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# Jerte Valley Cherry-Enriched Diets Improve Nocturnal Rest and Increase 6-Sulfatoxymelatonin and Total Antioxidant Capacity in the Urine of Middle-Aged and Elderly Humans

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Tryptophan, serotonin, and melatonin, present in Jerte Valley cherries, participate in sleep regulation and exhibit antioxidant properties. The effect of the intake of seven different Jerte Valley cherry cultivars on the sleep–wake cycle, 6-sulfatoxymelatonin levels, and urinary total antioxidant capacity in middle-aged and elderly participants was evaluated. Volunteers were subjected to actigraphic monitoring to record and display the temporal patterns of their nocturnal activity and rest. 6-sulfatoxymelatonin and total antioxidant capacity were quantified by enzyme-linked immunosorbent assay and colorimetric assay kits, respectively. The intake of each of the cherry cultivars produced beneficial effects on actual sleep time, total nocturnal activity, assumed sleep, and immobility. Also, there were significant increases in 6-sulfatoxymelatonin levels and total antioxidant capacity in urine after the intake of each cultivar. These findings suggested that the intake of Jerte Valley cherries exerted positive effect on sleep and may be seen as a potential nutraceutical tool to counteract oxidation.

**Key Words:** Tryptophan—Serotonin—Melatonin—Cherry—Sleep.

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SINCE organisms cannot synthesize tryptophan, it is considered an essential amino acid that can only be obtained in the diet. This amino acid has sleep-enhancing properties (1) and it exerts antioxidant effects (2). In addition, it is the precursor of important metabolites such as serotonin and melatonin, which are molecules that influence the behavior of the organisms, exerting important functions such as the regulation of the sleep–wake rhythm, mood, and eating (3). A reduction in diurnal serotonin levels due to a low intake of tryptophan during the day causes alterations in nocturnal rest (4) and influences the regulation of the endogenous circadian clock (5). The indole melatonin is considered a final product in the metabolism of tryptophan in the anabolic pathway of biogenic amines. Melatonin, like its precursor, exerts a sleep-enhancing effect and improves sleep and certain pathological conditions associated with sleep (6). Indeed, age-related disturbances in the sleep–wake and temperature rhythms have been correlated with age-related reductions in the amplitude of the nocturnal melatonin peak (7). In fact, the loss of melatonin in advanced age leads to disturbances in the circadian pacemarker, which causes internal temporal desynchronization inducing a variety of chronopathologies and leads to generalized deterioration of health (8,9).

Besides its regulatory effects on the sleep–wake rhythm, melatonin is a potent free radical scavenger and antioxidant (1,8,10,11) that not only scavenges especially highly toxic hydroxyl radicals, but also performs indirect antioxidant actions via its ability to stimulate antioxidant enzymes (12), diminishing free radical formation at the mitochondrial level by reducing the leakage of electrons from the electron transport chain (13). Moreover, melatonin is a lipophilic–hydrophilic molecule that diffuses widely into cellular compartments, thus providing on-site protection against free radical–mediated damage to biomolecules (8).

Although long thought to be exclusively present in vertebrates, melatonin has been reported to be a ubiquitous, evolutionarily conserved compound in all living beings, including plants (14,15). The presence of melatonin's precursor, the amino acid tryptophan, as well as the immediate metabolite in its biosynthetic pathway, serotonin, has also been noted in a number of fruits and vegetables (16,17). As in animals, melatonin has been reported to provide protection against oxidative stress in plants (18).

Consuming foodstuffs containing melatonin alters circulating levels of the indole (19) and increases 6-sulfatoxymelatonin (aMT6-s) concentrations in the urine (20). This may have implications for aging or diseases where exacerbated production of free radicals occurs because the production of the pineal indole wanes with increasing age leading some to speculate that its loss contributes to the aging process (9).

