Polymorphisms of the TUB Gene Are Associated with Body Composition and Eating Behavior in Middle-Aged Women

Jana V. van Vliet-Ostaptchouk, N. Charlotte Onland-Moret, Ronit Shiri-Sverdlov, Patrick J. J. van Gorp, Anne Custers, Petra H. M. Peeters, Cisca Wijmenga, Marten H. Hofker, Yvonne T. van der Schouw

1 Department of Pathology and Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, 2 Department of Molecular Genetics, Maastricht University, Maastricht, The Netherlands, 3 DBG-Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands, 4 Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands, 5 Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Background. The TUB gene, encoding an evolutionary conserved protein, is highly expressed in the hypothalamus and might act as a transcription factor. Mutations in TUB cause late-onset obesity, insulin-resistance and neurosensory deficits in mice. An association of common variants in the TUB gene with body weight in humans has been reported. Methods / Findings. The aim was to investigate the relationship of single nucleotide polymorphisms (SNPs) of the TUB gene (rs2272382, rs2272383 and rs1528133) with both anthropometry and self-reported macronutrient intake from a validated food frequency questionnaire. These associations were studied in a population-based, cross-sectional study of 1680 middle-aged Dutch women, using linear regression analysis. The minor allele C of the rs1528133 SNP was significantly associated with increased weight (+1.88 kg, P = 0.022) and BMI (+0.56 units, P = 0.05). Compared with non-carriers, both AG heterozygotes and AA homozygotes of the rs2272382 SNP derived less energy from fat (AG: −0.55 ± 0.28%, P = 0.05, AA: −0.95 ± 0.48%, P = 0.047). However, both genotypes were associated with an increased energy intake from carbohydrates (0.69 ± 0.33%, P = 0.04 and 1.68 ± 0.56%, P = 0.003, respectively), mainly because of a higher consumption of mono- and disaccharides. Both these SNPs, rs2272382 and rs1528133, were also associated with a higher glycemic load in the diet. The glycemic load was higher among those with AG and AA genotypes for the variant rs2272382 than among the wild types (+1.49 (95% CI: −0.27–3.24) and +3.89 (95% CI: 0.94–6.85) units, respectively). Carriers of the minor allele C of rs1528133 were associated with an increased glycemic load of 1.85 units compared with non-carriers. Conclusions. Genetic variation of the TUB gene was associated with both body composition and macronutrient intake, suggesting that TUB might influence eating behavior.

Citation: van Vliet-Ostaptchouk JV, Onland-Moret NC, Shiri-Sverdlov R, van Gorp PJJ, Custers A, et al (2008) Polymorphisms of the TUB Gene Are Associated with Body Composition and Eating Behavior in Middle-Aged Women. PLoS ONE 3(1): e1405. doi:10.1371/journal.pone.0001405

INTRODUCTION

The hypothalamus plays a central role in the control of energy balance and the regulation of body weight and food intake [1–3]. The tubby protein is highly expressed in the paraventricular (PVN), ventromedial (VMH), and arcuate nuclei (ARC) of the hypothalamus that regulate satiety and appetite [4–6]. Loss-of-function mutations in tubby result in late-onset obesity, insulin resistance and neurosensory deficits in mice [7]. Further investigations showed that the expression of different neuropeptides was altered. Lower levels of agouti-related protein (AGRP) and proopiomelanocortin (POMC) mRNA levels in ARC were paralleled by increased levels of neuropeptide Y (NPY) in the dorsomedial-ventromedial hypothalamus (DMH/VMH) as well as preproorexin mRNA in the lateral hypothalamus (LHA) in mature and juvenile tubby mice [8–10]. Such multiple derangements in the neural system lead to hyperphagia in the adult animals [8].

Tubby homologs are highly conserved among vertebrate genomes [11] and in C. elegans the important role of the tubby ortholog, tub-1, in fat storage regulation has been shown [12–14]. Several studies have suggested that tubby may function as a transcription factor and/or as an adaptor molecule for downstream signaling of insulin and/or G-protein-coupled receptors [15–17].

Previously, we reported a significant association between variants in the TUB gene and body mass index (BMI) in a Dutch population of type 2 diabetes patients [18]. The minor alleles of single nucleotide polymorphisms (SNPs) rs2272302, rs2272383 and rs1528133 were associated with an average of 1.5 kg/m² higher BMI, and were 1.3 times more frequent among obese people (BMI > 30 kg/m²) than lean individuals (BMI < 25 kg/m²).

