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Study The Effects of Circadian Rhythms on Some Liver Function Parameters in Local Iraqi Sheep

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Abstract

This experiment aims to study the effects of the circadian rhythm of common liver function tests. Ten adult sheep (male and female) were divided into two groups, T1 (male) and T2 (female), and placed in two separate rooms. Serum was isolated from T1 and T2 blood samples taken at 7, 12 a.m. and 12 p.m. hours, respectively. Results: Serum alanine aminotransferase (ALT) and creatinine levels in both groups increased significantly during daylight hours (12:00). Aspartate aminotransferase (AST) showed a different value in a male that increased significantly at 12 a.m. but in a female that was elevated at 7 a.m. as compared with other times. Serum urea concentration during the night hours was expressed at a high level in both males and females as compared to the concentration during the day hours. In addition, the results showed differences and variation between males and females. There is a significant increase in ALT, urea, and creatinine in females (T2) compared to males (T1) during the day and night hours. Furthermore, at 12 a.m. and 12 p.m., males had significantly higher AST levels than females. Liver functions showed variations between day and night hours, as well as between males and females, because of metabolism and activities. Comprehensive study is required to reduce and overlap this stress.
Keywords: AST, ALT, Circadian cycles, liver function, Sheep.

Introduction

Circadian rhythms are biological cycles created by the organism and have durations close to 24 hours long. These oscillations can be expressed in behavioral, physiological, or molecular patterns. Circadian timers are found in nearly all cells in animals. The suprachiasmatic nucleus (SCN) is a dual organization in the hypothalamus. It is the crucial pacemaker of the natural clock system, regulating most of the body's circadian rhythms (1). The organism's internal and external circadian rhythms are strongly linked to the external

environment's rhythm variations. Animals' neurological and endocrine systems will alter in response to changes in environmental circumstances, resulting in changes in their behaviors, lifestyles, and physiological states. (2)

The liver is a vital metabolic organ that monitors the body's energy processing. It performs as a metabolic point for various tissues, including skeletal muscle and adipose tissue. (3). The liver filters all of the blood that leaves the stomach and intestines, which is in charge of processing this blood. It disintegrates, restores harmony, and generates nutrients. It also converts medications into simpler forms for the remainder of the body. Toxins are removed from the body by the liver via sinusoid channels, which are guarded by immune cells known as Kupffer cells as well as the bulk of cytochrome P450 enzymes involved in xenobiotic metabolism and some conjugating enzymes, which are found in the endoplasmic reticulum of liver ER. Furthermore, Bile is a greenish-yellow fluid generated by liver cells that serves two purposes: transport waste products and the breakdown of lipids during digestion. (4)

Like many other organs in the body, the liver has an internal timing mechanism called the circadian clock, which adapts physiological activities to the appropriate time of day. The liver uses this mechanism uses this mechanism uses this mechanism uses this mechanism uses this mechanism to predict periodic systemic and environmental changes and to function proactively (5). Plenty of transcriptomics investigations have also linked circadian genes to essential metabolic enzymes and their regulating proteins (6); (7), and (8), demonstrating the strong relationship between circadian control and metabolism. Likewise, data from proteomics and phosphoproteomics indicate that many rhythmic proteins with circadian times play a role in metabolism (9) and (10). While numerous interactions between the core clock and metabolic processes and routes have been discovered, the dynamic coordination of circadian liver metabolism is still unknown.

The aminotransferases belong to the most sensitive and widely used liver enzymes. Aspartate aminotransferase and alanine aminotransferase are two of them. These enzymes are normally found primarily in liver cells and, to a lesser extent, in muscle cells. When the liver is injured or harmed, the liver cells release these enzymes into the bloodstream, increasing AST and ALT levels and indicating liver disease (11).

Other indicators of liver activity include urea, which is produced in the liver and is a protein metabolite. The breakdown of amino acids produces ammonium ions. Excess ammonium ions are converted to urea and used to synthesize nitrogen compounds (12).

Creatinine is commonly used as a measure of kidney function. Indeed, itit was used as a screening test to describe the relation shape between liver and muscle metabolism. Creatinine is an outcome of creatine metabolism, which is generated in the liver from three amino acids, arginine, glycine, and methionine, and stored in muscle to be phosphorylated and used as an energy source (13). Once the circadian clock is interrupted, animals are more susceptible to diseases, particularly metabolic abnormalities. However, little is known

about the circadian changes in liver functioning in domestic animals, as well as how they react to external stimuli. In the present study, we investigated the rhythms of liver function indicators in sheep subjected to phase shifts in the environmental light-dark cycle. Our objectives were to determine environmental and physiological ingredients that influence circadian rhythms, which will assist in developing novel targets and corresponding approaches to optimize farm animal production efficiency.

