ORIGINAL RESEARCH

**FTO knockdown in rat ventromedial hypothalamus does not affect energy balance**

Margriet A. van Gestel, Loek E. Sanders, Johannes W. de Jong, Mienieke C. M. Luijendijk & Roger A. H. Adan

Brain Center Rudolf Magnus, Department of Translational Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands

**Keywords**
Feeding, FTO, obesity, ventromedial hypothalamus.

**Correspondence**
Roger A. H. Adan, Brain Center Rudolf Magnus, Department of Translational Neuroscience, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands. Tel: +31 (0) 88 75 68517 Fax: +31 (0) 88 75 69032 E-mail: r.a.h.adan@umcutrecht.nl

**Funding Information**
This work was supported by a grant from TI Pharma, project T5-210-1.

Received: 21 May 2014; Revised: 9 August 2014; Accepted: 21 August 2014

doi: 10.14814/phy2.12152

**Physiol Rep, 2 (12), 2014, e12152, doi:10.14814/phy2.12152**

**Abstract**

Single nucleotide polymorphisms (SNPs) clustered in the first intron of the fat mass and obesity-associated (FTO) gene have been associated with obesity. FTO expression is ubiquitous, with particularly high levels in the hypothalamic area of the brain. To investigate the region-specific role of FTO, AAV technology was applied to knockdown FTO in the ventromedial hypothalamus (VMH). No effect of FTO knockdown was observed on bodyweight or parameters of energy balance. Animals were exposed twice to an overnight fast, followed by a high-fat high-sucrose (HFHS) diet for 1 week. FTO knockdown did not result in a different response to the diets. A region-specific role for FTO in the VMH in the regulation of energy balance could not be found.

**Introduction**

Overweight and obesity are increasingly important health problems worldwide. The World Health Organization reports that 1.4 billion adults are overweight and approximately one-third of them are obese. During 1980 and 2008, obesity rates nearly doubled (Finucane et al. 2011). Obesity has been implicated as a major risk factor for cardiovascular diseases (Garrison et al. 1987; Manson et al. 1995; Ogden et al. 2007) and diabetes (Field et al. 2001; Oguma et al. 2005). Furthermore, obesity was associated with depression (Luppin et al. 2010). An environment that promotes high caloric food intake and discourages physical activity contributes to the occurrence of obesity. Obesity-associated genes might explain why individuals respond differently to this obesogenic environment. Indeed, family, twin and adoptions studies point to a strong genetic basis for the development of obesity (Stunkard et al. 1986a,b; MacDonald 1990; Maes et al. 1997).

In 2007, studies confirmed the fat mass and obesity-associated (FTO) gene as the first genome-wide association study (GWAS)-identified obesity susceptibility gene (Dina et al. 2007; Frayling et al. 2007; Scuteri et al. 2007). Common variants in the first intron of the FTO gene were associated with an increase in body mass index (BMI) of approximately 0.4 kg/m² per risk allele (Frayling et al. 2007). Variations in the FTO gene seem to influence energy balance by increased energy intake (Cecil et al. 2008; Speakman et al. 2008; Timpson et al. 2008; Haupt et al. 2009; Tanofsky-Kraff et al. 2009; Wardle et al. 2014).
FTO Knockdown in the Ventromedial Hypothalamus

M. A. van Gestel et al.

2009) and not by decreased physical activity (Berentzen et al. 2008; Do et al. 2008; Speakman et al. 2008; Goossens et al. 2009; Hakonen et al. 2009; Haupt et al. 2009; Wardle et al. 2009; Liu et al. 2010). FTO was identified as a 2-oxoglutarate-dependent nuclear acid demethylase and is involved in the demethylation of single-stranded DNA and RNA (Gerken et al. 2007; Jia et al. 2008). It is suggested that FTO may regulate transcription of genes involved in energy balance by demethylation (Gerken et al. 2007).