Since it was discovered in plants, there has been an ever-growing number of studies reporting the detection of melatonin in a variety of fruits (14), including tart cherries (21). This is also the case of Jerte Valley cherries where high levels of melatonin and/or serotonin (22) as well as elevated concentrations of tryptophan (23) have been recently reported in seven different cultivars of these fruits (Bourlat, Navalinda, Van, Ambrunés, Pico Limón, Pico Negro, and Pico Colorado). Thus, the consumption of these fruits may be beneficial as they may enhance the health effects mediated by melatonin against oxidative processes or disorders of sleep, in which a reduction of the indole has been suggested as a possible cause (9,24). It was, therefore, hypothesized that during a 3-day intake of the aforementioned seven Jerte Valley cultivars, the participants (middle-aged and elderly individuals with expected reduced levels of circulating melatonin) would experience improved nocturnal rest and increased urinary total antioxidant capacity as a consequence of increasing their melatonin levels. Also, because the cultivars contain different amounts of tryptophan (23), serotonin, melatonin (22), anthocyanin pigments, and phenolic compounds (25), the participants may experience differences in terms of sleep parameters or the antioxidant status of their urine. Thus, the purpose of this study was to evaluate whether the consumption of Jerte Valley cherry fruits for 3 days positively influences the sleep–wake cycle and the total antioxidant capacity as well as augments the levels of aMT6-s (a metabolite that is considered to reflect the nocturnal melatonin concentration), found in first-void morning urine in middle-aged and elderly participants.

## Methods

### *Participants and Experimental Design*

The study was carried out on six middle-aged (35–55 years old, $n = 6$) and six elderly (65–85 years old, $n = 6$) volunteers whose body mass index was 23.5±7.5, weight 60.0 ± 12.3 kg and height 162 ± 15 cm. Participants were of Caucasian ethnicity.

This study was approved by the Ethical Committee of the University of Extremadura (Badajoz, Spain) in accordance with the Declaration of Helsinki, the Council of Europe, and the Universal Declaration of UNESCO on human rights, biomedicine, and human genome. Each participant was ascertained to be in good health by means of their medical history and a clinical examination including routine laboratory tests and screening. The participants all were nonsmokers, were not using any medication, and abstained from alcohol. Informed consent was obtained from all participants.

Data from this prospective study were collected from May 2007 to July 2007. Participants were recruited through word of mouth. They consumed 200 g twice a day as the lunch and dinner desserts of seven different cultivars of whole cherries (*Prunus avium* L.) grown in the Jerte Valley (Cáceres, Extremadura, Spain): Stalked cherries (Bourlat, Navalinda, and Van) and unstalked cherries or 'picotas' (Ambrunés, Pico Limón, Pico Negro, and Pico Colorado). Participants ingested these cherries for three trial days (72 hours), repeating the protocol with each different cherry cultivar tested, but maintaining a washout period of at least 1 week between cultivars.

### *Sleep Parameter Measurements*

Actigraphic monitoring was used to record and display the temporal patterns of the individuals' activity and rest (Actiwatch; Cambridge Neurotechnology Ltd, Cambridge, UK). Each participant wore a wrist actimeter that logged activity for 3 days before the beginning of the trial (72 hours, basal values) and during the 3 days of the trial (72 hours, trial values). These actimetry data were then analyzed with the sleep analysis (Cambridge Neurotechnology Ltd) software package to give the following parameters: sleep efficiency (sleep percentage while the participant was in bed); actual sleep time (assumed sleep minus awake time); number of awakenings (number of high activity episodes during sleep); total nocturnal activity (total activity pulses during sleep); sleep latency (time period measured from going to bed until the onset of sleep); assumed sleep (difference between the onset and the final awakening); immobility (minutes when mobility is zero).

### *Urine Sample Collection*

First-void morning urine was collected before the trial (basal values) and the day after the last ingestion of cherries (trial). The samples were stored at −20°C until biochemical assay.

