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The use of moderated mediated analysis to study the influence of hypo-hydration on working memory

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Abstract

Introduction: To date, dehydration has been typically reported to influence psychological parameters when there has been at least a 2% loss of body mass, although there has been little examination of those going about their everyday lives, those who have lost less than 1% of body mass. In such situations factors such as the initial hydration status and individual differences in the response to a reduced fluid intake are likely to be influential. Yet to study the complexity added by such additional variables novel methods of statistical analysis are required.

Objectives: The present study describes the use of moderated mediation, an approach that asks various questions: firstly, is drinking influential?; secondly, does a mediator (*e.g.*, thirst) sit between an independent and dependent variable?; and thirdly, does an effect only occur under certain conditions such as initial osmolality?

Method: In the study, 118 subjects were exposed to 30 °C for four hours during which they half drank 300 ml water. The serial sevens test of working memory was performed before and at the end of the procedure.

Results: A 0.6% loss of body mass reduced the efficiency of working memory. Those who consumed water had better working memory; working memory was worse in participants who lost more body mass or became thirstier, but only in those with higher levels of baseline osmolality.

Conclusions: Small variations in hydration status influenced cognitive functioning although there were individual differences in the response. The parameters that influence an adverse response to hypo-hydration need to be established to allow giving appropriate advice.

Key words: Hypo-hydration. Moderated mediation analysis. Water. Working memory.

OBJECTIVES

In the popular press there is often the suggestion that dehydration is a common and largely unrecognized problem, with advice such as we should “drink eight 8-ounce glasses of water a day” or we should examine the color of our urine to monitor to what extent we are well hydrated. In fact, there is very little evidence that hypo-hydration is an everyday problem. Changes in mood and cognitive functioning are often the first symptoms of minor nutritional deficiencies (1), yet a review of the influence of dehydration on psychological parameters concluded that any evidence of an adverse effect occurred when there had been at least a 2% loss of

body mass (2). However, as it has also been suggested that those going about their everyday lives are not likely to lose 1% of their body mass (2), it might be argued that hypo-hydration will rarely be a concern. However, with minor differences in hydration status, factors such as the initial hydration status and individual differences in the response to a reduced fluid intake may be influential. The present study describes a novel approach in this area: the use of moderated mediation (3). The way in which this approach allows the study of the interaction between relevant variables is illustrated. It is reported that a 0.6% body mass loss reduced the efficiency of working memory.

Moderated mediation (3) can be conceived as a series of questions (Fig. 1). First, we have the “what question”: for example, does drinking as opposed to not drinking influence a dependent variable such as mood? Secondly, the “how question”: does a mediator sit between an independent and dependent variable. Thus an independent variable, such as drinking, may influence a mediator variable, for example thirst. In turn, the mediator influences the dependent variable mood. This is termed an indirect effect. In this example, drinking as such does not influence mood. Drinking rather influences thirst and thirst then influences mood. Finally, the “when question”: moderated mediation implies that a mediator is only influential at certain levels of another variable, the moderator. This is termed a conditional indirect effect, as the influence of a mediator depends on another variable. With the present hypothetical example, thirst may mediate the effect of drinking but this may only occur under certain conditions; for example, it may depend on an individual's initial osmolality.

This suggested statistical approach will be illustrated, and the findings in this area extended, by examining a test of working memory, using regression equations and moderated mediated analysis. It is important to distinguish working memory from short-term memory. Short-term memory involves storing information for a short period but, critically, it does involve the manipulation of that information or organizing how it is held in memory. In

contrast, working memory holds, but also processes, information while reasoning or calculating takes place. Thus, working memory involves the temporary storage and manipulation of information.

In the present context, the choice of working memory as the dependent variable offers the advantage that it is a suitable measure to consider whether there is a need to look at individual differences when considering hypo-hydration. If there exist sub-groups who are susceptible to hypo-hydration, how should they be characterized? Although there are reports stating that a loss of body mass greater than 2% disrupts working memory (4,5), this finding has not always been replicated (6-8). Working memory has never been reported to be disrupted by a loss of 1-2% of body mass (4,8-10). As such, if the use of moderated mediation can demonstrate that a small difference in hydration can be disruptive under some conditions, the value of this novel statistical approach would be demonstrated.

