Emerging Function of Fat Mass and Obesity-Associated Protein (Fto)

Timo D. Müller, Matthias H. Tschöp, Susanna Hofmann

1 Institute for Diabetes and Obesity, Helmholtz Zentrum München, Munich, Germany, 2 Department of Medicine, Technische Universität München, Munich, Germany, 3 Institute of Experimental Genetics, Helmholtz Zentrum München, Munich, Germany

Genome-wide association studies (GWAS) are a laborious but powerful tool to identify genetic risk factors associated with complex polygenic traits such as obesity [1], diabetes [2], or coronary artery disease [3]. The link between genetic variation in FTO and obesity was first described in a GWAS for type 2 diabetes [1] and was later independently confirmed in different populations all over the world. First described in 2007, genetic variation in FTO has since become one of the most solidly confirmed risk factor for polygenic obesity in humans; yet, information about how FTO affects metabolism is still scarce. Bioinformatic analyses suggest FTO codes for a Fe(II)- and 2-oxoglutarate-dependent nucleic acid demethylase [4,5] that catalyzes demethylation of 3-methylthymine in single-stranded DNA [5]. However, how this proposed function of FTO is integrated into the complex network of energy metabolism control remains the object of intense scientific investigation.

Analyses of genetically engineered mouse models, in which the function of Fto is either eliminated [6–8] or enhanced [9], support a role of Fto in energy metabolism but are inconsistent as to whether Fto modulates caloric intake, energy expenditure, or both. In 2009, global lack of Fto was reported to result in a lean phenotype as a consequence of increased energy expenditure [7]. Similar results were reported by another study [8], but both studies share two pitfalls. First, interpreting energy expenditure differences can be challenging when body composition differences are also present (see below). Second, germline loss of Fto causes perinatal lethality and growth retardation, which may give rise to secondary effects that are unrelated to the mechanism by which variation in FTO affects human metabolism [7,8]. Notably, homozygous mice carrying a loss-of-function missense mutation in the C-terminal domain of Fto (367F) show no signs of perinatal lethality or growth retardation; they are lean, exhibit normal food intake and, when normalized by body weight, show increased energy expenditure [6]. While these studies appear to point to a potential role of Fto in regulating energy expenditure rather than food intake, mice globally overexpressing Fto are obese, hyperphagic, and exhibit normal energy expenditure when corrected for body fat or lean tissue mass [9]. In line with these data, most human studies report that obesity-predisposing FTO alleles are associated with increased food intake, but not energy expenditure (Table 1) [10–13].

In summary, despite intense scientific discussion about whether Fto primarily affects energy expenditure or food intake [14], it remains unclear what role Fto plays in early development compared to adult life and which tissues and/or brain regions are involved in mediating the effects seen in the global Fto knock-out (ko) mice. An important step in solving these questions has now been taken by Roger Cox and colleagues. In the current issue of *PLOS Genetics*, McMurray et al. [15] report a series of elegant studies further elucidating the complexity of Fto with respect to how, when, and where it is most relevant for energy metabolism and shedding new light on the recently proposed role of Fto in protein metabolism [16].

In their manuscript, the authors recapitulate that germline loss of Fto leads to perinatal lethality, growth retardation, and a lower body weight that is accompanied by decreased body fat and lean tissue mass. However, in contrast to previous reports, the authors convincingly show that there is no difference in energy expenditure when the data are interpreted correctly, i.e., using a regression approach that takes into account potential confounding by differences in lean body mass. Several review articles have recently highlighted this issue [17,18], and it is now clear that simply dividing raw energy expenditure results by lean body mass can lead to spurious conclusions. (This is illustrated quite nicely in Figure 3 of McMurray et al., where an apparent difference of energy expenditure per gram of lean body mass vanishes upon regression adjustment using ANCOVA.) Interestingly, total food intake was not changed in the germline Fto ko mice, whereas the ratio between CO2 production and O2 consumption (respiratory exchange ratio; RER) was decreased, suggesting that Fto ablation promotes protein and/or fat utilization.

