

IN VIVO NUCLEAR MAGNETIC RESONANCE IMAGING

CONTRACT NAS9-17256

FINAL REPORT MAY 30, 1986

DEPARTMENT OF MEDICINE

BAYLOR COLLEGE OF MEDICINE

(NASA-CR-179902) IN VIVO NUCLEAR MAGNETIC
RESONANCE IMAGING Final Report (Baylor
Coll. of Medicine) 34 p CSCL 20H

N87-12328

Unclas
G3/72 44882

PRINCIPAL INVESTIGATOR: Adrian LeBlanc, Ph.D.

SUMMARY

During the past year the Woodlands Baylor Magnetic Resonance Imaging (MRI) facility became fully operational. A detailed description of this facility is part of this report. One significant instrument addition this year was the 100 MHz, 40cm bore superconducting imaging spectrometer. This instrument gives us the capability to acquire high energy phosphate spectra. This will be used to investigate ATP, phosphocreatinine and inorganic phosphate changes in normal and atrophied muscle before, during and after exercise. An exercise device for use within the bore of the imaging magnet is under design/construction.

A study of T_1 and T_2 changes in atrophied muscle in animals and human subjects was completed. A report of these results is attached and is scheduled for publication in the Journal of Medical Physics in August, 1986.

We have completed the imaging and analysis of the lower leg of 15 research subjects before and after 5 weeks of complete bedrest. A compilation of these results are attached and will be submitted to the Journal of Magnetic Resonance in Medicine next month.

Our bedrest studies scheduled to begin this summer will, in addition to our previous measurements, include multiple component T_2 analysis, T_1 data and high energy phosphate measurements.

**BAYLOR COLLEGE OF MEDICINE
MRI FACILITY**

The Baylor College of Medicine Magnetic Resonance Center is a multidisciplinary facility founded by the College in 1980 with the express purpose of implementing and developing in vivo MRI techniques including morphologic imaging and chemical spectroscopy. It has an annual budget of 8 professionals, 6 technicians and appropriate clerical help. The laboratory occupies 6,900 square feet of space with the appropriate animal facilities, shielded rooms, and basic biochemistry laboratory.

The Magnetic Resonance Center contains five magnets:

- 1) 6 MHz (0.15 Tesla) BMT 1000 1 meter ("whole body") bore sensitive magnet imaging system.
- 2) 12 MHz (0.24 Tesla) BMT 1100 1 meter bore ("whole body") resistive magnet imaging system.
- 3) 100 MHz (2.35 Tesla) Med. Spec 40cm bore superconducting magnet imager/spectrometer.
- 4) 200 MHz (4.7 Tesla) CXP 200 98mm bore superconducting magnet mini imager/spectrometer.
- 5) 400 MHz (9.4 Tesla) AM 400 WB 89mm bore superconducting magnet high resolution spectrometer.

The Bruker consoles include an Aspect 3000 computer with 960K memory and peripherals which include dual floppy disc drives and 80 MByte hard disc. The computer controls all data acquisition and storage of raw FIDs, Fourier transformed FIDs and displays all images and spectra.

In addition to the console computers in the Bruker imaging systems, the Baylor MRI Center has several other computers available for simulation, post processing, and experiment control. The computer systems are summarized below.

COMPUTER SYSTEMS HARDWARE SUMMARY

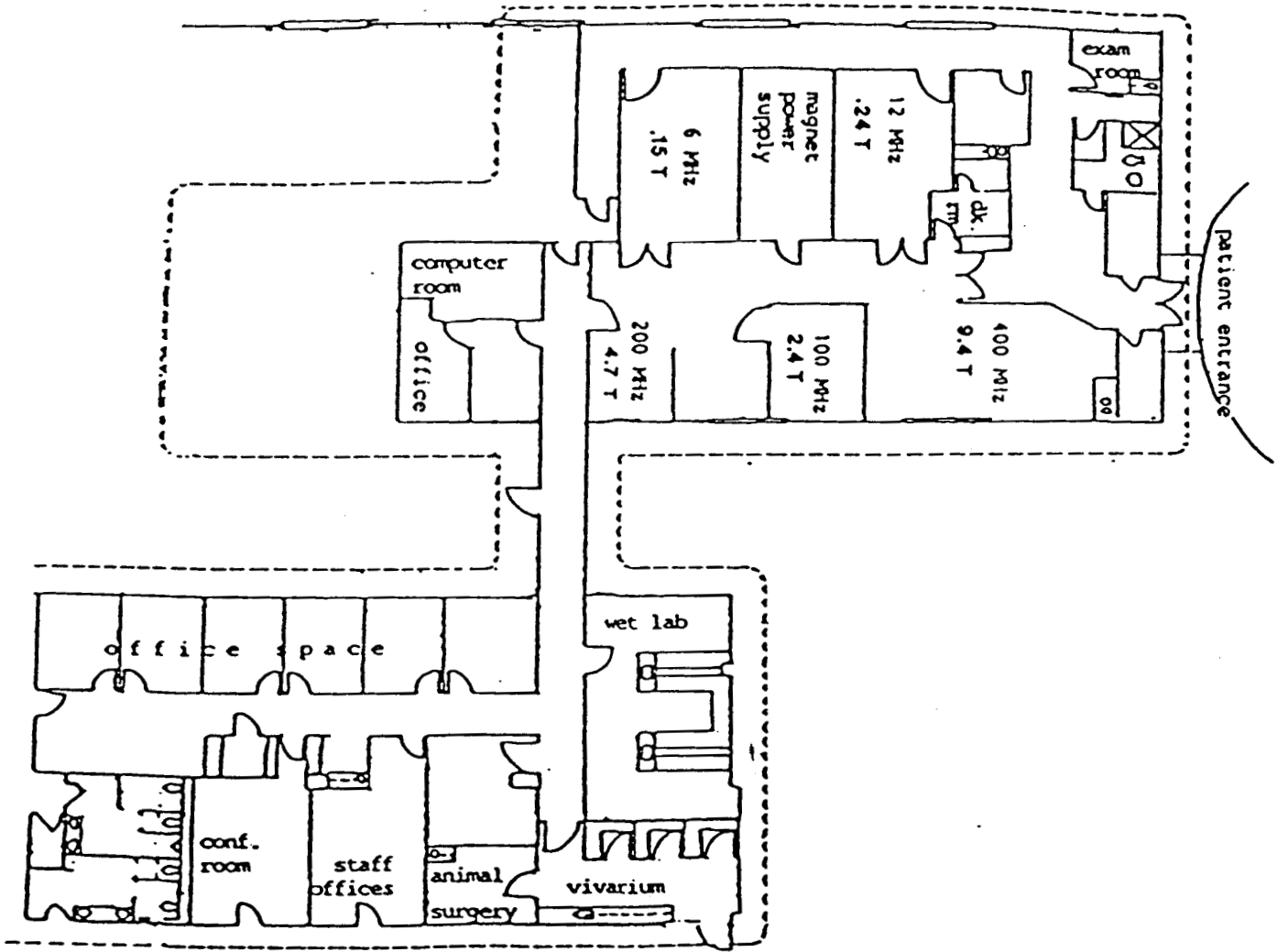
<u>SYSTEM</u>	<u>MANUFACTURER</u>	<u>MEMORY</u>	<u>DISKS</u>	<u>PERIPHERALS</u>
VAX-11/750	Digital Equip.	4Mb	456Mb	Floating Point Accelerator DR-11W, DMF-32, 5 graphics and 5 text terminals, and two dial-up ports.
System/9000	IBM Instruments	1.3Mb	10Mb	DR-11W Emulator, Sensor I/O board
System/9000	IBM Instruments	1.3Mb	20Mb	DR-11W Emulator, Sensor I/O, Vector Arithmetic Processor, RasterTech ONE/20 Image Processing System.
Apect 3000	Bruker (Console Computer)	2.1Mb	96Mb	Fourier Transform Processor, Array Processor, DR-11 Emulator