### *Urinary 6-Sulfatoxymelatonin and Antioxidant Capacity*

For the quantification of urinary aMT6-s, a commercial enzyme-linked immunosorbent assay kit (IBL, Hamburg, Germany) was used according to the manufacturer's instructions. To adjust for variation in the dilution of urine, aMT6-s concentrations were expressed as urinary aMT6-s:urine

Table 1. Trial Study: Sleep Parameters Obtained After a 3-Day Consumption of Different Varieties of Jerte Valley Cherries Twice a Day with Respect to Their Basal Values in Middle-Aged (M) and Elderly (E) Volunteers

| Cherry Varieties | Sleep Efficiency | | Actual Sleep Time | | Number of Awakenings | | Total Nocturnal Activity | | Sleep Latency | | Assumed Sleep | | Immobility | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | E | M | E | M | E | M | E | M | E | M | E | M | E |
| Bourlat | 1.06 ± 0.05 | 0.99 ± 0.01 | 1.18 ± 0.03* | 1.23 ± 0.07* | 0.82 ± 0.3 | 0.85 ± 0.3 | 0.74 ± 0.07* | 0.84 ± 0.05* | 0.99 ± 0.33 | 0.93 ± 0.16 | 1.03 ± 0.13 | 1.14 ± 0.03* | 0.99 ± 0.13 | 1.17 ± 0.03 |
| Navalinda | 1.06 ± 0.06 | 1.02 ± 0.05 | 1.17 ± 0.05* | 1.17 ± 0.03* | 1.03 ± 0.3 | 0.9 ± 0.1 | 0.87 ± 0.4 | 0.98 ± 0.02 | 0.54 ± 0.10* | 0.51 ± 0.07* | 1.04 ± 0.12 | 1.15 ± 0.03* | 1.06 ± 0.12 | 1.15 ± 0.32 |
| Van | 1.12 ± 0.02* | 0.99 ± 0.05 | 1.19 ± 0.05* | 1.21 ± 0.05* | 1.02 ± 0.3 | 0.99 ± 0.1 | 1.02 ± 0.09 | 0.99 ± 0.1 | 0.99 ± 0.12 | 0.91 ± 0.17 | 1.15 ± 0.05* | 1.09 ± 0.08 | 1 ± 0.09 | 1.11 ± 0.16 |
| Ambrunés | 1.01 ± 0.07 | 1.04 ± 0.03 | 1.02 ± 0.05 | 1.14 ± 0.03* | 0.9 ± 0.2 | 0.83 ± 0.01 | 0.97 ± 0.11 | 0.94 ± 0.16 | 0.92 ± 0.1 | 0.88 ± 0.07 | 0.99 ± 0.04 | 1.05 ± 0.11 | 1.08 ± 0.13 | 1.17 ± 0.03* |
| Pico Limón | 1.03 ± 0.06 | 0.99 ± 0.06 | 1.15 ± 0.05* | 1.14 ± 0.03* | 0.78 ± 0.05* | 0.88 ± 0.34 | 0.96 ± 0.03 | 0.71 ± 0.07* | 0.91 ± 0.32 | 0.87 ± 0.19 | 1.02 ± 0.1 | 1.04 ± 0.05 | 1.10 ± 0.26 | 1.13 ± 0.17 |
| Pico Negro | 1.06 ± 0.05 | 1.06 ± 0.07 | 1.45 ± 0.07* | 1.33 ± 0.05* | 0.91 ± 0.1 | 0.85 ± 0.05 | 0.94 ± 0.1 | 0.81 ± 0.05* | 1.02 ± 0.15 | 0.7 ± 0.01* | 1.07 ± 0.08 | 1.16 ± 0.03* | 1.09 ± 0.13 | 1.31 ± 0.05* |
| Pico Colorado | 1.05 ± 0.05 | 1.08 ± 0.08 | 1.18 ± 0.07* | 1.21 ± 0.03* | 0.89 ± 0.2 | 0.75 ± 0.01* | 0.96 ± 0.01 | 0.55 ± 0.04* | 0.95 ± 0.22 | 0.72 ± 0.23 | 1 ± 0.13 | 1.09 ± 0.06 | 1.12 ± 0.18 | 1.25 ± 0.08* |

*Notes*: Each value represents the mean ± *SD* and was expressed as fold increase over the basal levels.
*$p < .05$. Significant increase or decrease with respect to the values obtained for the respective basal values.

creatinine ratio; creatinine concentration was determined by means of the Jaffe test, as described elsewhere (26). Total antioxidant capacity was evaluated by means of a colorimetric assay kit (Cayman, MI, USA), according to the manufacturer's instructions. This assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulfonate]) to ABTS by metmyoglobin. The capacity of the antioxidants in the sample to prevent ABTS oxidation was compared with that of Trolox, a water-soluble tocopherol analogue, and quantified as millimolar Trolox equivalents.