Materials And Methods

The present research utilized ten adult sheep aged 6 to 12 months. They were grouped into two groups: T1 (males) and T2 (females), and they lived in two air-conditioned rooms (20-25 C°). The animals were given two weeks to adapt to the conditions of the experiment. The blood samples were collected via jugular vein from every animal at 7, 12 a.m., and 12 P.M. The serum was separated to estimate test AST and ALT (IU/L), (14) as well as concentrations of Urea mg/dl (15) and Creatinine mg/dl. (16) These parameters were used for this study using a colorimetric assay Kit (Agape Diagnostics Switzerland for AST and ALT), (Sam Diagnostic, DubaiUAE for Urea) and (Biolabo SAS, France for Creatinine). Two-way ANOVA (SPSS program) was used to statistically analyze all the data and show differences between the groups and times. The values were reflected using means and standard errors $P \leq 0.05$. (17) The work done in Iraq Veterinary College of Bagdad University.

Results

The liver enzyme activities in the serum of both sexes of sheep during the day and night times the results demonstrated that ALT activity increases significantly ($P<0.05$) in both males and females at 12 a.m. during daylight hours compared to 7 a.m. and 12 p.m. during night hours. Simultaneously, serum AST levels in T1 at 12 a.m. were significantly higher than at other times. AST activity in T2 (females) increased significantly ($P<0.05$) in the morning (7 a.m.) compared to another also significant decrease at 12 a.m. compared to 12 p.m. The same sheep show a significant variation in ALT and AST between groups (males and females). In all periods, females exhibit higher T2 levels in ALT and AST compared to males.

Discussion

Many studies have been conducted to explain how the circadian clock affects numerous elements of homeostasis and how organs coordinate their activity (18), (19), and (20). The liver's circadian clock regulates the metabolism of medications and energy sources such as glucose, proteins, and lipids. (21)

In the current experiment, we need answers about the communication between the circadian rhythms and hepatic functions outside the brain, and we can see that this communication between liver and muscle is altered by diurnal day and sex in Iraqi Sheep.

Table (1): The liver enzymes (ALT and AST) in the blood serum of different groups of sheep at 7, 12 a.m. and 12 p.m.

Time Parameters	Groups	At 7 a.m.	At 12 a.m.	At 12 p.m.	LSD
	T1(Male)	10±0.36	25.8±0.35	25.5±0.63	2.2
ALT		Bb	Ab	Ab	
(IU/l)	T2(Female)	19.7±0.53	33.3±1.1	30.9±0.44	
		Ca	Aa	Ba	
	T1(Male)	39.1±0.47	47.8±0.36	43.5±0.29	1.25
AST		Cb	Aa	Ba	
(IU/l)	T2(Female)	43.8±0.19	34.8±0.14	37.76±0.45	
		Aa	Cb	Bb	

Mean ± SE, Number of sheep (n=5). The different big letters = significant variations in times within the group. The different small letters have significant variations in groups within different groups.

Table (2): The circadian rhythm of urea and creatinine in the blood serum of different groups of sheep at 7, 12 a.m., and 12 p.m.

Time Parameters	Groups	At 7 a.m.	At 12 a.m.	At 12 p.m.	LSD
	T1(Male)	20.9±0.36	21.4±0.4	59.3±0.39	1.26
Urea		Bb	Bb	Ab	
(mg/dl)	T2(Female)	25.9±0.13	33.4±0.4	62±0.25	
		Ca	Ba	Aa	
	T1(Male)	0.4±0.05	0.59±0.08	0.44±0.012	0.04
Creatinine		Cb	Aa	Ba	
(mg/dl)	T2(Female)	0.43±0.02	0.6±0.07	0.42±0.01	
		Ba	Aa	Ba	

Mean ± SE, Number of sheep (n=5). The different big letters = significant variations in times within the group. The different small letters have significant variations in groups within different groups.

The results express liver enzymes (ALT and AST) at mid-daylight and mid-night during the day.

Food plays a vital part in regulating circadian clocks in peripheral tissues. Thus, chrono nutrition refers to the concept of controlling the timing of food consumption and food composition. (22).

The mammals are diurnal, meaning they do most of their work during the day and sleep at night, so this daily variation in light intensity results in overt rest-activity and feeding fasting cycles (23).

As a result, the feeding fasting cycles are synchronized by circadian clocks, resulting in daily changes in metabolic processes, which sustain energy intake. Recent research has shown that the expression of several genes in various organs (including the liver) exhibits circadian rhythmicity in mammals, allowing anabolism and catabolism to be regulated (24) and (25).

Indeed, Hepatocyte functions, including nutrition uptake, processing, assimilation, and detoxification, show significant diurnal fluctuations. The circadian clocks and timing signals like light and food interact

dynamically to create and maintain these metabolic rhythms (25) and (26). So, the ALT and AST enzymes are considered markers of liver activities. These are elevated when the liver is under stress.

