## <u>ORIGINAL RESEARCH</u>

# Humor's Effect on Short-term Memory in Healthy and Diabetic Older Adults

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### ABSTRACT

**Context** • With aging, the detrimental effects of stress can impair a person's ability to learn and sustain memory. Humor and its associated mirthful laughter can reduce stress by decreasing the hormone cortisol. Chronic release of cortisol can damage hippocampal neurons, leading to impairment of learning and memory.

**Objectives** • The study intended to examine the effect of watching a humor video on short-term memory in older adults.

**Design** • The research team designed a randomized, controlled trial.

**Setting** • The study took place at Loma Linda University in Loma Linda, CA, USA.

**Participants** • The study included 30 participants: 20 normal, healthy, older adults—11 males and 9 females and 10 older adults with type 2 diabetes mellitus (T2DM)— 6 males and 4 females.

**Intervention** • The study included 2 intervention groups of older adults who viewed humorous videos, a healthy group (humor group), aged  $69.9 \pm 3.7$  y, and the diabetic group, aged  $67.1 \pm 3.8$  y. Each participant selected 1 of 2 humorous videos that were 20 min in length, either a Red Skeleton comedy or a montage of *America's Funniest Home Videos*. The control group, aged  $68.7 \pm 5.5$  y, did not watch a humor video and sat in quiescence.

**Outcome Measures** • A standardized, neuropsychological, memory-assessment tool, the Rey Auditory Verbal Learning Test (RAVLT), was used to assess the following abilities: (1) learning, (2) recall, and (3) visual recognition. The testing occurred twice, once before (RAVLT1) and once after (RAVLT2) the humorous video for the humor and diabetic groups, and once before (RAVLT1) and once after (RAVLT2) the period of quiescence for the control group. At 5 time points, measurements of salivary cortisol were also obtained. The Kruskal-Wallis test was used to measure significance of the data based on the 3 groups.

**Results** • In the humor, diabetic, and control groups, (1) learning ability improved by 38.5%, 33.4%, and 24.0%, respectively (P=.025); (2) delayed recall improved by 43.6%, 48.1%, and 20.3%, respectively (P=.064); and (3) visual recognition increased by 12.6%, 16.7%, and 8.3%, respectively (P=.321). For levels of salivary cortisol, the research team found significant and borderline decreases for the humor group between baseline and (1) post-RAVLT1 (P=.047), (2) postvideo (P=.046), and (3) post-RAVLT2 (P=.062). The diabetic group showed significant decreases between baseline and (1) post-RAVLT1 (P=.047), (2) postvideo (P=.034). The study found no significant changes for the control group.

**Conclusion** • The research findings supported potential clinical and rehabilitative benefits for humor that can be applied to whole-person wellness programs for older adults. The cognitive components—learning ability and delayed recall—become more challenging as individuals age and are essential to older adults for providing a high quality of life: mind, body, and spirit. Because older adults can experience age-related memory deficits, complementary, enjoyable, and beneficial humor therapies should be implemented for them. (*Altern Ther Health Med.* 2015;21(3):16-25.)

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Corresponding author: Gurinder Singh Bains, MD, PhD E-mail address: gbains@llu.edu Ithough the world's population is growing and life expectancies are increasing, the health-related wellbeing of the population is lagging.<sup>1</sup> As a result of the upsurge in life expectancy, an obligation exists for the health care system to develop a heightened awareness of the need to provide whole-person care that meets the needs of the mind, body, and spirit. A salutogenic paradigm of health care prevention, education, and promotion—is necessary to promote overall human wellness.<sup>2,3</sup> Salutogenic models focus on factors that support human health and well-being rather than on factors that cause disease.

Because of a projected increase in the population of older adults and the resulting demands that health care systems will face, commitment to promotion of wellness for the mind, body, and spirit should target that population. They face abundant challenges with regard to age-related loss of short-term memory<sup>4,5</sup> that affect quality of life. These challenges are encountered by individuals, their families, and the society in which the older adults live. This issue is complicated by the fact that older adults encounter ageassociated diseases, such as diabetes mellitus, that also affect short-term memory.<sup>6</sup> The growth of the world's population of older adults is a major influencing factor in a current pandemic of type 2 diabetes mellitus (T2DM).7 Therapies for short-term memory loss need to be a crucial component of whole-person wellness for older adults as well as an element applied in clinical settings.

In older adults, issues with short-term memory become notably apparent through a lack of health care compliance. For example, noncompliance can manifest as reduced compliance with therapeutic exercise, inaccurate timings and dosages of medications, and neglect of health care appointments.<sup>8</sup> These issues of behavioral and physical compliance are observed in home-rehabilitation treatment programs and are also noted in day-to-day events by families and friends. Noncompliance often leads to an escalation of T2DM outcomes and, thus, a diminished quality of life among older adults.

To manage diabetes successfully and avoid a diminished quality of life, individuals need to comply with physicians' recommendations,<sup>6</sup> yet poor memory remains a barrier that often impedes compliance.<sup>9</sup> Subsequently, this factor directly affects society's ever-increasing health care costs and the efficacy of treatments.<sup>10</sup> Memory impairments in older adults can lead to mild cognitive decline.<sup>11</sup> Further, those deficits, which impose a 3-fold greater risk of dementia, can be precursors in a progression toward that disability.<sup>12</sup> Also, in T2DM individuals, the risk for dementia is approximately doubled.<sup>13</sup>

Numerous approaches have been developed and employed to improve short-term memory and cognition in older adults. Studies have shown that cognitive training can enhance short-term memory.<sup>14,15</sup> Programs, such as cognitive stimulation that incorporate increased physical activity, proper nutrition, advancement of quality of life, increased social interaction, and the integration of daytime naps, have been shown to have benefits for that population.<sup>16-18</sup> Humor and the resultant mirthful laughter affect the human body in numerous beneficial ways. In addition to promoting cognition, humor and mirthful laughter can protect against disease,<sup>19</sup> decrease stress,<sup>20</sup> improve the quality of life in depressed older adults,<sup>21</sup> and boost the immune system.<sup>22</sup> Humor therapy, through mirthful laughter, elicits positive emotions that have been used widely as a component of complementary therapy.<sup>23</sup> It has also been shown to improve vascular function and has been shown to promote similar physiological changes as those observed during physical activity.<sup>24,25</sup>

