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CIRCADIAN VARIATIONS IN THE RESPONSIVENESS OF HUMAN GALLBLADDER TO SULFATED MINERAL WATER

Chr. Gutenbrunner,[1,*] A. El-Cherid,[2] A. Gehrke,[3] and M. Fink[3]

[1]Institute of Balneology and Medical Climatology,
Hanover Medical School, Hanover, Germany
[2]Department of Rehabilitation and Balneology,
Bad Wildungen, Germany
[3]Department of Physical Medicine and Rehabilitation,
Hanover Medical School, Hanover, Germany

ABSTRACT

It is well known that the intake of sulfate-containing natural mineral waters leads to contraction of the gallbladder, probably induced by the release of cholecystokinin (CCK). As early as 1959, there were some hints in the literature of circadian variations in gallbladder response; to find out whether this applies with sulfate as a stimulus, a pretest for basic information about gallbladder reaction to sulfate-containing mineral water was carried out on 19 healthy volunteers. On this basis, 15 healthy subjects of both sexes were then studied. After 6h of fasting, 500 mL of a sulfate-containing mineral water (2800 mg SO_4^{2-}/L) were ingested within 5 min. The size of the gallbladder was registered ultrasonographically before and 15, 30, 60, and 120 min after drinking. The experiments were carried out seven times at different hours of the day for each volunteer. After the intake of the mineral water, the mean gallbladder size decreased significantly, followed by an increase after 60 min ($P < .001$). Significant circadian spontaneous variation in gallbladder size was detected (acrophase around 09:00; amplitude was 30.0% of daily average, $P < .001$). The contraction induced by the sulfate-containing

*Corresponding author. Prof. Christoph Gutenbrunner, M.D., Institut für Balneologie und Medizinische Klimatologie, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

water was most marked in the early morning hours and minimal around midday; the amplitude of this variation accounting for 29.0% of the daily average ($P < .01$). In contrast, the postdrinking relaxation was maximal around 18:00 and minimal around 9:00 (amplitude 38.5%, $P < .001$). These results show that the basal size of the gallbladder and its reaction to stimuli show a marked circadian variation: Whereas contractibility is maximal in the morning, dilatation is stronger in the afternoon. (*Chronobiology International, 18(6), 1029–1039, 2001*)

Key Words: Circadian variations; Gallbladder response; Sulfate intake.

INTRODUCTION

The literature provides little information about circadian variations in the motoric responsiveness of gastrointestinal organs in humans. It has been reported that the gastric emptying time is shorter in the morning than in the evening (1,2), and that the transportation velocity of the small bowel (3), as well as the excretion of gastrointestinal regulatory peptides (4–6), depend on the time of the day. Circadian variation in the gallbladder responsiveness to various stimuli was reported in the 1950s (7), but systematic investigations of this topic are lacking.

The intake of sulfate-containing mineral waters or Epsom salts triggers gallbladder contraction (8–11), probably due to release of cholecystokinin (CCK) from the bowel. The CKK release is induced by the increase in intraluminal volume in the duodenum (12,13), which is more marked after the intake of sulfate because of its osmotic capabilities and slow intestinal absorption.

Because of these properties, natural sulfate-containing mineral water is used for the therapy of gallbladder dysfunctions. It also follows that sulfate responsiveness could be a suitable method to test for circadian variations in the sensitivity of the gallbladder to stimulants.

EXPERIMENTAL

For the experiment, (1) to determine the reaction of the gallbladder to sulfate-containing mineral water and (2) to examine possible circadian variations in the gallbladder response, two test series were performed. After informed consent was given, the first test (pretest) included 19 healthy subjects, and the main test included 15 healthy subjects. All subjects were our employees, so it was possible to set a comparable time frame of daily work (08:00–16:30).

Oral history and a clinical examination were assessed and blood samples were taken from the volunteers for baseline data. Results of baseline data are shown in Table 1. The volunteers were synchronized by being asked to go to bed between 22:00 and 23:00 for at least 1 week prior to and throughout the experiment.

