV and RV were of epicardial origin. Even more surprising, complementary numbers of nonepicardium-derived fibroblasts (64% in the IVS and 12% in the LV and RV) were found to be of endocardial/endothelial origin, characteristic of valvular interstitial cells (13). Together, in healthy ventricles, 95% of all GFP⁺ fibroblasts were of endocardial or epicardial origin, and none of them were hematopoietically derived, as the *Vav-Cre* driver exclusively labeled CD45⁺ or PECAM1⁺ cells. After aortic bending, both epicardial- and endocardial-derived fibroblasts underwent transient prolifera-

tion while maintaining their relative proportions within the GFP+ population and spatial distributions in the LV, RV, IVS, interstitial and perivascular regions. Furthermore, the resulting fibroblast accumulation was not the result of de novo activation of the epicardium or endothelium/endocardium (in contrast to ref. 10) or caused by differentiation of hematopoietic cells. By performing additional lineage tracing, Moore-Morris and colleagues showed that the newly identified endocardial fibroblast lineage in the adult myocardium is created in early embryos by endothelial-to-mesenchymal transition of atrioventricular canal endocardium during cushion formation (Figure 1A).

Taken together, these results strongly suggest that the vast majority of fibroblasts in the adult mouse heart derive from two resident lineages, a previously characterized epicardial-derived population and a newly described endocardial-derived population (Figure 1B). These two fibroblast lineages have a specific spatial distribution in different regions of the heart as well as in perivascular and interstitial space. During pressure overload (Figure 1C), localized proliferation of these fibroblasts (without substantial migration) and their persistence, rather than de novo epicardial and endothelial activation or differentiation of hematopoietic cells is the main driver of fibrosis and thus has potential as a therapeutic target. The origin of the remaining GFP+ fibroblasts (5%–6% of fibroblasts) that are not derived from the epicardium or endocardium is currently unknown, and, as suggested by Moore-Morris et al., these cells could arise from other resident or nonhematopoietic circulating progenitors and may also contribute to fibrosis. An inducible collagen1a1-*Cre* mouse line, when available, could further help elucidate the fibroblast fate in cardiac pathology.

Future studies and therapeutic implications

As with any good study, the findings by Moore-Morris et al. open multiple avenues for future investigation. Surprisingly, even after 4 weeks of TAC, none of the perivascular fibroblasts and only 15% of interstitial fibroblasts expressed the myofibroblast marker αSMA, suggesting that, unlike in healing myocardial infarcts (14), myofibroblasts in pressure overload hypertrophy are not abundant or associated with the collagen accumulation. Furthermore, as recently shown by

Braitsch et al. (15) and confirmed in the study by Moore-Morris and colleagues, pressure overload does not induce epicardial activation and thickening observed in myocardial infarction. Together, these results suggest that the specific fibrogenic roles of resident fibroblasts and myofibroblasts (through proliferation and/or migration), epicardial and endothelial activation, and pericyte- or bone marrow–derived progenitors may differ depending on the underlying heart pathology (e.g., myocardial infarction, congenital cardiomyopathy, diabetes, hypertension, etc.). While disease-specific differences in the mechanical environment (16) or the level of inflammation in the heart may favor a particular fibrotic response, detailed studies in the mouse lines used by Moore-Morris et al. are warranted and will help to answer these important questions.

Curiously, Moore-Morris and colleagues found that fibroblast proliferation peaks at 4 to 7 days after TAC and ceases thereafter, as previously reported (17). Simultaneously, the degree of fibrosis remained steady, and while ventricles developed hypertrophy, cardiac function decreased only slightly, without signs of cardiomyocyte apoptosis. Taken together, the 4-week remodeling after aortic banding in the study by Moore-Morris et al. appears to be mostly adaptive rather than maladaptive. On the other hand, molecular changes in cardiac fibroblasts associated with irreversible maladaptive remodeling may represent the most appropriate targets for fibroblast-specific therapies. It will be important for future investigations to examine the fate of cardiac fibroblasts in the later phases of hypertrophic disease, including accelerated pathological remodeling and subsequent heart failure. Furthermore, transcriptional profiling at 7 days after TAC revealed that both the epicardial and endocardial fibroblast lineages underwent similar gene expression changes in response to pressure overload, including upregulation of anti-death–associated genes. This observation implies that timely proapoptotic targeting of all resident cardiac fibroblasts, by inhibition of miR-21 or activation of miR-29 (18) for example, has potential as a promising strategy to alleviate fibrosis in the heart. It should be noted that, despite the global nature of pressure overload, the resulting fibrotic sequela occurs in distinct interstitial and perivascular lesions. Why these particular regions of the myocardium are

especially prone to fibrogenesis remains unknown. In fact, one could even imagine the existence of "fibrogenic niches" throughout the heart in which certain highly responsive fibroblasts and/or surrounding biochemical or biomechanical cues locally drive cardiac fibrosis.