Psychoneuroimmunology (PNI) can explain the beneficial effects that mirthful laughter induces in the human body. PNI is a discipline that explains the network of communication pathways between the brain and the and immune neuroendocrine systems. During electroencephalography, mirthful laughter stimulates the gamma-wave band of the brain.<sup>26</sup> This stimulation leads to the development of higher levels of cognitive activity and has been shown to decrease cortisol, the neuroendocrine stress hormone.<sup>20</sup> In addition, PNI supplements the knowledge base about the relationship between the salutogenic, wholeperson, wellness lifestyle and mirthful laughter.27

Cortisol is released via the hypothalamic pituitary adrenal axis. Cortisol can be neurotoxic to the hippocampus, the site of consolidated memory, and a positive eustress experience can reduce stress and, thus, decrease the levels of cortisol.<sup>20,28</sup> However, during a state of chronic excess secretion of cortisol due to continuous stressful events, the hippocampus can become damaged. This damage in turn can lead to impairment in emotion, learning, memory,<sup>5,29</sup> and recall of prior stored memory.<sup>30</sup>

Cortisol modulates brain function through alterations in brain-neuron structure.<sup>31</sup> However, the brain can build structural plasticity (ie, new neuron formation) in response to those alterations.<sup>32</sup> This protective feature may prevent permanent damage caused by acute and chronic stress.<sup>31</sup> Functional imaging has shown permanent changes in brain neurons as a result of stressors, such as a test requiring an individual to count backward.<sup>33</sup> In addition, research has shown that the volume of hippocampal gray matter can decrease due to depression.<sup>34</sup> poor glucose control in T2DM,<sup>35,36</sup> and chronic life stress.<sup>37</sup> During testing on memorization of lists of words by older adults, the anterior left hippocampus plays a vital role in memory encoding and recall.<sup>38</sup>

Studies have revealed that cognitive function can decline in individuals with T2DM.<sup>39-42</sup> The exact mechanism of T2DM-associated cognitive dysfunction has not yet been clarified.<sup>43</sup> However, numerous concepts have been suggested. Poor glucose control, as evidenced by higher hemoglobin  $A_{1c}$ (Hb $A_{1c}$ ) and elevated triglyceride levels in the plasma, can contribute to a decline in cognition.<sup>39,40,42</sup> Greenwood et al<sup>13</sup> showed that poor glycemic control was related to lower memory-test scores. Umegaki et al<sup>44</sup> showed that T2DM individuals in a 3-year span can experience cognitive dysfunction due to increased insulin and Hb $A_{1c}$ . Elevated glucose concentrations can have harmful effects on brain neurons through the formation of advanced glycation end products (AGEs).<sup>44,45</sup>

AGEs increase arterial rigidity and enhance oxidative stress.<sup>46</sup> Oxidative stress leads to the formation of free radicals that can eventually lead to cell death. In addition, oxidative stress has been implicated in the genesis of Alzheimer's disease,<sup>47</sup> cardiovascular plaque formation, and blood vessel complications in diabetics.<sup>48,49</sup> T2DM individuals without dementia have shown tendencies toward cognitive decline and have limitations in activities of daily living.<sup>50</sup> Healthy brain function, particularly in diabetics, is one essential component of the salutogenic, whole-person wellness program for older adults.

Several studies have shown a therapeutic relationship between humor and its resultant mirthful laughter with regard to behavioral and physiological connections, such as increase in stroke volume, increase in pain tolerance, improved mood, decreased stress, improved depression, and increased in antiinflammatory cytokines.<sup>22,24</sup> However, no studies have shown a link-beneficial or not-between mirthful laughter, humor, and improvement in short-term memory among healthy or diabetic older adults. The aim of the research discussed in this article was to examine the effects of watching a humor video on short-term memory in healthy and diabetic groups of older adults. The research team hypothesized that those healthy and diabetic groups who watched a humorous video would demonstrate enhancement in their short-term memories (ie, in learning ability), delayed recall, and visual recognition. In addition, the team proposed that variations in levels of the cortisol, the stress hormone, at predetermined time points, should be measured.

#### METHODS

#### Participants

Thirty older adults were enrolled in the study. Participants were recruited through word of mouth from Loma Linda University's campus, the Loma Linda University's Diabetes Treatment Center, and neighboring communities, including Loma Linda University's faculty and staff and their spouses and residents of a local, senior retirement center.

Participants were excluded if they had impaired hearing that would prevent them from understanding a researcher's verbal instructions. In addition, participants were questioned and excluded if they had any of the following conditions: (1) cognitive impairments, (2) neurological disorders, (3) psychiatric disorders, or (4) history of substance abuse. Further, participants were excluded if they were taking a corticosteroid. Participants were given the Mini Mental State Exam (MMSE) by the investigator to check for cognitive ability and excluded if their score was less than 24. MMSE is a highly validated exam that tests for cognitive ability.<sup>51</sup> It is divided into 5 components: (1) orientation—10 points, (2) registration—3 points, (3) attention and calculation—5 points, (4) recall—3 points, and (5) language and praxis—9 points. The exam is scored out of a total of 30 points, with

11 questions. A score of 24 to 30 indicates no cognitive impairment; 18 to 23 shows mild cognitive impairment; and 0 to 17 represents severe cognitive impairment. The duration of the exam is approximately 10 minutes. All potential participants were found to be free of cognitive impairments based on the test.

Due to the fact that many older adults do take medications for various conditions, medications were recorded. The informed consent was read by all participants and verbally explained to each person. All questions regarding the study were answered to the participant's satisfaction. Participants signed a statement of informed consent prior to joining the study. The Institutional Review Board of Loma Linda University approved all procedures.

The investigators were blinded as to participants' allocation to the humor and control groups. After signing the informed consent, all nondiabetic participants were instructed to choose 1 slip of paper from 2 in an envelope to determine randomly to which group he or she would be assigned. One slip had the words *Control Group*, and the other slip had the words *Humor Group*.