In order to ascertain the validity of our previous results in a population-based sample, and to explore whether macronutrient intake may be involved in the development of overweight and/or obesity, the present study investigated the effects of genetic variants in the TUB gene on food intake and body composition in middle-age Dutch women.

Academic Editor: Philippa Talmud, University College London, United Kingdom

Received June 1, 2007; Accepted October 1, 2007; Published January 9, 2008

Copyright: © 2008 van Vliet-Ostaptchouk et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: We thank the Dutch Diabetes Foundation, the European Vascular Genomics Network, and SenterNovem (IGE05012) for financial support. The Prospective-EPIC study was financed by the European Commission—Europe Against Cancer (WHO AEP/90/05), the Dutch Ministry of Health, the Dutch Prevention Fund, the UK Research Fund, and the World Cancer Research Fund (WCRF 98A04 and WCRF 2000/30).

Competing Interests: The authors have declared that no competing interests exist.

* To whom correspondence should be addressed. E-mail: Y.T.vanderSchouw@umcutrecht.nl
MATERIALS AND METHODS

Subjects
The women included in this study are Dutch participants in the European Prospective Investigation into Cancer and Nutrition (EPIC), conducted in Utrecht, the Netherlands (Prospect-EPIC) [19]. Between 1993 and 1997, 17,357 women aged 49–70 years and residing in or near Utrecht were recruited through a regional, population-based, breast cancer screening program. All the women signed written informed consent and the study was approved by the Institutional Review Board of the University Medical Center Utrecht.

At recruitment, each participant filled out a general questionnaire on lifestyle factors, gynecological and obstetric history, and past and current morbidity, as well as a validated semi-quantitative food frequency questionnaire (FFQ) with the aim of capturing the habitual diet during the year preceding enrolment. In addition, pulse rate, blood pressure and anthropometric measurements were taken and a blood sample was donated and stored at −196°C. A random sample of 1736 (10%) women was taken for biochemical analyses. Buffy coat samples were missing for 56 women, so the study population comprised 1680 women.

Anthropometry variables
Body height was measured to the nearest 0.5 cm with a wall mounted stadiometer (Lameris, Utrecht, the Netherlands). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg with a floor scale (Seca, Atlanta, GA, USA). Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Waist and hip circumferences were measured to the nearest 0.5 cm with a standard household tape measure, and the waist-to-hip ratio was calculated.

Food frequency questionnaire
The FFQ contains questions on the usual frequency of consumption of 77 main food items during the year preceding enrollment. Further information was sought on consumption frequency for different sub-items, preparation methods, and additions. Color photographs were used to estimate portion size for 28 food items. Overall, the questionnaire allows the estimation of the average daily consumption of 175 foods, by asking about sub-items for several foods, like fruit and vegetables, in additional questions. The FFQ was validated in pilot studies prior to the start of our study [20]. We calculated glycemic load by multiplying the glycemic index of a food with its carbohydrate content, then multiplying this value with the frequency of consumption of this food item [21]. The glycemic index of a food with its carbohydrate thus reflects the amount of carbohydrate consumed [21]. Such expression of dietary glycemic index per gram of carbohydrate thus reflects the overall quality of the daily carbohydrate intake.

Genotyping
DNA was extracted from whole blood using the QiAamp® Blood Kit (Qiagen Inc., Valencia, CA, USA). SNPs rs2272382, rs2272383 and rs1528133, previously reported to be associated with BMI [18], were genotyped using Taqman assay-on-demand (Applied Biosystems, Foster City, CA, USA). Assays were performed according to the manufacturer’s specifications (assays C_86243_10, C_271936_1, C_9597097_1, respectively). The genotypes were analyzed using a TaqMan 7900 HT (Applied Biosystems, Foster City, CA, USA). The DNA samples were processed in 384-well plates, each containing 4 negative controls and 12 genotyping controls (four duplicates of three different samples obtained from the Centre d’Etude du Polymorphisme Humain or CEPH). The genotype success rates were always >95% (95.7% for rs2272382, 96.5% for rs2272383 and 96.4% for rs1528133). The SNPs did not significantly deviate from Hardy-Weinberg equilibrium ($\chi^2$ = 0.41, $p = 0.52$ for rs2272382, $\chi^2$ = 1.38, $p = 0.24$ for rs2272383 and $\chi^2$ = 0.24, $p = 0.63$ for rs1528133). There were no discordances in the genotypes of any of the CEPH samples.