FTO is widely expressed throughout the brain, especially in the hypothalamic arcuate (ARC), paraventricular, dorsomedial (DMH), and ventromedial (VMH) nuclei (Gerken et al. 2007; McTaggart et al. 2011). In this study, we focused on the role of FTO on energy balance in the VMH, a hypothalamic nucleus involved in obesity, fear, and female reproductive behavior (Brobeck et al. 1943; Mathews and Edwards 1977; Satoh et al. 1997; Trogrlic et al. 2011). A microRNA-expressing AAV was injected into the VMH of rats and bodyweight, food intake, locomotor activity, and body temperature were monitored. No effect of FTO knockdown was found on bodyweight or parameters of energy balance. We previously showed that exposure to a restricted feeding schedule results in increased expression of FTO in the ARC and the VMH (Boender et al. 2012). To examine the effect of fasting on bodyweight and food intake, animals with FTO knockdown were exposed to an overnight fast twice. We did not observe an effect of fasting on bodyweight or on refeeding after restriction. Finally, a high-fat high-sucrose (HFHS) diet was introduced to the animals. Again, no differences were seen between the controls and the VMH FTO knockdown animals in their response to the HFHS diet. FTO in the VMH seems to have no impact on bodyweight or energy balance.

Material and Methods

Cell lines

Human embryonic kidney (HEK) 293T cells were maintained at 37°C with 5% CO2 in growth medium (Dulbecco’s modified Eagle medium, DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (FCS) (Lonz, Basel, Switzerland), 2 mmol/L glutamine (PAA, Gölbe, Germany), 100 units/mL penicillin (PAA), 100 units/mL streptomycin (PAA), and nonessential amino acids (PAA).

Construction of plasmids

A FTO-Renilla fusion plasmid was constructed as previously described (Van Gestel et al. 2014). Experiments were conducted using miRNAs targeting FTO, a control miRNA targeting Hcrtr1 and a control miRNA targeting Firefly Luciferase. pAAVs-expressing miRNAs were generated using the Gateway cloning technology (Invitrogen) as previously described (White and Nolan 2011). Briefly, miRNA sequences targeting FTO and Hcrtr1 were designed using the ‘Block-iT RNAi Designer’ (Invitrogen) (Table 1). The oligos were annealed and ligated into the synthetic intron of PSM155 (Du et al. 2006). A cassette containing the intronic miRNA upstream of enhanced green fluorescent protein (EGFP) was then amplified using B3 and B4 primers and recombined to generate the entry vectors pENTR-R4-miFTO1-EGFP-R3, pENTR-R4-

Table 1. Overview of oligonucleotides used in this study. Overview of oligonucleotides that were used to obtain miRNAs targeting FTO and Hcrtr1 mRNA and to perform a qPCR.

| Oligonucleotide | Forward | Reverse |
|-----------------|---------|---------|
| miFTO#1         | TGCCTTTAGGATATTTCAGCCAGTTTTGGCCACTGAGCTGCTGACGAGCTAAATATCCTAAA | CCTGCCAGCAGCCACGACGAGCTGCTGACGAGCTAAATATCCTAAA |
| miFTO#2         | TGCCTTTAGGATATTTCAGCCAGTTTTGGCCACTGAGCTGCTGACGAGCTAAATATCCTAAA | CCTGCCAGCAGCCACGACGAGCTGCTGACGAGCTAAATATCCTAAA |
| miFTO#3         | TGCCTTTAGGATATTTCAGCCAGTTTTGGCCACTGAGCTGCTGACGAGCTAAATATCCTAAA | CCTGCCAGCAGCCACGACGAGCTGCTGACGAGCTAAATATCCTAAA |
| miHcrtr         | TGCCTTTAGGATATTTCAGCCAGTTTTGGCCACTGAGCTGCTGACGAGCTAAATATCCTAAA | CCTGCCAGCAGCCACGACGAGCTGCTGACGAGCTAAATATCCTAAA |
| GFP             | CACAGAGCGTGGAGGAGAAC | CCCCTGAACCTGAAACATAAA |
| FTO#1 qPCR      | GAGCCGGAGAAGCTAAGAAACTG | CTGCTGCGAGTGGAGGAGAAC |
| FTO#3 qPCR      | CGCACGGAGAAGCTAAGAAACTG | AGTCAGCTGAGTGGAGGAGAAC |
| CycA qPCR       | AGCCTGGAGAAGGAGATT | AGCCTGGAGAAGGAGATT |
miFTO2-EGFP-R3, pENTR-R4-miFTO3-EGFP-R3, and pENTR-R4-miHcrtr1-EGFP-R3. Each entry vectors was recombined with pENTR-L1-ESYN-L4, pENTR-L3-oPRE-L2, and pAAV-R1-R2 to generate pAAV-ESYN-miFTO1-EGFP (pAAV-miFTO#1), pAAV-ESYN-miFTO2-EGFP (pAAV-miFTO#2), pAAV-ESYN-miFTO3-EGFP (pAAV-miFTO#3), and pAAV-ESYN-miHcrtr1-EGFP (pAAV-miHcrtr1). pAAV-miLuc was a kind gift of M.F. Nolan (White and Nolan 2011).

