CELL BIOLOGY

Correction for "Mice lacking asparaginyl endopeptidase develop disorders resembling hemophagocytic syndrome," by Chi-Bun Chan, Michiyo Abe, Noriyoshi Hashimoto, Chunhai Hao, Ifor R. Williams, Xia Liu, Shinji Nakao, Akitsugu Yamamoto, Shi-Yong Li, Ikuko Hara-Nishimura, Masahide Asano, and Keqiang Ye, which appeared in issue 2, January 13, 2009, of *Proc Natl Acad Sci USA* (106:468–473; first published December 23, 2008; 10.1073/pnas.0809824105).

The authors request that Chengyun Zheng, Jan-Inge Henter, Marie Meeths, and Magnus Nordenskjold be added to the authors list between Akitsugu Yamamoto and Shi-Yong Li. Chengyun Zheng designed research and analyzed data, Jan-Inge Henter designed research, Marie Meeths performed research and analyzed data, and Magnus Nordenskjold analyzed data. The online version has been corrected. The corrected author and affiliation lines and related footnotes appear below.

Chi-Bun Chan[a,1], Michiyo Abe[b,1], Noriyoshi Hashimoto[b], Chunhai Hao[a], Ifor R. Williams[a], Xia Liu[a], Shinji Nakao[c], Akitsugu Yamamoto[d], Chengyun Zheng[e,f], Jan-Inge Henter[e], Marie Meeths[e,f], Magnus Nordenskjold[f], Shi-Yong Li[a], Ikuko Hara-Nishimura[g], Masahide Asano[b,2], and Keqiang Ye[a,2]

[a]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, 615 Michael Street, Atlanta, GA 30322; [b]Division of Transgenic Animal Science, Advanced Science Research Center, and [c]Department of Cellular Transplantation Biology, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan; [d]Department of Bio-Science, Nagahama Institute of Bio-Science and Technology, 1266 Tamura-machi, Nagahama 526-0829, Japan; [g]Department of Botany, Graduate School of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan; [e]Department of Woman and Child Health, Childhood Cancer Research Unit, and [f]Department of Molecular Medicine and Surgery, Clinical Genetics Unit, Karolinska Institute, Karolinska University Hospital, SE-141 86 Stockholm, Sweden

Author contributions: C.-B.C., M. Abe, N.H., C.H., I.R.W., C.Z., J.-I.H., M. Asano, and K.Y. designed research; C.-B.C., M. Abe, N.H., C.H., I.R.W., X.L., S.N., A.Y., and M.M. performed research; I.H.-N. contributed new reagents/analytic tools; C.-B.C., M. Abe, N.H., C.H., I.R.W., C.Z., M.M., M.N., S.-Y.L., M. Asano, and K.Y. analyzed data; and C.-B.C., M. Asano, and K.Y. wrote the paper.

[1]C.-B.C. and M. Abe contributed equally to this work.

[2]To whom correspondence may be addressed. E-mail: asano@kiea.m.kanazawa-u.ac.jp or kye@emory.edu.

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BIOCHEMISTRY

Correction for "Crystal structure of a near-full-length archaeal MCM: Functional insights for an AAA+ hexameric helicase," by Aaron S. Brewster, Ganggang Wang, Xian Yu, William B. Greenleaf, José María Carazo, Matthew Tjajadia, Michael G. Klein, and Xiaojiang S. Chen, which appeared in issue 51, December 23, 2008, of *Proc Natl Acad Sci USA* (105:20191–20196; first published December 10, 2008; 10.1073/pnas.0808037105).

The authors note that the author name **Matthew Tjajadia** should have appeared as **Matthew Tjajadi**. The online version has been corrected. The corrected author line appears below.

Aaron S. Brewster, Ganggang Wang, Xian Yu, William B. Greenleaf, José María Carazo, Matthew Tjajadi, Michael G. Klein, and Xiaojiang S. Chen

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NEUROSCIENCE

Correction for "Caloric restriction improves memory in elderly humans," by A. V. Witte, M. Fobker, R. Gellner, S. Knecht, and A. Flöel, which appeared in issue 4, January 27, 2009, of *Proc Natl Acad Sci USA* (106:1255–1260; first published January 26, 2009; 10.1073/pnas.0808587106).