#### Interventions

Ten individuals, 6 males and 4 females with a mean age of  $68.7 \pm 5.5$  years, were in the control group and did not see a humor video. Ten healthy individuals, 5 males and 5 females with a mean age of  $69.3 \pm 3.7$  years, were in one intervention group and saw a humor video (humor group). Ten diabetics, 6 males and 4 females with a mean age of  $67.1 \pm 3.8$  years, were in a second intervention group and saw a humor video (diabetic group). The diabetic participants had a mean diabetes duration of  $7.7 \pm 6.5$  years and a mean glycated hemoglobin (HbA<sub>1c</sub>) of  $6.8 \pm 1.0$ .

Determination of which humor video each participant in the intervention groups watched was established by asking the person to select 1 of 2 humorous videos: a Red Skelton comedy or a montage of *America's Funniest Home Videos*. Three participants in the humor group selected *America's Funniest Home Videos*, and 7 participants in that group selected the Red Skelton comedy. Seven participants in the diabetic group selected *America's Funniest Home Videos*, and 3 participants in that group selected the Red Skelton comedy. Older adults in the control group did not watch a video and were not permitted to read, speak, or use their cell phones for 20 minutes, which corresponded to the amount of time that the intervention groups spent watching the videos.

The Red Skelton video was a segment from the digital video disc *Double Feature: The Lucy Show/More Red Skelton* (Vina Distributor, Garden Grove, CA, USA), a video from a genre of comedy called the American variety show. The participants watched chapters 1 to 3 for a total of 20 minutes: The chapter 1 duration was 6 minutes and 33 seconds; the chapter 2 duration was 2 minutes and 52 seconds, and the chapter 3 duration was 11 minutes and 35 seconds. The video was selected because participants were older adults and could relate to the humor from that era of comedy. Chapter 1 consisted

of Red Skelton's monologue. Chapters 2 and 3 consisted of Red Skelton with guest stars performing comedy sketches.

America's Funniest Home Videos was a collection of 2 videos on YouTube. They were entitled "100 Falling People, Part 1—America's Funniest Home Videos," part 538; and "100 Falling People, Part 2—America's Funniest Home Videos," part 540. The durations of the 2 videos were 11 minutes and 11 seconds for part 1 and 8 minutes and 50 seconds for part 2, for a total of 19 minutes and 1 second. Both videos consisted of short clips of babies, children, and adults falling in various comical situations.

#### **Outcome Measures**

The study used 2 measurements of outcomes: (1) the Rey Auditory Verbal Learning Test (RAVLT) and (2) a sampling of salivary cortisol. The Kruskal-Wallis test was used to measure significance of the data based on the 3 groups.

Rey Auditory Verbal Learning Test. The RAVLT was used to determine the abilities of participants in 3 areas: (1) learning, (2) delayed recall, and (3) visual recognition. The test was printed by Western Psychological Services (Torrance, CA, USA). It has been used in clinical practice as well as in research studies and is a widely used method of neuropsychological assessment that was developed by Andre Rey. A number of studies have shown high test-retest reliability<sup>52</sup> and validity for the RAVLT.<sup>53,54</sup> It consists of a 15-item word list that is presented 5 times. The instructions are straightforward and can be understood easily by older adults. Examples of words on list A are drum, curtain, school, color, house, and river. The longest word on list A is curtain, which is 7 letters. For list B, examples of words are stove, desk, bird, shoe, and church. Mountain is the longest word on list B with 8 letters. In the current study, the RAVLT was administered twice: (1) immediately before the humor/quiescence period (RAVLT1) and (2) immediately after the humor/quiescence period (RAVLT2).

**Salivary Cortisol Sampling.** The functioning form of cortisol in blood and saliva is its unbound form,<sup>55</sup> and salivary flow rates do not have any effect on levels of salivary cortisol.<sup>56</sup> A strong correlation exists between salivary and serum levels of cortisol.<sup>57-59</sup> To control for confounding results, the timing of the samplings of salivary cortisol was taken into consideration. When faced with a stressful situation, cortisol levels increase separately from the diurnal rhythm.<sup>60</sup> However, in deference to the diurnal rhythm of cortisol, levels were taken between 10:30 AM and 4:00 PM.<sup>61,62</sup> Products for sampling salivary cortisol were purchased from Salimetrics, LLC (State College, PA, USA). Supplies included cryostorage boxes, oral swabs, swab storage tubes, and barcoded sample labels.

### **General Procedures**

A bottle of water was provided to each potential participant. On arrival, the individual entered a private sitting area and was seated for 10 minutes to acclimatize to the room and surroundings, which were kept at 22°C. The testing area that was used was a quiet and comfortable room to preclude possible distractions. No interruptions occurred during sessions. After informed consent was obtained, a few minutes were devoted to the investigator's building rapport with the participant to make him or her feel comfortable. Next, the first of 5 samples of salivary cortisol was obtained. This first sample was the prebaseline sample.

After that first sampling, the investigator administered the MMSE. Upon the investigator reaching the conclusion that a participant's cognitive ability was intact, a nondiabetic participant randomly chose a slip of paper that determined whether he or she would be in the humor group or the control group. After allocation to a group, participants in the humor and diabetic groups chose the videos that they wanted to view, either the Red Skelton Comedy or *America's Funniest Home Videos*. When the investigator and participant were ready, a second sample of salivary cortisol was obtained (ie, the baseline cortisol) and the RAVLT1 was administered to check for learning ability, delayed recall, and visual recognition. Two investigators administered the test.

Investigator 1 read aloud the words, while Investigator 2 kept a tally of the words repeated by the participant on a premade word-list form. Investigator 1 and the participant were seated face to face at a table to facilitate better hearing and understanding of the spoken words spoken. Investigator 2 sat to the side and at a distance from the participant and investigator 1 to prevent the participant's seeing or hearing the tallying of the correctly repeated words. For reliability purposes, throughout the study, the same investigator 1 was used to speak, and the same investigator 2 was used to record. After the RAVLT1 was administered and before the start of the 20 minutes of either watching a humor video or sitting calmly, a third salivary cortisol sample was obtained. When the 20 minutes had passed, the fourth sample of salivary cortisol was obtained, and then the RAVLT2 was administered. Last, after the RAVLT2 was finished, the final salivary cortisol sample was obtained.

