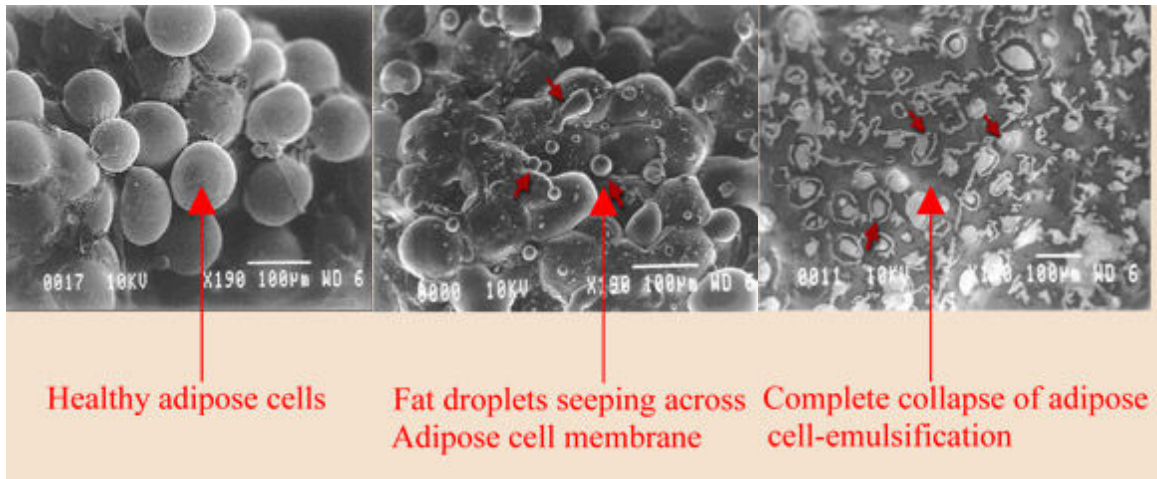


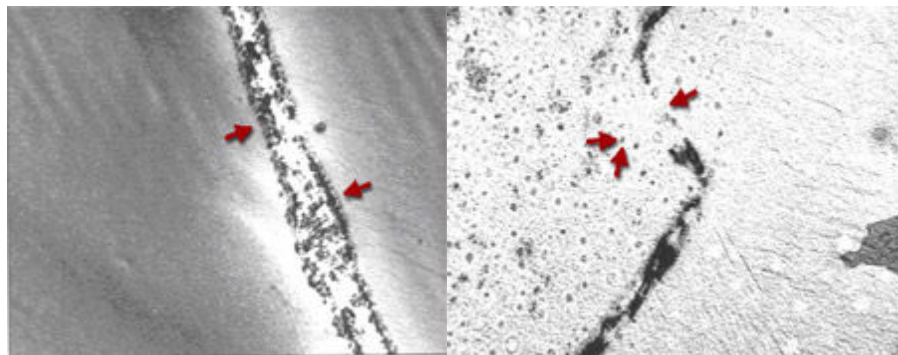
# Process Overview

## Erchonia® LipoLASER™ Biochemical Effects on Adipose Tissue



The stunning series of photographs above impressively demonstrates the low level laser's ability to emulsify adipose tissue. The pictures show the collapse of the rigid adipose cell and the secretion of triglycerides and fatty acids. These remarkable images immediately reveal why laser-assisted liposuction can serve as a great benefit for the physician and the patient.

### Why do Triglycerides and Fatty Acids Seep Across the Membrane?



These images reveal the formation of a transitory pore forming in the bi-lipid membrane of an adipose cell. This pore formation is the direct result of laser stimulation, and the reason why the triglycerides and fatty acids move into interstitial space. The pore is not damaging to the cell, but simply serves as a means for the fatty contents of the cell to evacuate. The formation of the transitory pore is the product of a series of secondary reactions originating in the mitochondria.