The authors note that the following two sentences should have been included on page 1257, left column, first full paragraph, after the sentence ending in the fifth line: "Note that one subject had to be excluded from the correlation analysis for insulin and one subject had to be excluded for hs-CRP due to a measurement error in insulin or hs-CRP, respectively. Therefore, analyses were conducted with 9 of initially 10 subjects defined by weight loss of >2 kg." This error does not affect the conclusions of the article.

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Caloric restriction improves memory in elderly humans

A. V. Witte[a], M. Fobker[b], R. Gellner[c], S. Knecht[a], and A. Flöel[a,d,1]

Departments of [a]Neurology and [c]Internal Medicine, [b]Center for Laboratory Medicine, and [d]Interdisciplinary Center of Clinical Research, University of Münster, Albert-Schweitzer-Strasse 33, 48149 Münster, Germany

Edited by Fred Gage, The Salk Institute, San Diego, CA, and approved December 19, 2008 (received for review September 4, 2008)

Animal studies suggest that diets low in calories and rich in unsaturated fatty acids (UFA) are beneficial for cognitive function in age. Here, we tested in a prospective interventional design whether the same effects can be induced in humans. Fifty healthy, normal- to overweight elderly subjects (29 females, mean age 60.5 years, mean body mass index 28 kg/m^2) were stratified into 3 groups: (*i*) caloric restriction (30% reduction), (*ii*) relative increased intake of UFAs (20% increase, unchanged total fat), and (*iii*) control. Before and after 3 months of intervention, memory performance was assessed under standardized conditions. We found a significant increase in verbal memory scores after caloric restriction (mean increase 20%; $P < 0.001$), which was correlated with decreases in fasting plasma levels of insulin and high sensitive C-reactive protein, most pronounced in subjects with best adherence to the diet (all r values < -0.8; all P values <0.05). Levels of brain-derived neurotrophic factor remained unchanged. No significant memory changes were observed in the other 2 groups. This interventional trial demonstrates beneficial effects of caloric restriction on memory performance in healthy elderly subjects. Mechanisms underlying this improvement might include higher synaptic plasticity and stimulation of neurofacilitatory pathways in the brain because of improved insulin sensitivity and reduced inflammatory activity. Our study may help to generate novel prevention strategies to maintain cognitive functions into old age.

aging | cognition | diet | unsaturated fatty acids

Because of the constant growth of the elderly population in today's societies worldwide (1), the search for new prevention and treatment strategies to maintain higher brain functions throughout life is of major economic and medical importance (see for example ref. 2). In the last 3 decades, numerous studies suggested that modifiable lifestyle factors including a low-calorie diet (caloric restriction, CR), and specific micro- and macronutrients like unsaturated fatty acids (UFA), might exert beneficial effects on the aging brain (3–7). In animal models of aging and neurodegenerative diseases, CR protected hippocampal, striatal, and cortical neurons, and ameliorated functional decline (8–18). In longitudinal observations in humans, it was found that a CR diet, as consumed by residents of the city of Okinawa, Japan, contributed to healthy aging and longevity (19). Conversely, obesity as a result of high energy intake has been shown to increase the risk of age-related cognitive decline (20).

A diet rich in mono- and polyUFA has been demonstrated to enhance cognitive performance in rats (21). It has been further proposed by epidemiological studies in humans that UFA, provided e.g., by olive oil and sea-fish in the traditional mediterranean diet, exert a risk-lowering effect for AD and cognitive impairment (22–27). Recently, 2 interventional studies reported a significant cognitive improvement in patients suffering from mild cognitive impairment (MCI) after intake of omega-3 polyUFA supplements vs. placebo (28, 29). However, inconclusive or negative findings from animal and observational studies have also been reported for CR (e.g., 30, 31, 32) and UFAs (33, 34).

Taken together, potential benefits of specific "brain-healthy diets" have been proposed, but have not been confirmed unequivocally by animal experiments and human epidemiological studies. Evidence drawn from prospective interventional trials in humans is still missing (CR) or scarce (UFA, 28, 29). Therefore, the aim of the present study was to elucidate cognitive effects of a diet low in calories or high in UFAs in healthy elderly individuals (for a flowchart, see Fig. 1). Because memory impairment is an early indication of AD and its precursor, MCI (35), we considered the ability to remember and learn new contents as our primary outcome measure, in accordance with previous studies on lifestyle interventions (36, 37). Moreover, we tried to identify potential mechanisms underlying the positive effects of these dietary interventions. Metabolic factors like insulin-resistance or low-grade inflammation might contribute to age-related cognitive impairments (38, 39), and improvement of metabolic state should result in acute improvement of cognition, in addition to long-term deceleration of cognitive decline. Therefore, we assessed peripheral blood levels for insulin, glucose, and markers of inflammation. Neuronal function may also be enhanced via neurotrophic factors (4), which are suggested to be activated by moderate stressors like CR via adaptive cellular stress response pathways (5). This possibility was tested by assessing neurotrophic levels in peripheral blood.

