VEGF and GLUT1 are highly heritable, inversely correlated and affected by dietary fat intake: Consequences for cognitive function in humans

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ABSTRACT

Objective: Reduction of brain glucose transporter GLUT1 results in severe neurological dysfunction. VEGF is required to restore and maintain brain glucose uptake across the blood brain barrier via GLUT1, which was shown to be acutely diminished in response to a high fat diet (HFD) in mice. The genetic and HFD-related regulation and association of VEGF and GLUT1 (SLC2A1) in humans was investigated in the NUtriGenomic Analysis in Twins (NUGAT) study.

Methods: 92 healthy and non-obese twins were standardized to a high-carbohydrate low-fat diet for 6 weeks before switched to a 6-week HFD under isocaloric conditions. Three clinical investigation days were conducted: after 6 weeks of low-fat diet and after 1 and 6 weeks of HFD. Serum VEGF and other cytokine levels were measured using ELISA. Gene expression in subcutaneous adipose tissue was assessed by quantitative Real-Time PCR. Genotyping was performed using microarray. The Auditory Verbal Learning Task was conducted to measure cognitive performance.

Results: In this human study, we showed that the environmental regulation of SLC2A1 expression and serum VEGF by HFD was inversely correlated and both factors showed strong heritability (>90%). In response to the HFD containing 45% fat, serum VEGF levels increased (P = 0.002) while SLC2A1 mRNA expression in adipose tissue decreased (P = 0.001). Higher BMI was additionally associated with lower SLC2A1 expression. AA-genotypes of the rs9472159 polymorphism, which explained ~39% of the variation in circulating VEGF concentrations, showed significantly reduced serum VEGF levels (P = 6.4 × 10⁻¹¹) but higher SLC2A1 expression (P = 0.009) in adipose tissue compared to CC/CA-genotypes after 6 weeks of HFD. Memory performance in AA-genotypes declined in response to the HFD compared to CC- and CA-genotypes.

Conclusions: The results provide evidence to suggest the translatability of the dietary regulation of VEGF and GLUT1 from mouse models to humans. Our data demonstrate that HFD induces a genetically determined and correlated decrease of GLUT1 and increase of VEGF which may affect memory performance.

Clinical Trial Registration Number: NCT01631123

Keywords VEGF; GLUT1; High fat diet; Cognition

1. INTRODUCTION

Cognitive function is known to be affected by macronutrient intakes, with high fat diets and obesity being particularly deleterious [1,2]. In mice, inflammatory responses in the central nervous system (CNS) to high fat diet (HFD) and subsequent brain insulin resistance appear to occur within days after initiating a high fat diet [3]. Although the pathways involved in central insulin resistance have been investigated in considerable detail, knowledge as to the precise mechanisms which transmit HFD associated signals are only partially understood [1,2,4]. The CNS depends on glucose as its energy substrate, and glucose needs to be transported across the blood brain barrier. Brain glucose uptake is mediated by facilitated transport via the glucose transporter GLUT1, and an important role of GLUT1 in cognitive function is becoming increasingly apparent [5,6]. Disturbances in GLUT1 function due to genetic aberrations of the SLC2A1 gene lead to seizures, motor

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2.2. Serum concentrations of VEGF and inflammatory parameters

VEGF, IL6, IL8, and TNFα concentrations were measured in the serum of all participants at each CID using human ELISA Kits (R&D Systems Inc., Minneapolis, MN, USA). The human VEGF immunosassay ELISA had a lower detection limit of 9 pg/ml (VEGF intraassay coefficient of variation <6.7%, interassay coefficient of variation <8.8%; R&D Systems Inc, Minneapolis, MN, USA).