Urinary aMT6-s and antioxidant capacity are expressed as percentages (each participant served as its own control), taking the basal urinary aMT6-s or antioxidant capacity values as 100%; the values obtained on the days of the trial are percentages with respect to their corresponding basal values, determined by multiplying the aMT6-s or antioxidant capacity values recorded for a given individual by 100 and dividing by the basal aMT6-s or antioxidant capacity values recorded for the same individual.

### Statistical analysis

The Kolmogorov–Smirnov test was applied for examining normality of the distribution of results. Once confirmed that the data did not fulfill a normal distribution, Friedman and Kruskal–Wallis nonparametric tests followed by Dunns' multiple comparison tests were used to analyze the results. Each value represents the mean ± *SD* from six different volunteers, which were analyzed in duplicate. The degree of significance was set at $p < .05$. All analyses were performed using GraphPad Prism (version 5.0, 2007; GraphPad Software, Inc; San Diego, CA).

### Results

Table 1 lists the sleep parameters obtained during the intake of the different cultivars of Jerte Valley cherries with respect to the basal values in the middle-aged and the elderly participants. Sleep efficiency only increased significantly ($p < .05$) in middle-aged volunteers after the intake of Van cherries. With the exception of the middle-aged Ambrunés cherry-fed individuals, actual sleep time rose significantly ($p < .05$) after the consumption of each of the seven cherry cultivars. Additionally, number of awakenings decreased significantly ($p < .05$) after the intake of Pico Limón and Pico Colorado cultivars in middle-aged and elderly volunteers, respectively. Likewise, the total nocturnal activity parameter experienced a significant ($p < .05$) reduction in elderly volunteers after the intake of Bourlat, Pico Limón, Pico Negro, and Pico Colorado cherries, and in the middle-aged Bourlat cherry-fed individuals. Sleep latency diminished significantly ($p < .05$) in both middle-aged and elderly volunteers after the consumption of Navalinda cherries and after the intake of the Pico Negro cultivar in the elderly participants. A significant elevation ($p < .05$)