Statistical analysis
All statistical analyses were performed using the SPSS program, version 13.0 for Windows (SPSS, Chicago, IL, USA). Means with standard deviation, median and range (for the not-normally distributed characteristics) or frequencies (where appropriate) of baseline characteristics were presented for the entire population. The genotype frequencies were tested for Hardy-Weinberg equilibrium using a $\chi^2$ test with 1 df. The association between genotypes of SNPs and the cohort’s anthropometrical characteristics and macronutrient intakes were calculated using a linear regression model. Individuals homozygous for the common allele served as the reference group.

Linkage disequilibrium (LD) among SNPs was computed using Haplovew program, version 4.0RC1 [22].

RESULTS
We genotyped the three SNPs (rs2272382, rs2272383 and rs1528133) reported to be significantly associated with BMI in our previous study [18]. Figure 1 shows the structure of the TUB gene and its 3’ flanking region and the position of the genotyped SNPs. The allele frequencies of the variants as well as the pairwise linkage disequilibrium among SNPs were comparable with those reported by Shiri-Sverdlov et al (18) (Figure 1B,C). The haplotype analysis of the three genotyped SNPs revealed five common haplotypes (Figure 1D).

Food intake and anthropometrical characteristics of the 1680 women are shown in Table 1. First, we investigated the association of genetic variants of the TUB gene with different measures of body composition. The variant rs1528133 was significantly associated with weight and BMI (Table 2). The carriers of the minor allele C had an average increase in weight of 1.88 kg (95% CI: 0.27–3.48) and of 0.36 units in BMI (95% CI: 0.0–1.12) compared with non-carriers. The same trend was observed for waist and hip circumferences, but it was less pronounced.

Table 3 shows intake of macronutrients, expressed as a percentage of total energy intake, according to the TUB gene SNPs. The rs2272382 variant was significantly associated with fat and carbohydrate intake. In comparison with non-carriers, the percentage of energy derived from fat was 0.55% (95% CI: −1.10–0.00) less in heterozygotes and 0.95% (95% CI: −1.88–−0.01) less in homozygotes for the minor allele A. The observed differences were mainly due to a lower percentage of energy derived from saturated and monounsaturated fats. However, both AG- and AA-genotypes showed significant association with higher carbohydrate intake compared with GG-genotype (0.69% (95% CI: 0.03–1.34) and 1.68% (95% CI: 0.58–2.78), respectively), mainly because of a higher consumption of mono- and disaccharides. A similar but weaker pattern of association with a lower percentage of energy from fat and higher percentage of energy from carbohydrates was found for the minor allele of...
rs2272383. No differences were observed in total energy intake and the intake of other macronutrients (data not shown). Adjustment for age and BMI did not alter the results (data not shown).

The analysis of association between SNPs of the TUB gene and glycemic load (GL) and glycemic index (GI) is shown in Table 4. Two SNPs, rs2272382 and rs1528133, were associated with a higher GL in the diet. The GL was higher among those with AG and AA genotypes for the variant rs2272382 than among the wild types (+1.49 [95% CI: -0.27–3.24] and +3.89 [95% CI: 0.94–6.85] units, respectively). Carriers of the minor allele C of rs1528133 were also associated with an increased GL of 1.85 units compared with non-carriers. We observed no association of the TUB gene SNPs with glycemic index.

DISCUSSION
This study shows association between polymorphisms in the TUB gene and body composition, fat and carbohydrate intake, and glycemic load in a Dutch female population.

In a previous study, we found that common variants of the TUB gene can influence body weight and contribute to obesity in diabetics [18]. In this study, we have confirmed that the minor allele for rs1528133 is significantly associated with increased weight and BMI, and thus show that the relation of TUB with body composition can be extended to a general population.