2.3. Analysis of gene expression in subcutaneous adipose tissue

A biopsy of subcutaneous adipose tissue was performed lateral to the umbilicus by fine needle aspiration. About 500 mg of adipose tissue were homogenized (Speed Mill, Analytik Jena, Jena, Germany), and total RNA was extracted by using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). Gene expression was analyzed by quantitative Real-Time PCR. High-Capacity cDNA Reverse Transcription Kit™ (Applied Biosystems by Life Technologies, Carlsbad, CA, USA) was used to synthesize cDNA from 1 μg of RNA. Samples were labeled by Power SYBR Green Master Mix and measured in triplicates in optical 384-well plates with the ABI ViiATM 7 Real-Time PCR System (Applied Biosystems by Life Technologies, Carlsbad, CA, USA). Samples were normalized to ribosomal protein large P0 (RPLP0) and for evaluation the standard curve method was used. The primer sequences were: EMR1 forward primer GCC TGT TCG GAC GAC ATA CTT, reverse primer TCT TAT CCC CTT GCC TAC CA; VEGFA forward primer CAT CTT CAA GCC ATC CTG TGT G, reverse primer CGG CAT AAT CTG CAT GGT GAT; KDR (VEGFR2) forward primer TGC GAA GTA CCT TGG TTA CCC A, reverse primer TAA TCG TCA GTT CAT GCC CGC; RPLP0 forward primer GTC TGG AGG GTG TCC, reverse primer GGA CTC GTG TGT ACC GTG T.

2.4. Genotyping

Genomic DNA was extracted fromuffy coats (NucleoSpin, Macherey— Nagel, Düren, Germany). Genotyping was performed using Human-OmniExpressExome BeadChips (Illumina, Inc., San Diego, CA, USA) at the Interdisciplinary Center for Clinical Research (IZKF, Leipzig, Germany). Genotype frequencies were analyzed for deviation from Hardy—Weinberg equilibrium by chi-square test using R 3.1.2 plus Hardy-Weinberg package 1.5.5.

2.5. Auditory Verbal Learning Task (AVLT)

The German version of the Auditory Verbal Learning Task (AVLT [13,14]; was performed at the end of the LF dietary intervention and again at the end of the HFD period. A list containing 15 unrelated nouns was read to the participants, and they were asked to recall as many words as possible after the presentation irrespective of right order. This procedure was repeated 4 times (trial 1—5). After a delay of 30 min, the list had to be recalled by participants without further presentation (trial 7). Subsequently, the participants had to recognize...
2.6. Heritability

Heritability was estimated by applying the ACE structural equation model. This model analyzes covariance based on comparing the degree of concordance within and between monozygous vs. dizygous twin pairs. The proportion of variance is decomposed into (A) additive genetic influences, (C) common environmental, and (E) individual environmental influences. The ACE model was calculated using R 2.15.0 plus OpenMX package.

2.7. Statistical analysis

The Kolmogorov–Smirnov test was used to assess variables for normal distribution. Non-normally distributed variables were natural logarithm (ln)-transformed. Mean values for continuous data were compared using repeated measures or one-way ANOVA followed by Bonferroni adjusted posthoc test. The Kruskal–Wallis test as non-parametric equivalent of the ANOVA was used to verify significant results for non-normally distributed data. To compare two, not normally distributed, independent or dependent variables, the nonparametric Mann–Whitney-U and Wilcoxon test was used respectively. Statistical analyses were processed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and p < 0.05 were considered significant. Values are expressed as mean ± SEM, unless otherwise stated.

3. RESULTS

3.1. Heritability of serum VEGF concentrations and SLC2A1 expression in AT

According to minimum and maximum values the fasting levels of VEGF varied 46-fold at LF6 (clinical investigation day (CID) after 6 weeks of low-fat diet), 32-fold at HF1 (CID after 1 week of HFD), and 19-fold at HF6 (CID after 6 weeks of HFD) and were highly correlated among the monozygous (LF6: ρ = 0.933, p = 9.9 × 10⁻¹⁶, Figure 1A) but not among the dizygous twin pairs (LF6: ρ = 0.476, p = 0.118, Figure 1B), indicating a strong heritability of 94%.

The mRNA expression of SLC2A1 as assessed by quantitative Real-Time PCR (qPCR) in subcutaneous AT biopsies also showed a very high correlation among the monozygous (LF6: ρ = 0.771, p = 6.1 × 10⁻⁶) but not the dizygous twin pairs (LF6: ρ = 0.373, p = 0.259) with an estimated heritability of A = 0.938 at LF6 (A, proportion of variance due to additive genetic effects). For comparison, the gene expression of GLUT4 and GLUT5 was not heritable (A = 0 at LF6), confirming that GLUT1 shows a unique, strong heritability among the glucose transporters.