Luciferase assay

HEK293T cells in a 24-well plate were transfected with 5 ng pcDNA4/TO-luc, 500 ng pBabe-FTO-Renilla, and 1500 ng pAAV-miFTO or pAAV-miHcrtr1 using polyethyleneimine (PEI) (Polysciences, Eppelheim, Germany). Three days after transfection, cells were lysed in passive lysis buffer and analyzed with a dual luciferase reporter assay according to manufacturer’s protocol (Promega, Madison, WI). Firefly and Renilla luciferase activity were assessed; values were corrected for transfection efficiency using Firefly Luciferase activity and normalized to pAAV-miHcrtr1 knockdown.

Virus production and purification

Virus was generated and purified as previously described (De Backer et al. 2010). Briefly, HEK293T cells were co-transfected with pAAV-miRNA and pDP1 (Plasmid Factory, Bielefeld, Germany) in fifteen 15 × 15 cm dishes using PEI. Sixty hours after transfection, cells were collected, pelleted, and resuspended in ice-cold buffer (150 mmol/L NaCl, 50 mmol/L Tris, pH 8.4). Cells were lysed by three freeze–thaw cycles and incubated for 30 min at 37°C with 50 U/mL benzonase (Sigma, Zwijndrecht, the Netherlands). The lysate was loaded over onto a 15%, 25%, 40%, and 60% iodixanol gradient. After centrifugation at 500,000 g for 60 min at 18°C, the 40% layer was extracted and used for ion-exchange chromatography. AAV positive fractions were determined by quantifying virus titers using an Amicon Ultra 15 mL filter (Millipore, Amstrecht, the Netherlands). Titer was determined by qPCR on GFP (Table 1) and concentrated using a centrifugal concentrator. The lysate was loaded onto a packed column of Sephadex G-50 superfine, and virus was eluted with 150 mmol/L NaCl, 50 mmol/L Tris pH 8.4. Virus was administered by placing a syringe needle into the VMH (coordinates from Bregma: −2.1 AP, +1.5 ML, −9.9 DV, at a 5° angle). A total of 1 μL virus (1 × 10^{12} genomic copies/mL) were injected at a rate of 0.2 μL/min. Rats received a transmitter in the abdominal cavity for the recording of locomotor activity and body temperature (TA10TA-F40, Data Science International, New Brighton, MN).

Surgical procedures

Rats were anesthetized using fentanyl/fluanisone and midazolam and mounted onto a stereotaxic apparatus. Virus was administered by placing a syringe needle into the VMH (coordinates from Bregma: −2.1 AP, +1.5 ML, −9.9 DV, at a 5° angle). A total of 1 μL virus (1 × 10^{12} genomic copies/mL) were injected at a rate of 0.2 μL/min. Rats received a transmitter in the abdominal cavity for the recording of locomotor activity and body temperature (TA10TA-F40, Data Science International, New Brighton, MN).