Results

Dietary Compliance. Details of physiological measures and serum levels at baseline and after intervention are shown in Table S1. As intended by the intervention, there was a significant weight loss ($F_{(2, 46)} = 7.25$, $P = 0.002$; $t_{(18)} = 3.24$, $P = 0.005$; Fig. 2; Table S1) and body mass index (BMI) reduction ($F_{(2, 46)} = 7.24$, $P = 0.002$; $t_{(18)} = 3.33$, $P = 0.004$; Fig. 2, Table S1) in the CR group (group 1). In addition to significant weight loss and reduction of fasting insulin levels, CR subjects' postintervention questionnaire on adherence to dietary guidelines demonstrated that they followed the instructions (16 of 18 answered "definitely yes," or "predominantly yes"). The remaining 2 subjects of the CR group (n = 18, 1 subject did not complete the questionnaire) answered they changed their dietary habits "at least half of the time. Asked whether they changed their physical activity during the study, 18 of 18 answered with "no." No significant changes emerged for body fat and waist-to-hip ratio in this group, nor for parameters of lipid metabolism ($P > 0.05$; Table S1). For the UFA enhancement group (group 2) and for the control group no

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The authors declare no conflict of interest.

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[1]To whom correspondence should be addressed at: Department of Neurology, University of Münster, Albert-Schweitzer-Strasse 33, 48129 Münster, Germany. E-mail: floeel@uni-muenster.de.

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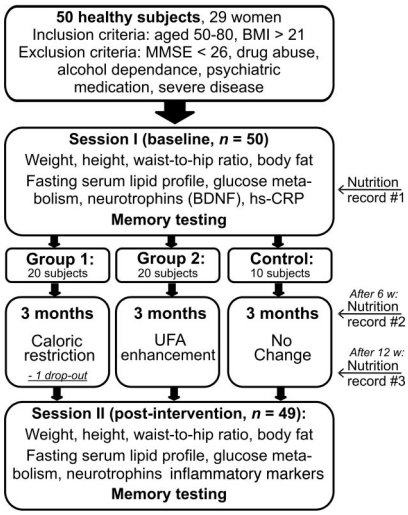

Fig. 1. Flow-chart of the study. 50 healthy elderly subjects were initially included in the study and performed baseline measurements of physiological parameters, fasting serum levels, and memory tests (session I). Based on age, sex, and BMI, subjects were stratified into 3 groups to follow either a specific diet, namely caloric restriction ($n = 20$, group 1) or unsaturated fatty acids (UFA) enhancement ($n = 20$, group 2), or not to change previous eating habits (control, $n = 10$). Dietary instructions were provided by clinical dieticians. One women from group 1 was not available for posttesting. After a period of 3 months, participants again underwent measurements of physiological parameters, fasting serum levels, and memory tests (session II). At baseline, after 6 and after 12 weeks, subjects additionally completed nutrition diaries over 7 consecutive days.

significant changes emerged for weight, BMI, body fat, and waist to-hip ratio, nor for serum levels of triglycerides, cholesterol, HDL, LDL, or their ratio (all $P > 0.05$; Fig. 2; Table S1).

Dietary intake at baseline and after the intervention (self-reported) is shown in Table S2. Dietary records revealed that all groups increased the proportional intake of UFAs significantly ($F_{(1,46)} = 92.45$, $P < 0.001$; $t_{(48)} = -10.06$, $P < 0.001$; Table S2), yet with highest increase in the UFA enhancement group (+18%, close to the aim of + 20%). The UFA-to-saturated fatty acids ratio was significantly improved only in the UFA enhancement group ($F_{(2, 46)} = 3.32$, $P = 0.045$; $t_{(19)} = -4.58$, $P < 0.001$; Fig. 2; Table S2). Considering marine sources of omega-3 UFAs, intake of eicosapentaenoic acid (EPA) and docosahexaeinoic