To circumvent the challenge of perinatal lethality and growth retardation, McMurray et al. used tamoxifen-inducible ubiquitin-cre mice to delete Fto at the time of sexual maturity. These adult onset Fto ko mice showed no increased lethality or growth retardation but had a lower body weight accompanied by a decreased lean mass and, interestingly, an increased body fat mass. No changes were observed in energy expenditure or total food intake, but, similar to the germline Fto ko, RER decreased in adult onset Fto ko mice, an effect also noted in a recent human study [19].

As central nervous system (CNS)-specific Fto deletion was recently reported to recapitulate the phenotype of the germline Fto ko mice [8], McMurray et al. further used an adenoviral associated approach to specifically knock out Fto in the mediobasal hypothalamus (MBH). Interestingly,
these adult onset hypothalamic Fto ko mice showed no change in total body weight or body composition compared to sham controls but displayed a slightly decreased body weight gain that was accompanied by decreased food intake without any change in energy expenditure or RER. Taken together, these data indicate that although perturbation of Fto signaling in the MBH can impact food intake, sites other than the hypothalamus may be more important for Fto’s influence on body composition and energy homeostasis.

In summary, Cox and colleagues with their current publication have made several important contributions that allow for a better understanding and potentially improved targeting of Fto signaling in the control of energy homeostasis. The authors make a convincing case that Fto may not directly affect energy expenditure in mice, thereby also shedding some light on a complex methodological question. The authors furthermore show that adult, rather than perinatal, loss of Fto is well tolerated, enabling the analysis of Fto effects without the confounding factors associated with perinatal lethality or growth retardation. The authors also show that lack of Fto in the hypothalamus explains only a small part of the phenotype observed in the global Fto ko mice, indicating that Fto promotes its biological effects through other, non-hypothalamic pathways. Finally, the observation that RER is decreased in the germline and adult onset Fto ko mice points to a role of Fto in regulating peripheral metabolism and substrate utilization. The current paper by Cox and colleagues along with the other studies reviewed here highlight both the considerable challenges of, and the need for, careful and often time-consuming functional studies before the value of GWAS candidate genes can be truly appreciated.

Table 1. Overview about the most relevant Fto studies analyzing food intake and energy expenditure in mice and humans.

| Species       | Individuals/Assessed Phenotype | SNP Analyzed | BW (Mouse) | Energy Intake | EE | Reference                                      |
|---------------|--------------------------------|--------------|------------|---------------|----|-----------------------------------------------|
| Mouse         | Global germline Fto ko         | n/a          | ↓          | ↑             | ↑  | Fischer et al., (2009) Nature 458: 894–898    |
| Mouse         | Global germline Fto ko         | n/a          | ↓          | →↑           | ↑  | Gao et al., (2010) PLOS ONE 5: e14005          |
| Mouse         | CNS-specific Fto ko            | n/a          | ↓          | →↑           | ↑  | Gao et al., (2010) PLOS ONE 5: e14005          |
| Mouse         | Global LOF Fto missense mutation | n/a          | ↓          | →           | ↑  | Church et al., (2009) PLOS Genet 5: e1000599 |
| Mouse         | Global Fto overexpression      | n/a          | ↑          | ↑             | →  | Church et al., (2010) Nat Genet 42: 1086–1092 |
| Human         | 150 Scottish Caucasians        | rs9939609    | ↑          | ↑             | →  | Speakman et al., (2008) Obesity (Silver Spring) 16: 1961–1965 |
| Human         | 151 German subjects            | rs8050136    | ↑          | ↑             | →  | Haupt et al., (2009) Exp Clin Endocrinol Diabetes 117(4): 194–197 |
| Human         | 97 Scottish children           | rs9939609    | ↑          | ↑             | →  | Cecil et al., (2008) N Engl J Med 359(24): 2588–66. |
| Human         | 2,075 participants from the Look AHEAD (Action for Health in Diabetes) clinical trial | rs1421085, rs3751812, rs9222708 | n/a       | ↑   | n/a  | McCaffrey et al. (2012) Am J Clin Nutr 95(6): 1477–86 |
| Human         | 711 Korean children            | rs9939973, rs9939609 | ↑          | ↑            | n/a | Lee et al., (2010) Clin Chim Acta 411(1–2): 1716–22. |
| Human         | 1978 European- and African-American youth | rs9939609 | ↑          | →            | n/a | Liu et al., (2010) BMC Med Genet 11: 57          |
| Human         | 438 Healthy participants of the STRIP Study | rs9939609 | ↑          | →            | n/a | Hakanen et al., (2009) J Clin Endocrinol Metab 94(4): 1281–7 |
| Human         | 234 obese and 323 controls from Copenhagen | rs9939609 | ↑          | n/a         | →  | Berentzen et al., (2008) J Clin Endocrinol Metab 93(7): 2904–8 |
| Human         | 908 individuals from the Quebec City metropolitan area | rs17817449 | ↑          | n/a         | →  | Do et al., (2008) Diabetes 57: 1147–1150       |
| Human         | 908 individuals from the Quebec City metropolitan area | rs1421085 | ↑          | n/a         | →  | Do et al., (2008) Diabetes 57: 1147–1150       |

