Mechanical properties of the aortic arterial wall during 24 hours: a preliminary study in conscious sheep

S. Graf\textsuperscript{1,2}, D. Craiem\textsuperscript{1,3}, M. Valero\textsuperscript{1}, M. Alfonso\textsuperscript{1}, J. G. Barra\textsuperscript{1,2}, R.L. Armentano\textsuperscript{1}

\textsuperscript{1} Fac. de Ingeniería y Ciencias Exactas y Naturales, Universidad Favaloro
\textsuperscript{2} Dto. de Ciencias Fisiológicas, Universidad Favaloro
\textsuperscript{3} CONICET, Buenos Aires, Argentina

E-mail: graf@favaloro.edu.ar

Abstract. Previous experiences in animals showed a different behavior between the variability of pressure, arterial diameter and elasticity when they were registered for a couple of hours. To better understand arterial mechanics variability, we propose to measure simultaneously aortic pressure and diameter during 24 hours in a sheep. For that purpose, we developed a portable prototype device. It allows continuously recording physiological signals throughout the day and storing them in a solid state memory for later analysis. Pulse wave velocity and Peterson modulus were assessed beat-to-beat as arterial stiffness indexes. We identified 53,762 heart beats during 24 hours that were separated into 2 groups: below or above median mean pressure (71 mmHg). Mean diameter, pulse wave velocity and Peterson modulus increased for higher pressure values (p<0.05) whereas heart rate slowed down (p<0.05). Pressure-diameter loops were successfully recreated all along the experience. This new methodology sets the basis for further experiences involving the estimation of 24 hours arterial mechanics variability.

1. Introduction
The alterations of the arterial system can be evaluated from a biomechanical perspective analyzing the function and structure of the arteries. Previous studies from our group applied mathematical models to assess the geometric, elastic and viscous properties of the arteries. They were carried out starting from pressure-diameter loops in chronically instrumented conscious animals \cite{1, 2}. Although the heart rate variability was extensively studied, insufficient information is available about the variability of the arterial mechanical properties during the day and night. We showed that pressure, diameter and elasticity variability were dissimilar during short term (one hour) experiences performed in sheep \cite{3, 4}. Particularly, elasticity did not followed pressure variations during all the time. On the basis of these observations, we propose to extend the analysis to 24 hours, measuring simultaneously arterial pressure and diameter and pulse wave velocity (PWV). The online assessment of these properties could help to better understand the different regulatory mechanisms that control blood flow and pressure during day and night. Recently, we developed an autonomous and portable prototype device, which allows continuous recording and digital storage of physiological signals for analysis. The device was validated in vitro and contrasted against synthesized (simulated) signals \cite{5}. Using this device, the aortic blood pressure, diameter and consequently arterial stiffness evolution can be estimated beat to beat. To validate arterial stiffness
measurements, PWV was also calculated using two arterial diameter sensors [6]. This work presents the methodology, the system implementation and the preliminary results from an animal experience.

2. Materials and methods

2.1. Prototype description

The portable prototype device is based on a dsPIC30F6014A (Microchip, USA) microcontroller (figure 1). Basically, it includes a signal conditioning stage (filtering and amplification), a 12-bit A-D converter stage and a data storage stage (4GB SD type flash memory card). The signals can be monitored in real time by a liquid crystal display, or via serial communication using a computer.

![Figure 1](attachment:image.png)

**Figure 1.** (a) Block diagram and (b) picture of the implemented prototype.

The equipment allows acquiring and storing up to 4 channels simultaneously, at a configurable sampling frequency of 0.5, 1, 2 and 4 KHz and at configurable time intervals. In order to verify the proper operation, several tests were conducted for at least 24 hours. The memory storage capability was tested using synthetic signals resulting in no loss of data. Keeping a constant signal on each input channel, it was demonstrated that the variation throughout a day was less than 1%. Settling time of the device was less than 5 minutes [5].

2.2. Animal instrumentation

A healthy Corrediale crossbreed sheep of 47 kg and 1.5 year-old was used for the experiment. Surgery was performed under general anesthesia premedicated with acepromazine (0.2 mg/kg) induced with thiopental sodium (20 mg/kg) and maintained with isoflurane (2.5 % in pure oxygen at 2 L/min) with assisted mechanical ventilation (Neumover 910, Córdoba, Argentina). During surgery, blood pressure, electrocardiogram, capnography and heart rate were monitored (Siemens Sirecust 404-1). Two thoracotomies in the fourth and seventh left intercostal spaces were performed. The proximal and distal thirds of the aorta were exposed to implant pressure and diameter sensors. The solid-state pressure microtransducer of 2.5 mm (Konigsberg Instruments, Inc., Pasadena, CA) was implanted in the proximal third. The pressure microtransducer has been previously calibrated in vitro in saline at 37°C against a pressure caliper (Xcaliber, Viggo-Spectramed, Oxnard, CA). Both in proximal and distal regions of the aorta, ultrasonic crystals (5 MHz, 4 mm) were sutured with 6-0 silk on the adventitia in two places diametrically opposed, to measure outer diameter by sonomicrometry (Triton Technology Inc., San Diego, CA). The first pair was sutured to the proximal third of the vessel at 5 mm of the pressure sensor to avoid interference and the second pair was...
sutured in the distal third (the distance between the two sensors were 14.7 cm). The optimal placement of the crystals was verified by the digital oscilloscope screen (Tektronix TOS 220, Tektronix Inc. Beaverton, USA). The correct recording of the sensors was verified during operation by monitoring the signals in real time with the prototype connected to a PC before and after closure of thorax. After sensors implantation, cables were tunneled to emerge through the skin on the back of the sheep, which was then sutured. Finally, the incisions were closed by layers.