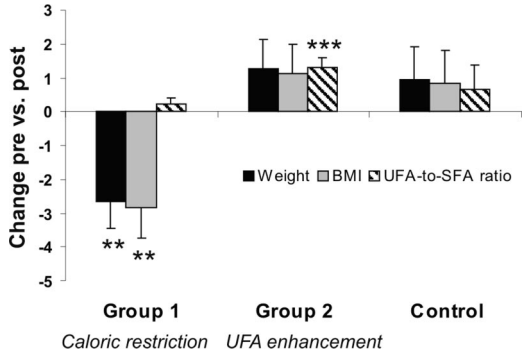

Fig. 2. Percentage changes in weight (black bars), BMI (gray bars), and changes in unsaturated fatty acids (UFA)-to-saturated fatty acids (SFA) ratio (striped bars) after caloric restriction (group 1), UFA enhancement (group 2), and control condition. Note that caloric restriction led to a significant decrease in weight, BMI, and UFA enhancement to a significant increase in the UFA-to-SFA-ratio. Error bars indicate standard error. ***, $P < 0.001$; **, $P < 0.01$ according to $ANOVA_{RM}$ posthoc testings.

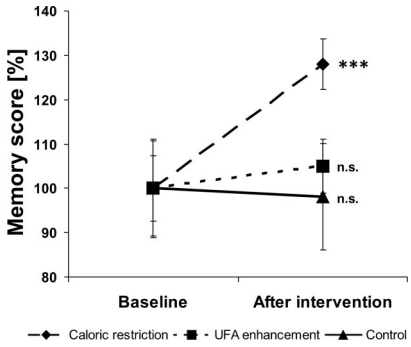

Fig. 3. Percentage memory scores normalized to baseline values before and after caloric restriction (dashed line), unsaturated fatty acid (UFA) enhancement (dotted line), and control (solid line). Note that after caloric restriction, a highly significant improvement in memory scores can be seen. Baseline memory scores were not significantly different. Dots give means, bars indicate standard error. ***, $P < 0.001$.

acid (DHA) was relatively low at baseline (mean 0.2 g/day; max. 1.6 g/day) and did not increase over the intervention ($P > 0.05$).

Intervention Effects. $ANOVA_{RM}$ showed a significant TIME x GROUP interaction on memory scores ($F_{(2, 46)} = 5.42$, $P = 0.008$). Subsequent t tests revealed that this was due to significant differences in memory scores in the CR group before and after intervention, with higher scores after the intervention ($t_{(18)} = -4.73$, $P = 0.0002$; Fig. 3). Likewise, there was a significant difference for memory scores uncorrected for false-positive misidentifications ($t_{(18)} = -2.85$, $P = 0.011$) and for "number of false-positives" ($t_{(18)} = 2.62$, $P = 0.018$). In summary, subjects remembered more words and made fewer mistakes after caloric restriction. No differences were shown for memory scores in the UFA enhancement group (all P values ≥ 0.31) or in the control group (all P values > 0.62).

In the CR group, inverse associations emerged between changes in several laboratory parameters [insulin, fasting glucose, hs-CRP, and tumor necrosis factor-alpha (TNF-α)] and changes in memory score:

Increases in memory score were correlated with decreases in insulin levels ($r = -0.45$, $P = 0.06$). Focusing the analysis on those individuals with best adherence to the intervention (defined as weight loss >1 SD of mean weight loss of the control group, resulting in a weight loss of >2 kg, $n = 9$), a highly significant inverse association emerged (Bonferroni corrected, $r = -0.78$, $P = 0.014$; Fig. 4). In addition, there was a trend for

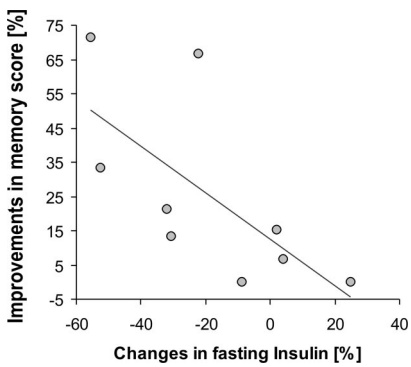

Fig. 4. Inverse correlation (Spearman, $r = -0.81$, $P = 0.014$) between changes in insulin levels and memory score improvements after caloric restriction in those subjects with best adherence to the diet ($n = 9$). Line indicates regression fit.

