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## Short Report

# A nutraceutical product based on Jerte Valley cherries improves sleep and augments the antioxidant status in humans<sup>☆</sup>

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## SUMMARY

**Background & aims:** The relationship between diet and health has led to intense research into bioactive compounds in foods. The present study evaluated the effect of the consumption twice a day of a nutraceutical product made with Jerte Valley cherries (Cáceres, Extremadura, Spain) on nocturnal rest, urinary 6-sulfatoxymelatonin (aMT6-s), and urinary antioxidant capacity in young (20–30 yr-old), middle-aged (45–55 yr-old), and elderly (65–75 yr-old) subjects.

**Methods:** In volunteers ( $n = 6$  per age group), the temporal patterns of the individuals' activity and rest were recorded by actigraphic monitoring. For the quantification of aMT6-s, a commercial ELISA kit was used, and total antioxidant capacity was evaluated by means of a colorimetric assay kit.

**Results:** The intake of the product increased significantly the Actual Sleep Time and Immobility, and decreased significantly the Total Nocturnal Activity in all subjects. Moreover, it increased significantly the urinary aMT6-s and antioxidant capacity in all subjects with respect to their basal values.

**Conclusions:** The consumption of a nutraceutical product made with Jerte Valley cherries improves nocturnal rest and has an antioxidant effect in humans.

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The amino acid tryptophan, the neurotransmitter serotonin, and the indole melatonin are present in various fruits and vegetables.<sup>1</sup> These bioactive compounds participate in the physiological regulation of sleep as well as in the improvement of antioxidant defences.<sup>2–4</sup> Melatonin possesses immunoregulatory properties<sup>5</sup> and is also a potent free radical scavenger<sup>6</sup> whose presence has been described in tart cherries.<sup>7</sup> In the Jerte Valley cherries (Cáceres, Extremadura, Spain), we recently reported high levels of tryptophan,<sup>8</sup> serotonin, and/or melatonin<sup>9</sup> in seven different cultivars (Bourlat, Navalinda, Van, Ambrunés, Pico Limón, Pico Negro, and Pico Colorado), and that the consumption of fresh cherries from these cultivars had positive effects on nocturnal rest as well as elevating the levels of aMT6-s and antioxidants in the

urine of middle-aged and elderly subjects (unpublished data). The aim of the present study was to evaluate the effect of the intake of a nutraceutical product made with the four Jerte Valley cherries cultivars (Spanish patent no. 200803761) that contain the highest levels of tryptophan, serotonin, and melatonin (Bourlat, Navalinda, Pico Negro and Pico Colorado) on the sleep-wake cycle and aMT6-s, a metabolite that is considered to reflect the nocturnal melatonin concentration, and total antioxidant capacity levels found in first-void morning urines in young, middle-aged, and elderly volunteers.

The administrations were of 27.85 g powdered freeze-dried nutraceutical product mix diluted in 125 ml of water. The product mix consisted of 18.85 g freeze-dried cherries in equal amounts of the Bourlat, Navalinda, Pico Negro, and Pico Colorado varieties of Jerte Valley cherries (equivalent to 141 g fresh cherries, because 13.37 g freeze-dried product is obtained from 100 g fresh fruit), 7.5 g maltodextrin, and 1.5 g ascorbic acid. This quantity of nutraceutical product was ingested twice a day (as lunch and dinner desserts) for three consecutive days by young ( $n = 6$ ), middle-aged ( $n = 6$ ), and elderly ( $n = 6$ ) volunteers. Actigraphic monitoring was

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used to record and display the temporal patterns of the individuals' activity and rest (Actiwatch®, Cambridge Neurotechnology Ltd., UK). Each subject wore on a wrist an actimeter that logged activity for 3 days before the beginning of the trial (basal values), during the 3 days of the trial, and 1 day following its termination. These actimetry data were then analyzed with the Sleep Analysis® (Cambridge Neurotechnology Ltd., U.K.) software package to give the following parameters: Sleep Efficiency, Actual Sleep Time, Number of Awakenings, Total Nocturnal Activity, Sleep Latency, Assumed Sleep, and Immobility. For the quantification of aMT6-s, a commercial ELISA kit (IBL) was used according to the manufacturer's instructions. To adjust for variation in the dilution of urine, the aMT6-s concentrations were expressed as urine aMT6-s/urine creatinine. Creatinine was determined by means of the Jaffe test, as described elsewhere.<sup>10</sup> Total urine antioxidant capacity was evaluated by means of a colorimetric assay kit (Cayman), according to the manufacturer's instructions. Data were expressed as mean  $\pm$  standard error of the mean (SEM) of the number of determinations. The results were analyzed using Friedman's test, and the degree of significance was set at  $p < 0.05$ .