doi:10.1371/journal.pgen.1003223.t001
References

1. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, et al. (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316: 889–894.

2. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, et al. (2007) Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 39: 857–864.

3. (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447: 661–678.

4. Gerken T, Girard CA, Tung YC, Webby CJ, Saudack V, et al. (2007) The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318: 1469–1472.

5. Han Z, Niu T, Chang J, Lei X, Zhao M, et al. (2010) Crystal structure of the FTO protein reveals basis for its substrate specificity. Nature 464: 1203–1209.

6. Church C, Lee S, Bagg EA, McTaggart JS, Deacon R, et al. (2010) A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene. PLoS Genet 5: e1000599. doi:10.1371/journal.pgen.1000599

7. Fischer J, Koch L, Emmertling C, Vierkotten J, Peters T, et al. (2009) Inactivation of the Fto gene protects from obesity. Nature 458: 894–898.

8. Gao X, Shin YH, Li M, Wang F, Tong Q, et al. (2010) The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice. PLoS ONE 5: e14035. doi:10.1371/journal.pone.001405

9. Church C, Moir L, McMurray F, Girard C, Banks GT, et al. (2010) Overexpression of Fto leads to increased food intake and results in obesity. Nat Genet 42: 1086–1092.

10. Speakman JR, Rance KA, Johnstone AM (2008) Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. Obesity (Silver Spring) 16: 1961–1965.

11. Do R, Bailey SD, Desbiens K, Belisle A, Mouniprit A, et al. (2006) Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. Diabetes 57: 1147–1150.

12. Haupt A, Thamer C, Staiger H, Tschritter O, Kirchhoff K, et al. (2009) Variation in the FTO gene influences food intake but not energy expenditure. Exp Clin Endocrinol Diabetes 117: 194–197.

13. Cecil JE, Tavendale R, Watt P, Hetherington MM, Palmer CN (2008) An obesity-associated FTO gene variant and increased energy intake in children. N Engl J Med 359: 2558–2566.

14. Speakman JR (2010) FTO effect on energy demand versus food intake. Nature 464: E1; discussion E2.

15. McMurray F, Larder R, Nicholson G, Wells S, et al. (2013) Adult onset global loss of the Fto gene alters body composition and metabolism in the mouse. PLoS Genet 9: e1003166. doi:10.1371/journal.pgen.1003166

16. Cheung MK, Galati P, O’Rahilly S, Yeo GS (2012) FTO expression is regulated by availability of essential amino acids. Int J Obes (Lond) Epub ahead of print 22 May 2012. doi:10.1038/ijo.2012.77

17. Butler AA, Kozak LP (2010) A recurring problem with the analysis of energy expenditure in genetic models expressing lean and obese phenotypes. Diabetes 59: 323–329.

18. Tschop MH, Speakman JR, Arch JR, Auwerx J, Brunning JC, et al. (2012) A guide to analysis of mouse energy metabolism. Nat Methods 9: 57–63.

19. Kowalska I, Adamska A, Malecki MT, Karczewska-Kucuzewska M, Nikolajuk A, et al. (2012) Impact of the FTO gene variation on fat oxidation and its potential influence on body weight in women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 77: 120–125.