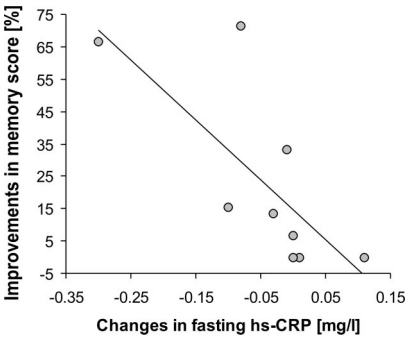

Fig. 5. Inverse correlation (Spearman, $r = -0.83$, $P = 0.005$) between changes in high sensitive C-reactive protein (hs-CRP) and memory score improvements after caloric restriction in those subjects with best adherence to the diet ($n = 9$). Line indicates regression fit.

a significantly reduced mean value of fasting glucose serum levels in the CR group after the intervention ($t_{(18)} = 1.82$, $P = 0.086$), yet $ANOVA_{RM}$ failed to reach significance ($F_{(2, 46)} = 1.98$; $P = 0.15$).

Furthermore, increases in memory score were correlated with decreases in hs-CRP levels (trend; $r = -0{,}41$, $P = 0.083$). Again, if including only those subjects with best adherence to the diet, a highly significant inverse association emerged (Bonferroni corrected, $r = -0.83$, $P = 0.005$; Fig. 5). A weak correlation was also found for increases in memory score and decreases in TNF-α in the CR group ($r = -0.39$, $P = 0.102$), more obvious in those subjects with best adherence to the diet ($r = -0.59$, $P = 0.094$).

For serum levels of BDNF, IGF-1, and IL-1β, no significant correlations with memory scores emerged ($P > 0.05$), nor for these or any of the other parameters in the UFA enhancement group or in the control group ($P > 0.05$). Likewise, no significant effects for GROUP $\times$ TIME was detected by $ANOVA_{RM}$.

Discussion

In this prospective interventional study in healthy normal to overweight elderly individuals, we found a significant improvement in memory performance after a caloric restricted diet (CR) over a period of 3 months. Memory improvement was correlated with decreases in fasting insulin and hs-CRP, most pronounced in those individuals with best adherence to the CR diet. In contrast, no significant changes in memory performance emerged after a diet rich in UFA or after control conditions.

Caloric Restriction. The findings of this interventional trial in humans support experimental animal studies (4) and epidemiological observations in humans (19, 40) that have suggested beneficial effects of CR on the aging brain. For example, CR has been demonstrated to enhance spatial memory performance in rats (17), and even CR over a period of 4 months sufficed to reduce age-related impairments in motor- and learning tasks in mice (9). Moreover, Fontan-Lozano and colleagues (41) reported that a CR diet, using an intermittent fasting regime enhanced learning and consolidation processes in mice, probably via higher expression of an NMDA-receptor subunit in the hippocampus. Interestingly, adult-onset short-term CR over 7 weeks in rats attenuated the effects of excitotoxic insults in hippocampal slices compared with ad libitum control diet (8). These results concur with the current study, because we found that even moderate CR over a period of 3 months improves cognition in healthy elderly subjects.

Unsaturated Fatty Acids. Considering UFA, observational studies in elderly cohorts (23, 25, 42) and small clinical trials in patients suffering from MCI or AD (43, 44) suggested that a diet high in mono- or omega-3 UFA might postpone cognitive decline. In rats, it has been demonstrated that a diet rich in mono- and di-UFA enhanced spatial memory performance (21). Conversely, higher intake of omega 3-fatty acids did not improve cognitive performance in epidemiological studies (for example, see ref. 33). The latter results are consistent with our study that failed to detect a beneficial effect of a 3-month dietary intervention high in UFA on cognitive performance. Several possible explanations may account for these negative findings: First, our results could be due to low adherence to the UFA diet, or to insufficiently high UFA dosage in the dietary protocol, rather than to a lack of positive effects of UFA on cognition per se. Because our dietary protocol promoted a self-prepared diet arranged independently at home, the present study does not allow us to distinguish between these possibilities. According to dietary records, however, there was a significant increase in the UFA-to-saturated fatty acids ratio in the UFA enhancement group, which nearly met the protocol aim of 20% UFA increase. However, self-reported dietary information is prone to errors (45).