METHODS

PROCEDURE

The procedure was approved by the local Ethics Committee and participants gave written informed consent. From an under-graduate population, 118 (61 male) subjects were recruited by a circular email. Applicants were excluded if they reported a major neurological or psychological disorder or had, in the last week, used a sleeping tablet, anti-histamines, a decongestant or a painkiller. Among the females, 70% were taking an oral contraceptive. Participants were non-smokers aged 18 to 30 years with an average age of 20.4 years.

They were exposed to a temperature of 30 °C for four hours, after randomly being allocated to groups that either did or did not drink 300 mL of pure water. At baseline, those who subsequently did or did not drink were well matched: they did not differ in urine osmolality (no water condition 688.5 milliosmoles/kg [SD 51.6]) or BMI (no water 24.0 [16.7-33.6] v water consumed 23.6 [16.4-33.3]). They were blind as to the nature of the experiment (they were informed it was a study of the effect of heat) and unaware of the fact that others did or did not consume water. To capture the full range of habitual hydration status on the day of testing participants arrived having consumed their usual breakfast.

Upon arrival, body temperature and body mass were measured and a urine sample was collected. Subjects remained in a room at 30 °C for four hours, during which on two occasions they performed a battery of tests (Table I). The temperature of the room varied from 30 to 31 °C, with an average humidity of 53% (depending on testing day it ranged from 43% to 62%). Half of the participants received two 150 ml glasses of water. At the end of the procedure, body temperature and body mass were measured again and a second urine sample was collected.

WORKING MEMORY - SERIAL SEVENS

A computerized version of the serial sevens task was used, in which subjects were required, from a starting number between 800

Table I. Outline of experimental procedure

0 min	Urine sample, body temperature, body weight
15 min	Working memory
1 h 30 min	150 mL of water for those who drank
3 h	150 mL of water for those who drank
3 h 45 min	Working memory
3 h 50 min	Temperature, body weight (before urination)
4 h	Urine sample, body weight (after urination)

and 999, to say whether a second number was exactly seven less. One of two buttons that corresponded to yes or no was pressed. The test is scored as the time taken, in milliseconds, to perform each subtraction. A change in functioning was obtained by subtracting the time taken at baseline from the time taken at the end of the procedure.

BODY MASS

Body mass was measured using an electronic scale (Kern KMS-TM, Kenr and Sohn GmbH, Germany) that, to avoid problems associated with movement, took 50 assessments over a 5 second period and produced an average value. It was sensitive enough to weigh to within 5 grams (17% of an ounce). Subjects were weighed on arrival (after urinating) and after having emptied the bladder for a final time after 240 minutes. Changes in mass from baseline to 240 minutes reflected water loss due to perspiration, breathing and urine excretion. The total body mass lost due to urination, perspiration and breathing was calculated as the change percentage in body mass from the beginning to end of the study. The data presented were obtained wearing the same light clothing throughout the procedure, as preliminary studies found that these data were virtually identical to when measured naked.

OSMOLALITY

Urine osmolality was assessed using an Osmomat 3000 freezing point osmometer (Gonotec GmbH, Berlin, Germany).

BODY TEMPERATURE

Body temperature was measured using a TH8 Infrared Ear Thermometer (Radiant Innovation, Taiwan).

STATISTICAL ANALYSIS

Cook's distance: detection of possible outliers

As with regression analysis, a particular observation may exert undue influence, Cook's distance (11) was calculated, establishing

the extent to which model residuals would change if a particular data point was excluded. Larger Cook's distance values indicate a greater influence. The threshold for determining influential observations was set as $4/N$ in line with previous recommendations (12). On the occasions that a case had a Cook's distance that exceeded this threshold, it was excluded and the data was re-analyzed. On no occasion did this affect the outcome: therefore as there was no reason to suspect that these cases were unusual, the reported results included all cases.