#### **Sampling Salivary Cortisol**

Twenty-four hours prior to their appointments with investigators, potential participants were informed not to eat or engage in strenuous exercise for 1 hour prior to their appointment times.<sup>61,63</sup> They were directed not to drink any coffee or alcohol, to abstain from smoking upon awakening, and to avoid falling asleep again after awakening.<sup>64,66</sup> Upon a participant's arrival, verbal instructions were given to him or her explaining the protocol for sampling of salivary cortisol. All questions were answered to the participant's satisfaction.

As shown in Figure 1, samples were taken at 5 predetermined time points throughout the study. An oral swab was placed directly under a participant's tongue by an investigator, and the individual was instructed to keep his or her mouth closed for 90 seconds, which was timed with a stopwatch. Subsequently, the oral swab was removed by the investigator and placed directly into an oral-swab tube, capped, and then immediately stored in the -20°C freezer of



Abbreviation: RAVLT, Rey Auditory Verbal Learning Test.

a refrigerator. After the participant completed the study and all samples of salivary cortisol were obtained, the storage tubes were immediately placed in a -80°C freezer. Each of the storage tubes was prelabeled by Salimetrics with a barcode that included the participant's number and the predetermined time point at which the sample was taken. The same investigator obtained all samples of salivary cortisol.

Participants were allowed to drink water throughout the research study. However, when a sampling of salivary cortisol was approaching, participants were informed 10 minutes prior to it not to take any sips of water until after the sample was made. When all experiments were completed, samples were shipped overnight to Salimetrics for analysis. Two cortisol readings ( $\mu$ g/dL) were analyzed per sample, and an average was reported.

#### Administering the RAVLT

**Learning Ability.** As shown in Figure 2, investigator 1 read aloud a list of 15 words (list A), instructing participants were to listen carefully, and not interrupt the sequence. The words were spoken at a rate of approximately 1 word per second. When all 15 words had been read, the participant was instructed to repeat as many words as he or she could recall.

The order of words repeated by the participant was not of importance. To facilitate the jogging of their memories, participants were encouraged to speak the words aloud repeatedly after they could not recall words to any further extent. They were allowed to repeat words previously spoken. Investigator 2 used a premade list of words from list A and list B and checked the list as words were spoken by a participant. When the participant could no longer recall any words, the same words were tested again in the same procedure, for a total of 5 trials. The research team moved to the next trial after the participant stated that he or she could not recall any additional words or until a minute of silence, at most, had passed. No time break existed between any of the 5 trials.

After the fifth trial, investigator 1 immediately read aloud a set of 15 new words from a different list (list B) and instructed participants to repeat as many of the list B words as they could recall. Investigator 2 again tallied the correct words. As per the RAVLT protocol, list B was given to confuse the participant intentionally.

Following the reading of list B, investigator 1 asked the participant to repeat the words from the original list (list A). At this juncture, the imperative point was that investigator 1 did not speak the words prior to having the participant recall and

Table 1. Mean (SD) of Demographic Characteristics by Group (N=30)

	Control (n=10)	Humor (n=10)	Diabetic (n=10)	P Value <sup>a</sup>
Gender %				
Male	60	50	60	.873 <sup>b</sup>
Female	40	50	40	
AGE <sup>b</sup> (y)	68.7 (5.5)	69.3 (3.7)	68.3 (3.2)	.490
Height <sup>b</sup> (cm)	170.5 (8.8)	168.8 (9.6)	174.3 (9.9)	.958
Weight <sup>b</sup> (kg)	76.9 (17.7)	83.9 (19.8)	87.7 (16.2)	.326
BMI <sup>b</sup> (kg/m <sup>2</sup> )	26.2 (4.1)	29.5 (6.8)	28.8 (4.0)	.287

Abbreviations: SD, standard deviation; ANOVA, analysis of variance.

<sup>a</sup>Kruskal-Wallis ANOVA. <sup>b</sup>Pearson's χ<sup>2</sup>.





Abbreviations: SDs, standard deviations; RAVLT, Rey Auditory Verbal Learning Test.

state the words. The participant was then given a 10-minute break. During the break, the participant was not allowed to sleep, read, or talk on their cell phones or to the investigators. After the 10-minute break concluded, delayed recall was tested.

**Delayed Recall.** Investigator 1 once more asked the participant to recall and repeat the words from list A. Once again, prior to the participant stating the words, investigator 1 did not read the list A words. The participant was then given a 10-minute break. During the break, the participant again was not allowed to sleep, talk on a cell phone, talk to investigators, or read. After the 10-minute break was completed, visual recognition was tested.

**Visual Recognition.** Investigator 2 handed the participant a pen and a 1-page form consisting of a list of words. The participant was instructed to locate the words that were spoken to and learned and recalled by him or her from list A and to check off those words. Participants were told to check off a maximum of 15 words. The form listed 50 words and consisted of words from list A, list B, and various other words. Participants were given a maximum of 3 minutes to complete this task.

#### Watching the Video and Retesting

Humor and Diabetic Groups. After being administered the RAVLT1, each participant watched the comedy video that he or she had chosen for 20 minutes on a laptop. The participant was left alone in the room to watch the video and wore noise-reduction headphones. After the 20-minute viewing session was completed, the RAVLT2 was given, following the same procedure as earlier and using the same words as did the RAVLT1.

**Control Group.** After being administered the RAVLT1, participants in the control group were instructed to sit calmly for 20 minutes. The research team informed participants that they would be left alone for that time period. Participants were not allowed to read, sleep, or talk on a cell phone. After the 20 minutes of sitting calmly were finished, the RAVLT2 was given following the same procedure as described earlier, using the same words as did RAVLT1.