Table 1. Anthropometrical and food intake characteristics of 1680 women from the EPIC Cohort.

| Characteristic             | Mean ± SD  | 95% CI      | P-value  |
|---------------------------|------------|-------------|----------|
| Age (y)                   | 57.22 ± 6.06 |            |          |
| Height (cm)               | 164.20 ± 6.02 |            |          |
| Weight (kg)               | 69.56 ± 6.00 |            |          |
| BMI (kg/m²)               | 25.89 ± 6.01 |            |          |
| Hip (cm)                  | 105.13 ± 6.01 |            |          |
| Waist (cm)                | 83.30 ± 6.01 |            |          |
| Waist-to-hip ratio        | 0.79 ± 0.06 |            |          |
| Total energy intake (kcal/day) | 1798.69 ± 6.00 |            |          |
| Protein intake (% of energy) | 16.17 ± 6.01 |            |          |
| Fat intake (% of energy)  | 35.45 ± 6.01 |            |          |
| Carbohydrate intake (% of energy) | 44.77 ± 6.01 |            |          |
| Alcohol intake (% of energy) | 3.71 ± 6.01 |            |          |
| Glycemic load (GL/day), energy adjusted | 100.26 ± 6.01 |            |          |
| Glycemic index, energy adjusted | 0.52 ± 6.01 |            |          |

1 All values are mean ± SD

Table 2. Anthropometrical characteristics of 1680 women from the EPIC study by genotype for rs1528133 SNP in the TUB gene.

| Genotype | Mean ± SD  | β  | 95% CI      | P-value  |
|----------|------------|----|-------------|----------|
| AA (n = 1392) |            |    |     |          |
| Weight (kg) | 69.56 ± 6.00 | 1.88 | 0.27–3.48 | 0.022    |
| Waist (cm)  | 83.30 ± 6.00 | 1.23 | 0.17–2.64 | 0.09     |
| Hip (cm)    | 105.13 ± 6.00 | 1.10 | 0.07–2.27 | 0.06     |
| BMI (kg/m²) | 25.81 ± 6.00 | 0.56 | 0.11–1.12 | 0.050    |
| Waist-to-hip ratio | 0.79 ± 6.00 | 0.00 | 0.00–0.01 | 0.00     |

1 All values are mean ± SD

doi:10.1371/journal.pone.0001405.t0001

doi:10.1371/journal.pone.0001405.t0002
To be added
In conclusion, our findings suggest that the TUB gene may play a role in modulating food intake. The results of the present study show, for the first time, that genetic variation of the TUB gene might influence food preferences in humans. To our knowledge, no explicit studies of the “tub effect” on feeding behavior in animals have been performed either, except for the reported hyperphagia in the adult tubby mice compared to their normal littermates [8]. Thus, it is unknown how TUB can stimulate the selective intake of macronutrients. Nevertheless, there are several possible mechanisms that might explain our observations (Figure 2). First, TUB may exert its effect through NPY, which is known to be positively linked to an animal’s voluntary selection of carbohydrates and it has been suggested it plays a role in the signaling of deficiency of glucose availability, stores or utilisation [40]. Second, TUB may influence the intake of fat through AGRP, shown to increase the preferences towards a high-fat diet in animals [41,42]. Third, the possible interconnections between TUB and orexin, which is highly responsive to changes in circulating and dietary nutrients [40], are indicated by its high upregulation in the young tubby mice. Moreover, orexin was reported to interact with serotonin (5-HT) in appetite regulation [43]. Interestingly, tub has also been shown to translocate from the plasma membrane to the nucleus after serotonin activation of 5-HT2c receptors [15], which could point to a function of tub as a downstream effector of these receptors.

An important question remains how a high-carbohydrate diet can influence body weight. Observational studies have suggested that the type of carbohydrates consumed may be related to body weight. Observational studies have suggested that the type of carbohydrates consumed may be related to body weight. Observational studies have suggested that the type of carbohydrates consumed may be related to body weight. Observational studies have suggested that the type of carbohydrates consumed may be related to body weight. Observational studies have suggested that the type of carbohydrates consumed may be related to body weight. Observational studies have suggested that the type of carbohydrates consumed may be related to body weight.
weight \[44–46\], and this was supported by the results of a recent trial in which both high-protein and low-glycemic index (GI) regimens led to a greater loss of body fat, but cardiovascular risk reduction was optimized by a high-carbohydrate, low-GI diet \[47\].