Mediation analysis

A number of possible mediators of the effect of hydration on cognition have been suggested, including changes in thirst, fluid loss, osmolality and body temperature. In order to consider these mechanisms a moderated multiple mediation analysis was carried out, using the SPSS PROCESS macro (model 8) (3), using bootstrapped sampling to estimate the indirect mediation effect. In the present analysis, 5,000 bootstrapped samples were drawn with replacement from the dataset, to estimate a sampling distribution for the indirect mediation pathway. A conceptual diagram of the model is presented in figure 1. A dummy variable was created for the dichotomous independent variable, drinking vs not drinking (X), with the change in speed of working memory as the dependant variable (Y). Change in thirst, change in body temperature, amount of fluid lost and change in osmolality were parallel mediators (M). The total effect of X on Y (denoted by c in figure 1), can be expressed as the sum of the direct effect (denoted by c') and indirect effects, that is the product of the a and b paths (denoted by ab), such that $c = c' + ab$. Indirect effects and 95% confidence

intervals are reported. Given the large variation in participants urine osmolality (and hence hydration status) upon arrival at the laboratory, this factor was entered as a potential moderator (W) of both the direct and indirect effects (Fig. 1).

RESULTS

The effect of drinking water on each of the mediators was considered (path a) and also whether the effect of drinking on each of the mediators depended on baseline osmolality.

PERCENTAGE OF WEIGHT LOST

As expected, those who consumed water lost significantly less weight than those who did not ($\beta = .235$ 95% CI LL -.822, UL -.653); on average, 0.6% of their body mass. Those who arrived with the highest osmolality lost less weight, irrespective of whether they had drunk ($\beta = .445$ 95% CI LL -.001, UL -.002). The interaction drink X osmolality was also significant ($\beta = .227$ 95% CI LL .001, UL .002); when no drink was consumed those who arrived with a higher osmolality lost less weight.

THIRST

Participants reported greater thirst if they had not drunk water ($\beta = -.245$ 95% CI LL -18.624, UL -4.477), but this did not depend on baseline osmolality.

CHANGE IN OSMOLALITY

As expected, participants who consumed water had a lower increase in osmolality ($\beta = -.369$ 95% CI LL -.242.049, UL -.17.775). In addition, those who arrived with the highest osmolality had the smallest increase ($\beta = -.464$ 95% CI LL -.524, UL -.298) but there was no interaction between drinking and baseline osmolality.

BODY TEMPERATURE

Those who arrived with the highest osmolality tended to have an increase in body temperature ($\beta = .153$ 95% CI LL .001, UL .002), however, drinking did not influence body temperature ($\beta = -.095$ 95% CI LL -.237, UL -.055) and this did not depend on baseline osmolality.

EFFECTS ON WORKING MEMORY

Next, the effect of drinking water on working memory was considered (path c) and then the influence of each mediator (path b).

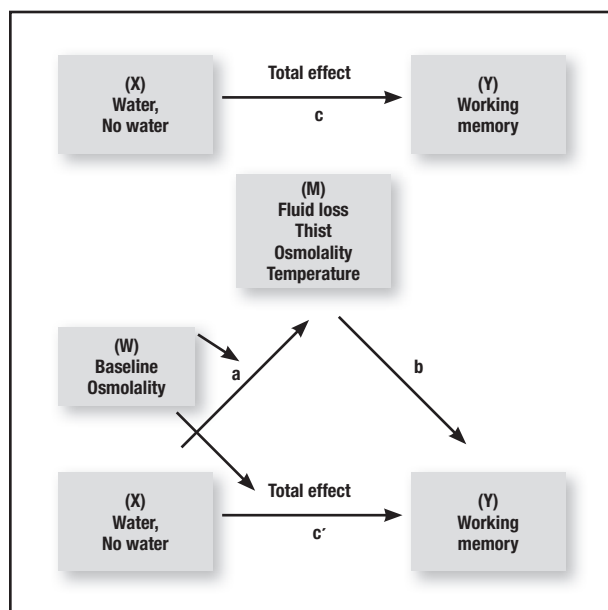


Figure 1.

Schematic of the moderated mediation analysis.