COMPUTER SYSTEMS SOFTWARE SUMMARY

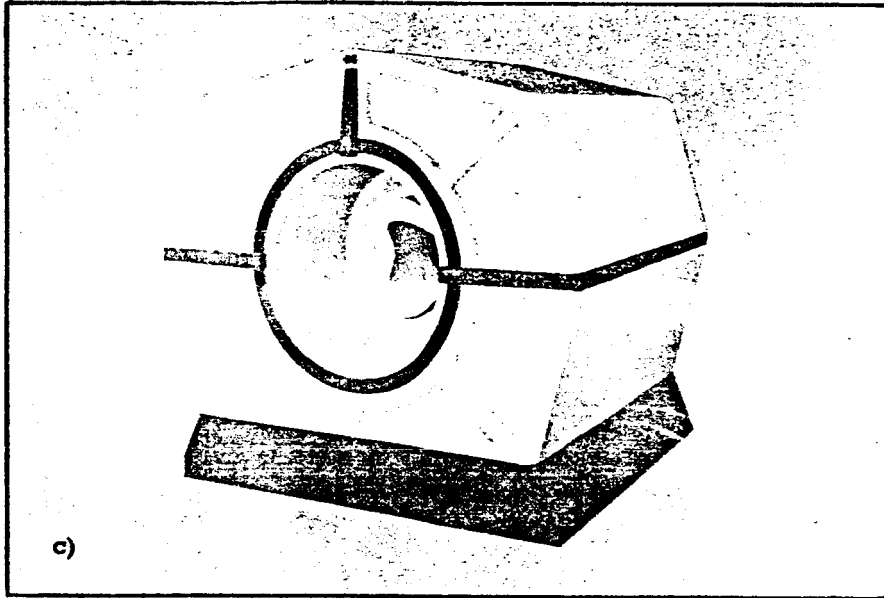
<u>SYSTEM</u>	<u>OPERATING SYSTEM</u>	<u>LANGUAGES</u>	<u>SOFTWARE</u>
VAX	VMS with Phoenix (Unix4.1BSD Emulator)	VAX Assembler, C, FORTRAN, Lisp	Bloch Eqns; T1,T2,SD images;image display
S/9000	IBM CSOS	68000 Assembler, C, FORTRAN, Pascal, BASIC	Backprojection, 2DFT reconstruction, image display, spectrum analysis
Aspect	Adakos	Aspect Assembler Pascal	Bruker reconstruc- tion and display

The main computer is a Digital Equipment Corporation (DEC) VAX-11/750. It is used for simulations, calculation of true T_1 , T_2 and spin density (SD) parametric images, and the display and archiving of image data. VAX/VMS is the primary operating system, with Phoenix, a Unix 4.1BSD emulator, running as a VMS process. Also available are two IBM instruments System/9000 laboratory instrumentation computers. Each of the four Bruker consoles has an Aspect computer system. A current project is the interconnection of the MR Center's computers, with the VAX as a central hub of a star configuration, using DR-11W parallel interfaces as the spokes between the VAX and each of the Bruker consoles and the System/9000x. Data are interchangeable among all of the computers. The VAX can read and write magnetic tapes written by the Bruker consoles. The 9000s can communicate with the VAX by RS-232 and DR-11 interfaces and with the Bruker consoles by reading and writing Bruker formatted floppy discs.

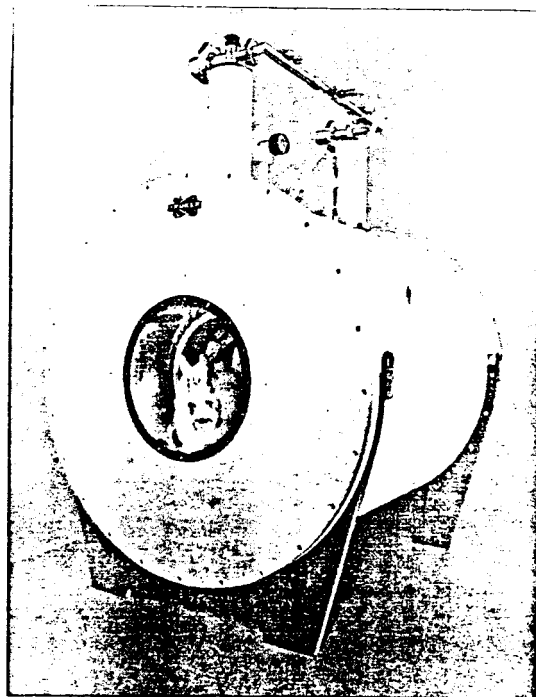
FLOORPLAN - MAGNETIC RESONANCE CENTER



ORIGINAL PAGE IS
OF POOR QUALITY



BMT 1100 WHOLE BODY IMAGER



MED SPEC 24/40 IMAGER SPECTROMETER

RELAXATION TIMES OF NORMAL AND ATROPHIED MUSCLE

Adrian LeBlanc Harlan Evans Ernesto Schonfeld
Joseph Ford Cherri Marsh
Victor Schneider Philip Johnson