Table 1. Baseline Data (Mean ± Standard Deviation)

Primary Parameters	Pretest (Female = 10, Male = 9)	Test (Female = 7, Male = 8)
Age (years)	31.3 ± 8.4	36.3 ± 7.9
Height (cm)	169.4 ± 6.0	173.4 ± 7.3
Weight (kg)	75.3 ± 11.2	67.8 ± 7.8
Body mass index (BMI)	26.2 ± 3.1	25.2 ± 1.7
γ-Glutamyl-transferase (U/L)	16.5 ± 12.0	18.7 ± 7.6
Glutamate oxaloacetal transaminase (U/L)	11.8 ± 3.7	10.5 ± 2.4
Glutamate pyruvate transaminase (U/L)	15.2 ± 4.8	17.7 ± 3.2

On the day when the reaction of the gallbladder was tested in the pretest, the volunteers received 500 mL of the sulfate-containing mineral water or plain water at 06:30 after overnight fasting; a third test was carried out without drinking. The test fluids had to be ingested within 5 min; the temperature of the water was 18°C–20°C. The mineral water contained approximately 2800 mg SO_4^{2-}/L (Table 2). The volunteers received a standardized breakfast 30 min after the fluid intake; the breakfast consisted of one bread roll, 20 g butter, and 1 cup of water (50 mL), which is equivalent to the test meal used for the so-called Pancreolauryl®-Test (14). The meal had to be eaten within 7 min (for further details, see Ref. 15).

For the main test, the procedure was the same as in the pretest for the tap water control and the test meal. The experiments were carried out seven times, at different hours of the day (Table 3); the minimum time span between two measurements was 12h, and the minimum prior fasting period was 5h. With the exception of the nocturnal measurements, the volunteers' circadian habits were left unchanged.

Table 2. Analysis of Test Waters Used

	Sulfate-Water	Tap Water
Cations		
Sodium (Na^+)	3469.0 mg/L	6.2 mg/L
Calcium (Ca^{++})	745.0 mg/L	90.0 mg/L
Magnesium (Mg^{++})	78.0 mg/L	23.5 mg/L
Anions		
Chloride (Cl^-)	5030.0 mg/L	25.0 mg/L
Sulfate (SO_4^-)	2842.0 mg/L	28.8 mg/L
Bicarbonate (HCO_3^-)	317.0 mg/L	292.0 mg/L
Gas		
Carbon dioxide (CO_2)	77.0 mg/L	18.7 mg/L

Table 3. Experimental Timetable

Number of Test Persons	Time of Day						
	06:00	09:00	12:00	15:00	18:00	21:00	0:00
1	M1	M2	M3	M4	M5	M6	M7
2	M7	M1	M2	M3	M4	M5	M6
3	M6	M7	M1	M2	M3	M4	M5
4	M5	M6	M7	M1	M2	M3	M4
5	M4	M5	M6	M7	M1	M2	M3
. . .							
17	M6	M7	M1	M2	M3	M4	M5
18	M5	M6	M7	M1	M2	M3	M4
19	M4	M5	M6	M7	M1	M2	M3

M1, M2, . . . , M6 = number of measurements.

The ultrasonic measurement of gallbladder volume is a well-established method and is especially suitable for repeated measurements because of the lack of radiation exposure. The specificity and reliability of this method have been proven to be sufficient for chronobiological experiments, as well as for pharmacological studies (16–18). The quantification of gallbladder volume using the Simpson formula is the most exact method available (15). Interindividual differences in the results were minimized in the present study by having one experienced investigator carry out all measurements.

The gallbladder volume in the present experiments was measured ultrasonographically (Siemens Sonoline SL1, Munich, Germany; transducer frequency 3.4 MHz) by the same investigator, with measurements made before and 15, 30, 60, and 120 min after drinking. The position of the transducer was standardized to the maximum diameter of the gallbladder, and a second measurement was taken after a 90° rotation of the transducer. The gallbladder volume was calculated using the Simpson formula: $V = [(\pi \times h):4] \times (D_1^2 + D_2^2 + \ldots D_n^2)$ (16).

Gallbladder volume is expressed as a percentage of the predrinking value. The effect of the sulfate-containing water 15 min after drinking was compared using analysis of variance (ANOVA); the longitudinal effects were tested by repeated measures ANOVA. To calculate the mean peak and trough times of the circadian rhythm, individual curves were smoothed by the formula $x_g = (x - 1 + 2x + x + 1):4$, as performed elsewhere (19). The individual peak and trough times were read from the resulting curves, and the mean values were calculated. The SPSS software package (version 10.0) was used for all statistical calculations.

RESULTS

Mean gallbladder volume decreased significantly, by about 50%, within 15 min after the intake of the sulfate-containing mineral water ($P < .001$), whereas

in controls and after tap water, no contraction occurred (Fig. 1). The amount of contraction was similar to that registered after the test meal.

After 60 min, dilatation to about 135% of the original size was recorded (Fig. 2; $P < .001$), which even increased 120 min after fluid intake. This effect might be due to stimulation of the bile secretion by sulfate (20).

In Fig. 3, the spontaneous circadian variations in the gallbladder volume are shown. The maximum values were recorded at 09:00, whereas minimal values occurred between 18:00 and 24:00; the circadian variation of roughly plus or minus 30% was statistically significant ($P < .001$).

