

The 1D1 of Burning Calories

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You have probably daydreamed about eating as much as you want – but instead of becoming overweight or obese, you would burn the calories off effortlessly. We admit that we have dreamed of such a scenario – more than once. In the current issue of *Nature Genetics*, Chadt et al. [1] show that they did a lot more than just dream; they actually report results from a series of elegant studies, suggesting that the enhanced and balanced burning of excess calories may not be impossible. They report that a loss-of-function mutation in the so-called *Tbc1d1* gene of the lean SJL mouse strain confers resistance from high-fat-diet-induced obesity, most likely by increased skeletal muscle substrate utilization.

Genome-wide linkage analysis based on cross-breeding experiments is a tedious but powerful tool to identify the chromosomal regions (quantitative trait loci; QTLs) underlying polygenic traits. More than 270 QTLs for traits related to obesity and body weight have been mapped in crosses between various mouse lines [2]. For human obesity, analyses of such mouse models are of general clinical importance: each QTL explains a part of the variance of the trait under investigation and might point to homologous regions in the human genome. The New Zealand obese (NZO) strain is considered an ideal mouse model for the identification of obesity-related polygenes because this model reflects various features of the human metabolic syndrome, including polygenic obesity, insulin resistance, hypertension, and hypercholesterolemia [3]. In contrast, mice of the Swiss/Jim-Lambert (SJL) strain are lean, normoglycemic, and resistant to diet-induced obesity [4, 5].

In a previous genome-wide linkage study based on female mice of a NZO x F1 (NZOxSJL) backcross, Joost and colleagues [4–6] identified a major QTL (LOD score 7.9) for high-fat-diet-induced obesity on chromosome 5; this QTL was termed New Zealand obese 1 (Nob1). Along with being at an increased risk for obesity, carriers of the NZO allele at Nob1 exhibited a pronounced acceleration of the development of

hyperglycemia and insulin resistance upon exposure to HFD [5, 6].

In their new report, Al-Hasani, Joost and colleagues [1] assessed the body weight gain of several other cross-bred populations (NZOxNZB and NZOxC57BL/6J). They observed neither a correlation of the Nob1 genotype with HFD-induced body weight gain, nor linkage of body weight gain to chromosome 5. These results indicated that Nob1 represents an obesity suppressor gene from the lean SJL strain. The authors narrowed down the critical region of Nob1 by introgression of a 24 Mb segment of Nob1 from SJL into a mixed NZO/C57BL/6J background. Compared to control animals, both homozygous and heterozygous carriers of the Nob^{SJL} allele showed markedly reduced body weight and blood glucose levels upon HFD exposure. Microarray and subsequent qRT-PCR analysis of various tissues further revealed that only two transcripts in the critical 24 Mb region of Nob1 were differentially expressed (>two-fold) between NZO and SJL mice. Among these, *Tbc1d1*, a gene with high sequence homology to the insulin-signalling protein Tbc1d4 (AS160), mapped exactly into the Nob1 peak region and was three-fold down-regulated in the skeletal muscle of lean SJL mice. Further cloning and sequencing of *Tbc1d1* led to the identification of a SJL-specific loss-of-function mutation in *Tbc1d1*. This mutation leads to a truncated protein that lacks most invariant residues of the GTPase-activating (Rab GAP) domain.

Does *Tbc1d1* underlie the Nob1 linkage peak and entail resistance to HFD-induced obesity in SJL mice? If so, how might Tbc1d1 affect energy balance? Some evidence points to an implication of Tbc1d1 in glucose metabolism. Tbc1d1 comprises of two N-terminal phosphotyrosine-binding (PTB) domains, a central calmodulin-binding domain, and a C-terminal Rab GAP domain [7]. Tbc1d1 shows high sequence homology to Tbc1d4 (AS160), which is involved in insulin signalling. GLUT4 translocation, the insulin-induced trafficking of the

glucose transporter GLUT4 to the plasma membrane, is partly provoked by an Akt (protein kinase B)-mediated phosphorylation of Tbc1d4 [7, 8]. The high sequence homology between Tbc1d1 and Tbc1d4 raises the question of whether Tbc1d1 also might be implicated in insulin-mediated GLUT4 translocation.