2.3. Experimental protocol
The experiment was carried out after 7 days of the surgical instrumentation to ensure proper postoperative recovery. The first channel of the prototype was adjusted to work with the pressure microtransducer, while the two following channels were conditioned to receive the sonomicrometer diameter signals. The prototype device was configured to acquire and store in memory three signals over 24 hours continuously for 5 minutes, separated by an interval of 5 minutes. Sampling frequency was set to 1 KHz. The activity of the sheep was monitored using digital images of 512×512 pixels taken with a digital camera (Logitech, USA), connected to a PC. The day after the study, data from the memory card was downloaded and signals were processed using proper software developed in our laboratory.

For each cardiac cycle, systolic, diastolic and mean values from pressure and diameter signals were automatically obtained. PWV was assessed as the ratio of the distance between the two diameter sensors and the resulting temporal delay between the onsets of the two diameter waveforms, identified by the tangent method [7]. At a sampling frequency of 1 KHz and considering a distance of 14.7 cm between sensors, the estimated error in PWV was ~3.4%. Heart rate was calculated from the pressure signal. The pressure-strain elastic (Peterson) modulus \( E_p \) was calculated as defined in equation (1):

\[
E_p = \frac{\Delta P}{\Delta D} \cdot D_d,
\]  

where \( \Delta P \) is the difference between systolic and diastolic pressure, and \( \Delta D \) is the difference between systolic and diastolic diameter \( D_d \).

2.4. Statistical analysis
Data are expressed as mean ± standard deviation. In order to characterize the mechanical behavior of the arterial wall, the total amount of identified beats along 24 hours were separated into 2 groups: below or above the median mean pressure. Differences were compared using Student t test. A value of \( p<0.05 \) was considered significant.

3. Results
The instrumented animal during the experience is shown in figure 2. Note the sensors wires emerging from the back of the animal. The sheep was free to move using a custom hose-spring system hanging from the ceiling. The prototype device and the sonomicrometer were located on the other side of the wall. The animal was isolated and remained calmed during the experience. All measurements were made without sedation with the animal in conscious state. It was free to eat and rest in any moment and did not present discomfort during the experience.

Aortic pressure, diameter and PWV temporal evolution for 24 hours are shown in figure 3. The pressure-diameter measures in the aorta are shown in figure 4, with the excursion of the diastolic, mean and systolic values during 24 hours. Pressure values ranged from 40 to 100 mmHg and diameter values from 19.5 to 23.5 mm.
We identified 53,762 heart beats during 24 hours that were separated into 2 groups according to the median pressure value, which resulted in 71 mmHg. The hemodynamic parameters of each group are detailed in table 1. Mean diameter, PWV and $E_p$ increased during high pressure group (p<0.05) whereas heart rate slowed down (p<0.05).

**Figure 2.** Conscious instrumented sheep and associated equipment used for signals recordings during 24 hours.

**Figure 3.** Temporal evolution of pressure, diameter and PWV every 10 minutes during 24 hours. The plotted values represent the median from 5-minutes continuous recordings. For pressure and diameter the systolic, mean and diastolic values are shown. For the PWV, an additional trend line is shown.
Table 1. Hemodynamic parameters during 24 hours separated by groups.

|                         | Low pressure group n=26,881 beats | High pressure group n=26,881 beats |
|-------------------------|-----------------------------------|-----------------------------------|
| Mean pressure (mmHg)    | 66 ± 4                            | 75 ± 4 *                          |
| Mean diameter (mm)      | 21.56 ± 0.51                      | 21.67 ± 0.55 *                    |
| PWV (cm/s)              | 461 ± 34                          | 478 ± 37 *                        |
| Heart rate (bpm)        | 98 ± 18                           | 93 ± 15 *                         |
| $E_p$ (mmHg)            | 285 ± 64                          | 299 ± 70 *                        |

* p<0.05 (Student t test) between low and high pressure group.