3.2. Increase in circulating VEGF concentrations in response to HFD

VEGF serum concentrations significantly increased in response to the HFD (repeated measures ANOVA, p = 0.002) with significantly higher levels after 6 weeks of the high fat dietary phase (mean ± SD: LF6 271.5 ± 173.1 pg/ml, HF1 268.8 ± 167.9 pg/ml, HF6 292.8 ± 193.1 pg/ml; Bonferroni adjusted posthoc test: HF6 vs. LF6: ρ = 0.023, HF6 vs. HF1: p = 0.008; Figure 2).

3.3. VEGF, VEGFR2, and GLUT1 gene expression in AT in response to HFD

VEGFA gene expression was significantly reduced in subcutaneous adipose tissue after 6 weeks of HFD (repeated measures ANOVA, p = 4.3 × 10⁻⁷; Bonferroni adjusted posthoc test LF6 vs. HF6: p = 9.5 × 10⁻⁵, HF1 vs. HF6: p = 0.030; Figure 3A). Gene expression levels did not correlate with circulating levels of VEGF, which suggests that circulating levels are not determined by adipose tissue derived VEGF (LF6: ρ = 0.112, p = 0.315; HF1: ρ = 0.017, p = 0.877; HF6: ρ = 0.056, p = 0.617). Gene expression of VEGF receptor 2 (VEGFR2, KDR), the primary receptor for VEGF, significantly decreased in response to the HFD (repeated measures ANOVA, p = 0.003; Bonferroni adjusted posthoc test LF6 vs. HF6: p = 0.011, LF6 vs. HF1: p = 0.031; Figure 3B).

Figure 1: VEGF levels are highly correlated in monozygous twins. Strong intrapair correlation of VEGF serum concentrations in (A) monozygous twins and no correlation in (B) dizygous twins. **p < 0.01.
The mRNA expression of SLC2A1 in AT was significantly downregulated after 6 weeks of HFD (repeated measures ANOVA, p = 0.001; Bonferroni adjusted posthoc test LF6 vs. HF6: p = 4 × 10^−5) and negatively correlated with serum levels of VEGF at LF6 (r = 0.296, p = 0.007). The correlation between SLC2A1 and VEGF became more stringent upon switching to the HFD (HF1: r = 0.428, p = 5.4 × 10^−5; HF6: r = −0.311, p = 0.005).

This raised the question whether GLUT1 expression is linked to inflammatory stimuli from macrophages as suggested by the study of Jais et al [7]. Indeed, an extraordinary correlation was observed between SLC2A1 expression in adipose tissue and the macrophage marker EMR1 (EGF-like module-containing mucin-like hormone receptor-like 1, human homolog of F4/80; LF6: ρ = 0.656, p = 3.0 × 10^−11; HF1: ρ = 0.629, p = 1.5 × 10^−10; HF6: ρ = 0.583, p = 1.2 × 10^−5), supporting the idea that stimuli emanating from macrophages upregulate GLUT1. Notably, SLC2A1 did not correlate with circulating levels of IL6, IL8, or TNFα, demonstrating that the correlation was rather exceptional.

Further, it was brought into question whether there may be a similar regulation of GLUT1 in adipose tissue and blood brain barrier (BBB) cells. Therefore, this was tested in the mouse model in which a positive correlation of VEGF and HFD had been shown [7]. We could not observe a statistically significant reduced Slc2a1 mRNA expression in white adipose tissue of wildtype mice fed a high fat diet for 4 weeks (p = 0.251, Figure S1).

Myeloid cells represent a significant source of VEGF. Therefore, we isolated peripheral blood mononuclear cells and circulating monocytes from the participants (n = 30) and determined VEGFA gene expression. Remarkably, there was no change in response to HFD (Figure S2), and the gene expression was not correlated with circulating levels of VEGF (LF6: p = 0.806), suggesting that circulating VEGF does not significantly originate from circulating monocytes.

Stimulation of primary human macrophages, adipocytes, or cocultures, respectively, with VEGF for 24 h did not influence gene expression of GLUT1 (Figure S3).
3.4. Altered SLC2A1 gene expression in normal weight vs. overweight subjects