Those who consumed water performed the task more quickly at the end of the morning ($\beta = -.265$ 95% CI LL -497.932 , UL -132.416) (Fig. 2). In addition, reaction times became longer in those who lost the greatest percentage of body weight ($\beta = -.237$ 95% CI LL -117.760 , UL -45.824) and those who became the more thirsty ($\beta = -.226$ 95% CI LL -14.269 , UL -2.449). There were no effect of changes in body temperature or osmolality on working memory.

CONDITIONAL DIRECT AND INDIRECT EFFECTS

Finally, the direct (path c') and indirect effect of drinking water on working memory, through each mediator, was considered. Whether these effects were conditional upon baseline osmolality (Fig. 1) was also examined.

The direct effect of drinking on working memory was not significant and baseline osmolality did not moderate this effect (Table I). Neither change in osmolality nor body temperature mediated the effect of drinking on working memory. However, both thirst and loss of body mass mediated the effect but only in those with higher levels of baseline osmolality. Those who lost more body mass had a poorer working memory, as did those who became most thirsty; these effects were prevented by drinking water but only in those with higher levels of osmolality at baseline (Table I). For both fluid loss ($-.301$, 95% CI LL $-.688$, UL $-.091$) and thirst ($.163$, 95% CI LL $.0149$, UL $.424$) the index of moderated mediation (the indirect effect of highest order product) (3) was significant.

SUMMARY

- Those with a higher baseline osmolality tended to have a greater increase in body temperature and lost less weight, irrespective of whether they drank or not.
- Those who consumed water had better working memory.
- Working memory was worse in those who lost more body mass or became more thirsty, but only in those with the higher levels of baseline osmolality.

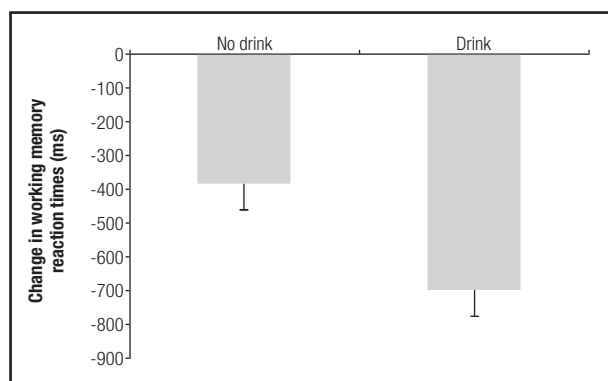


Figure 2.

The effect of drinking water on working memory.

DISCUSSION

There is a parallel between drinking water preventing a decline in working memory (Fig. 2) and previous findings: using the same paradigm, drinking prevented a decline in episodic memory and focussed attention (13). Yet the present report contrasts with previous reports in that working memory was not disrupted by a loss of 1-2% of body mass (4,8-10) and that even a loss of more than 2% has only sometimes (4-8) been found to be disruptive.

Various factors may account for present positive response to drinking. Firstly, the sample size was by far the largest in this area and previous studies may have used a sample that was too small to pick up a change of the size that is likely to result (2).

Sample size is likely to be fundamental, as both psychological and physiological measures in this area are subject to considerable individual variability with the consequence that large samples are needed to achieve the required statistical power. Rather than trying to study subjects who had been treated identically, the present study considered subjects who had consumed their normal breakfast and whose hydration status reflected the habitual fluid intake.

In the event baseline osmolality varied from 115 to 1,168 milliosmoles/kg. In those who had not drunk, the identical hypo-hydration inducing situation produced a loss of body mass that ranged from -1.52% to -0.24% . In those who had drunk, the change in body mass varied from -1.51% to $+0.54\%$. Those with a higher baseline osmolality tended to have a greater increase in body temperature; they also lost less weight, irrespective of whether they drank water.

A difference in response, depending on baseline osmolality, suggested the possibility that physiological adaptations may have occurred to the level of habitual water intake: that is, those used to a low level of intake responded differently. Similarly, with both attention and episodic memory the loss of a greater percentage of body mass resulted in poorer memory and attention (13). If the basic phenomenon is an interaction between hypo-hydration, and individual differences in physiological status, this is a reason to use a moderated mediation analysis.