#### **Statistical Methods**

Statistical analysis was performed using the statistical package SPSS for Windows, version 22 (IBM, Armonk, NY, USA). Data were summarized using frequencies and means and standard deviations. The demographic characteristics of the 3 groups of older adults were compared using the Kruskal-Wallis analysis of variance (ANOVA) and Pearson's  $\chi^2$  test. The Kruskal-Wallis ANOVA was also used to compare the RAVLT scores among the 3 groups. The Wilcoxon signed-rank test was

conducted to assess changes in cortisol levels as a result of the intervention. The significance level was set at  $P \le .05$ .

#### RESULTS

The demographics of all 30 participants are displayed in Table 1. No significant differences existed among the control group, humor group, and diabetic group in terms of gender, age, height, weight, and body mass index (BMI). Percentage changes in learning ability, delayed recall, and visual recognition due to the 20-minute humor interventions for the humor group and diabetic group and the 20-minute quiescent period for the control group, with no humor, are shown in Figure 3. Results indicated that learning ability, delayed recall, and visual recognition were enhanced in all groups.

Regarding the differences between pre- and postintervention percentage changes in scores on the RAVLT, significant changes occurred in learning ability among the 3 groups (P = .025), as shown in Figure 3. The humor group when compared with the control group demonstrated the greatest improvement in learning ability. The humor group showed a 38.5% increase in learning ability after watching a humor video as compared with a 33.4 % increase in the diabetic group and a 24.0% increase in the control group, as presented in Figure 3. The percentage increase in the humor group was more than 1.5 times that of the control group. The effect of the humor video seems to have been most influential in improving learning ability. The results for learning ability were statistically significant at P = .025, and even though the percent changes were greater in delayed recall among the 3 groups, they were borderline significant at P = .064. As shown in Figure 3, although the control group increased its delayed recall by 20.3%, the humor and diabetic groups had greater increases, 43.6% and 48.1%, respectively. Although a percentage change in visual recognition occurred in all 3 groups-8.3% in the control group, 12.6% in the humor group, and 16.7% in the diabetic groupthis increase was not significantly different among groups (P = .321).

Percentage changes within each group for the 3 parts of the RAVLT are shown in Figure 4. For the control group, the greatest percentage change was seen in learning ability (24.0%) versus delayed recall (20.3%) (P = .099). The opposite was observed in the humor and diabetic groups. The humor group had a statistically significant difference between the increase in delayed recall, 43.6%, and the increase in learning ability, 38.5% (P = .002). The diabetic group had a 48.1% increase in delayed recall versus a 33.4% increase in learning ability (P = .090).

The means (standard deviations [SDs]) of the salivary cortisol levels ( $\mu$ g/dL) at the 5 predetermined time points for all 3 groups are shown in Table 2. The cortisol level for one control participant appeared to be an outlier, and after using Grubbs's

test, it was determined to be 2.8 SDs away from the mean and, thus, was deleted. Watching a humor video had a significant effect on levels of salivary cortisol in the humor and diabetic groups at all but the last time point for the humor group, with the levels dropping after watching a humor video. The *P* value for that last time point was borderline in significance.

The most relevant drop in levels of salivary cortisol for the humor and diabetic groups occurred from baseline measurements to immediately after watching the humor



**Table 2.** Mean (SD) Cortisol Level ( $\mu$ g/dL) by Study Group (N = 29)

	Control (n=9)	Humor (n=10)	Diabetic (n=10)
(A) Prebaseline	.20 (.08)	.25 (.20)	.18 (.09)
(B) Baseline/ pre-RAVLT1	.20 (.08)	.27 (.20)	.18 (.13)
(C) Post-RAVLT1/prehumor or quiescence	.22 (.12)	.23 (.14)	.13 (.05)
(D) Posthumor or quiescence/ pre-RAVLT2	.17 (.10)	.21 (.13)	.12 (.03)
(E) Post-RAVLT2	.14 (.06)	.17 (.07)	.11 (.03)
	P value	P value	P value
A-B	.257	.242	.242
B-C	.477	.047	.047
B-D	.130	.046	.025
B-E	.047	.062	.034

Abbreviations: SD, Standard deviation; ANOVA, analysis of variance.

Note: Determined using Wilcox signed-rank test.

video, P = .046 and P = .025, respectively. For the control group during the same time frame, no significant changes in levels of salivary cortisol occurred, P = .130. In addition, a significant change in the levels of salivary cortisol occurred for the humor and diabetic groups from baseline to just immediately before watching the humor video, P = .047 and P = .047, respectively.

Also, no significant change in levels of salivary cortisol occurred during the same time frame for the control group,

P = .477. Although, significant changes occurred for the control group from baseline to immediately after administering the RAVLT2 at the end of study (P = .047), the greatest statistically significant change was observed in the diabetic group (P = .034). In addition, the change observed for the humor group during the same time frame was borderline in significance (P = .062). Prebaseline measurements compared to baseline measurements were not significant for the control, humor, and diabetic groups—P = .257, P = .242, and P = .242, respectively.

### DISSCUSSION

As a result of the fact that the population of older adults is increasing at a fast rate, a growing need exists to address the challenges of short-term memory deficiencies. The damaging effects of aging and stress can impair the ability to learn and sustain memory. Salutogenic, whole-person wellness programs for older adults must include a component on improving short-term memory.

Previous studies have shown that various interventions, such as cognitive-stimulation programs, exercise, and multivitamin and herbal supplementation can improve short-term memory.<sup>16,17,67</sup> In the current study, the research team examined whether short-term memory in healthy and diabetic older adults could be improved by watching a humor video and, in addition, whether cortisol could be modulated in the course of the study. To the team's knowledge, its current research was the first to examine the effects of humor and its resultant mirthful laughter on the enhancement of short-term memory in healthy and diabetic older adults.

The results of the current study showed that the older adults in the humor and diabetic groups had a substantial increase in learning ability and delayed recall when compared with the control group. In addition, the findings showed that prebaseline cortisol levels, taken 10 minutes before baseline measurements that were taken immediately before administration of RAVLT1, were not significantly different for the 3 groups. This finding suggested that no indication of an anticipatory effect existed at the onset of the memory tests for the 3 groups.