### How does low level laser treatment affect cells?

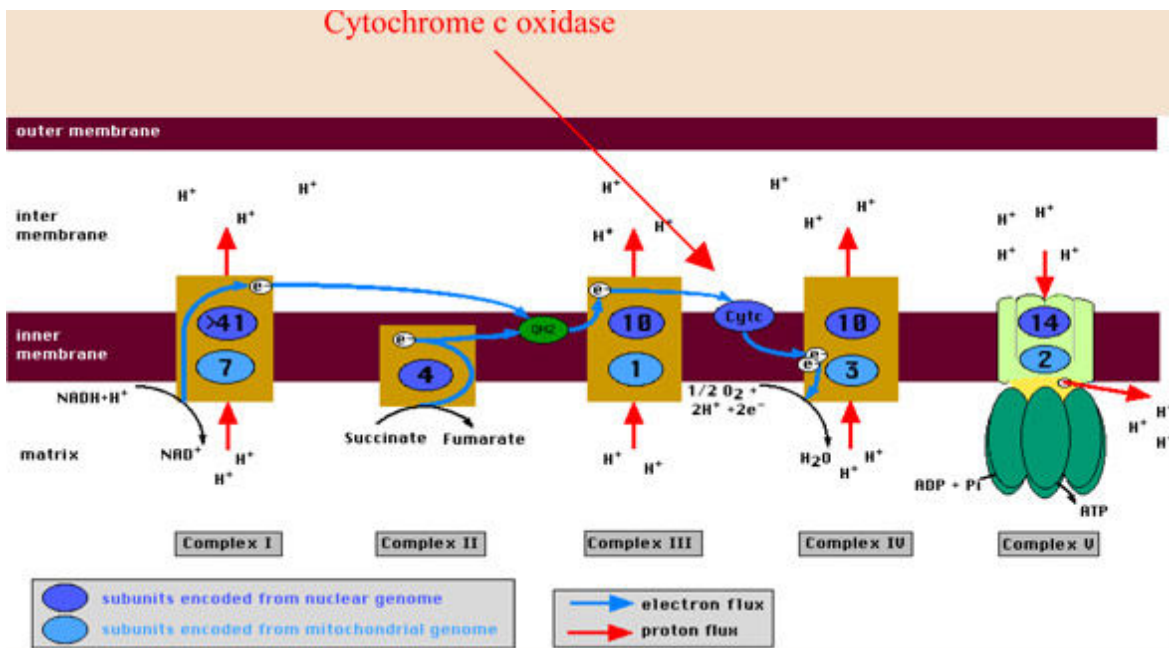
I will begin to walk you through a series of primary and secondary reactions that originate in the mitochondria of a single cell and can migrate throughout the whole body!

The mitochondrion is an energy station for all eukaryotic cells, and that energy produced in the mitochondrion is what provides life to the entire organism. The mitochondrion is where adenosine tri-phosphate (ATP) is produced, an essential molecule driving many reactions.

The mitochondrion is the specific target for Erchonia® low level laser devices. Specifically, cytochrome c oxidase, a terminal enzyme, is targeted by the low level laser. Cytochrome c oxidase is a photoacceptor, absorbing light at a peak spectrum of 630-670 nm (red spectrum). This

particular molecule is responsible for ensuring that the Respiratory Chain goes to completion. The Respiratory Chain harvests electrons from O<sub>2</sub> and NADH passing them along through a series of redox reactions, ultimately producing ATP and H<sub>2</sub>O. Cytochrome c oxidase promotes the electron flow along the Respiratory Chain between Complexes III and IV.

Low level laser is proposed, based on a study performed in 2005, to stimulate photoexcitation of certain reaction centers in the cytochrome c oxidase molecule (like CuA and CuB) influencing the redox state of these molecules, and consequently, the rate of the electron flow in the molecule; meaning, photoexcitation of cytochrome c oxidase may lead to redox changes and modulations of biochemical reactions through a cascade of reactions called photosignal transduction (stimulation of other reactions).



### The Biochemical Chain of Reactions!

Light stimulation at 632nm for mitochondrion (cytochrome c oxidase) photoexcitation.

↓

Photexcitation induces change in photoacceptor (cytochrome c oxidase).

↓

ATP levels are significantly elevated altering cellular metabolism.

↓

Redox homeostasis occurs.