Baylor College of Medicine
and
Medical Sciences Division
Johnson Space Center, NASA
Houston, Texas

Send Correspondence to:

Adrian LeBlanc, Ph.D.
The Methodist Hospital
6560 Fannin, MS ST396
Houston, Texas 77030

ABSTRACT

Magnetic Resonance Imaging is being used to investigate physiological changes induced by microgravity. Using human (bed rest) and animal (tail suspension) models simulating zero gravity, muscle atrophy was studied. Despite significant physiological changes in muscle mass, distribution of blood flow and muscle water, no changes in muscle proton relaxation times were found at several different resonant frequencies (6, 10, 20, 200 MHz). These results suggest that observed changes in relaxation times as reported in pathologic studies is likely due to the pathological changes and not the accompanying muscle atrophy.

INTRODUCTION

Magnetic resonance imaging (MRI) permits the investigation of in-vivo biochemical mechanisms, some of which cannot be adequately studied by in-vitro techniques (1). In addition MRI is non-invasive and does not involve radiation exposure. For these reasons we are investigating the application of MRI to elucidate some of the physiological changes that occur during space flight. Some of the known physiological changes that occur during space flight are: decreased red cell mass and red cell production; decreased plasma volume; negative calcium, nitrogen, and phosphorus balances; cephalad fluid shift; cardiovascular deconditioning; skeletal muscle loss; and vestibular dysfunction (2-4). Studies using bed rest, believed to be an analog of weightlessness, have shown similar changes (5-8). Space flight and ground based simulation animal studies have demonstrated additional changes, e.g., differential muscle fiber atrophy (slow twitch greater than fast twitch), muscle electrolyte concentration changes, increased marrow fat, and decreased bone formation rate (9-13). This paper reports our magnetic resonance results in atrophied muscle using human and animal analogs of microgravity.

METHODS

Animal Studies:

The purpose of these animal studies was to compare T_1 and T_2 data with physiological changes associated with disuse muscle atrophy, i.e., muscle wet weight, water content, and muscle perfusion. To simulate space flight, rear limb suspension of the rat was employed. This model has been shown to cause a number of physiological changes observed in animals flown in space

(12,13). Similar to space flight, this model, which has been previously described, reduces limb loading without immobilization (14). Female Sprague-Dawley rats beyond the rapid growth phase weighing 300-400 grams were rear limb suspended for 30-90 days and compared to non suspended control animals. Since the physiological changes reached their maximum value by 30 days of suspension, the data for 30-90 days of suspension were combined in this report. At the end of the experimental period, and just prior to sacrifice, some of the suspended and controls were injected via the left ventricle with radioactive labeled Ru-103 microspheres (15um, 700k particles). About 5 minutes after injection, the animals were sacrificed and the soleus and gastrocnemius muscles were removed and weighed. The muscle tissue from the left limb was dried to constant weight in an oven at 100°C for 5-10 days and the percent water calculated from the weight difference. Muscles from the opposite limb were used either for determining Ru-103 activity or for magnetic resonance measurements. The percent uptake of Ru-103 microspheres was determined by comparison with standards prepared at the time of injection. The percent uptake was divided by the wet muscle weight to give percent cardiac output per gram of muscle as an index of tissue perfusion.

EXPERIMENT NO. 1:

Whole soleus or gastrocnemius muscles from four suspended and five control animals were placed in plastic vials and analyzed in a Bruker 200MHz magnet interfaced to a CXP 200 console. T_1 data was obtained using an inversion recovery sequence with a T_1 varying from 1 to 7000msec. T_2 was obtained by Carr-Purcell-Meiboom-Gill (CPMG) spin echo technique with 2 msec between the 180° pulses. Measurements were made at room temperature (about 27°C) from 30 minutes to several hours after sacrifice and removal

from the animal. Repeat measurements of samples at different times after removal showed no significant change in relaxation times. This observation has been documented by others (20).

EXPERIMENT NO. 2:

Samples of soleus and gastrocnemius muscles from three control and two suspended animals were measured in a prototype bench top proton spin analyzer operating at 20 MHz. The instrument used the spin echo technique for T_2 obtained from the average of 16 events and several repeat measurements of the same sample.

EXPERIMENT NO. 3:

The whole gastrocnemius from 6 controls and 5 suspended animals were measured in an IBM PC-10 table top minispectrometer operating at 10MHz. T_1 was measured with an inversion recovery sequence and T_2 by CPMG. Typically, 2-4 data sets were collected and averaged. Because of small sample size, the soleus muscle could not be analyzed by this instrument. The sample time was as described in experiment 2. This instrument heats the sample to a constant temperature of 40°C prior to data collection.

Human Studies:

Our laboratory is conducting a series of human bed rest studies investigating countermeasures against disuse bone loss. Nine male volunteers, age 23-41 years, have completed the bed rest protocol with MRI of the right lower limb. The experimental protocol consists of 5 weeks of ambulatory control followed by five weeks of complete bed rest. The

subjects were allowed to get up five minutes per day for bowel movements, otherwise they remained recumbent. During the 10 weeks of the study the subjects were fed a regulated nutritionally adequate diet. MRI was performed twice just before bed rest at the end of the 5 week ambulatory control period and twice immediately after bed rest.