In situ hybridization (ISH)

For the ISH, cryostat sections of 20 μm thickness from fresh, frozen brains were mounted onto slides. Sections were fixed in 4% paraformaldehyde for 20 min, washed in phosphate-buffered saline, acetylated for 10 min and washed again. The following steps differed between the ISH and the locked nucleic acid (LNA) ISH.

For the ISH, sections were prehybridized in hybridization solution (50% formamide, 5 × SSC, 5 × Denhardt’s, 250 μg/mL tRNA Baker’s yeast, 500 μg/mL sonicated salmon sperm DNA) for 2 h at room temperature. The hybridization solution containing 400 ng/mL 720-bp long digoxigenin (DIG)-labeled EGFP riboprobe (antisense to NCBI gene DQ768212) was then applied to the slides followed by overnight incubation at 68°C. After a quick wash in 68°C prewarmed 2 × SSC, slides were transferred to 68°C prewarmed 0.2 × SSC for 2 h. After blocking for 1 h with 10% FCS in B1 (0.1 mol/L Tris pH 7.5/0.15 mol/L NaCl), DIG was detected with an alkaline phosphatase-labeled antibody (1:5000, Roche, Mannheim, Germany) after overnight incubation at RT using NBT/BCIP as a substrate. Sections were dehydrated in ethanol, cleared in xylene, and embedded in Entellan.
The LNA ISH hybridization is performed as previously described (Kan et al. 2012). Briefly, sections were prehybridized in hybridization solution (50% formamide, 5 × SSC, 5 × Denhardts, 200 mg/mL tRNA Baker’s yeast, 500 mg/mL sonicated salmon sperm DNA, 0.02 g/mL Roche blocking reagent) for 1 h at RT. Hybridization was performed with 10 nmol/L double-DIG (3’ and 5’)-labeled LNA probe for human miR-124 (Exiqon, Vedbaek, Denmark) for 2 h at 55°C. After a quick wash in 60°C prewarmed 5 × SSC, slides were transferred to 60°C prewarmed 0.2 × SSC for 2 h. After blocking for 1 h with 10% FCS in B1 (0.1 mol/L Tris pH 7.5/0.15 mol/L NaCl), DIG was detected with an alkaline phosphatase-labeled antibody (1:2500, Roche) after overnight incubation at RT using NBT/BCIP as a substrate. Slides were further processed for immunohistochemistry.

**RNA isolation and qPCR in vivo knockdown**

The VMH was dissected from fresh frozen cryostat sections. RNA was extracted by adding 0.5 mL Trizol to the tissue. After 5 min incubation at RT, 100 μL chloroform was added followed by 2 min incubation at RT. After centrifugation for 15 min at 16,200 × g, RNA was precipitated from the aqueous layer by adding 0.25 mL isopropanol. After 10 min incubation at RT, samples were centrifuged for 10 min at 13,000 rpm. Next, the pellet was washed with 0.5 mL 100% ethanol. After another centrifugation step, the pellet was dissolved in water. Expression of FTO was detected using quantitative PCR using primers for each miRNA sequence and a housekeeping gene, CycA (Table 1). The difference between Ct values of FTO and CycA was calculated for each sample. Next, the difference between the AAV-mirFTO#1/2 samples and the AAV-mirLuc samples was calculated to determine fold change.

**Statistical analyses**

All data were presented as means ± SEM. Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA). A P-value of <0.05 was considered to be significant.

**Results**

**In vitro knockdown efficiency**

Three different miRNA sequences targeting FTO were cloned into an AAV2 vector and their in vitro knockdown efficiency was determined using a dual luciferase assay. pAAV-miHcrtr1 was used as a control. pAAV-miFTO#1 and pAAV-miFTO#2 were selected for further study because of their 69% and 74% silencing efficacy, respectively (Fig. 1A).