Directly following RAVLT1, the cortisol levels of the diabetic and humor groups significantly decreased compared with baseline. Given those results, the research team must ask whether the anticipation of watching and enjoying humor might have lessened the potential stress response associated with knowledge of the memory test and, therefore, have affected test scores. The decrease might be attributed to an anticipatory effect caused by the awareness that participants would be in a mirthful state. In contrast, in the control group, an increase in cortisol levels followed RAVLT1.

A stressful state can be either a eustress or distress state. As shown by their decreased cortisol levels, the results for the diabetic and humor groups appear to suggest they underwent a state of eustress while participating in the memory test. On the other hand, as indicated by their increased cortisol levels, the control group seems to have experienced a state of distress. Despite having the knowledge that a second memory test (RAVLT2) was expected, cortisol levels decreased, as expected, during the 20-minute time frame when the diabetic and humor group were watching the humor video, and the control group was seated in quiescence. Further, the study found that humor and the associated mirthful laughter decreased cortisol levels just as readily as a quiescent state did.<sup>20</sup> For the diabetic and humor groups, the effect that humor and laughter had on modulating cortisol levels when compared with baseline was more surprising (Table 2). Cortisol levels for the diabetic and humor groups, after watching the humor video, were significantly lower than their baseline levels. Although the cortisol levels for the control group decreased while seated in a quiescence state, the difference was not significant.

The research team feasibly proposes that the hippocampus, the site of consolidation of short-term memory, endured a lower amount of overall suppression induced by cortisol in the diabetic and humor groups, possibly explaining the improved cognitive outcomes.<sup>5,29</sup> A consistent decrease in cortisol levels from baseline occurred, throughout each time point, in the diabetic and humor groups. By the conclusion of the study (ie, the completion of RAVLT2), the diabetic group showed a statistically significant decrease in cortisol from baseline. In addition, the humor group showed a decrease in cortisol from baseline measurements that was borderline for statistical significance (Table 2).

Moreover, the research team proposes that the diabetic and humor groups were in a continuous state of eustress. This effect possibly can justify the greater overall scores on the memory tests in both groups after the humor and laughter sessions. Significantly greater scores on memory tests were observed in the diabetic and humor groups, although cortisol levels had decreased in the all 3 groups during the 20-minute period (Table 2). Watching the humor video had the greatest influence on delayed recall in the diabetic and humor groups, where the percentage increase was more than double for those groups as compared with that of the control group. Also, the diabetic and humor groups showed a significantly greater improvement in learning ability than did the control group.

In addition, the control group showed, throughout the research study, variations in cortisol levels. Currently, the research team lacks adequate data to suggest that this variability did or did not affect a hippocampal effect over cognition. Although the control group exhibited a significant decrease in cortisol levels between baseline measurements and RAVLT2, the research team observed that this decrease was not associated with increases in scores on the memory tests (ie, the control group's scores for learning ability and delayed recall were negatively affected).

Within the humor group, delayed recall was tested after learning ability and delayed recall showed a more significant increase than that observed for learning ability. Following a learning session, delayed recall should increase. Due to the continuous eustress state of the humor group, the enhancement to learning ability would be expected to translate into better delayed recall.

In contrast, within the diabetic group, no significant differences were observed between the increases in learning ability and delayed recall. The expectation of the enhancement of delayed recall due to an improvement in learning ability was not observed in the diabetic group. The research team further suggests that the diabetic group was not able to overcome the damaging effects of the end products of previous advanced glycation because the RAVLT acted as an acute short-term stressor. Oxidative stress has been linked to cognitive impairment.<sup>68</sup> In the brain, oxidative stress results in a decrease in neurotransmitters (ie, acetylcholine, which in turn leads to poor communication between brain neurons). In addition, acetylcholine plays a major role in the development of memory and learning.<sup>69</sup> This finding further illustrates the importance of having humor therapy as a component in a salutogenic, whole-person wellness program for older adults.

In addition, within the control group, possibly due to the continuum of distress, no significant difference existed between the increase in learning ability and delayed recall. This conclusion supports the assertion that humor may play a role in improving short-term memory—specifically, learning ability and delayed recall in older adults.

**Limitations.** Potential limitations existed for this study. The sample size was small; however, based on the results of a pilot study, the current sample size had a 90% power. Nevertheless, a larger sample size would better represent the older adult population. The individuals who participated were limited to older adults, healthy and diabetic. Future studies should include groups of older adults with other medical conditions, such as obesity and poorly controlled diabetes. Obese individuals should be involved because obesity has been associated with greater risks for cognitive deficiencies.<sup>70</sup>

The degree to which individuals, healthy or not, can handle their allostatic loads (ie, wear and tear on the body) is dependent on quality of life.<sup>71</sup> Individuals are able to modulate their cortisol levels during stress based on prior experiences, upbringing, and habituation.<sup>72</sup> Social interactions with family and friends and positive self-esteem have been shown to have a beneficial effects on allostasis, resulting in a lower allostatic load. Being able to return to homeostasis through a decreased level of allostatic load can lead to an improved quality of life.

#### CONCLUSIONS

The research team's research findings offer suggestive clinical and rehabilitative benefits that can be applied to salutogenic, whole-person wellness programs for older adults. The cognitive components, learning ability and delayed recall, become more challenging as individuals age. Learning ability and delayed recall are essential to older adults for an improved quality of life: for health, social interactions, and economic status. To prevent development of age-related memory deficits in older adults, complementary, enjoyable, and beneficial humor therapies should be considered.