The importance of glycemic load (GL) compared with glycemic index (GI) was emphasized before \[48\]. GI indicates carbohydrate quality, and ranks how rapidly a particular food turns into sugar, whereas POMC/CART neurons have the opposite effect. Both signals project onto LHA neurons that express MCH and orexin (important stimulators of food intake), which are also mediated by dopamine, serotonin and endocannabinoids. The loss of the TUB function in tubby mice causes alteration in the expression of different neuropeptides, such as AGRP, NPY and POMC in ARC, as well as upregulation of NPY by \(-30\)-fold in DMH/VMH and orexin by \(-60\)-fold in LHA (highlighted in red). The image of tubby mice was provided by J. Naggert and P. Nishina (Nat. Genet. 39, 149; 2007).

**Figure 2.** The interactions between the neuropeptides in the hypothalamus regulating energy homeostasis and its alteration in tubby mice. Signals related to diet and circulating nutrients (shown at the bottom), which stimulate (+) or inhibit (−) the production of peptides, are received by the various hormonal receptors in the ARC nuclei, which contain NPY/AGRP- and POMC/CART-producing groups of cells. The activation of NPY/AGRP neurons promotes food intake, whereas POMC/CART neurons have the opposite effect. Both signals project onto LHA neurons that express MCH and orexin (important stimulators of food intake), which are also mediated by dopamine, serotonin and endocannabinoids. The loss of the TUB function in tubby mice causes alteration in the expression of different neuropeptides, such as AGRP, NPY and POMC in ARC, as well as upregulation of NPY by \(-30\)-fold in DMH/VMH and orexin by \(-60\)-fold in LHA (highlighted in red). The image of tubby mice was provided by J. Naggert and P. Nishina (Nat. Genet. 39, 149; 2007). Abbreviations: AGRP, agouti-related protein; ARC, arcuate nucleus; CART, cocaine–amphetamine-regulated transcript; DMH and VMH, dorsomedial and ventromedial hypothalamus; LHA, lateral hypothalamus; MCH, melanin-concentrating hormone; NPY neuropeptide Y; POMC, pro-opiomelanocortin.

doi:10.1371/journal.pone.0001405.g002

In conclusion, this study has shown for the first time that polymorphisms of the TUB gene, previously shown to influence body weight, are also associated with differences in macronutrients intake and glycemic load and could thus be related to eating behavior in humans. However, replication studies in other populations are necessary to confirm our findings. Further functional studies of TUB could also clarify the observed effects on food intake and reveal the genetic component influencing eating preferences towards the high-GL diet, which results in the increased weight and BMI. Additional investigations should be carried out in other populations to understand the exact mechanism of TUB on food intake.
ACKNOWLEDGMENTS
We would like to thank Jackie Senior for editing the manuscript, and Laurens Voogd and Frans Banky for helping with DNA aliquots.

Author Contributions
Conceived and designed the experiments: Yv CW MH. Performed the experiments: Jv. Analyzed the data: Yv. Jv NO. Contributed reagents/materials/analysis tools: PP AC. Wrote the paper: Jv. Other: Contributed to conception and design: MH Yv CW. Critical revision of the article for important intellectual content: Yv MH CW PP RS NO. Contributed to statistical expertise: NO Yv. Provided study materials: PP. Contributed to the interpretation of the data: RS Yv NO.