Most importantly, the relevance of the findings by Moore-Morris et al. for human heart disease and therapy remains to be determined (19, 20). Undoubtedly, an improved understanding of cardiac fibroblast origin, fate, and interaction with other cell types in the heart as well as the complex roles of cardiac fibroblasts in cardiac development, function, and disease will be fundamental to our ability to use these cells as a future therapeutic tool. The study by Moore-Morris et al. is a step in the right direction.

Address correspondence to: Nenad Bursac, Department of Biomedical Engineering, Duke University, 3000 Science Drive, Hudson Hall, Rm 136, Durham, North Carolina 27708-90281, USA. Phone: 919.660.5510; Fax: 919.684.4488; E-mail: nbursac@duke.edu.

1. Souders CA, Bowers SL, Baudino TA. Cardiac fibroblast: the renaissance cell. *Circ Res*. 2009; 105(12):1164–1176.
2. Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. *Circ Res*. 2009; 105(10):934–947.
3. Biernacka A, Frangogiannis NG. Aging and cardiac fibrosis. *Aging Dis*. 2011;2(2):158–173.
4. Hirsch E, Nagai R, Thum T. Heterocellular signalling and crosstalk in the heart in ischaemia and heart failure. *Cardiovasc Res*. 2014;102(2):191–193.
5. Sadoshima J, Weiss JN. Cardiac fibroblasts: The good, the bad, the ugly, the beautiful. *J Mol Cell Cardiol*. 2014;70:1.
6. Krenning G, Zeisberg EM, Kalluri R. The origin of fibroblasts and mechanism of cardiac fibrosis. *J Cell Physiol*. 2010;225(3):631–637.
7. Moore-Morris T, et al. Resident fibroblast lineages mediate pressure overload–induced cardiac fibrosis. *J Clin Invest*. 2014;124(7):2921–2934.
8. Yata Y, et al. DNase I-hypersensitive sites enhance alpha1(I) collagen gene expression in hepatic stellate cells. *Hepatology*. 2003;37(2):267–276.
9. Smith CL, Baek ST, Sung CY, Tallquist MD. Epicardial-derived cell epithelial-to-mesenchymal transition and fate specification require PDGF receptor signaling. *Circ Res*. 2011;108(12):e15–e26.
10. Zeisberg EM, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med*.

2007;13(8):952–961.

11. Osterreicher CH, et al. Fibroblast-specific protein 1 identifies an inflammatory subpopulation of macrophages in the liver. *Proc Natl Acad Sci U S A.* 2011; 108(1):308–313.

12. Kong P, Christia P, Saxena A, Su Y, Frangogiannis NG. Lack of specificity of fibroblast-specific protein 1 in cardiac remodeling and fibrosis. *Am J Physiol Heart Circ Physiol.* 2013;305(9):H1363–H1372.

13. Eisenberg LM, Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. *Circ Res.* 1995;77(1):1–6.

14. Shinde AV, Frangogiannis NG. Fibroblasts in myocardial infarction: A role in inflammation and repair. *J Mol Cell Cardiol.* 2014;70C:74–82.

15. Braitsch CM, Kanisicak O, van Berlo JH, Molkentin JD, Yutzey KE. Differential expression of embryonic epicardial progenitor markers and localization of cardiac fibrosis in adult ischemic injury and hypertensive heart disease. *J Mol Cell Cardiol.* 2013; 65:108–119.

16. Hinz B. The myofibroblast: paradigm for a mechanically active cell. *J Biomech.* 2010;43(1):146–155.

17. Souders CA, Borg TK, Banerjee I, Baudino TA. Pres-

sure overload induces early morphological changes in the heart. *Am J Pathol.* 2012;181(4):1226–1235.

18. Zhu H, Fan GC. Role of microRNAs in the reperfused myocardium towards post-infarct remodelling. *Cardiovasc Res.* 2012;94(2):284–292.