**In vivo knockdown efficiency and hypothalamic injections**

The plasmids were encapsidated into an AAV1 coat and AAV-miFTO#1 (n = 8), AAV-miFTO#2 (n = 8) and AAV-miLuc (n = 8) were stereotactically injected into the ventromedial hypothalamus. After 5 weeks, their brains were analyzed for in vivo knockdown efficiency and the placement of the hypothalamic injection. In vivo knockdown of FTO in the VMH was confirmed by qPCR. Due to technical problems, tissue from four animals could not be studied for knockdown efficiency. AAV-miFTO#1 (n = 6) and AAV-miFTO#2 (n = 8) decreased FTO mRNA levels by 82% and 68% compared to controls (n = 6), respectively (Fig. 1B). The viral vector contained a GFP cassette allowing the transduced area to be precisely identified. An ISH to detect GFP mRNA expression was used to analyze transduction efficiency. Figure 2A

![Figure 1. In vitro and in vivo knockdown efficiency of FTO constructs. In vitro knockdown efficiency of a DNA FTO-Renilla fusion construct by pAAV-miFTO1/2 relative to the control pAAV-miHcrtr1. pAAV-miFTO#1 and pAAV-miFTO#2 were selected for in vivo use based on their silencing efficacy of 69% and 74%, respectively (A). In vivo knockdown efficiency of pAAV-FTO#1 and pAAV-FTO#2 in the VMH as measured by qPCR for FTO (B).](image-url)
and C represent typical examples of injections targeting the ventromedial hypothalamus. To ensure that the hypothalamic injections did not result in toxicity due to oversaturation of the microRNA pathway as observed after AAV-mediated short hairpin RNA expression in the VMH (Van Gestel et al. 2014), we performed a miRNA 124 LNA ISH, as described previously (Van Gestel et al. 2014). No decreased miRNA 124 levels were observed after AAV transduction (Fig. 2B and D), indicating no oversaturation of the miRNA pathway.

**Bodyweight and energy balance**

Groups were matched for bodyweight and chow intake prior to surgery. After injection of the AAVs into the VMH, bodyweight and chow intake were measured for 5 weeks. No effect of FTO knockdown was found on bodyweight (two-way ANOVA, \( f = 1.363, P = 0.2057 \)) or chow intake (two-way ANOVA, \( f = 1.215, P = 0.2992 \)) (Fig. 3A and B). Locomotor activity and body temperature were measured in the third week after surgery and used as a measure for energy expenditure. No differences in locomotor activity (dark phase: two-way ANOVA, \( f = 1.452, P = 0.1371 \); light phase: two-way ANOVA, \( f = 0.8037, P = 0.6640 \)) or body temperature were observed (dark phase: two-way ANOVA, \( f = 0.5881, P = 0.8709 \); light phase: two-way ANOVA, \( f = 1.015, P = 0.4420 \)) (Fig. 3C and D).

**High-fat high-sucrose diet and fasting**

Animals were fasted two times for 16 h and refeeding was measured. Fasting had no effect on bodyweight (two-way ANOVA, \( f = 1.845, P = 0.1393 \)) and no differences were observed in refeeding (two-way ANOVA, \( f = 0.3633, P = 0.8329 \)) (Fig. 4A and B). To examine the effect VMH FTO knockdown on high caloric food intake, animals were exposed to a high-fat high-sucrose diet for 1 week. No effect was observed on bodyweight (two-way ANOVA, \( f = 0.9387, P = 0.4743 \)) and total caloric intake (two-way ANOVA, \( f = 0.4805, P = 0.7499 \)) (Fig. 5A and B).
Figure 3. Bodyweight and parameters of energy balance after FTO knockdown in the VMH. Relative bodyweight (A) and chow intake (B) were measured for 5 weeks. No differences in relative bodyweight (two-way ANOVA, $f = 1.363, P = 0.2057$) or chow intake (two-way ANOVA, $f = 1.215, P = 0.2992$) were observed after FTO knockdown in the VMH. In the third week, locomotor activity (C) and body temperature (D) were assessed. FTO knockdown in the VMH did not affect locomotor activity (dark phase: two-way ANOVA, $f = 1.452, P = 0.1371$; light phase: two-way ANOVA, $f = 0.8037, P = 0.6640$) or body temperature (dark phase: two-way ANOVA, $f = 0.5881, P = 0.8709$; light phase: two-way ANOVA, $f = 1.015, P = 0.4420$) in the dark phase or in the light phase.