Second, the amount of marine omega-3 UFA (mainly EPA, DHA) did not increase over the intervention period according to dietary records, mainly because there was no significant increase in fatty seafish meals. Because these sources of omega-3 fatty acids are suggested to be most effective in delaying cognitive decline (23, 28, 29), a low EPA/DHA-intake might have contributed to our negative findings, yet the evidence is still unequivocal (see e.g., 46 for positive results on olive oil, however, this might also depend on other, non-UFA ingredients in olive oil). Therefore, future studies have to further evaluate the effects of different UFAs on cognitive functions on the aging brain in health and disease.

Mechanisms of Diet-Induced Cognitive Changes. ***Insulin.*** In the present study, we found a decrease of fasting peripheral insulin in the CR group, in accordance with studies in healthy rats (47) and monkeys (48–50) after CR, and with clinical data in obese patients (e.g., 51). Reducing peripheral insulin levels should result in increased insulin sensitivity and central insulin levels (3), because higher levels of peripheral insulin lead to a down-regulation of insulin transport at the bloodbrain-barrier and thus to central hypoinsulinemia (52, 53). Importantly, improved insulin signaling in the brain has been suggested to have neuroprotective effects (38, 52, 54,), whereas increased peripheral circulating insulin may promote the development of cognitive impairments and AD (52, 54, 55).

Furthermore, the observed correlation between decrease in peripheral insulin and increase in memory points to a possible role of insulin in mediating the beneficial effects of CR on memory functions (for review, see ref. 7). Levels of insulin, insulin receptors, and insulin-regulated pathways in the brain are involved in glutamate- and GABA-mediated synaptic plasticity and in gene expressions required for long-term memory consolidation (38, 56). For example in the hippocampus, insulin has been shown to induce NMDA receptor phosphorylation (57), and to increase channel activities of NMDA receptors (58), which play an important role in learning and memory formation (for example, see ref. 59). Thus, it has been convincingly demonstrated that insulin signaling exerts neuroprotective and neuromodulatory effects in the brain, although the molecular machinery linking insulin and cognitive improvement, for example the exact role of kinase molecules in learning and memory, needs to be further elucidated (38).

In summary, the present study lends experimental support to a model derived from animal studies in which reduced fasting insulin levels due to CR led to lower insulin resistance, higher insulin sensitivity, subsequently to improved insulin signaling in

the brain and to increased synaptogenesis and neuronal survival (3). Higher insulin sensitivity due to CR in our subjects, with subsequently improved insulin signaling in the brain, would be a plausible explanation for the observed memory improvements in the current study.

Neurotrophic factors. Neuronal function may also be enhanced via neurotrophic factors (5, 38), which are suggested to be activated by moderate stressors like exercise and CR via adaptive cellular stress response pathways (e.g., heat shock protein 70; for details, see refs. 4 and 60). Neurotrophic factors, such as IGF-1 and BDNF, are widely known to be involved in neuronal growth and neurogenesis and might also protect mature neurons from degeneration (61). IGF-1 is also a ligand for insulin receptors (62), thus activating insulin pathways in the brain. Both IGF-1- (63) and BDNF-levels (64) have been suggested to be enhanced after CR in rodents. Our results did not show a significant difference in either IGF-1 or BDNF induced by dietary interventions. One explanation might be that we could only assess peripheral levels of IGF-1 and BDNF. Even though both peripheral IGF-1 (65) and BDNF (66) have been shown to pass the blood–brain barrier, these measures may not be a perfect reflection of brain concentrations. In addition, other neurotrophic molecules such as glia-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) might have changed in adaptation to CR (3), which were not assessed in the current study. To clarify these issues, measurements of these factors could be additionally assessed in future studies.

Inflammation. CR has been shown to exert anti-inflammatory effects (4), including down-regulation of hs-CRP levels in rodents (67) and TNF-α in humans (68, 69), in line with the present data. With regard to cognition, several studies have proposed that ''inflammatory activity,'' as indicated by serum markers of inflammatory responses, is negatively correlated with neuropsychological performance and cognitive decline (70, 71). For example, an observational study by Teunissen and colleagues (72) found significant inverse correlations of serum levels of CRP and haptoglobin with performance in a verbal learning task (congruent to the memory test used in the current study) in healthy elderly individuals. The current study is the first to confirm these findings, and to extend the proposed association for TNF-α in an interventional design. However, anti-inflammatory pathways linking CR and memory remain to be further elucidated (4), e.g., by including a larger number of subjects with elevated levels of inflammatory markers at baseline, and testing an extended range of markers. Interestingly, TNF-α has been demonstrated to promote insulin resistance in experimental animal studies (73, 74). Therefore, a reduction of TNF-α by CR might additionally contribute to maintain cognitive functions via improved insulin signaling (3).