Since the pre and post bed rest images are separated by 5 weeks, we constructed a jig, Figure 1, to permit a direct calibration of transverse images as well as to rigidly hold the limb during a scan. Each slice required 5 minutes for acquisition. In addition to rigidly holding the leg, the jig image determines slice location, pixel size and slice thickness. This is accomplished by a series of machined grooves in the holder which are filled with an MRI sensitive material. After experimenting with a number of substances, vegetable shortening was chosen to fill the grooves. Four grooves (Figure 1, A-D) are used for calibrating the pixel size and hence the x-y plane dimensions. The image length produced from the "E" grooves after simple geometric calculation gives slice thickness. The image of the series of grooves labeled "F" and the position of the "E" groove images is used to calibrate the precise location on the jig where the MRI slice is acquired. This permits exact anatomical repositioning by carefully positioning the leg in the jig in the same manner each time. Calibration is accomplished by noting the distance between grooves "A", "E", and "F". During analysis a computer program automatically computes the distance from heel to MRI slice. A digital histogram through the two spots of the "F" grooves gives a crude estimate of image resolution. The large tube labeled "G" is used to obtain standard relaxation times which are used to monitor system stability for comparing tissue relaxation times.

Each scan consisted of 5 slices, 2cm apart starting at a point approximately over the belly of the soleus where the gastrocnemius muscle mass is negligible. This point is determined and the distance from the heel recorded and is used as the starting point for all subsequent scans.

All imaging was done using a 6MHz Bruker magnet, CXP 200 console with the head coil inserted. The MRI technique was spin echo using a 180° slice selective preparation pulse followed 20ms later by a 90° read pulse and 16, 180°, spin echo pulses with a T_e of 9ms. A delay of one second is used between the end of the spin echoes and the next slice select pulse.

Reconstruction was accomplished from 120 projections at 1.5° intervals in a collection matrix of 256 x 256. For each projection a series of 16 echoes were collected and combined into four echoes by summing successive echoes in groups of four. After reconstruction, each of these are displayed as separate images. From these four images, T_2 and pseudo spin density images are calculated. The methodology and documentation for producing accurate calculated T_2 images has been previously published (15). In this work we have improved the T_2 calculation by fitting the T_2 decay by the weighted least squares method. From this image the T_2 of muscle is determined by computing the T_2 mean and standard error from several small (1cm²) areas within the gastrocnemius and soleus muscles.

The muscle area was determined by integrating pixels with a T_2 range from 40-60msec either for the entire slice or for an area which corresponded to the gastrocnemius and soleus muscles. This range of T_2 values was sufficient to exclude other tissues, i.e., fascia fat, bone marrow and blood. It was found that transverse slices taken approximately through the belly of the gastrocnemius were the most reproducible as demonstrated from repeat scans obtained on the same day. This is because

leg diameter is changing less rapidly with distance at this location and therefore, slight errors in repositioning are less critical. Muscle atrophy was determined from 2 contiguous slices through this region and the duplicate measurements averaged pre and post bed rest. The mean and standard deviations are therefore calculated from four measurements.

RESULTS

Table 1 summarizes the animal data. Both the soleus and gastrocnemius lost significant mass, 56% and 26% respectively. Resting cardiac output to the soleus and gastrocnemius decreased significantly in excess of the decrease in muscle atrophy, i.e., percent cardiac output per gram of muscle decreased. Percent water decreased significantly in the soleus, 5.1%, but was unchanged in the gastrocnemius. The relaxation times (T_1 and T_2) did not differ significantly between the soleus and gastrocnemius and were not altered by muscle atrophy.

Table 2 gives the pre and post bed rest muscle area measurements. Each value is the mean \pm SD of 4 values, 2 slices repeated in the same day. The precision for total muscle area measurement was approximately 3%. An unpaired t test was used to test significance. The gastrocnemius and soleus lost an average of 11% in area while the total cross sectional muscle area lost 7%. A paired t test of the differences was significant ($p = 0.04$).

Table 3 are the T_2 values before and after bed rest. There was no significant change in muscle T_2 either individually or on average.

DISCUSSION

A review article by Bottomley et al. tabulated published T_1 and T_2 values of various tissues including a number of species obtained from both

in-vivo and in-vitro measurements (16). Using these data they derived mathematical relations between T_1 and frequency. Using the skeletal muscle constants from their paper, calculated T_1 values for 10 and 200MHz are 398 and 1403 ms, very close to the values obtained in this paper, Table 1. Even though the relation was developed from data obtained at frequencies below 100MHz, it appears to be valid up to 200MHz for muscle. The T_2 values for the bed rest subjects are very close to the average T_2 computed by Bottomley, 47ms. Our rat in-vitro T_2 values are lower but within 1.5 SD of the mean reported by Bottomley except for the 20MHz data which is believed to be too low. These data, from a prototype instrument, were felt to be useful and therefore included in this report because our main objective was to detect changes in relaxation time with atrophy.

The soleus is a slow twitch muscle while the gastrocnemius is predominately fast twitch. Rat experiments have shown that immobilization of slow twitch muscle causes speeding by an apparent change in fiber type from slow to fast (17). Our data show no significant difference in relaxation time between soleus and gastrocnemius before or after atrophy.

Despite significant muscle atrophy in both human and animal models, no change in T_1 or T_2 was demonstrated in this study. This would at first appear disappointing considering that differences in relaxation times between normal and physiologically altered tissue is the key to proton MRI and its clinical utility. In both the animal and human experiments, physiology is significantly altered, i.e., muscle atrophy, decreased muscle blood flow, and fiber type changes. However, it is reasonable to assume that these changes are a consequence of decreased loading and that the resulting muscle is "normal" but simply reduced in amount. Muscle atrophy is an accompanying result of most muscle diseases. Muscle T_1 is reported to be significantly elevated in animals with inherited muscular dystrophy

suggesting altered muscle pathology is detectable by MRI (18,19). Although we did not measure T_1 in-vivo in the human studies, our animal data suggests that both T_1 and T_2 are not affected by disuse atrophy. Our data suggests that observed changes in relaxation times is likely due to a pathological condition and not due to the concomitant atrophy.