4. Discussion
In this work we show for the first time the continuous assessment of aortic elastic properties in a sheep during 24 hours. Aortic pressure and diameter were recorded using a custom portable prototype especially designed in our laboratory. The device successfully acquired and stored more than 50,000 heart beats throughout one day. In this particular experience, pressure varied ~30% and diameter only ~10% with respect to 24 hours mean values. Aortic stiffness was independently estimated using two indexes: the Peterson modulus $E_p$, calculated from pressure-diameter loops, and the PWV, estimated using 2 diameter sensors separated with a known distance. Although the animal was calm during the experiment, the arterial pressure showed an important variation (figure 3). As expected, aortic wall stiffness was positively associated to pressure changes. To study the association between pressure values and arterial mechanics, we divided all the measured beats in 2 groups using the median mean pressure as a threshold. Arterial stiffness was higher for pressure beats above the threshold: for a 15% of pressure change, PWV and $E_p$ increased ~5%. However, pressure and stiffness were not permanently correlated during the experience. In fact, we found that the relationship between arterial pressure and diameter was not systematic, evidencing the complexity of the arterial system. As shown in figure 4, pressure-diameter values do not lie on the same unique elasticity curve during 24 hours. This reflects the natural biological variability of the arterial wall properties, where the active components of the wall (vascular smooth muscle) continuously regulate the vessel size. In previous works of our group, different beat-to-beat elastic models were tested in vivo [8, 9]. So, we are encouraged to initiate a new set of experiments aimed to analyzing the variability of the mechanical properties in aorta. Analyzing the animal activity along the 24 hours,
we concluded that could not follow a circadian behavior. As can be seen from the figure 3, there is not any comprehensible day/night pattern. That might be related to the permanent availability of food. The digestive system of ruminants might generate recurrent pressure maneuvers that could modulate the arterial pressure. Also, the activity of the animal could be altered by the hose-spring system. Further experiments are necessary to explore these suggestions. Some issues remain to be solved to ensure the full portability of the prototype. First, although the system supports the direct connection of the pressure microtransducer, it requires an external sonomicrometer to generate the diameter signals. A new portable sonomicrometry system is being designed in our laboratory and will be incorporated in the near future. Secondly, we are reducing the size of the prototype using surface mount components and small batteries that will allow a better portability. Then, the device will become completely autonomous without requiring any external connection, allowing the animal to carry the whole system on his back.

5. Conclusions
Through a beat to beat 24 hours analysis we studied the influence of pressure on the aortic mechanical properties of a sheep. In general, when pressure increased, diameter, PWV and $E_p$ concomitantly increased whereas heart rate decreased. Pressure-diameter loops were successfully recreated all along the experience, confirming the capability of assessing true arterial wall behavior during 24 hours. These findings encourage us to program new experiments aimed to analyze the arterial mechanical properties throughout the day.

6. Acknowledgments
This work was in part subsidized by the project PIP number 112-200901-00734 (CONICET) and project PICTO 31355 (ANPCyT).

References
[1] Armentano R L, Barra J G, Levenson J, Simon A and Pichel R 1995 Arterial wall mechanics in conscious dogs: Assesment of viscous, inertial, and elastic moduli to characterize aortic wall behaviour Circ. Res. 76(3) 468-78
[2] Barra J G, Armentano R L, Levenson J, Cabrera-Fisher E I, Pichel R H and Simon A 1993 Assesment of smooth muscle contribution to descending thoracic elastic mechanics in conscious dogs Circ. Res. 73 1040-50
[3] Gamero L G, Armentano R L and Levenson J 2002 Arterial wall diameter and viscoelasticity variability Compu. in Card. 29 513-6
[4] Craiem D, Graf S, Salvucci F, Chironi G, Megnien J L, Simon A, Armentano R L 2010 Physiological principles of the ambulatory arterial stiffness index explained by the non-linearity of arterial elasticity Physiol. Meas. 31(7) 1037-46
[5] Alfonso M 2009 Sistema portable para el cálculo y registro de parámetros fisiológicos. Thesis. FICEN. Universidad Favaloro
[6] Laurent S et al. 2006 Expert consensus document on arterial stiffness: methodological issues and clinical applications Eur. Heart J. 27 2588 – 605
[7] Chiu C Y, Arand P W, Schroff A, Feldman T and Carroll J 1991 Determination of pulse wave velocities with computerized algorithms Am. Heart J. 121 126
[8] Armentano R L, Barra J G, Pessana F M, Craiem D, Graf S, Santana D B and Sanchez R A 2007 Smart smooth muscle spring-dampers. Smooth muscle smart filtering helps to more efficiently protect the arterial wall IEEE Eng. Med. Biol. Mag. 26 62-70
[9] Gamero L G, Armentano R L, Barra J G, Simon A and Levenson J 2001 Identification of arterial wall dynamics in conscious dogs Exp. Physiol. 86 519-28