SLC2A1 gene expression significantly correlated with BMI (LF6: \( p = -0.381, p = 3.8 \times 10^{-4} \); HF1: \( p = -0.302, p = 0.005 \); HF6: \( p = -0.322, p = 0.003 \)). SLC2A1 gene expression in AT was significantly lower in overweight subjects (BMI \( \geq 25 \text{ kg/m}^2 \) and \(<30 \text{ kg/m}^2 \)) compared to normal weight subjects (BMI \( < 25 \text{ kg/m}^2 \); LF6: \( p = 0.001 \); HF1: \( p = 0.003 \); HF6: \( p = 0.003 \); Figure 4). SLC2A1 gene expression also correlated with age (LF6: \( p = -0.289, p = 0.008 \); HF1: \( p = -0.263, p = 0.016 \); HF6: \( p = -0.250, p = 0.024 \)). In contrast, VEGF serum levels only significantly correlated with age (LF6: \( p = 0.288, p = 0.005 \); HF1: \( p = 0.341, p = 0.001 \); HF6: \( p = 0.248, p = 0.017 \)).

3.5. Polymorphism rs9472159 is associated with serum VEGF concentrations and SLC2A1 gene expression in AT

In view of the extraordinarily high heritability of VEGF, we analyzed polymorphisms in the NUGAT study previously reported in the literature to account for the variation in circulating VEGF levels [8,9]. Of 10 markers covered by the array, 2 variants, rs9472159 and rs9369434, were significantly associated with VEGF serum levels (\( p = 8.6 \times 10^{-10} \); HF6: corrected \( R^2 = 0.339, p = 1.4 \times 10^{-11} \)). An increase in VEGF serum concentrations in response to the high saturated fat diet was confirmed only for wildtype CC-genotypes (repeated measures ANOVA; CC p = 0.043, CA p = 0.067 and AA p = 0.490; \( \Delta \text{VEGF ANOVA p} = 0.009, \text{CC vs. CA and AA genotype, p} = 0.011 \) and p = 0.024, Figure 5B). Most notably, rs9472159 was also linked to the adipose tissue gene expression of SLC2A1 (LF6: \( p = 0.009 \); HF1: \( p = 0.030 \); HF6: \( p = 0.009 \); Figure 5C) suggesting that the correlation of the two proteins has some genetic determinants.

Genotype frequencies for SNP rs9369434, also located intergenically and in proximity to rs9472159 on chromosome 6, were CC = 26, CT = 55 and TT = 11 and were in Hardy–Weinberg disequilibrium (\( \chi^2 = 4.80, p = 0.029 \)). The frequency of the T allele was 0.42. Carriers of the T-allele had significantly lower VEGF serum levels compared to carriers of the major allele C (LF6: CC vs. CT vs. TT; 375.1 ± 42.2 pg/ml vs. 254.9 ± 18.0 pg/ml 109.1 ± 13.5 pg/ml, \( p = 2.2 \times 10^{-5} \); HF1: \( p = 3.7 \times 10^{-5} \); HF6: \( p = 6.0 \times 10^{-5} \)). Up to 23% of the variation in circulating VEGF levels could be explained by the rs9369434 polymorphism (LF6: corrected \( R^2 = 0.204, p = 4.0 \times 10^{-6} \); HF1: corrected \( R^2 = 0.196, p = 7.0 \times 10^{-6} \); HF6: corrected \( R^2 = 0.229, p = 8.5 \times 10^{-7} \)). An increase in VEGF serum concentrations in response to the high saturated fat diet was again only observed for the wildtype CC-genotypes (repeated measures ANOVA; CC p = 0.005, CT p = 0.157 and TT p = 0.901). Polymorphism rs9369434 was also associated with adipose tissue gene expression of SLC2A1 (LF6: \( p = 0.134 \); HF1: \( p = 0.030 \); HF6: \( p = 0.039 \)).

3.6. Auditory Verbal Learning Task (AVLT)

In an exploratory approach, the German version of the Auditory Verbal Learning Task (AVLT [10,11,14]) was conducted to evaluate whether the HFD affected learning and memory performance. We did not observe a significant decline of learning and memory performance in response to the 6 weeks of HFD but a significant improvement learning score, delayed recall score and consolidation memory score (Wilcoxon test, \( p = 1.3 \times 10^{-5} \); \( p = 0.036 \); Table S1 and Figure 6A) most likely as effect of repetitive testing. Recognition did not change in response to the HFD (Wilcoxon test, \( p = 0.817 \), Table S1).

However, after stratification by the rs9472159 genotype, we observed that the consolidation memory score, which is per definition adjusted for the initial learning rate and therefore less prone to test-retest/ ceiling effects, declined in homozygous carriers of the polymorphism in response to the HFD compared to wildtype CC and heterozygous carriers CA (Kruskal–Wallis test \( p = 0.009 \); Mann-Whitney-U test CC vs. AA \( p = 0.021 \), CA vs. AA \( p = 0.003 \); Figure 6B and Table S2).