Indeed, Roach et al. [7] recently showed that ectopic expression of Tbc1d1 in 3T3-L1 adipocytes blocked insulin-stimulated GLUT4 translocation. In addition, they showed that insulin led to the phosphorylation of Tbc1d1 on a binding site for Akt that is conserved between Tbc1d1 and Tbc1d4. Moreover, Tbc1d1, like Tbc1d4, mediated GLUT4 translocation through an Akt-mediated decrease of Rab GAP activity, indicating that a high level of Rab GAP activity causes suppression of GLUT4 translocation (and thus glucose uptake), whereas a decrease of Rab GAP activity does the opposite [7, 9]. In light of this observation, the identified SJL-specific loss-of-function mutation in *Tbc1d1* by Chadt et al. [1] would thus be expected to contribute to decreased levels of blood glucose through an increased GLUT4 translocation. Indeed, as shown in their new paper, carriers of the Nob1^{SJL} allele show markedly reduced blood glucose levels as compared to carriers of the Nob1^{NZO} allele. Even though this result points to Tbc1d1 as a missing link between GLUT4 translocation and glucose uptake, it warrants further investigation as to whether GLUT4 translocation is indeed affected by this mutation. One potential explanation to be considered is that the lower blood glucose levels might at least in part be a mere reflection of the lower body weight of the Nob1^{SJL} allele carrier.

Additionally, the question remains of how a truncated Tbc1d1 protein might contribute to resistance to HFD-induced obesity. In line with earlier results [10], Chadt et al. [1] show that Tbc1d1 is predominantly expressed in skeletal muscle, a main tissue of glucose action in vivo. Skeletal muscle glucose up-

take is accompanied by increased GLUT4 translocation; both insulin and contraction independently stimulate GLUT4 translocation in vivo [10]. One of the main differences between Tbc1d1 and Tbc1d4 is the occurrence of an AMPK (AMP-activated protein kinase) phosphorylation site between the two PTB domains of Tbc1d1 [9]. The PTB domain seems to be relevant for the implication of Tbc1d1 in energy homeostasis, since a non-conservative mutation (R125W) in the PTB domain of *Tbc1d1* has recently repeatedly been shown to be associated with severe familial obesity in humans [11, 12]. Moreover, in vivo stimulation by insulin, contraction, and the AMPK activator AICAR has recently been shown to increase phosphorylation of Tbc1d1 (and thus glucose uptake) in skeletal muscle [10]. In their new paper, Chadt et al. [1] further provide data which suggest that carriers of the Nob1^{SJL} allele display an elevated skeletal muscle lipid oxidation as indicated by a decreased respiratory quotient (RQ) during the dark period. Accordingly, palmitate oxidation was increased by approximately 25% in soleus muscle of 12-week-old Nob1^{SJL/SJL} mice. In line with this observation, siRNA-mediated knock-down of *Tbc1d1* increased palmitate uptake and oxidation in C2C12 myotubes, whereas overexpression of Tbc1d1 in C2C12 myotubes had the opposite effect. These data indicate that Tbc1d1 might play a fundamental role in skeletal muscle substrate utilization.

The lean phenotype of the SJL mice might therefore be explained by a shift of skeletal muscle substrate utilization from glucose to fat due to the lack of Tbc1d1 activity. The scientific breakthrough by Chadt et al. [1] suggests that we may after all be able to one day lose body weight without the need to change our eating habits. However, the evidence that Tbc1d1 is implicated in the 101 of substrate utilization and glucose uptake in skeletal muscle, at least makes Tbc1d1 a hot drug target for the prevention and treatment of metabolic disease.

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On the Contents of This Issue

This fifth issue of *OBESITY FACTS* covers a wide range of obesity-related topics, addressing mainly anthropometric, epidemiological and etiological aspects of overweight and obesity across all age ranges. The respective authors stem from four different European countries (UK, Germany, Sweden, Spain).

Questionnaire-based identification of behavioral traits that explain variance of BMI should be very useful in many studies related to overweight and obesity. Previous attempts have mostly focused on a limited (and frequently topically related) number of such variables/traits. Chambers and Swanson [1] psychometrically tested a comprehensive measure of obesity risk factors using a 100-item, easy-to-use questionnaire developed within a previous pilot study. Exploratory factor analysis led to the identification of 74 items which showed a clear factor structure with 5 dietary factors, 5 activity factors and 8 unrelated factors. The results which based on 359 adult volunteers (71% female) aged 18–81 years who had completed the questionnaire revealed that younger subjects reported unhealthier behaviors. Upon adjustment for age, less healthy eating, more emotional eating, higher amounts eaten, less physical activity, more use of mechanized transport, and more/less successful dieting behavior were all strongly related to higher BMI. Two major limitations apply to the study: The volunteers, many of whom were students, were not representative of the general population. It is unknown to what extent the subjective perception of weight status influenced the responses. Nevertheless, the results are clearly promising and warrant further research in an attempt to easily and reliably subgroup probands based on behavioral traits reliably explaining variance of BMI. Furthermore, it would be valuable to know if and what subfactors explain variance of waist circumference (WC).