Figure 4. Overnight fasting. Animals were exposed to an overnight fast twice. Food was removed for 16 h from 1700 h to 900 h. Bodyweight at day 0 (the day before the overnight fast) was set at 100%. Fasting had no effect on relative bodyweight (two-way ANOVA, $f = 1.845, P = 0.1393$) (A). Cumulative food intake was measured 2, 4, and 24 h after refeeding. No effect of FTO knockdown in the VMH was found on refeeding (two-way ANOVA, $f = 0.3633, P = 0.8329$) (B).
Discussion

In 2007, single nucleotide polymorphisms in the first intron of FTO were associated with body mass index. FTO is ubiquitously expressed in the brain, with high levels in the hypothalamic nuclei, which are important contributors to energy homeostasis. Previously, we have shown that VMH FTO is upregulated after exposure to a restricted feeding schedule. In this study, we investigated whether FTO knockdown in the VMH affects energy balance.

AAV-miFTO was bilaterally injected in the VMH and the effect on energy balance was measured for 5 weeks. FTO knockdown in the VMH did not affect bodyweight, food intake, body temperature, or locomotor activity. Next, animals were exposed twice to an overnight fast, which did not result in differences in bodyweight or refeeding. Finally, animals received a high-fat high-sucrose diet for 1 week. Animals did not show a difference in caloric intake, nor was their bodyweight differently affected by the diet.

Mouse models with an (in)complete FTO loss-of-function demonstrate reduced body weight and fat mass, without a decrease in energy intake (Church et al. 2009; Fischer et al. 2009). Consistent with these findings, mice overexpressing FTO show increased bodyweight and fat mass on both chow and high-fat diet (Church et al. 2010). In contrast to the FTO deficient models, this effect on body weight was the result of an increase in food intake. Although it was shown that FTO-mediated regulation of energy balance is located in the brain (Gao et al. 2010), FTO knockdown in the VMH did not result in a change in bodyweight or parameters of energy balance. Despite the very efficient in vivo knockdown of 84% in this study, it seems that FTO in the VMH does not affect energy homeostasis. FTO knockdown in the arcuate nucleus or mediobasal hypothalamus resulted in a modest reduction in food intake in the first week (Tung et al. 2010) and a reduction in food intake and bodyweight gain (McMurray et al. 2013), respectively. In this study, FTO knockdown was not limited to the VMH and parts of the DMH and ARC were transduced as well in some rats. In this region, FTO expression is highest in the VMH (Boender et al. 2012) and therefore we targeted the VMH. As we did not observe a phenotype in any of the rats, we did not find evidence for a role of FTO in the DMH and ARC in energy balance. However, we cannot exclude that knockdown of FTO in these structures might counteract the consequences of FTO knockdown in the VMH. The latter seems less likely considering the outcome after FTO knockdown in the ARC (Tung et al. 2010). Furthermore, although the virus spread could be observed throughout the whole mediobasal hypothalamus, some regions in the rostro-caudal extent of the VMH might be spared or have an incomplete transduction. Although these regions are relatively small, theoretically these might compensate for the loss of FTO. Possibly, other factors implicated in transcription and translation can compensate for a reduction in FTO, resulting in the absence of an effect of FTO knockdown. Although AAV-mediated knockdown was very effective, we cannot rule out that there are still functional levels of FTO protein.

Animals did not respond differently to exposure to overnight fasting, although we previously observed upregulation of FTO after exposure to a restricted feeding schedule. An overnight fast might not be enough for the development of a phenotype, secondary to the altered mRNA levels. Although effects of diet manipulation on FTO levels vary a lot in general, we cannot rule out the possibility that FTO knockdown in the VMH will result in a different outcome after exposure to a restricted feeding schedule.