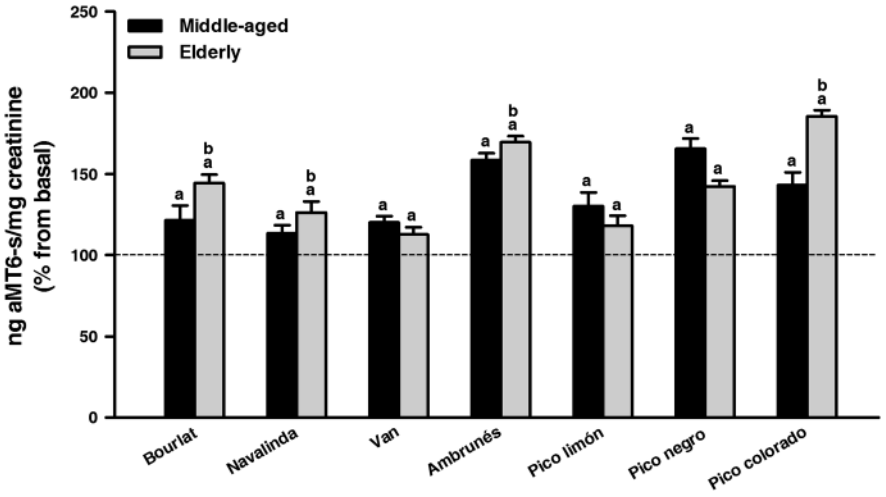


Figure 1. Effect of cherry intake on urinary aMT6-s levels expressed as nanogram aMT6-s per milligram creatinine in basal (urine sample obtained before the intake of 200 g of different cherry cultivars) and trial (urine sample taken after 3 days of intake of 200 g of different cherry cultivars) conditions in middle-aged and elderly participants. Results are expressed in percentages, with 100% being the value obtained in the basal group. Values regarding basal group were obtained from first-void morning urine collected before the trial. Each value represents the mean ± *SD* of the standardized urinary aMT6-s levels obtained from six different volunteers. Samples were analyzed in duplicate. *Notes*: [a]$p < .05$ with respect to the corresponding basal values; [b]$p < .05$ with respect to the corresponding values obtained in the middle-aged participants.

in assumed sleep in elderly participants after the intake of Bourlat, Navalinda, and Pico Negro cherries was also found. This parameter rose significantly ($p < .05$) in middle-aged individuals after the intake of the Van cultivar. Similarly, there was a significant increase ($p < .05$) in immobility in elderly participants after the intake of Ambrunés, Pico Negro, and Pico Colorado cherries.

The intake of each of the seven cherry cultivars produced a significant rise ($p < .05$) in urinary aMT6-s in both middle-aged and elderly volunteers with respect to the corresponding basal values. Also, it showed a significant ($p < .05$) increase in elderly participants with respect to the corresponding values obtained in the middle-aged participants after the intake of Bourlat, Navalinda, Ambrunés, and Pico Colorado cherries (Figure 1).

A significant rise ($p < .05$) was found in total antioxidant capacity in both middle-aged and elderly volunteers with respect to the basal values. Moreover, a significant increase ($p < .05$) was produced in elderly participants with respect to the corresponding values obtained in the middle-aged participants after the intake of Navalinda and Van cherries (Figure 2).

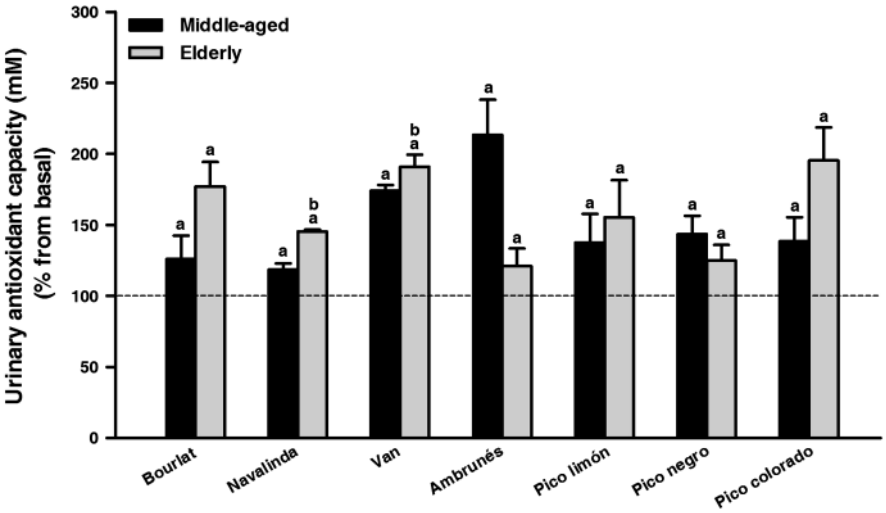


Figure 2. Effect of cherry intake on urinary antioxidant capacity (mM) measured in basal (urine sample obtained before the intake of 200 g of different cherry cultivars) and trial (urine sample taken after 3 days of intake of 200 g of different cherry cultivars) conditions in middle-aged and elderly participants. Results are expressed in percentages, with 100% being the value obtained in the basal group. Values regarding basal group were obtained from first-void morning urine collected before the trial. Each value represents the mean ± *SD* of the urinary antioxidant capacity obtained from six different participants. Samples were analyzed in duplicate. *Notes*: [a]$p < .05$ with respect to the corresponding basal values. [b]$p < .05$ with respect to the corresponding values obtained in the middle-aged participants.