REFERENCES

1. R.A. Meyer, M.J. Kushmerick, T.R. Brown, *Am. J. Physiol.* 242, C1 (1982).
2. A.E. Nicogossian, J.F. Parker, *Space Physiology and Medicine*. (NASA SP-447), Washington, D.C.: U.S. Government Printing Office 1982.
3. R.S. Johnston, L.F. Dietlein, *Biomedical Results from Skylab* (NASA SP-337), Washington, D.C.: U.S. Government Printing Office 1977.
4. R.S. Johnston, L.F. Dietlein, C.A. Berry, *Biomedical Results of Apollo*, (NASA SP-368), Washington, D.C.: U.S. Government Printing Office 1975.
5. A.R. Hargens, *Med. and Science in Sports and Exercise*. 15(5), 421 (1983).
6. J.E. Deitrick, G.D. Whedon, E. Shorr, *Am. J. Med.* 4, 3 (1948).
7. C.L. Donaldson, S.B. Hulley, J.M. Vogel, R.S. Hattner, J.H. Bayers, D.E. McMillan, *Metabolism* 19, 1071 (1970).
8. V.S. Schneider, J. McDonald, *Calcif. Tiss. Int.* 36, S151 (1984).
9. W. S. Jee, T.J. Wronski, E.R. Morey, D.B. Kimmel, *Am. J. Physiol.* 244, R310 (1983).
10. E.R. Morey, D.J. Baylink, *Science* 201, 1138 (1978).
11. X.J. Musacchia, D.R. Deavers, G.A. Meininger, T.P. Davis, *J. Appl. Physiol.* 48, 479 (1980).
12. T.J. Wronski, T.W. Halstead, E.R. Morey, *Aerosp. Med. Assoc. Preprint* 150 (1981).
13. G.H. Templeton, M. Padalino, J. Manton, M. Glasberg, C.J. Silver, P. Silver, D. DeMartino, T. LeConey, G. Klug, H. Hagler, J.L. Sutko, *J. Appl. Physiol.* 56, 278 (1984).
14. A. LeBlanc, C. Marsh, H. Evans, P. Johnson, V. Schneider, S. Jhingran, *J. Appl. Physiol.* 58 (5), 1669 (1985).
15. N. J. Schneiders, H. Post, P. Brunner, J. Ford, R.N. Bryan, M.R. Willcott, *Med. Phys.* 10(5), 642 (1983).
16. P.A. Bottomley, T.H. Foster, R.E. Argersinger, L.M. Pfeifer, *Med. Phys.* 11(4), 425 (1984).
17. F.W. Booth, *J. Appl. Physiol.* 52, 1113 (1982).
18. M.G. Luthra, W. S. Yamanashi, R.P. Munjaal, S.R. Amtey, *Magnetic Res. Imaging* 1, 75 (1982).
19. D.C. Chang, L.K. Misra, P.T. Beall, R.C. Fanguy, C.F. Hazelwood, *J. Cell Physiol.* 107, 139 (1981).

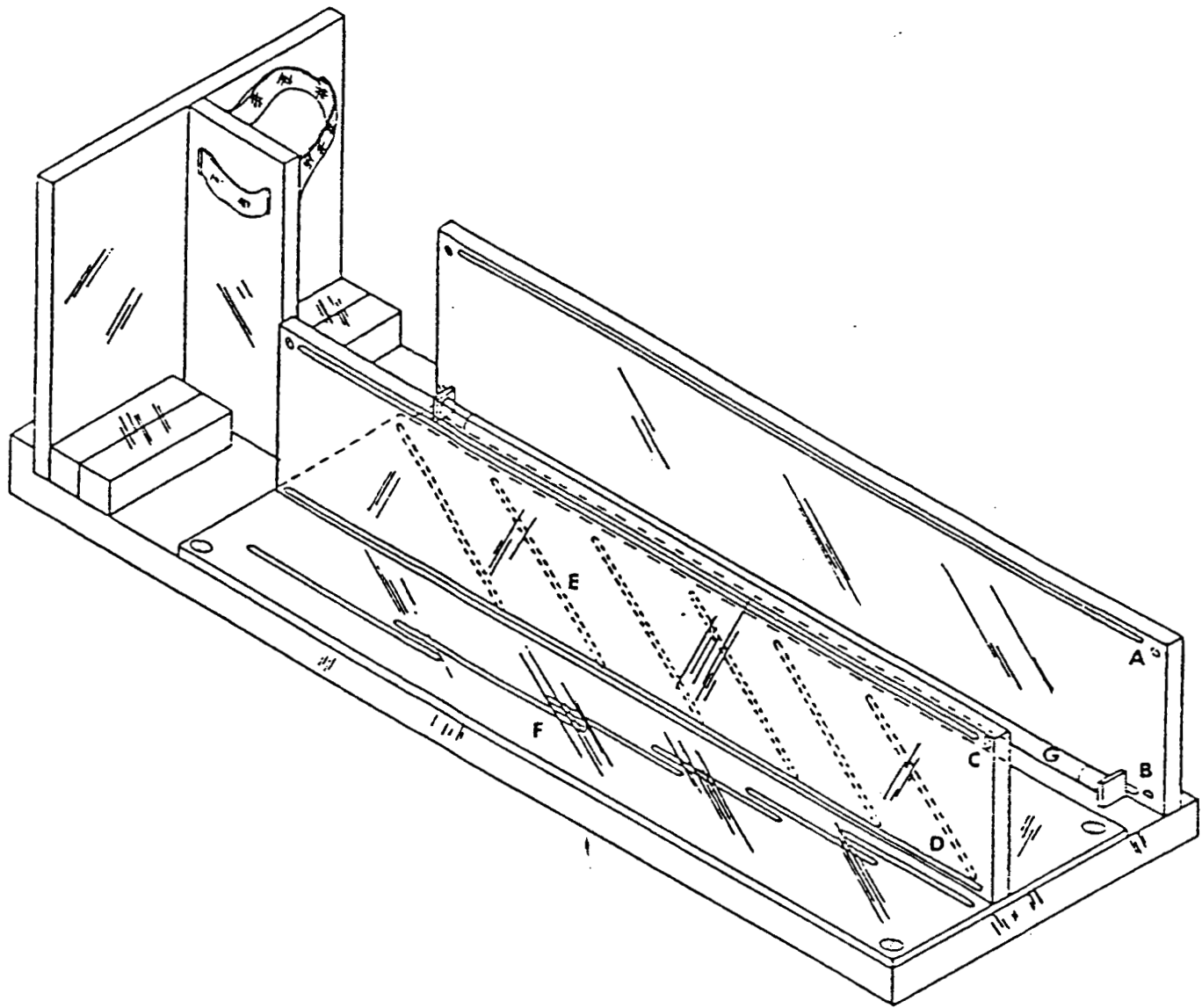


Fig. 1. Leg immobilization and calibration jig - see text for details.