The circadian rhythms of urea and creatinine concentrations in the serum of both sexes of sheep during the day and night.

The serum urea and creatinine concentrations in both males and females increased significantly ($P<0.05$) at 12 p.m. during the night hours compared to 7 a.m. and 12 a.m. during the day.

At the same time, sheep show a significant variation in urea concentration between groups (males and females). There is an increase in T2 (females) during the light and night hours of the day. In addition, there is no significant difference in creatinine levels between both sexes of sheep at 12 a.m. and 12 p.m. times.

Creatinine levels may be critical to identifying the core of the problem. The normal range is 10–20 mg/dl BUN to 1 mg/dl creatinine. A high Blood Urea Nitrogen (BUN) to creatinine ratio (more than 20:1) indicates prerenal disease, whereas a low ratio (less than 10:1) indicates renal disorder (27).

In this study, we investigated the diurnal variations of urea and creatinine in both sexes in local Iraqi sheep and how they relate to liver function. We observed that urea levels increased in the middle of the day and especially in the evening hours, implying that liver activity increased in these periods as a result of many factors such as diet, light, and darkness. Furthermore, the metabolic activities that occur throughout the night sleep, and sleep are closely linked to numerous hormonal and metabolic processes in the body. They are critical in maintaining metabolic homeostasis (28). In ruminants, urea is recycled into the rumen by salivary inclusion, which might lower urea levels in the morning.

On the other hand, at 12 a.m. on light days, both sexes have higher creatinine levels. Creatinine is a byproduct of muscle metabolism. It is formed by the spontaneous, non-enzymatic, irreversible cyclization of creatine and is released into the bloodstream before the kidneys remove it. So, the elevation in serum creatinine is a common laboratory finding as an indicator of kidney failure. (29) Their study revealed an elevation in serum creatinine in patients with liver cirrhosis that improved the relationship between liver activity and creatinine level. (30)

However, the diurnal variation of creatinine levels may be caused by or influenced by factors, particularly the diet, and is a strong influencer of the overall creatinine concentration in the body because the precursor of creatinine is creatine from external sources, and de novo synthesis takes place in liver. (31)

Conclusion

Circadian rhythms provide a novel viewpoint on liver activity and metabolism concerning environmental influences and daytime schedules. Circadian rhythms, which have developed in nearly every living organism, are found in virtually all tissues of mammals and help coordinate the time of metabolism of all organic compounds in the liver. On the other hand, we observed

changes in hepatic function marker levels between male and female sheep.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة تأثير الساعة البيولوجية على بعض معايير وظائف الكبد في أغنام العراق المحلية

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الخلاصة

هدفت دراسة لمعرفة تأثير الساعة البيولوجية في معايير وظائف الكبد الشائعة تم تقسيم عشرة أغنام بالغة (ذكر وأنثى) إلى مجموعتين، ت1 (ذكور) وت2 (إناث) وضعهم في غرفتين منفصلتين. تم عزل المصل من عينات الدم ت1 وت2 المأخوذة في الساعات 7 صباحًا و12 ظهرًا و12 ليلاً، على التوالي: زادت مستويات إنزيم الألانين الأميني انزيم ناقلة الامين انيلين والكرياتينين في المصل بشكل معنوي و في كلا المجموعتين بشكل ملحوظ خلال ساعات النهار (12 ظهرًا). أظهر إنزيم الأسبارتات أميني انزيم ناقلة الاسبارتات قيمة مختلفة في الذكور حيث ارتفع بشكل معنوي في الساعة 12 صباحًا، ولكن في الإناث كان مرتفعًا في الساعة 7 صباحًا مقارنة بالأوقات الأخرى. ظهر تركيز اليوريا في المصل خلال ساعات الليل على مستوى معنوي عال في الذكور والإناث مقارنة بالتركيز خلال ساعات النهار. بالإضافة إلى ذلك، اظهرت النتائج اختلافات معنويةبين الذكور والإناث. هناك زيادة في انزيم ناقلة الامين انيلين ، اليوريا، والكرياتينين في الإناث (ت2) مقارنة بالذكور (ت1) خلال ساعات النهار والليل. علاوة على ذلك، في الساعة 12 صباحًا و12 ظهرًا، كان هناك فروق ذات دلالة إحصائيةلدى الذكور في مستويات انزيم ناقلة الاسبارتات أعلى من الإناث و يتسبب النسق اليوميالبايولوجيبين ساعات النهار والليل وكذلك بين الذكور والإناثفي تغييرات في وظائف الكبد بسبب اختلافات الأيض والأنشطة.

الكلمات المفتاحية: انزيم ناقلة الاسبارتات, انزيم ناقلة الامين انيلين ، الساعة البيولوجية، وظيفة الكبد، أغنام.