Comparing measures of delayed recall and recognition elucidated a trend for better memory performance in CC/CA-genotypes compared to AA-genotypes, respectively (recessive model; Mann-Whitney-U test \( p = 0.078 \) and \( p = 0.102 \); Table S2). We did not observe any genotype-based difference with regard to learning score in response to the HFD (Kruskal–Wallis test \( p = 0.273 \); Table S2).

Polymorphism rs9369434 was also associated with cognitive impairment after 6 weeks of HFD (Table S3).

4. DISCUSSION

Stimulated by studies linking cognitive function to the dietary regulation of GLUT1 and its regulation by VEGF, we investigated the translatability of these findings into humans. Our data confirm an inverse link between the expression of GLUT1 and serum levels of VEGF.
through their regulation by food intake. In addition, we report extensive heritability of the expression of both factors and identified a genetic variant partially explaining the heritability. Moreover, this variant was linked to cognitive functions, which again concurs with the data from mice and provides possible links for the well-established role of high fat diet and in particular high saturated fat for the risk of developing dementia [16–19].

GLUT1 shows the highest expression in the blood brain barrier and the brain requires GLUT1 for adequate function [6,20–22]. Earlier studies demonstrated an upregulation of GLUT1 expression and glucose transport upon treatment with VEGF in rat brain cortical endothelia and in retinal endothelial cells [23,24] as well as in bovine aortic endothelial cells in which VEGF strongly upregulated glucose transport via GLUT1 while GLUT4 was unaffected [25]. The upregulation of GLUT1 by VEGF therefore was shown in several vascular tissues across different species. Adipocytes primarily express GLUT4 while GLUT1 shows a low level of expression and is not regulated by insulin or glucose [21]. Adipose tissue biopsies contain endothelial cells, macrophages, and preadipocytes, which account for about 50% of the cells present. We therefore tested whether GLUT1 is regulated by VEGF in primary human adipocytes and did not observe any regulation, suggesting that the changes might be related to other cell types present in the biopsies. The inverse correlation between VEGF and GLUT1 was rather exceptional and was not observed for other glucose transporters. The correlation was reproduced at all investigation days and increased upon switching to the HFD, confirming the solid relation of the two factors in humans. The increase in VEGF was not associated with increases in cytokines IL-6 and TNFα and thus was not part of a generalized inflammatory reaction suggesting a specific role of VEGF in regulating GLUT1 in humans.

An additional aspect was introduced by the extensive heritability of VEGF, which was the highest among several cytokines analyzed in our twin study including IL6 and TNFα, which showed no or a very modest heritability. A certain heritability of VEGF had been shown in family studies [26]. Our heritability estimates for VEGF are considerably higher since we chose twins without significant differences in body weight and standardized dietary intakes and thereby corrected for the significant environmental alterations of VEGF levels. This raises the possibility that the impact of VEGF may differ substantially in humans depending on the inherited level of expression.

The same applies to GLUT1, which is the only glucose transporter with a strongly inherited expression. GLUT1 was markedly downregulated by increased body weight, which is an important aspect closely linked to metabolism. The expression of both factors was additionally affected by HFD and correlated with age and in both cases again in opposite direction. Therefore, VEGF and GLUT1 are inversely regulated by unfavorable, frequently coinciding metabolic impacts on a strong genetic background of inheritance of basal levels.

GLUT1 mediates basal, insulin-independent glucose uptake, which might be reduced in adipose tissue given reduced GLUT1 mRNA expression in response to the HFD. In view of GLUT4, which is responsible for the majority of glucose uptake in adipose tissue, it is not known whether a reduced GLUT1 expression in adipose tissue exerts significant metabolic consequences.
A potentially helpful finding in our NUGAT study was that the rs9472159 polymorphism was associated with levels of VEGF and GLUT1. Being located in an enhancer- and promoter-associated histone mark region, it was assumed to potentially alter expression of surrounding genes, like VEGFA [15]. The additional association with GLUT1 concurs with the well-established regulation of GLUT1 by VEGF providing one possible explanation for the association. This genetic polymorphism should facilitate further studies on the role of VEGF and GLUT1 since this information is readily available in many human studies. Although Hardy–Weinberg equilibrium was not present in our sample, previous studies assessed its association with serum VEGF [9] suggesting that our sample was only slightly unbalanced. However, the role of this polymorphism certainly needs to be viewed with caution since earlier studies have shown the modest reproducibility of genetic associations derived from relatively small studies.