#### REFERENCES

- Lunenfeld B, Stratton P. The clinical consequences of an ageing world and preventive strategies. Best Pract Res Clin Obstet Gynaecol. 2013;27(5):643-659.
- Antonovsky A. The salutogenic model as a theory to guide health promotion. Health Promot Int. 1996;11(1):11-18.
- Lindstrom B, Eriksson M. From health education to healthy learning: implementing salutogenesis in educational science. Scand J Public Health. 2011;39(6)(suppl):85-92.
- Derouesne C. Complaints of memory loss in the elderly [in French]. Rev Prat. 1994;44(11):1432-1435.
- Mormino EC, Kluth JT, Madison CM, et al. Episodic memory loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects. *Brain.* 2009;132(pt 5):1310-1323.
- Dellasega C. Self-care for the elderly diabetic. J Gerontol Nurs. 1990;16(1):16-20.
  Ginter E, Simko V. Type 2 diabetes mellitus, pandemic in 21st century. Adv Exp
- Med Biol. 2012;771:42-50. 8. Jack K, McLean SM, Moffett JK, Gardiner E. Barriers to treatment adherence in
- Jack R. Herzpy outpatient clinics: a systematic review. Man Ther. 2010;15(3):220-228.
  Sluijs EM, Kok GJ, van der Zee J. Correlates of exercise compliance in physical
- therapy. Phys Ther. 1993;73(11):771-782; discussion 783-786.
- McLean SM, Burton M, Bradley L, Littlewood C. Interventions for enhancing adherence with physiotherapy: a systematic review. *Man Ther.* 2010;15(6):514-521.
- Han JW, Oh K, Yoo S, et al. Development of the ubiquitous spaced retrievalbased memory advancement and rehabilitation training program. *Psychiatry Investig.* 2014;11(1):52-58.
- Goldman WP, Morris JC. Evidence that age-associated memory impairment is not a normal variant of aging. Alzheimer Dis Assoc Disord. 2001;15(2):72-79.
- Greenwood CE, Kaplan RJ, Hebblethwaite S, Jenkins DJ. Carbohydrate-induced memory. impairment in adults with type 2 diabetes. *Diabetes Care*. 2003;26(7):1961-1966.
- Berry AS, Zanto TP, Clapp WC, et al. The influence of perceptual training on working memory in older adults. *PLoS One*. 2010;5(7):e11537.
- D'Esposito M, Gazzaley A. Can age-associated memory decline be treated? N Engl J Med. 2011;365(14):1346-1347.
- Segal SK, Cotman CW, Cahill LF. Exercise-induced noradrenergic activation enhances memory consolidation in both normal aging and patients with amnestic mild cognitive impairment. J Alzheimers Dis. 2012;32(4):1011-1018.
- Tardif S, Simard M. Cognitive stimulation programs in healthy elderly: a review. Int J Alzheimers Dis. 2011;2011:378934.
- Backhaus J, Junghanns K. Daytime naps improve procedural motor memory. Sleep Med. 2006;7(6):508-512.
- Kimata H. Differential effects of laughter on allergen-specific immunoglobulin and neurotrophin levels in tears. *Percept Mot Skills*. 2004;98(3, pt 1):901-908.
- Berk LS, Tan SA, Fry WF, et al. Neuroendocrine and stress hormone changes during mirthful laughter. Am J Med Sci. 1989;298(6):390-396.
- Walter M, Hanni B, Haug M, et al. Humour therapy in patients with late-life depression or Alzheimer's disease: a pilot study. Int J Geriatr Psychiatry. 2007;22(1):77-83.
- Bennett MP, Lengacher C. Humor and laughter may influence health, IV: humor and immune function. *Evid Based Complement Alternat Med.* 2009;6(2):159-164.
- Tan SA, Tan LG, Lukman ST, Berk LS. Humor, as an adjunct therapy in cardiac rehabilitation, attenuates catecholamines and myocardial infarction recurrence. *Adv Mind Body Med.* 2007;22(3-4):8-12.
- Mora-Ripoll R. The therapeutic value of laughter in medicine. Altern Ther Health Med. 2010;16(6):56-64.
- Sugawara J, Tarumi T, Tanaka H. Effect of mirthful laughter on vascular function. Am J Cardiol. 2010;106(6):856-859.
- 26. Berk LS, Pawar P, Alphonso C, Rekapalli N, Arora R, Cavalcanti P. Humor associated mirthful laughter enhances brain EEG power spectral density gamma wave band activity (31-40 Hz). Paper presented at: 2013 Annual Meeting of the Society for Neuroscience; November 9-13, 2013; San Diego, CA.
- Tanyi RA, Berk LS, Lee JW, Boyd K, Arechiga A. The effects of a psychoneuroimmunology (PNI) based lifestyle intervention in modifying the progression of depression in clinically depressed adults. *Int J Psychiatry Med.* 2011;42(2):151-166.
- Berk LS, Felten DL, Tan SA, Bittman BB, Westengard J. Modulation of neuroimmune parameters during the eustress of humor-associated mirthful laughter. Altern Ther Health Med. 2001;7(2):62-76.
- Meyer T, Smeets T, Giesbrecht T, Quaedflieg CW, Merckelbach H. Acute stress differentially affects spatial configuration learning in high and low cortisolresponding healthy adults. *Eur J Psychotraumatol.* 2013;4. doi:10.3402/ejpt. v4i0.19854.
- de Quervain DJ, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature*. 1998;394(6695):787-790.
- McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol.* 2008;583(2-3):174-185.