The results in Table 1 show that the intake of the cherry nutraceutical product increased ( $p < 0.05$ ) the Actual Sleep Time in the young ( $12.3 \pm 0.5\%$ ), middle-aged ( $10 \pm 0.2\%$ ), and elderly ( $18.2 \pm 1.6\%$ ) subjects with respect to their basal values. The Assumed Sleep increased ( $p < 0.05$ ) in the elderly ( $22.5 \pm 1.0\%$ ) subjects with respect to their basal values. There was an increase ( $p < 0.05$ ) in Immobility in both young ( $12.1 \pm 0.6\%$ ) and elderly ( $26.1 \pm 1.7\%$ ) subjects and a decrease ( $p < 0.05$ ) in Total Nocturnal Activity in both young ( $24.8 \pm 2.8\%$ ) and elderly ( $11.9 \pm 0.6\%$ ) subjects with respect to their basal values.

Fig. 1A shows that the intake of this nutraceutical product induced an increase ( $p < 0.05$ ) in urinary aMT6-s levels in all three groups of subjects with respect to their corresponding basal values. There was also an increase ( $p < 0.05$ ) in the urine antioxidant capacity in all the groups with respect to their basal values, with the elderly subjects presenting the greatest increase (Fig. 1B).

The relationship between diet and health has led to intense research into bioactive compounds in foods. The indole melatonin, the neurotransmitter serotonin, and the amino acid tryptophan are bioactive compounds with important functions in organisms, with nocturnal rest<sup>4</sup> being one of them. In this context, nutraceutical compounds are producing a new nutritional revolution, with potential uses to fight against major disorders. The present study has shown that the intake of a nutraceutical product made with equal amounts of four different Jerte Valley cherry cultivars (Bourlat, Navalinda, Pico Negro, and Pico Colorado from Cáceres, Extremadura, Spain), which contain high levels of tryptophan,<sup>8</sup> serotonin, and/or melatonin,<sup>9</sup> has beneficial effects on nocturnal rest in young, middle-aged, and elderly subjects, with the most positive effects being on Actual Sleep Time, Total Nocturnal Activity, and Immobility. It is known that increased levels of circulating melatonin, directly by exogenous administration or indirectly by supplementation with its precursor tryptophan<sup>11</sup> have a positive