The duration of the gallbladder contraction after the intake of sulfate-containing mineral water exhibited a similar circadian variation, about 30% (Fig. 4, upper part), with some ultradian superimpositions. The gallbladder dilatation, measured 120 min after the intake of the sulfate-containing mineral water, exhibited an inverse circadian pattern, with maximal values at 18:00 and minimal values at 09:00. This variation of plus or minus 38.5% was also statistically significant ($P < .001$; Fig. 4, lower part). These results show that the susceptibility of the gallbladder to stimuli depends on the time of day, with stronger contraction in the morning and maximum dilatation in the evening.

The calculated mean peak and trough times of the maxima and minima of the circadian variations of gallbladder volume, gallbladder contraction, and gallbladder dilatation are shown in Table 4.

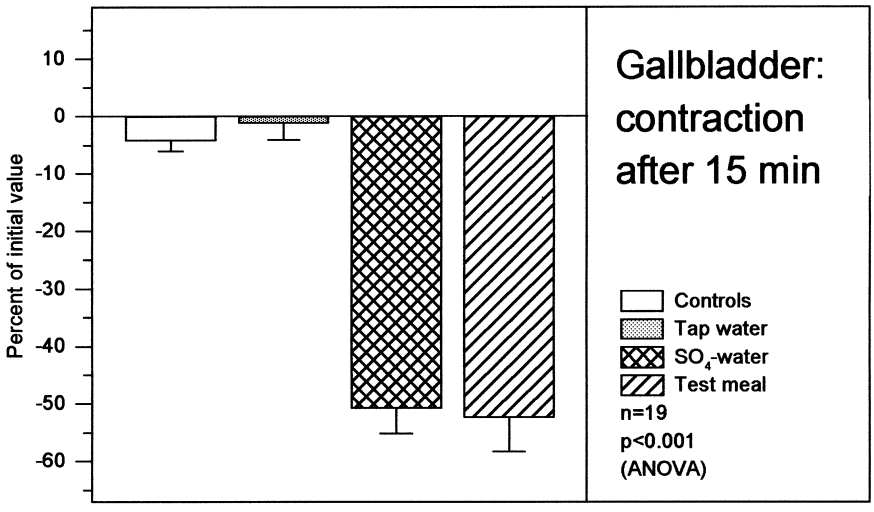

Figure 1. Mean change in the gallbladder volume within 15 min of intake of 500 mL of the natural sulfate-containing water, tap water, or a test meal (for composition, see text) in 19 healthy volunteers. In addition, the change in the gallbladder volume during a control period without eating or drinking is plotted. All values were calculated as percentage of the individual initial values. Brackets indicate standard errors.

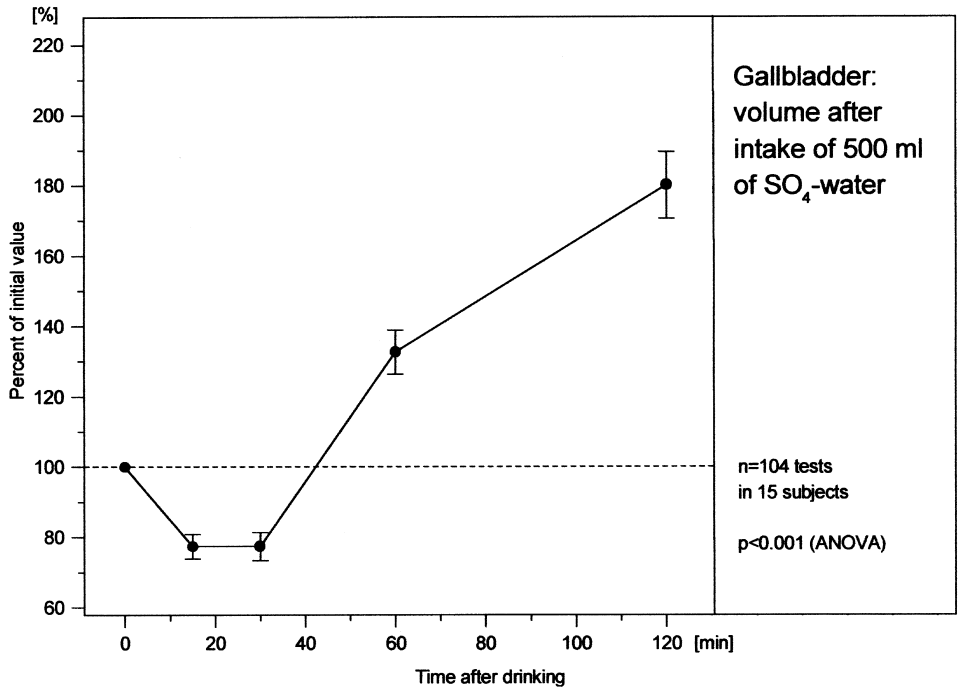

Figure 2. Mean time courses of the gallbladder volume after intake of 500 mL of the natural sulfate-containing water (104 tests in 15 healthy test persons). Values as percentage of the individual initial values before drinking. Brackets indicate standard errors.