- Popov VI, Bocharova LS. Hibernation-induced structural changes in synaptic contacts between mossy fibres and hippocampal pyramidal neurons. *Neuroscience*. 1992;48(1):53-62.
- Wang J, Rao H, Wetmore GS, et al. Perfusion functional MRI reveals cerebral blood flow pattern under psychological stress. *Proc Natl Acad Sci U S A*. 2005;102(49):17804-17809.
- Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. Am J Psychiatry. 2003;160(8):1516-1518.
- Gold SM, Dziobek I, Sweat V, et al. Hippocampal damage and memory impairments as possible early brain complications of type 2 diabetes. *Diabetologia*. 2007;50(4):711-719.
- Convit A, Wolf OT, Tarshish C, de Leon MJ. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc Natl Acad Sci U S A*. 2003;100(4):2019-2022.
- Gianaros PJ, Jennings JR, Sheu LK, Greer PJ, Kuller LH, Matthews KA. Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus. *Neuroimage*. 2007;35(2):795-803.
- Kircher T, Weis S, Leube D, et al. Anterior hippocampus orchestrates successful encoding and retrieval of non-relational memory: an event-related fMRI study. *Eur Arch Psychiatry Clin Neurosci.* 2008;258(6):363-372.
- Perlmuter LC, Nathan DM, Goldfinger SH, Russo PA, Yates J, Larkin M. Triglyceride levels affect cognitive function in noninsulin-dependent diabetics. J Diabet Complications. 1988;2(4):210-213.
- Helkala EL, Niskanen L, Viinamaki H, Partanen J, Uusitupa M. Short-term and long-term memory in elderly patients with NIDDM. *Diabetes Care*. 1995;18(5):681-685.
- Zhang J, Wang Y, Wang J, et al. White matter integrity disruptions associated with cognitive impairments in type 2 diabetes patients [published online ahead of print June 19, 2014]. *Diabetes.*
- Ravona-Springer R, Heymann A, Schmeidler J, et al. Trajectories in glycemic control over time are associated with cognitive performance in elderly subjects with type 2 diabetes. *PLoS One*. 2014;9(6):e97384.
- Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. *Endocr Rev.* 2008;29(4):494-511.
- Umegaki H, Kawamura T, Kawano N, Umemura T, Kanai A, Sano T. Factors associated with cognitive decline in elderly diabetics. *Dement Geriatr Cogn Dis Extra*. 2011;1(1):1-9.
- Valente T, Gella A, Fernandez-Busquets X, Unzeta M, Durany N. Immunohistochemical analysis of human brain suggests pathological synergism of Alzheimer's disease and diabetes mellitus. *Neurobiol Dis.* 2010;37(1):67-76.
- Yan HD, Li XZ, Xie JM, Li M. Effects of advanced glycation end products on renal fibrosis and oxidative stress in cultured NRK-49F cells. *Chin Med J (Engl)*. 2007;120(9):787-793.
- Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007;39(1):44-84.
- Ott C, Jacobs K, Haucke E, Navarrete Santos A, Grune T, Simm A. Role of advanced glycation end products in cellular signaling. *Redox Biol.* January 2014;2:411-429.
- Simm A. Protein glycation during aging and in cardiovascular disease. J Proteomics. October 2013;92:248-259.
- Christman AL, Vannorsdall TD, Pearlson GD, Hill-Briggs F, Schretlen DJ. Cranial volume, mild cognitive deficits, and functional limitations associated with diabetes in a community sample. *Arch Clin Neuropsychol.* 2010;25(1):49-59.
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-198.
- Uchiyama CL, D'Elia LF, Dellinger AM, et al. Alternate forms of the Auditory-Verbal Learning Test: issues of test comparability, longitudinal reliability, and moderating demographic variables. Arch Clin Neuropsychol. 1995;10(2):133-145.
- Moses JA Jr. Factor structure of Benton's tests of visual retention, visual construction, and visual form discrimination. *Arch Clin Neuropsychol.* 1986;1(2):147-156.
- Smith GE, Ivnik RJ, Malec JF, Kokmen E, Tangalos EG, Kurland LT. Mayo's Older Americans Normative Studies (MOANS): factor structure of a core battery. *Pyschol Assess*. 1992;4(3):382-390.
- Vining RF, McGinley RA, Symons RG. Hormones in saliva: mode of entry and consequent implications for clinical interpretation. *Clin Chem.* 1983;29(10):1752-1756.
- Vining RF, McGinley RA. The measurement of hormones in saliva: possibilities and pitfalls. J Steroid Biochem. 1987;27(1-3):81-94.
- Dorn LD, Lucke JF, Loucks TL, Berga SL. Salivary cortisol reflects serum cortisol: analysis of circadian profiles. Ann Clin Biochem. 2007;44(pt 3):281-284.
- Hiramatsu R. Direct assay of cortisol in human saliva by solid phase radioimmunoassay and its clinical applications. *Clin Chim Acta*. 1981;117(2):239-249.
- Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem.* 1983;20(pt 6):329-335.
- Krieger DT. Rhythms of ACTH and corticosteroid secretion in health and disease, and their experimental modification. J Steroid Biochem. 1975;6(5):785-791.
- Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*. 2009;34(10):1423-1436.

- Ice GH, Katz-Stein A, Himes J, Kane RL. Diurnal cycles of salivary cortisol in older adults. *Psychoneuroendocrinology*. 2004;29(3):355-370.
- Follenius M, Brandenberger G, Hietter B. Diurnal cortisol peaks and their relationships to meals. J Clin Endocrinol Metab. 1982;55(4):757-761.
- Badrick E, Bobak M, Britton A, Kirschbaum C, Marmot M, Kumari M. The relationship between alcohol consumption and cortisol secretion in an aging cohort. J Clin Endocrinol Metab. 2008;93(3):750-757.
- Badrick E, Kirschbaum C, Kumari M. The relationship between smoking status and cortisol secretion. J Clin Endocrinol Metab. 2007;92(3):819-824.
- Lovallo WR, Whitsett TL, al'Absi M, Sung BH, Vincent AS, Wilson MF. Caffeine stimulation of cortisol secretion across the waking hours in relation to caffeine intake levels. *Psychosom Med.* 2005;67(5):734-739.
- Macpherson H, Ellis KA, Sali A, Pipingas A. Memory improvements in elderly women following 16 weeks treatment with a combined multivitamin, mineral and herbal supplement : a randomized controlled trial [published online ahead of print October 18, 2011]. *Psychopharmacology (Berl)*. 2012;220(2):351-365.
- Liu R, Liu IY, Bi X, et al. Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. *Proc Natl Acad Sci U S A*. 2003;100(14):8526-8531.
- Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol. 2006;16(6):710-715.
- Gunstad J, Paul RH, Cohen RA, Tate DF, Gordon E. Obesity is associated with memory deficits in young and middle-aged adults. *Eat Weight Disord*. 2006;11(1):e15-e19.
- Seeman TE, Singer BH, Ryff CD, Dienberg Love G, Levy-Storms L. Social relationships, gender, and allostatic load across two age cohorts. *Psychosom Med.* 2002;64(3):395-406.
- Kirschbaum C, Prussner JC, Stone AA, et al. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosom Med.* 1995;57(5):468-474.