Limitations. Several limitations should be considered when interpreting our findings. First, dietary habits were self-reported only and thus prone to over- or underestimation (45). However, in the CR group, weight loss and BMI reduction demonstrated adherence to the intended dietary regime. Second, individuals in the control group did not receive the same amount of attention by dietary counsellors, and interaction with group members, as participants in the CR group. Better memory performance may thus be due to a Hawthorne (75) effect or an effect of ''enhanced environmental enrichment'' by social interaction in the CR group. However, the finding that individuals in the UFA enhancement group, receiving a similar amount of attention and social interaction, did not show memory improvements, renders this explanation highly unlikely.

Conclusion

To our knowledge, the current results provide first experimental evidence in humans that caloric restriction improves memory in the elderly. Our findings further point to increased insulin sensitivity and reduced inflammatory activity as mediating mechanisms, leading to higher synaptic plasticity and stimulation of neuroprotective pathways in the brain. Future studies incorporating measurements of additional neurotrophic and inflammatory markers, and brain imaging to assess structural changes (for example, see ref. 36), should provide further insights into potential mediators of improved cognition by changes in dietary habits.

The present findings may help to develop new prevention and treatment strategies for maintaining cognitive health into old age (3).

Materials and Methods

Subjects. Fifty healthy elderly subjects (age: 60.5 years $\pm$ 7.6 SD, BMI: 28 kg/m^2 $\pm$ 3.7 SD; 29 females) were recruited via newspaper advertisement. Inclusion criteria were age between 50 and 80 years, a BMI $>$ 21 to exclude potential underweight after intervention, and postmenopausal status for women. At screening visit, participants underwent a routine medical and neurological examination. Exclusion criteria were severe cardiac and pulmonary disease, diabetes or other metabolic disorder, psychiatric disorders, memory impairment based on a score of $<$26 on the MiniMental State Examination (MMSE; 76), and drug abuse, including alcohol dependence and heavy smoking. Psychiatric comorbidity was additionally monitored using the Beck's Depression Inventory (BDI, German version; 77) and Spielberger's State Trait Angst Inventar (STAI 1 and 2, German version; 78). One woman was not available for postassessment, leaving 49 participants for final analyses. Based on age, sex, and BMI, subjects were stratified into 3 groups: (*i*) Caloric restriction, (*ii*) increase of the amount of UFA (''UFA enhancement''), and (*iii*) control group; for details on groups see below. Demographic variables at baseline are given in Table S3. The study was conducted at the Department of Neurology at the University of Münster, Germany. All subjects provided written informed consent and received reimbursement for participation. The research protocol was approved by the Ethics Committee of the University Hospital of Muenster, Germany.

Caloric Restriction. According to previous recommendations based on studies of rodents and rhesus monkeys (49, 60), participants (n = 19) were instructed to reduce caloric intake aiming at a 30% reduction relative to previous habits, over a period of 3 months. The intended individual caloric intake was calculated a priori based on individual dietary records, because the aim of the caloric restriction intervention was to reduce each subject's individual caloric intake by 30%, compared with pretrial levels. To avoid cognitive changes due to malnutrition (79), minimal intake was set to 1,200 kcal per day.

Unsaturated Fatty Acids Enhancement. According to previous recommendations based on studies of rodents (21), participants (n = 20) were instructed to enhance intake of UFAs aiming at a 20% increase compared with previous habits, over a period of 3 months. They were instructed to keep the amount of total fat intake unchanged.

All subjects assigned to 1 of the 2 dietary interventions were trained on how to follow their respective diet by experienced clinical dieticians blinded to the underlying study hypothesis. Therefore, after completing baseline measurements, participants attended a 2-h tutorial (maximum 12 persons each). Additionally, they received dietary instructions in written forms. Group 1 additionally underwent 1-h individual schooling at baseline and a second 1-h tutorial carried out by the clinical dieticians after a period of 6 weeks. Moreover, subjects of the 2 intervention groups received supplementary dietary counseling via telephone if needed, so that any problems in adhering to the intervention could rapidly be addressed during the entire intervention period. To provide optimal supervision, dieticians obtained information about individual nutritional intake from the nutrition diaries and personal interviews. Adherence to the intervention was monitored by measures of weight, BMI, waist-to-hip ratio, amount of body fat, and fasting serum levels of triglycerides, cholesterol, and hs-CRP, because these parameters have been shown to decrease after caloric restriction and/or after a dietary enhancement of UFA (80–83). In addition, information on adherence to the diet was collected by a postintervention Questionnaire, and self-reported nutritional records were collected in the course of the intervention period.