DISCUSSION

As the first part of the study showed, the test water used was able to induce significant gallbladder contraction. Since the tap water controls did not produce a similar reaction, the composition rather than the volume of the ingested liquid caused the gallbladder reaction. The action of Epsom salts reported in the literature is probably due to this effect of sulfate (21,22), which has been described in several studies using natural mineral waters containing sulfate (8,12,20–24). The release of CCK from the duodenal mucosa as a result of specific chemical and/or mechanical dilatation stimuli is the most probable mechanism (25); the ability to release CCK was previously proven for natural sulfate waters (9,15,26). However, the sodium chloride contained in the mineral water may also play a role (13). The present findings also allow the possibility that tap water containing sulfate could have effects similar to those of the mineral water used. In an experiment with dogs, Sterczer et al. (27) showed that tap water with magnesium sulfate (500 mg as a 20% aqueous solution given orally) reduced the volume of the gallbladder by 24%.

According to the results of our experiments, the motility of the gallbladder is subject to marked circadian variation in the basal size and in the responsive-

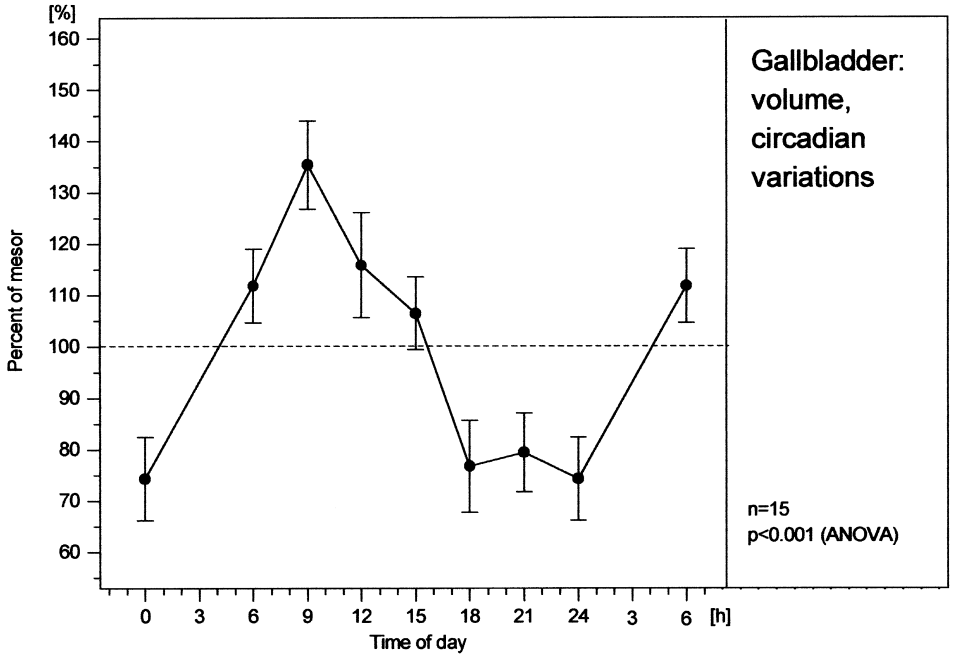

Figure 3. Mean circadian variation in the gallbladder volume measured by ultrasonic testing in 15 healthy subjects. All values were calculated as percentage of the individual daily means. Brackets indicate standard errors.

ness to standardized stimuli. The contractibility is maximal in the morning, whereas the dilatation is stronger in the afternoon.

Basically, the results of Zillmann et al. (28), who described maxima in gallbladder contraction at 08:00 and maxima in dilatation at 04:00, are in agreement with our measurements. However, these authors found more than one maximum during the 24h time span, indicating ultradian superimpositions. The investigation of ultradian rhythms requires more frequent measurements; hence, this question cannot be addressed with our data. Onodera et al. (29) described time dependency of the gallbladder size in healthy test persons, with a maximum contraction at 09:00. Whether this is due to endogenous circadian changes in gallbladder responsiveness is not clear as the meals were not adequately standardized.