It is possible that previous negative findings reflect attempts to experimentally produce a similar physiological state, when there is a need to consider the interaction between drinking and individual differences in physiological status. Alternatively, if physiological parameters had not been controlled, then large individual differences would greatly decrease the chance of statistical significance because of a greater variability in response. The finding that working memory was worse in those who lost more body mass, and became more thirsty, but only in those with the higher levels of baseline osmolality (Table II), illustrated the need to consider hypo-hydration in the context of individual physiological differences. The results demonstrate the value of using moderated mediation when considering such interactions.

A major problem when researching hydration is that it is inevitable that you know whether you have or have not drunk. As such, there is potentially a role for a placebo response; a problem as there is evidence that many factors associated with

Table II. Moderated mediation analysis of the effect of drinking on working memory

Baseline osmolality mOsm/kg	Conditional direct path c' (B, 95% CI)	Conditional indirect path ab (body mass) (B, 95% CI)	Conditional indirect path ab (thirst) (B, 95% CI)	Conditional indirect path ab (osmolality) (B, 95% CI)	Conditional indirect path ab (body temp) (B, 95% CI)
388.03	-266.24 (-550.90, 18.43)	-37.56 (-170.17, 37.32)	19.19 (-2.78, 106.40)	-47.30 (-201.76, 85.65)	17.62 (-4.30, 102.39)
669.92	-218.97 (-449.31, 11.44)	<i>-122.60</i> (-266.72, -34.66)	<i>65.32</i> (14.94, 153.40)	-35.633 (-148.07, 70.44)	-10.21 (-70.80, 7.59)
951.80	-171.70 (-484.71, 141.31)	<i>-207.64</i> (-423.51, -73.71)	<i>60.17</i> (35.58, 252.60)	-23.97 (-134.86, 37.63)	-38.03 (-173.69, 9.056)

Data are B and 95% CI for the direct effect of drinking and the indirect effects of drinking through the parallel mediators; fluid loss, change in thirst, osmolality and body temperature, moderated by baseline osmolality at +/- 1SD from the mean. Italics indicate that the path was significant.

drinking, rather than the actual drink, influence cognitive functioning. Amongst many other effects, telling the subject that drink improved performance resulted in a better outcome and even paying a discounted price for an energy drink decreased the ability to problem solve (14). Given the many widespread pre-existing assumptions about the benefits of remaining hydrated, it is to be expected that drinking, as such, would be beneficial. Benton and Young (13) suggested that one approach is not to study groups, who have or have not drunk, but rather to examine individual differences. As there is a limited ability of individuals to know their hydration status or to understand individual differences in the ability to deal with hypo-hydration, where objective measures such as osmolality can be obtained, these can be considered without the worry of a placebo response. The use of regression equations and moderated mediation considers continuous variables that are not simply related to whether a drink has been consumed. In this manner, the use of regression equations can prevent aspects of a placebo response driving the results.

The present and related findings (13) suggest that small variations in hydration status influence cognitive functioning. However, it cannot be assumed that when faced with the same hypo-hydration inducing situation that everybody will respond in the same way. To be able to offer appropriate advice, parameters that influence an adverse response to hypo-hydration need to be established and convenient methods of measurement developed. For example, three questions related to the frequency of drinking were found to predict the baseline osmolality of urine (15). Using the same paradigm as the present study, individuals who reported habitually drinking more fluid at the end of the morning had a lower urine osmolality and had lost more body mass (15).

A final question is whether the phenomena reported, although statistically significant, have practical significance? An important consideration is individual differences in habitual water intake and the ability to deal with hypo-hydration: these vary greatly to the extent that the benefit for some individuals is likely to be small, whereas in others it will be significant. A task that emerges is to establish the habitual pattern of fluid intake that prevents an acute negative response to a subsequent low fluid intake. Similarly, the

optimal pattern of consumption when faced with a hypo-hydration inducing situation needs to be established: how much should be consumed and how frequently? Another response is to accept that human behavior reflects the influence of a vast range of factors, all of which have a small effect. Although it is unrealistic to expect that a change in a single variable is going to have a major impact, a philosophy of looking for many "marginal gains" may make a difference: ensuring adequate hydration offers one such gain.

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