REFERENCES
1. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006) Central nervous system control of food intake and body weight. 443: 289–295.
2. Spiegelman BM, Flier JS (2001) Obesity and the regulation of energy balance. Cell 104: 531–543.
3. Williams G, Harrold JA, Cutler DJ (2008) The hypothalamus and the regulation of energy homeostasis: lifting the lid on a black box. Proc Nutr Soc 59: 385–396.
4. Ikeda A, Nishina PM, Naggett JK (2002) The tubby-like proteins, a family with roles in neuronal development and function. J Cell Sci 115: 9–14.
5. Sahly I, Gogat K, Kobetz A, Marchant D, Menasche M, et al. (1998) Prominent neuronal-specific tub gene expression in cellular targets of tubby mice mutation. Hum Mol Genet 7: 1477–1487.
6. Kleyv PW, Fan W, Kovats SG, Lee JL, Pulido JC, et al. (1996) Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. Cell 85: 281–290.
7. Coleman DL, Eicher EM (1990) Fat (fat) and tubby (tub): two autosomal recessive mutations causing obesity syndromes in the mouse. J Hered 81: 424–427.
8. Bachrach L, Madjig N, Ogren SO, Meister B (2004) Down-regulated expression of agouti-related protein (AGRP) mRNA in the hypothalamic arcuate nucleus of hyperphagic and obese tub/tub mice. Brain Res Mol Brain Res 125: 129–139.
9. Guan XM, Yu H, van der Ploeg LH (1998) Evidence of altered hypothalamic carbohydrate metabolism in mice homozygous for the tubby mutation. Hum Mol Genet 7: 263–272.
10. Wang Y, Seburn K, Bechtel L, Lee BY, Szatkiewicz JP, et al. (2006) Defective carbohydrate metabolism in mice homozygous for the tubby mutation. Physiol Genomics 24: 268–272.
11. Nishina PM, North MA, Ikeda A, Yan Y, Naggett JK (1998) Molecular characterization of a novel tubby gene family member, TULP3, in mice and humans. Genomics 34: 215–230.
12. Ashcroft F, Chang FY, Watts JL, Fraser AG, Kanath RS, et al. (2003) Genome-wide RNAi analysis of Caenorhabditis elegans fat regulatory genes. Nature 421: 263–272.
13. Mukhopadhyay A, Deplancke B, Wallajt A, Tissenbaum HA (2005) Characterization of a novel tubby gene family. Cell 85: 281–290.
14. Mak HY, Nelson LS, Basson M, Johnson CD, Ruvalk G (2006) Polygenic control of Caenorhabditis elegans fat storage. Nat Genet 38: 363–368.
15. Boggon TJ, Shan WS, Santagata S, Myers SC, Shapiro L (1999) Implication of G-protein domain-containing proteins in tubby proteins as transcription factors by structure-based functional analysis. Science 286: 2119–2125.
16. Santagata S, Boggon TJ, Baird CL, Gomez CA, Zhao J, et al. (2001) G-protein signaling through tubby proteins. Science 292: 2041–2050.
17. Kapeller R, Morriary A, Strauss A, Stubbish H, Therista K, et al. (1999) Tyrosine phosphorylation of tubby and its association with Src homology 2 signaling through tubby proteins. Science 292: 2041–2050.
18. Prentice AM, Black AE, Coward WA, Davies HL, Goldberg GR, et al. (1986) High levels of energy expenditure in obese women. Br Med J Clin Res Ed 292: 983–987.
19. Lichtmann SW, Pisarska K, Berman ER, Postme B, Dosding H, et al. (1992) Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. N Engl J Med 327: 1093–1098.
20. Rankinen T, Beauchard O (2006) Genetics of food intake and eating behavior phenotypes in humans. Annu Rev Nutr 26: 413–434.
21. Reed DR, Bachmanov AA, Beauchamp GK, Tredof MG, Price RA (1997) Heritable variation in food preferences and their contribution to obesity. Behav Genet 27: 373–387.
22. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haplowsies analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263–265.
23. Castillo M, Mulet J, Gutierrez LM, Ortiz JA, Castelan F, et al. (2006) Role of the R3G protein in trafficking of serotonin and nictinic acetylcholine receptors. J Mol Neurosci 30: 153–156.
24. Stubish H, Lynch GA, Morriary A, Fong T, Chicking E, et al. (2000) Targeted deletion of the tub mouse obesity gene reveals that tubby is a loss-of-function mutation. Mol Cell Biol 20: 678–682.
45. Lau C, Toft U, Tetens I, Richelsen B, Jørgensen T, et al. (2006) Association between dietary glycemic index, glycemic load, and body mass index in the Inter99 study: is underreporting a problem? Am J Clin Nutr 84: 641–645.
46. Hare-Braun H, Flint A, Heinmann BI, (2006) Glycemic index and glycemic load in relation to changes in body weight, body fat distribution, and body composition in adult Danes. Am J Clin Nutr 84: 871–879; quiz 952–873.
47. McMillan-Price J, Petocz P, Adkinson F, O’Neill K, Samman S, et al. (2006) Comparison of 4 diets of varying glycemic load on weight loss and cardiovascular risk reduction in overweight and obese young adults: a randomized controlled trial. Arch Intern Med 166: 1466–1475.
48. Livesey G (2005) Low-glycaemic diets and health: implications for obesity. Proc Nutr Soc 64: 105–113.
49. Willett W (2001) Eat, drink, and be healthy: the Harvard Medical School guide to healthy eating. New York, NY: Simon & Schuster Source.