The increments in prevalence rates of pediatric obesity are particularly worrisome. Craig et al. [2] assessed the implications of overweight defined via the international cut-offs specified by Cole and coworkers [3] and via high WC (≥ 91 st UK percentile) on blood pressure and lipid profile based on data obtained from the 1,944 participants (aged 4–18 years) of the National Diet and Nutrition Survey; the prevalence rate for overweight was compared with rates obtained in 1994 and 1998. Overweight and high WC were associated with increased systolic blood pressure, mean arterial pressure, low-density lipoprotein cholesterol and triacylglycerol, and decreased high-density lipoprotein cholesterol. Children who were both overweight and had a high WC had the most unfavorable cardiovascular risk profile. The prevalence rate for overweight obtained in the current study was twice as high as that in 1994, but similar to the

rate determined in 1998, thus seemingly paralleling recent US data indicative of the current attainment of a plateau for overweight/obesity rates.

Everyone who routinely measures WC in children and adolescents is concerned with differences in measurements according to anatomical site. There is currently no clear-cut consensus as to a standardized protocol for measuring WC; at least 14 different measurement sites have been reported in the literature. Only single studies have looked at the effects of measurement sites on the correlations of WC with BMI, fat mass and cardiovascular risk factors. Hitze et al. [4] have now assessed WC in 91 females and 89 males aged 6–20 years at four different sites: i) beneath the lowest rib (WCR), ii) 4cm above the umbilicus (WC4), iii) above the iliac crest (WCC) and iv) midway between WCR/WCC (WCM). All WCs were highly correlated; upon adjustment for age and pubertal status, WCs also revealed a high correlation to BMI; the correlations to % fat mass were within a similar range but lower than those to BMI. Furthermore, all WCs were comparably correlated with the obesity risk parameters (glucose/insulin levels, HOMA-IR, blood pressure and lipid profile). Hitze et al. discuss the minor differences which were somewhat more pronounced in females. In conclusion, the study of Hitze et al. can somewhat allay our concerns pertaining to different measurement sites for WC in young individuals.

Pettersson et al. [5] assessed the prevalence of obesity and abdominal obesity in the Swedish primary and occupational health care setting in 1,583 patients aged 18 to 65 years, who were consecutively referred to 29 primary care and 10 occupational health practices in Sweden in 2006. Patients were included irrespective of the reason for seeking the primary care physicians. Approximately 70 and 55% of male and female patients, respectively, were overweight (BMI ≥ 25). However, BMI ≥ 35 occurred more frequently in females (4.0 vs. 6.8%). More women than men were abdominally obese. Only 48 and 64% of the abdominally obese women and men, respectively, had a BMI ≥ 30 . The prevalence rates for overweight in both males and females exceeded those observed in the Swedish general population. The authors stress the importance of determining both BMI and WC in patients for the detection of conditions related with an elevated BMI/WC.

Browning et al. [6] investigated four inflammation markers (TNF- α , IL-6, monocyte chemoattractant protein 1 (MCP-1), C-reactive protein) in 54 females at baseline and after a 24-week weight loss intervention. The first three markers were measured using both a standard ELISA technique and a new multiplex technique, C-reactive protein was measured using a sensitive assay. Concentrations measured with the multiplex technique were poorly correlated to those obtained by ELISA; TNF- α was not detectable. Only

the ELISA-based concentrations were correlated with the independent inflammatory marker C-reactive protein. Taken together, these results suggest that caution is warranted upon interpretation of data obtained with the multiplex technique. The study suggests that circulating ELISA-based determinations of IL-6 and C-reactive protein, but not MCP-1 and TNF- α , are useful markers of obesity-related inflammation.

Several cross-sectional and longitudinal studies have linked a reduced amount of sleep with an elevated BMI in children and adults. In one of the few controlled intervention studies, Bomya-Westphal et al. [7] assessed 14 healthy women who were extensively investigated prior to and after 4 nights of consecutively increasing sleep curtailment (7 h, 6 h, 6 h and 4 h sleep/night) and after 2 nights of sleep recovery. Sleep reduction resulted in elevated energy intake, body weight, leptin/fat mass, free triiodothyronine, free thyroxine and glucose induced thermogenesis. No effect was discernible on mean resting and total energy expenditure, oral glucose tolerance, and ghrelin levels. The results add further evidence to the notion that reduced sleep causally entails an elevated BMI.

Holecki et al. [8] re-investigated the effect of vitamin D supplementation on weight loss of obese females who participated in a 3-month weight reduction program. No beneficial effect of the supplementation was observed. The au-

thors additionally provide a synopsis of human and rodent studies; the current study further substantiates that supplementation has no additional effect on weight loss.

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