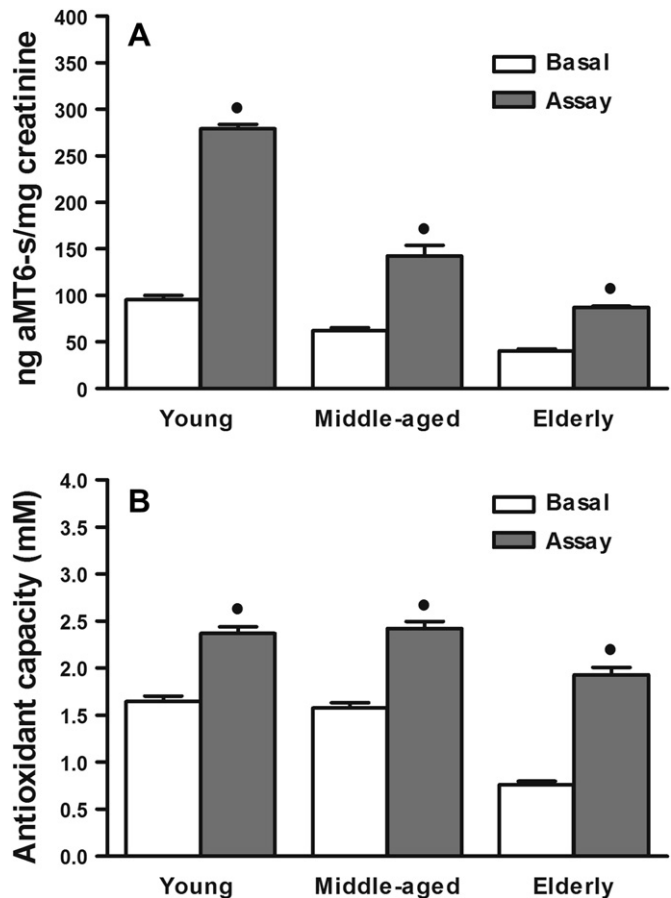


Fig. 1. Effect of nutraceutical product intake on standardized urinary aMT6-s levels expressed as ng aMT6-s/mg creatinine (A) or urinary antioxidant capacity (mM) (B) in basal (urine sample obtained before the intake of nutraceutical product) and trial (urine sample taken after three days of nutraceutical product intake) conditions in young, middle-aged, and elderly subjects. Each value represents the mean  $\pm$  SEM of the standardized urinary aMT6-s levels (A) or the urinary antioxidant capacity (B) obtained from six volunteers, performed in duplicate. \*  $p < 0.05$  with respect to their corresponding basal values.

effect on sleep,<sup>4</sup> as well as having an important physiological function as endogenous antioxidant.<sup>3</sup> The sleep-promoting and sleep/wake rhythm regulating effects of melatonin are attributed to its action on MT (1) and MT (2) melatonin receptors present in the suprachiasmatic nucleus of the hypothalamus while the antioxidant actions of this indole are related to its ability to stimulate antioxidant enzymes, to inhibit the prooxidative enzyme nitric oxide synthase, to diminish free radical formation at the mitochondrial level or to synergize with other antioxidants to protect against oxidative stress. Indirect evidence for an increase in circulating melatonin levels is a significant rise in urinary aMT6-s (the major urinary metabolite of melatonin) levels.<sup>10</sup> Indeed, it is known

**Table 1**  
Trial study: sleep parameters obtained during the intake of Jerte Valley cherry-based nutraceutical product twice a day (as lunch and dinner desserts) (trial values) with respect to their basal values in elderly, middle-aged, and young volunteers.

Age groups (n = 6)	Sleep efficiency	Actual sleep time	Number of awakenings	Total nocturnal activity	Sleep latency	Assumed sleep	Immobility
Elderly	↑ ns	↑ 18.2 $\pm$ 1.6*	↓ ns	↓ 11.9 $\pm$ 0.6*	↓ ns	↑ 22.5 $\pm$ 1.0*	↑ 26.1 $\pm$ 1.7*
Middle-aged	↑ ns	↑ 10 $\pm$ 0.2*	↓ ns	↓ ns	↓ ns	↑ ns	↑ ns
Young	↑ ns	↑ 12.3 $\pm$ 0.5*	–	↓ 24.8 $\pm$ 2.8*	–	↑ ns	↑ 12.1 $\pm$ 0.6*

Each value represents the mean  $\pm$  SEM of relative increases (↑) and decreases (↓) considering 100% as the basal values of the sleep parameters studied.

\*  $p < 0.05$  with respect to the respective basal values.

ns: Slight increase (↑) or decrease (↓) not significant with respect to the basal values.

– no change with respect to the basal values.

that the intake of melatonin-enriched vegetables increases urinary aMT6-s levels<sup>12</sup> as well as enhancing the antioxidant status.<sup>13</sup> In this sense, our study has demonstrated that the intake of this nutraceutical product significantly raises the urinary aMT6-s levels and antioxidant capacity in the young, middle-aged, and elderly subjects with respect to their basal values.

In sum, we demonstrated that the intake of this nutraceutical product made with Jerte Valley cherries (Cáceres, Extremadura, Spain) produces positive effects on sleep parameters, and increases urinary aMT6-s and antioxidant status in young, middle-aged, and elderly subjects. In this sense, we propose to carry out further studies to check the effect of this nutraceutical Jerte Valley cherry product acting against oxidative stress, sleep disorders, and depression.

### Conflict of interest

There are no conflicts of interest to disclose.

### Statement of authorship

MG<sup>a</sup> and JEP<sup>a</sup> carried out the experiments (sleep parameters and urine measurements). ML and DGG carried out the previous determinations of serotonin and melatonin in Jerte Valley cherry varieties. JC, AFTD and JIMM carried out the previous determinations of tryptophan in Jerte Valley cherry varieties. CB, MPT and JAP carried out the statistical analysis. JLM performed the level control of fruit maturity, harvesting, and delivery to our laboratory. ABR and SDP supervised the work, designed the experiments, and discussed the results. All authors have read and approved the final manuscript.

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