## Discussion

Many studies indicate that constituents of fruits and vegetables are protective against a variety of diseases (27). Thus, importance has been attached to the dietary indolamines, melatonin, and serotonin, in different plant foods, supporting the hypothesis that the health benefits associated with the Mediterranean diet are due to plant food chemical diversity (16,18,28,29).

In the present work, it was demonstrated that the intake of Jerte Valley cherry cultivars produced beneficial effects on sleep–wake rhythms in both middle-aged and elderly participants. These effects were different depending on the cherry cultivar ingested and the sleep parameters assessed (Table 1). Although some of the sleep parameters were unchanged with respect to their basal values, the intake of any of the Jerte Valley cherry cultivars produced no adverse effects in terms of the sleep parameters.

Several studies indicate that the consumption of cherries is health promoting, particularly in reducing the effects of some diseases (30,31,32). Currently, there is only a modicum of evidence indicating as to what ingredients may be responsible for the alleged beneficial properties. Anthocyanins and cyanidins present in cherries are known to play an important role as phytochemical antioxidants (33). However, other recently discovered molecules in this fruit, including melatonin (14,21,22), serotonin, or tryptophan (22,23), may act to improve or ameliorate certain disorders of sleep due to their sleep regulatory functions as has been documented elsewhere (1,6,34). In fact, the beneficial physiological effects provided by a sweet cherry–based nutraceutical product that are rich in these compounds has been reported (35).

These findings also show a significant increase in urinary aMT6-s levels in both groups of age with respect to the basal values. This is indirect evidence for a rise in circulating melatonin levels as a result of the intake of Jerte Valley cherries. These results are consistent with previous studies, which found that levels of urinary aMT6-s, the major urinary metabolite of melatonin, accurately reflect nocturnal plasma melatonin (20,26). In fact, Oba et al. (20) reported that increasing the consumption of vegetables that are high in melatonin content elevated morning aMT6-s concentrations in urine when compared with the levels of this metabolite in the urine of control participants who avoided these vegetables. Indeed, the association between vegetables and melatonin:aMT6-s concentration ratio is well known in both blood (19) and urine (36).

It is also known that endogenous melatonin production wanes with increasing age (9). This is reflected in an age-related difference in the levels of aMT6-s found in urine (37,38), a fact that was also observed in the samples tested in this study. In this sense, the significant rise in aMT6-s levels found in the elderly due to cherry intake, which may be considered a reflection of higher circulating melatonin levels, may be of importance for some sleep disorders. This could be related especially to those sleep disorders that are accompanied by a reduced level of melatonin, for example, sleep inefficiency associated with increased age. Because various systemic changes that occur in the rhythmic secretion of melatonin and serotonin with increasing age appear to relate to the worsening of sleep quality (39), the improvements in sleep induced by a cherry-enriched diet observed in the present work may be due to a preservation of blood levels of serotonin, melatonin, and their precursor, tryptophan, in the volunteers who participated in this study.

Additionally, a significant rise in urinary total antioxidant capacity was observed after the intake of each of the cherry cultivars in both the middle-aged and the elderly participants with respect to the basal values. This significant increase in urinary total antioxidant capacity was presumably due to the antioxidant role of the amino acid tryptophan, the neurotransmitter serotonin, and the indole melatonin (2,10,40), which are present in cherries. The possible contribution of phenols or anthocyanins, also contained in the cultivars used in the present research (25), or other bioactive compounds, including flavonoids, isoflavonoids, phytosterols, or phytic acid (32,41) cannot, however, be precluded. Similar findings were obtained by Reiter et al. (19) who showed that the consumption of walnuts, which are rich in melatonin, provoked a threefold increase in circulating melatonin levels and also improved serum antioxidant capacity measured in trolox equivalents.

These findings suggested that the intake of Jerte Valley cherries exerted positive effect on sleep and may be seen as a potential nutraceutical tool to counteract oxidation.

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