One of the limitations of a GWA study is that SNPs are associated that may link to a more distant gene than

Figure 5. HFHS diet. In the seventh week after surgery, animals received a HFHS diet for 1 week. FTO knockdown in the VMH had no implications for bodyweight (two-way ANOVA, $f = 0.9387$, $P = 0.4743$) (A) or total caloric intake (two-way ANOVA, $f = 0.4805$, $P = 0.7499$) (B) on a HFHS diet.
to the nearest gene. Recent studies have shown that the obesity-associated SNPs embedded in the first intron of FTO are influencing expression of different, distant genes, called RPGRIP1L and IRX3, instead of affecting FTO itself (Smemo et al. 2014; Stratigopoulos et al. 2014). This might explain the absence of a phenotype after FTO knockdown. More research needs to be conducted to clarify the role of RPGRIP1L and IRX3 in obesity and their relation to FTO.

**Conflict of Interest**

None declared.

**References**

Berentzen, T., S. I. I. Kring, C. Holst, E. Zimmermann, T. Jess, T. Hansen, et al. 2008. Lack of association of fatness-related FTO gene variants with energy expenditure or physical activity. J. Clin. Endocrinol. Metab. 93:2904–2908.

Boender, A. J., A. J. van Rozen, and R. A. H. Adan. 2012. Nutritional state affects the expression of the obesity-associated genes Etv5, Faim2, Fto, and Negrl. Obesity (Silver Spring) 20:2420–2425.

Brobeck, J. R., J. Tepperman, and C. N. H. Long. 1943. Experimental hypothalamic hyperphagia in the albino rat. Yale J. Biol. Med. 15:831–853.

Cecil, J. E., R. Tavernale, P. Watt, M. M. Hetherington, and C. N. A. Palmer. 2008. An obesity-associated FTO variant and increased energy intake in children. N. Engl. J. Med. 359:2558–2566.

Church, C., S. Lee, E. A. L. Bagg, J. S. McTaggart, R. Deacon, T. Gerken, et al. 2009. A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene. PLoS Genet. 5:e1000599.

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

De Backer, M. W. A., M. A. D. Brans, M. C. Luijendijk, K. M. Garner, and R. A. H. Adan. 2010. Optimization of adeno-associated viral vector-mediated gene delivery to the hypothalamus. Hum. Gene Ther. 21:673–682.

Dina, C., D. Meyre, S. Gallina, E. Durand, A. Körner, P. Jacobson, et al. 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat. Genet. 39:724–726.

Do, R., S. D. Bailey, K. Desbiens, A. Belisle, A. Montpetit, C. Bouchard, et al. 2008. Genetic variants of FTO influence adiposity, insulin sensitivity, leptin levels, and resting metabolic rate in the Quebec Family Study. Diabetes 57:1147–1150.

Du, G., J. Yonekubo, Y. Zeng, M. Osisami, and M. A. Frohman. 2006. Design of expression vectors for RNA interference based on miRNAs and RNA splicing. FEBS J. 273:5421–5427.

Field, A. E., E. H. Coakley, A. Must, J. L. Spadano, N. Laird, W. H. Dietz, et al. 2001. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. Arch. Intern. Med. 161:1581–1586.

Finucane, M. M., G. A. Stevens, M. J. Cowan, G. Danaei, J. K. Lin, C. J. Paciorek, et al. 2011. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet 377:557–567.

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

Frayling, T. M., N. J. Timpson, M. N. Weedon, E. Zeggini, R. M. Freathy, C. M. Lindgren, 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.

Gao, X., Y.-H. Shin, M. Li, F. Wang, Q. Tong, and P. Zhang. 2010. The fat mass and obesity associated gene FTO functions in the brain to regulate postnatal growth in mice. PLoS ONE 5:e14005.

Garrison, R. J., W. B. Kannel, J. Stokes 3rd, and W. P. Castelli. 1987. Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. Prev. Med. 16:235–251.