TABLE 1

PHYSIOLOGICAL CHANGES IN RAT MUSCLE AFTER 30-90 DAYS OF SUSPENSION

	n	Soleus			p ²	Gastrocnemius			p
		Control	Atrophy	Δ%		Control	Atrophy	Δ%	
Muscle Weight (g) $\bar{X} \pm SD$	21	0.143 ±0.015	0.063 ±0.013	-56	S	2.041 ±0.246	1.506 ±0.144	-26	S
% H ₂ O	21	74.0 ±0.8	70.2 ±1.3	-5.1	S	74.1 ±2.1	73.5 ±0.6	-0.8	NS
% C.O./g ¹	17	0.371 ±0.165	0.095 0.036	-74	S	0.166 ±0.039	0.074 ±0.015	-55	S
200 MHz (ms) T ₁	9	1441.	1436.	-0.3	NS	1451.	1493.	+3	NS
T ₂	7	35.2 ±2.8	34.6 ±1.6	-2	NS	37.3 ±2.6	35.2 ±2.3	-5	NS
20 MHz (ms) T ₂	5	22.7 ±0.5	20.0 ±1.1	-12	NS	20.8 ±1.2	20.8 ±0.1	0	NS
10 MHz (ms) T ₁	11	-	-	-	-	428.5 ±6.9	430.7 ±5.8	+0.5	NS
T ₂	11	-	-	-	-	30.5 ±2.8	32.7 ±3.8	+7	NS

(1) Percent cardiac output per gram of muscle

(2) Significance is $p \leq 0.05$

TABLE 2

TOTAL AND REGIONAL MUSCLE AREA (mm²) AFTER 5 WEEKS
OF BEDREST - 4cm SECTION THROUGH CENTER OF GASTROCNEMIUS

Subject	GASTROCNEMIUS + SOLEUS MEAN ± SD			
	Pre-Bedrest	Post-Bedrest	Δ%	p
36	6085 ± 446	5005 ± 328	-18	<0.001
37	*3970	4163 ± 57	5	NS
39	4548 ± 138	3697 ± 108	-19	<0.001
42	4517 ± 79	4291 ± 80	-5	NS
46	3558 ± 137	3384 ± 168	-5	NS
47	4456 ± 73	3956 ± 12	-11	<0.001
75	6061 ± 868	4491 ± 653	-26	0.008
76	5462 ± 110	4815 ± 100	-12	0.002
77	3874 ± 34	3480 ± 94	-10	<.001
\bar{x}			-11	
	TOTAL MUSCLE MEAN ± SD			
36	8980 ± 472	8121 ± 384	-10	<0.001
37	*6374	6519 ± 197	2	NS
39	6731 ± 281	6004 ± 211	-11	<0.001
42	7053 ± 70	6514 ± 140	-8	0.03
46	5326 ± 85	5307 ± 71	0	NS
47	6643 ± 9	6186 ± 6	-7	<0.001
75	7507 ± 304	6529 ± 581	-13	0.007
76	7999 ± 137	7244 ± 186	-9	0.002
77	6296 ± 216	5900 ± 273	-6	NS
\bar{x}			-7	

The difference between gastrocnemius + soleus and total muscle loss was significant (p=0.04)

*One measurement because of acquisition artifacts.

TABLE 3

T₂ RELAXATION TIMES OF LIMB MUSCLE
BEFORE AND AFTER 5 WEEKS OF BEDREST

Subject	Pre-Bedrest ms ± SD	Post-Bedrest ms ± SD
36	44 ± 1	52 ± 2
37	53 ± 2	53 ± 4
39	43 ± 2	46 ± 2
42	48 ± 2	47 ± 1
46	51 ± 3	48 ± 1
47	48 ± 1	50 ± 2
75	44 ± 2	51 ± 4
76	50 ± 1	49 ± 2
77	46 ± 2	52 ± 1
$\bar{X} \pm SE$	47 ± 1	49 ± 1
p		NS

**CHANGES IN NUCLEAR MAGNETIC RESONANCE (T_2) RELAXATION
OF LIMB TISSUE WITH BED REST**

Adrian LeBlanc, Harlan Evans, Ernesto Schonfeld, Joseph Ford
Victor Schneider, Satish Jhingran, Philip Johnson

Baylor College of Medicine
and
Johnson Space Center
Houston, Texas

Running Head:

Changes in Limb Tissue T_2 with Bed Rest

Send Proofs to:

Adrian LeBlanc, Ph.D.

6560 Fannin, MS S396

Houston, Texas 77030

ABSTRACT

Bed rest is used to simulate the effects of weightlessness on human physiology. Spin Echo was used to image the lower leg of 15 normal male volunteers before and after 5 weeks of horizontal bedrest. In addition to noninvasively measuring muscle size changes, accurate T_2 images were produced to investigate possible relaxation time changes immediately (2-4 hours) and 1-2 days after bedrest. Subcutaneous fat showed no change in T_2 , bone marrow showed a decrease while muscle showed no change immediately after bed rest but increased 1-2 days following reambulation.

CHANGES IN NUCLEAR MAGNETIC RESONANCE (T_2) OF LIMB TISSUE WITH BED REST

INTRODUCTION

Bed rest is a 1G analog used to simulate the physiological responses to weightlessness. It produces changes in a number of organ systems, including bone, skeletal muscle, endocrine, blood, as well as imbalances in fluid, electrolytes and other body minerals qualitatively similar to exposure to microgravity (1-3). Despite intense research, the extent of ultimate change, the mechanisms involved and the physiological consequences of most of these changes during long term exposure to weightlessness and bedrest are not known (4). Some of the changes occur rapidly and reach a new equilibrium within a few days, e.g., fluid shift, plasma volume, red cell mass. Other changes occur more slowly, e.g., muscle and bone atrophy, where the final new equilibrium state at present is unknown. Bone loss continued unabated throughout the longest U.S. mission to date, the 89 day Skylab 4 mission. Similarly, equivalent duration of bedrest causes continuous negative calcium balance (5). In microgravity, these changes may pose a limit to future long term flight unless countermeasures are designed. The development of countermeasures is hindered by a lack of knowledge of the mechanisms involved.