Control. Participants (n = 10) were instructed not to change previous eating habits over a period of 3 months. No specific dietary counselling was administered to avoid self-chosen/self-administered changes in dietary patterns that

have been reported after any dietary counselling (for example, ref. 84). For simplicity this condition is subsequently referred to as control intervention.

Nutritional records. Supply of nutrients, caloric-, and UFA-intake were documented by nutritional records at the beginning of the study (record 1), after 6 weeks intervention (record 2), and after 12 weeks intervention (record 3). Each record encompassed 7 days of protocol. For nutritional records, all subjects had to plot, on a daily basis, all food and drink intake in an in-house developed standard nutrition diary (University of Münster, Department of Internal Medicine) similar to records used in other studies (see e.g., 45). The diary contained numerous nutritional items presented in standard servings, and additional free lines to describe foods not listed in the diary. Subjects had to mark the respective items, with the possibility to adjust for individual servings. Nutrients, amount of calories, and amount of UFAs where quantified using the software EBISpro (Erhardt).

Physiological Parameters and Blood Sampling. Before and after 3 months of intervention (sessions I and II; see Fig. 1), the following variables were assessed: Weight (in kilograms; measured), height (in meters; self-reported), waist-to-hip ratio (in centimeters/centimeters, measured), body fat (percentage, measured), diastolic and systolic blood pressure, heart rate, fasting serum levels of triglyceride, total-, HDL- and LDL-cholesterol, insulin, glucose, insulin-like growth factor 1 (IGF-1), brain derived neurotrophic factor (BDNF), catecholamines (85), markers of inflammation [i.e., high sensitive-C-reactive protein (hs-CRP) and tumor necrosis factor-alpha (TNF-α)], and routine parameters (sodium, potassium, calcium, phosphate, protein, creatinine, urea; data not shown), for details, see *SI Text*.

Neuropsychological Testing. Before and after 3 months of intervention, subjects were tested on memory performance using the German version of the Rey Auditory Verbal Learning Task (AVLT) (84). The test was performed by a trained clinical neuropsychologist. Participants were asked to learn as many words as possible out of a list of 15 words. As primary outcome measure ''memory score,'' we considered the total number of retrieved words after 30 min (delayed memory), adjusted for false positive misidentifications (86, 87), in line with previous studies (36, 37, 88), and with signal detection theory (89). Additionally, analysis was conducted for total number of retrieved words without adjustment for false-positives, and for total numbers of false-positive errors. Two different but congruent versions were presented at the 2 test sessions to avoid test-retest effects.

To assess potential differences in attention or working memory due to the intervention, subjects completed trail making tests (TMT) A and B (90), and forward/backward digit span (WMS-R, 91), before and after the intervention. No significant differences emerged between pre and post intervention test sessions (all $P > 0.5$).

Statistical Analysis. Before data analysis, normal or near-normal distribution and homogeneity of variances were tested by the Kolmogorov–Smirnov test and the Levene's Test.

To monitor dietary compliance, individual physiological parameters and serum levels and dietary intake before and after the intervention were compared by a repeated measures analysis of variance ($ANOVA_{RM}$) with TIME (''baseline,'' ''after intervention period''), and between factor GROUP (''caloric restriction,'' ''UFA enhancement,'' ''control''), followed by post hoc paired *t* tests (2-tailed) as appropriate.

To assess intervention effects, an $ANOVA_{RM}$ on the outcome variable ''memory scores'' was conducted, with TIME and between factor GROUP. Depending on significance, post hoc paired *t* tests (two-tailed) were performed as appropriate.

Correlations between changes in memory score and changes in physiological parameters and serum levels from baseline to post intervention assessment were assessed using Spearman's correlation coefficient.

Significance was set at $P < 0.05$, all data are presented as mean with standard error of the mean, unless indicated otherwise.

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