Our results are also in accordance with the fact that the major storage of bile in the gallbladder occurs during the interdigestive period overnight (30). The circadian maximum in the gallbladder contraction that we found may be related to the maximum discharge from the gallbladder in the morning, the maximum dilatation in the afternoon, and to the maximal storage, as reported by these authors. These reactions are synchronous with the reported circadian variations in the release of CCK (4,5,31), indicating a causal mechanism. However, other

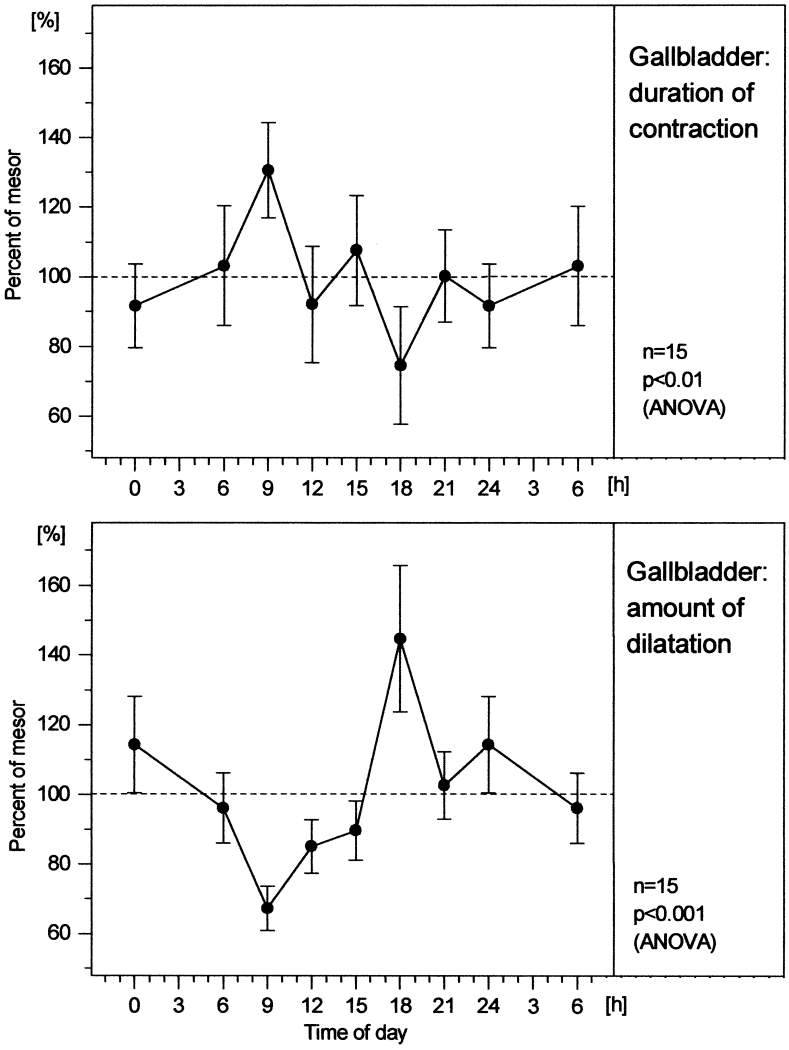

Figure 4. Mean circadian variation in the duration of the initial gallbladder contraction (above) and the consecutive gallbladder dilatation after intake of 500 mL of a natural sulfate-containing water in 15 healthy subjects. All values were calculated as percentage of the individual daily means. Brackets indicate standard errors.

hormonal influences on the tonus of the gallbladder, such as vasoactive intestinal peptides, gastrin, or neurotensin, could also be responsible for the circadian influences described (cf. 25). In addition, neuronal influences (especially vagal action) have to be discussed as a possible mechanism of the circadian variability of the motoric gallbladder sensitivity as well (25,32). Because of the different phases of these two rhythms, the last hypothesis however does not seem to be likely.

Table 4. Calculated Mean Peak and Trough Times (Mean Values and Standard Deviations)

Parameter	Maximum (h)	Minimum (h)
Gallbladder volume	9.8 ± 3.1	22.4 ± 2.2
Gallbladder contraction	11.6 ± 5.5	23.0 ± 7.1
Gallbladder dilatation	21.6 ± 4.7	10.0 ± 5.0

The present study indicates that—like other gastrointestinal functions (e.g., bile flow, bile composition, gastric emptying, gastric acid secretion) (33)—the motoric activity of the gallbladder exhibits a circadian pattern. This fact has to be considered in clinical diagnostics, as well as in pharmacological studies. An ongoing study is examining whether tap water that contains sulfate is as effective as the sulfate-containing mineral water.

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