The primary focus of magnetic resonance imaging (MRI) research has been in the field of diagnostic medicine. However, it has the potential to revolutionize the study of human physiology because of its ability to investigate in vivo chemical changes non invasively. We have been investigating the potential of MRI to measure chemical changes resulting

from exposure to bed rest. This paper reports on our measurements of proton T2 relaxation times in the lower limbs of normal male subjects before and after 5 weeks of complete bed rest.

METHODS

The bed rest protocol consisted of 5 weeks of ambulatory control followed by 5 weeks of horizontal bed rest. During the 10 week study the subjects were fed a regulated nutritionally adequate diet designed to maintain sodium and calcium balance. The details of the diet and blood and urine chemistries have been described previously (3). Fifteen male volunteers, age 23 - 41, completed the bed rest protocol with magnetic resonance imaging (MRI) of the lower right limb. MRI was normally performed twice just before bed rest during the ambulation control period, 2 - 3 hours after bed rest (R+0) and one or two days after re-ambulation (R+1,2). In 6 subjects it was not possible to obtain R+0 data and only R+1 or R+2 measurements were obtained. In four cases duplicate post bed rest images were obtained on R+ 0 only.

All scans were transverse and consisted of a series of slices through the mid calf area. Imaging was done with either a 6 MHZ resistive magnet*, or a 5 MHZ permanent magnet**. In both cases a head coil was used. The spin echo imaging acquisition and processing technique using the resistive system has been described (6). Briefly a 180° slice select pulse is followed 20 ms later by a 90° read pulse and 16, 180° spin echo pulses with a Te of 9ms and a Tr of 1 sec. Five slices were obtained spaced 2cm apart.

The imaging procedures on the permanent magnet was anisotropic 3D-FT. Each pulse sequence consisted of a 90° pulse followed by phase encoding in the Y and Z directions. Following the phase encoding, the X gradient was turned on and 8, 180° pulses were used to form spin echoes. The data from

the echoes at 24, 72, 120 and 168 ms (1st, 3rd, 5th, and 7th echos) was collected. Each data set consisted of a 4 dimensional array of 256 pixels in X, by 256 pixels in Y, by 15 pixels in Z, by 4 (number of spin echoes). The repetition times used ranged from 400 to 800 ms resulting in an acquisition time of 25 to 50 minutes. ^{Fin} Slices spaced 1cm apart were acquired.

A jig, previously described, was used to rigidly hold the limb during image acquisition and to accurately reposition the limb for each scan (6).

The T_2 calculation method described previously was used to calculate T_2 images for each slice (6). Multiple regions of interest of about 1 cm^2 were used to obtain average T_2 values in muscle, subcutaneous fat and bone marrow with $\bar{\mu}$ in each slice. Pre bed rest, the average T_2 value (\pm SD) was obtained from all slices from both the original and repeat measurements. The duplicate post bed rest measurements were averaged together and also separated according to when obtained, i.e., immediately after bed rest (R+0) or 1 to 2 days after ambulation (R+1,2).

RESULTS

Tables 1 - 3 give the individual and mean T_2 values before and after bed rest for subcutaneous fat, bone marrow, and muscle. The data for subjects 7, 8, and 9 were obtained with the 5 MHz system while all others were obtained with the 6 MHz system. Muscle demonstrated a significant increase in T_2 , bone marrow a significant decrease with fat showing no change. Table 4 shows the same data with the post bed rest data separated according to time of measurement. This shows that the decrease in bone marrow T_2 was present at R+0 and R+1,2. Muscle on the other hand does not show the change in T_2 immediately post bed rest (R+0) but this occurs after

re-ambulation on R+1,2. Figure 1 shows a T_2 image of a 1cm transverse slice through the mid calf of one subject. Images 1a and 1b are repeat measurements pre bed rest, 1c obtained at R+0, and 1d at R+1. The regional increase in T_2 at R+1 is obvious.

DISCUSSION

Weightlessness and bed rest result in fluid shift from the lower extremities to the upper torso most evident by the facial puffiness observed during spaceflight. While there is a negative water balance, the facial puffiness remains throughout flight. Loss of volume from the lower limbs has also been documented from limb circumference measurements in bed rest and spaceflight (5). Changes in relative size of the separate compartments, i.e., intravascular, interstitial, intracellular is less well documented. Significant fluid is undoubtedly lost from the calf muscles during 5 weeks of bed rest based on our cross sectional MRI measurements of muscle area. The total cross-sectional muscle area through the calf at the level of the gastrocnemius center was decreased -8%, $p < 0.001$, $n=15$. The T_2 measurements, however, do not decrease as one would expect from tissue dehydration, but remain unchanged. This suggests that the relationship between bound and free water has reached the pre bed rest equilibrium in the slice by the 5th week of bed rest. There is less tissue but the distribution of water appears unchanged. Re-ambulation however, alters this equilibrium, at least temporarily, as the muscle appears to have excess water reflected by an increased T_2 after re-ambulation. Intuitively we would expect a drop in T_2 1-2 days after the start of bed rest with a return to pre bed rest values sometime later. Multiexponential decay of spin echo data believed to represent multiple compartments has been

reported by several investigators (7-9). The first two compartments, representing crystalline and hydration water, have T_2 s of approximately 10-20 usec and 5-10 usec respectively, so short that they are not observable in most spectrometers. The other two compartments representing extracellular and intracellular water have T_2 s of about 147 and 44 msec respectively (7). We did not observe multiexponential components in our data probably because the acquisition time was too short relative to the long component. Presumably our data reflect predominantly the shorter component representing intracellular water which is also 4 times more abundant than the extracellular water compartment. The changes in T_2 after reambulation however could be due to changes in either compartment or both. We plan to investigate this in the near future using long echo trains with optimum signal to noise imaging.