19. Hasenfuss G. Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovasc Res.* 1998;39(1):60–76.

20. Masci PG, et al. Incremental prognostic value of myocardial fibrosis in patients with non-ischemic cardiomyopathy without congestive heart failure. *Circ Heart Fail.* 2014;7(3):448–456.

Fatness and fertility: which direction?

Stephanie B. Seminara

Harvard Reproductive Sciences Center and Reproductive Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA.

Metabolic status has long been thought to determine reproductive status, with abnormal metabolic phenotypes altering reproductive cascades, such as the onset of puberty. In this issue of the *JCI*, Tolson and colleagues provide evidence that kisspeptin, a hormone that promotes sexual maturation, regulates metabolism. Female mice lacking the kisspeptin receptor (KISS1R) gained more weight than control animals, and this weight gain was caused not by increased food consumption, but by an overall decrease in energy and metabolism. While this study provides a direct link between the kisspeptin pathway and metabolic output, more work will need to be done to determine whether alterations in this pathway contribute to human obesity.

Leptin and kisspeptin: linking metabolism and reproduction?

The concept that a minimum weight-to-height ratio is necessary for the onset and maintenance of menstrual cycles was first introduced in the 1970s; however, uncovering the physiologic pathways that connect reproduction and metabolism has been challenging (1, 2). In the 1990s, the discovery that patients deficient for leptin (a cell-signaling hormone critical for weight regulation) or leptin signaling have abnormal pubertal development led many to hail leptin as the long-sought link between energy stores and reproductive function (3, 4). Then in 2003, inactivating mutations in the gene encoding the kisspeptin receptor (*KISS1R*) were found to promote hypogonadotropic hypogonadism in mice and humans, catapulting the kisspeptin signaling pathway into the spotlight as a critical stimulus for activating gonadotropin-releasing hormone (GnRH) secretion and initiation of the hypothalamic-pituitary-gonadal cascade (5, 6). Through numer-

ous neuroanatomic, electrophysiologic, pharmacologic, and genetic studies, the kisspeptin system was proven to be a key element in reproductive maturation and function. Soon after the discovery of kisspeptin's role in regulating reproduction, investigators began to explore the possibility of a link between energy status and the kisspeptin system. States of negative energy balance, such as food deprivation, were found to induce suppression of the hypothalamic kisspeptin system, while administration of kisspeptin ameliorated the reproductive failure associated with undernutrition (7). Furthermore, leptin administration to leptin-deficient mice increased kisspeptin expression (8); therefore, momentum grew for the hypothesis that nutritional signals, such as leptin, acted through kisspeptin-expressing neurons to determine the timing of sexual maturation.

Enthusiasm for the hypothesis that leptin and kisspeptin coordinate the onset of sexual maturation dampened in 2011 following a report that selective deletion of leptin receptors from kisspeptin-expressing neurons did not affect sexual maturation and fertility (9). This surprising finding suggested that direct leptin action on kisspeptin neurons is not required for puberty

onset in mice; however, this study did not eliminate that possibility that leptin-dependent effects on reproduction are indirectly transmitted through kisspeptin-expressing neurons, potentially adding layers of complexity to the regulation of these hypothalamic networks.

Metabolic status determines reproduction — or does it?

As work on the relationship between metabolism and kisspeptin progressed, it appeared that the directional arrow in this association begins with the metabolic status of the organism (i.e., undernutrition, overnutrition, or lactation), which then leads to abnormal phenotypes in the reproductive cascade (delayed puberty or infertility). In this issue of the *JCI*, Tolson et al. have turned this paradigm on its head and present data suggesting that perturbations in kisspeptin signaling affect metabolism (10). Female, but not male, mice lacking KISS1R weighed significantly more than control animals. Furthermore, *Kiss1r⁻/⁻* females had increased fat mass, hyperleptinemia, higher fasting glucose, and impaired glucose tolerance in the setting of reduced metabolism and energy expenditure. Moreover, ovariectomized KISS1R-deficient females weighed more than ovariectomized controls, which suggests that the obesity phenotype is independent of differences in gonadal steroids due to loss of kisspeptin signaling.

Notably, weight differences between *Kiss1r⁻/⁻* females and control animals began to emerge at eight to ten weeks of age and continued to increase out to 18 weeks (10). Unfortunately, there are