The discovery that GLUT1 in the microvasculature represents an important target of VEGF thereby affecting brain function is highly relevant. The work of Jais et al. identified VEGF as a determining player in the regulation of cognitive performance and provides an important link to the unfavorable effects of obesity and HFD for the risk of developing dementia [7]. Peripheral insulin resistance was suggested to represent an attempt to improve brain glucose supply by reducing peripheral glucose utilization [7,27]. Indeed, in our study insulin sensitivity decreased in response to HFD as suggested by a significant increase in HOMA-IR values [29].

Cognitive function, operationalized by a sensitive verbal memory task, was not directly affected by the high fat diet and was unrelated to VEGF serum concentrations and SLC2A1 gene expression in AT. Improvements in measures of learning, delayed recall, and consolidation memory were most likely affected by the repetitive testing. The large variation of basal expression levels of SLC2A1 and VEGF apparently did not translate into differences in these tests in healthy and non-obese subjects. However, after stratification for rs9472159 genotypes, we observed significantly declined consolidation memory scores in carriers of the polymorphism (AA) compared to non- or heterozygous carriers (CC/CA). Consolidation memory score is generally considered to represent a quite reliable measure of delayed memory function [10,11,13,14]. Homozygous carriers of the polymorphism (AA) showed the lowest concentrations of serum VEGF and highest levels of GLUT1 gene expression in AT. The observation therefore warrants further investigation and the availability of a single SNP facilitates testing in more adequate larger cohorts. Notably, it was shown that under insulin-induced hypoglycemia, increases in serum VEGF were significantly correlated with preserved cognitive performance in healthy man [29]. Furthermore, low VEGF has been identified as a biomarker for Alzheimer’s disease in cerebrospinal fluid [30].

We attempted to identify a possible source of the elevated serum VEGF levels. The absence of any correlation of circulating VEGF with its expression in circulating PMBCs, monocytes or adipose tissue suggests that other sources might be involved. Jais and coworkers established a myeloid source in mice using genetic deletion and suggested that perivascular macrophages might provide the increased VEGF [7]. The same may apply in humans. A difference remains that we did not observe a rapid upregulation of VEGF upon the introduction of high fat intake, which may imply a slower regulation of the system in humans. Our intervention was relatively modest since we used an isocaloric high fat diet, which induces mild insulin resistance in contrast to paradigms of hypercaloric high fat diets which very rapidly elicit massive insulin resistance [31] and also corresponds to the dietary approach in the animal experiments. Moreover, responses may be more pronounced in obese subjects.

In summary, our data indicate VEGF as a determining factor linking dietary fat intake to cognitive function in humans.

**AUTHOR CONTRIBUTIONS**

R.S. researched data and substantially contributed to analysis and interpretation of the data. N.S., M.A.O., V.W., A.F., A.B., A.J., J.C.B., and T.F. contributed to data acquisition and evaluation. S.H. and M.K. contributed to study design, subject recruitment and data collection. A.F.H.P. conceived, designed and supervised the NUGAT study and significantly contributed to data interpretation and critical review of the manuscript. R.S. and A.F.H.P. drafted the manuscript, which was critically reviewed by all authors.

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**Figure 6:** Consolidation memory scores according to the Auditory Verbal Learning Task (A) before and after 6 weeks of HFD and (B) before and after HFD stratified by rs9472159 genotype. Consolidation memory score was defined as the number of correct words recalled after the fifth trial subtracted from the number of correct words recalled after 30 min delay, multiplied by $-1$ to create positive relations. Data are shown as mean ± SEM, *p* < 0.05, **p** < 0.01. LF6, investigation day after 6 weeks of the low fat diet; HF6, investigation day after 6 weeks of the HFD.
role neither in designing the study nor with respect to analysis or interpretation of the data.

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**CONFLICT OF INTEREST**

None.

**APPENDIX A. SUPPLEMENTARY DATA**

Supplementary data related to this article can be found at https://doi.org/10.1016/j.molmet.2018.02.004.

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