Gerken, T., C. A. Girard, Y.-C. L. Tung, C. J. Webby, V. Saudek, K. S. Hewitson, et al. 2007. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318:1469–1472.

Goossens, G. H., L. Petersen, E. E. Blaak, G. Hul, P. Arner, A. Astrup, et al. 2009. Several obesity- and nutrient-related gene polymorphisms but not FTO and UCP variants modulate postabsorptive resting energy expenditure and fat-induced thermogenesis in obese individuals: the NUGENOB study. Int. J. Obes. 2005 33:669–679.

Hakanen, M., O. T. Raitakari, T. Lehtimäki, N. Peltonen, K. Pahkala, L. Sillanmäki, et al. 2009. FTO genotype is associated with body mass index after the age of seven years but not with energy intake or leisure-time physical activity. J. Clin. Endocrinol. Diabetes 117:194–197.

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

Jia, G., C.-G. Yang, S. Yang, X. Jian, C. Yi, Z. Zhou, et al. 2008. Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and RNA by mouse and human FTO. FEBS Lett. 582:3313–3319.

Kan, A. A., S. van Erp, A. A. H. Derijck, M. de Wit, E. V. S. Hessel, E. O’Duibhhr, et al. 2012. Genome-wide
FTO Knockdown in the Ventromedial Hypothalamus

M. A. van Gestel et al.

microRNA profiling of human temporal lobe epilepsy identifies modulators of the immune response. Cell. Mol. Life Sci. CMLS [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22535415. (accessed 2012 July 2013).

Liu, G., H. Zhu, V. Lagou, B. Gutin, I. S. Stallmann-Jorgensen, F. A. Treiber, et al. 2010. FTO variant rs9939609 is associated with body mass index and waist circumference, but not with energy intake or physical activity in European- and African-American youth. BMC Med. Genet. 11:57.

Luppino, F. S., L. M. de Wit, P. F. Bouvy, T. Stijnen, P. Cuijpers, B. W. J. H. Penninx, et al. 2010. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch. Gen. Psychiatry 67:220–229.

MacDonald, A. 1990. AS. Body-mass indexes of British separated twins. N. Engl. J. Med. 322:1530.

Maes, H. H., M. C. Neale, and L. J. Eaves. 1997. Genetic and environmental factors in relative body weight and human adiposity. Behav. Genet. 27:325–351.

Manson, J. E., W. C. Willett, M. J. Stampfer, G. A. Colditz, D. J. Hunter, S. E. Hankinson, et al. 1995. Body weight and mortality among women. N. Engl. J. Med. 333:677–685.

Mathews, D., and D. A. Edwards. 1977. The ventromedial nucleus of the hypothalamus and the hormonal arousal of sexual behaviors in the female rat. Horm. Behav. 8:40–51.

McMurray, F., C. D. Church, R. Larder, G. Nicholson, S. Wells, L. Teboul, et al. 2013. Adult onset global loss of the fto gene alters body composition and metabolism in the mouse. PLoS Genet. 9:e1003166.

McTaggart, J. S., S. Lee, M. Iberl, C. Church, R. D. Cox, and F. M. Ashcroft. 2011. FTO is expressed in neurones throughout the brain and its expression is unaltered by fasting. PLoS ONE 6:e27968.

Ogden, C. L., S. Z. Yanovski, M. D. Carroll, and K. M. Flegal. 2007. The epidemiology of obesity. Gastroenterology 132:2087–2102.

Oguma, Y., H. D. Sesso, R. S. Paffenbarger Jr, and L.-M. Lee. 2005. Weight change and risk of developing type 2 diabetes. Obes. Res. 13:945–951.

Satoh, N., Y. Ogawa, G. Katsuura, T. Tsuji, H. Masuzaki, J. Hiraoka, et al. 1997. Pathophysiological significance of the obese gene product, leptin, in ventromedial hypothalamic (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. Endocrinology 138:947–954.

Scuteri, A., S. Sanna, W.-M. Chen, M. Uda, G. Alibai, J. Strait, et al. 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet. 3:e115.