The decrease in bone marrow T_2 is compatible with a decrease in water and/or an increase in fat. It is interesting that marrow fat is inversely related to red cell production and a decrease in red cell production rate is a known effect from bed rest and spaceflight (10). The decrease in bone marrow T_2 as a consequence of the bed rest is not readily reversed since it was significant at R+0 and did not recover by 1-2 days of re-ambulation. We would expect then that this change occurs slowly and would not be observed shortly after the beginning of bed rest and may be related to decrements in hemopoietic marrow. The decreased marrow T_2 occurred without changes in subcutaneous fat T_2 suggesting that the decrease in marrow T_2 is not due to a shift in T_2 of the fat itself. However, since marrow fat and extra osseus fat are not identical this possibility cannot be ruled out.

*Bruker, CXP 20 Console

** NMR Imaging Inc., Permascan

ACKNOWLEDGEMENT

We would like to thank Dr. Robert Wilcott and Gary Mee of NMR Imaging, Inc. for the use of the PermaScan imager for these studies and also for their help in acquiring the scans in the appropriate format.

REFERENCES

1. J.E. Deitrick, G.D. Whedon, E. Shorr, *Am. J. Med.* 4,3 (1948).
2. C.L. Donaldson, S.B. Hulley, J.M. Vogel, R.S. Hattner, J.H. Bayers, D.E. McMillan, *Metabolism* 19, 1071 (1970).
3. V.S. Schneider, J. McDonald, *Calcif. Tiss. Int.* 36, S151 (1984).
4. A.E. Nicogossian, J.F. Parker, *Space Physiology and Medicine. Special Publication, SP-447*, GPO, Washington, DC, 1982.
5. R.S. Johnston, L.F. Dietlein, *Biomedical Results from Skylab. Special Publication, SP-337*, GPO, Washington, DC, 1977.
6. A. LeBlanc, H. Evans, E. Schonfeld, J. Ford, C. Marsh, V. Schneider, P. Johnson, *Med. Phys.* 13(4), In Press (1986).
7. P.S. Belton, K.J. Packer, *Biochem. Biophys. ACTA* 354, 305 (1974).
8. C.F. Hazelwood, D.C. Chang, R.L. Nichols, *Biophys. J.* 14, 583 (1974).
9. Y. Mauss, D. Grucker, D. Fornasiero, J. Chambron, *Magn. Res. Med.* 2, 187 (1985).
10. M. Tavassoli, *Exp. Hematol.* 12, 139 (1984).

TABLE 1.

SUBCUTANEOUS FAT T₂ BEFORE AND AFTER
5 WEEKS OF HORIZONTAL BED REST

SUBJECT	PRE BED REST (msec)	POST BED REST (msec)
1	*99 ± 2	97 ± 2
2	99 ± 2	96 ± 2
3	107 ± 1	104 ± 2
4	103 ± 1	94 ± 1
5	101 ± 2	--
6	101 ± 2	104 ± 9
7	78 ± 2	83 ± 3
8	107 ± 5	88 ± 5
9	98 ± 6	93 ± 2
10	94 ± 2	94 ± 4
11	97 ± 2	105 ± 2
12	99 ± 2	98 ± 1
13	116 ± 1	114 ± 1
14	104 ± 1	104 ± 1
15	103 ± 2	99 ± 6
$\bar{X} \pm SD$	100 ± 8	98 ± 8

†P

NS

* Mean ± SD of individual slices

† 2 tailed paired t test

TABLE 2.

BONE MARROW T₂ BEFORE AND AFTER
5 WEEKS OF HORIZONTAL BEDREST

SUBJECT	PRE BED REST (msec)	POST BED REST (msec)
1	*135 ± 6	114 ± 7
2	147 ± 11	129 ± 17
3	149 ± 8	115 ± 2
4	145 ± 8	116 ± 2
5	124 ± 3	104 ± 23
6	126 ± 6	127 ± 17
7	95 ± 1	105 ± 2
8	122 ± 1	106 ± 4
9	115 ± 8	104 ± 2
10	130 ± 4	128 ± 4
11	132 ± 2	124 ± 3
12	129 ± 5	126 ± 5
13	132 ± 1	126 ± 2
14	129 ± 5	135 ± 4
15	127 ± 6	121 ± 30
$\bar{X} \pm SD$	129 ± 13	119 ± 10

†P

0.006

* Mean ± SD of individual slices

† 2 tailed paired t test

TABLE 3.

MUSCLE T₂ BEFORE AND AFTER
5 WEEKS OF²HORIZONTAL BEDREST

SUBJECT	PRE-BED REST (msec)	POST BED REST (msec)
1	*44 ± 1	52 ± 2
2	53 ± 2	53 ± 4
3	51 ± 3	48 ± 1
4	48 ± 1	50 ± 2
5	41 ± 4	61 ± 25
6	42 ± 4	50 ± 13
7	45 ± 4	49 ± 3
8	60 ± 4	55 ± 3
9	53 ± 4	50 ± 4
10	43 ± 2	46 ± 2
11	48 ± 2	47 ± 1
12	44 ± 2	51 ± 4
13	50 ± 1	49 ± 2
14	46 ± 2	52 ± 1
15	40 ± 4	54 ± 23
$\bar{X} \pm SD$	47 ± 5	51 ± 4

+P

0.04

* Mean ± SD of individual slices

+ 2 tailed paired t test

TABLE 4.

T_2 CHANGES IN LIMB TISSUES OF NORMAL MALES
(n=15) BEFORE AND AFTER BED REST

T_2 RELAXATION TIMES msec ($\bar{X} \pm SD$)

TISSUE	PRE-BED REST		POST BED REST(R+0)		POST BED REST (R+1,2)
Fat	100 \pm 8		97 \pm 10		98 \pm 8
+P		NS		NS	
Marrow	129 \pm 13		117 \pm 10		117 \pm 12
+P		0.03		NS	
Muscle	*47 \pm 5		49 \pm 3		53 \pm 3
+P		NS		0.01	

* 49 \pm 5 for values paired with R + 0 data

+ 2 tailed unpaired t test