FTO effect on energy demand versus food intake

Arising from: J. Fischer *et al. Nature* **458,** 894–898 (2009)

An intronic single nucleotide polymorphism (SNP) (rs9939609) close to the fat mass and obesity associated gene (*FTO*) was the first SNP to be discovered with common variants linked to body mass index[1]; at least seven studies in humans have implicated this SNP with variations in food intake and satiety[2–8], and four studies have rejected an effect on energy expenditure normalized for body weight[2,5,6,8]. Fischer *et al.*[9] recently constructed a mouse in which the homologous *Fto* gene was inactivated ($Fto^{-/-}$) and showed that these mice were protected from obesity. This observation strongly implicates the effects of the intronic SNP rs9939609 as arising due to an effect on the closest gene (*FTO*). However, the suggested mechanism underlying this effect in mice was opposite to that in humans. The $Fto^{-/-}$ mice showed no significant differences in food intake relative to wild-types litter-mates[9] but had an elevated metabolic rate. The apparent contrasting effects of the gene in humans and mice is worthy of closer investigation.

The difference in body fatness between $Fto^{-/-}$ and $Fto^{+/?}$ mice was about 3.5 g in males and 1 g in females at 20 weeks old (Fig. 3 in ref. 9). One gram of fat is equal to about 39 kJ of energy. Assuming the mice weaned at 3 weeks of age, then over 17 weeks (120 days) the energy imbalance necessary to generate this difference is about 0.33 kJ day^{-1} for females and about 1.16 kJ day^{-1} for males. The energy density of standard rodent chow is about 17 kJ g^{-1}. Hence the difference in food intake between genotypes required to generate the observed differences in body fatness was about 0.02 g day^{-1} in the females and 0.07 g day^{-1} in males. Food intake in rodents is potentially much easier to study than in humans because food intake in rodents can be measured accurately, they can be fed from a single food source removing the influence of differences in macronutrient composition and they can be monitored for much longer periods—exceptionally over their entire lives. Nevertheless, given that individual variability in food intake in mice has a standard deviation of about 0.3 g day^{-1} then the required sample size to detect these effect sizes, using a 3 level one-way ANOVA (as analysed in ref. 9) with a power of 80% and $\alpha = 0.05$ is 355 per group for males, and 4,337 for females. Fischer *et al.*[9] only presented data for food intake in female mice. They used a total sample of 31 female animals. The post hoc estimated power to detect the effect size of 0.02 g day^{-1} in food intake was only 5.1%. Many studies of the effects of genes on food intake are underpowered and this critique could have been levelled at any number of recent studies. An example of a correctly powered study is that of ref. 10 on the effects of insulin receptor substrate-1 (*Irs1*) null mice. In that study using a sample of 12 null and 12 wild-type mice it was shown that the effect of disruption of Irs1 on longevity did not come about because of an effect of the inactivation of Irs1 on food intake. Although the sample size in this latter study may not seem very different from that used by Fischer *et al.*[9], the key difference between the studies is the magnitude of the effect size that is being detected. In ref. 10 the effect size is 50× greater than that being detected by Fischer *et al.*[9], consequently the power to detect or reject this effect was 98%. Sample sizes in experiments like those conducted in ref. 9 need to be sufficient to detect the effect size that generates the observed difference in fatness. Clearly this experiment was underpowered and the rejection of an effect of the Fto genotype on food intake has a strong likelihood of being a type II error.

The second potential issue with the findings of Fischer *et al.*[9] relates to the estimated energy expenditure. First, there is the same power issue highlighted above with respect to food intake, except that there is even less power in the expenditure measures as the total sample is only 23 individuals. More importantly, to evaluate the role of expenditure differences Fischer *et al.* (Fig. 4 in ref. 9) made a simple division of the metabolism by the lean body mass (LBM). This is a common practice to attempt to normalize for body size effects. However, simple division by LBM can generate a spurious elevation of metabolic rate if the intercept of the relationship between metabolism and LBM is not zero[11], as is often the case. The suggested increased metabolism in the $Fto^{-/-}$ animals is potentially an artefact of using this analysis method. The metabolic rates of these animals may only seem higher because the expenditure has been divided by a smaller lean body mass. If these data had been analysed using ANCOVA[11] the effect of genotype would very probably disappear (but then the absence of an effect could also be a type II error because of the power issue).

Overall the construction of the $Fto^{-/-}$ mouse is a great achievement that identifies the *FTO* gene as the prime candidate being affected by the intronic rs9939609 SNP. Given the roles of many genes on both intake and expenditure it is entirely possible that the mechanisms by which *Fto* influences energy balance in the mouse, as claimed by Fischer *et al.*[9], really do contrast the effects in humans[2–8]. Unfortunately the issue of lack of power and the complexity of normalization of energy expenditure measurements mean that at present it is impossible to judge.

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Fischer et al. *reply*

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The human studies on *FTO* reported an association of an intronic single nucleotide polymorphism (SNP) with obesity. Our report of mice with the targeted inactivation of the *Fto* gene demonstrated a direct role of Fto in energy homeostasis[1]. We have shown that the absence of Fto protein results in leanness and that Fto deficiency affects energy homeostasis. Speakman[2] exemplifies that the experiments performed in mice[1] conflict with results in humans carrying the *FTO* risk allele presenting hyperphagia and increased caloric intake[3–9].

The majority of studies examining the effects of *FTO* risk SNPs and determinants of adiposity in humans have reported association with food intake and/or appetite, and none has reported a significant effect on energy expenditure. However, the effects are modest in size and the precise measurement of the components of energy balance in human is fraught with difficulty, so it would therefore be premature to state conclusively that FTO affects human adiposity only through an effect on food intake.

With respect to the analysis of energy expenditure in our mouse model, correction of energy expenditure, that is, O_2 consumption to lean body mass, represents at this point a standard procedure in mouse phenotyping. This kind of data analysis was specifically requested by the reviewers of our manuscript. Another group has since reported the generation and analysis of a mouse model carrying a point mutation in the murine *Fto* gene as a consequence of *N*-ethyl-*N*-nitrosourea mutagenesis[10]. The phenotype of this mouse line closely resembles the knockout phenotype[1], although the alterations in overall body size are much more moderate.

However, this model also presents reduced fat mass and resistance to high-fat-induced obesity. Notably, in line with our report, even in the absence of major weight differences and with unaltered lean mass, mice carrying the *Fto* point mutation show increased energy expenditure as reported for the knockout model[10].

Taken together, our report on Fto-deficient mice provides a direct study on the role of the Fto protein in the absence of dysregulated *Ftm* (also known as *Rpgrip1l*) expression, the key findings of which have already been reproduced in an independent mouse model with altered Fto function. The observed reduction in fat mass does represent an important step towards the further understanding of FTO biology and we are confident that this and other mouse models will provide important additional insights into the function of FTO in the near future.

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