↓

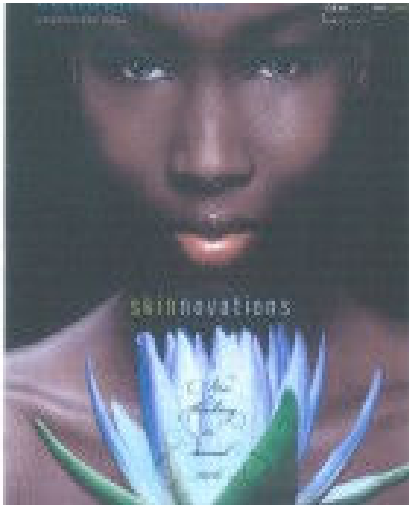
Redox transcription factors are activated and cellular signaling homeostatic cascades from the cytoplasm via the plasma membrane to the nucleus.

↓

Genes are activated - perhaps stimulating the formation of the transitory pore allowing for the evacuation of the triglycerides and fatty acids.

# Research

Dr. Gregory Roche, MD, one of the leaders in the advancement of cosmetic surgery practices, explains how the Zerona™ device and the non-invasive Zerona™ treatments are revolutionizing aesthetic protocols. Click on image to read full article



In 2006, Erchonia® Medical completed an IRB-approved, multi-site clinical trial on the body contouring protocol, which has since been trademarked as ZERONA™. The clinical trial proved ZERONA™ as a viable non-invasive process for reshaping the body through lost inches. See the amazing results of the study in this paper presented at ASLM 2008.



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## BODY CONTOURING WITH A MULTIPLE DIODE LASER SYSTEM

Ryan Maloney

San City West, AZ

**Background and Objectives:** Transmission electron microscopic images have shown the formation of a transitory pore in the adipocyte membrane followed by complete deflation of adipocytes subsequent to laser exposure. The intent of this study was to evaluate the application of a multiple 635 nm and 7.5 mw exit power per diode laser for the application of body contouring of the waist, hips, and thighs.

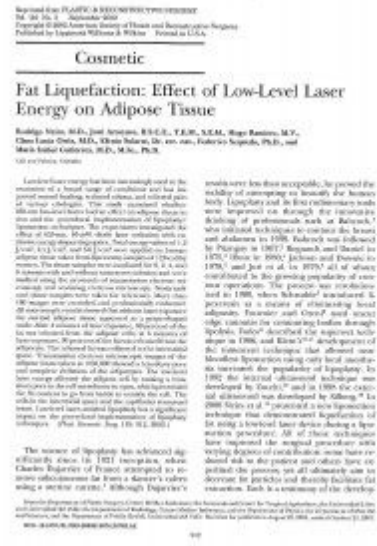
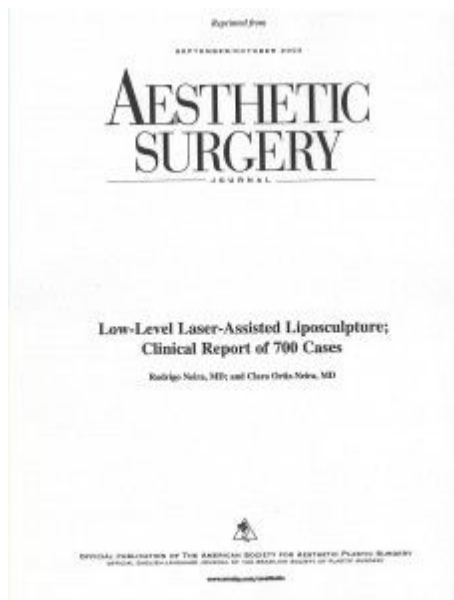
**Study Design/Materials and Methods:** The study recruited 16 test subjects for a non-randomized, non-blinded single group study. Each subject received six total treatments with a multiple 635 nm and 7.5 mw exit power per diode laser (EML, manufactured by Erchonia Medical Laser) across a consecutive two-week treatment administration phase; three treatments per week. The laser was applied to the front area of the subject's abdomen, hips, and right and left thighs for 20 minutes. The same procedure was repeated for the lower back, posterior portion of the hips and right and left thighs. Prior to each treatment, the subjects Body Mass Index (BMI) and circumference in inches of waist, hips, and each of the left and right thighs were evaluated.

**Results:** Preliminary data has indicated an average decrease in inches of -1.38 inches for the waist; -1.34 inches in hips, and -1.73 inches in the right thigh and -1.50 inches in the left thigh at the end of week two. 87.5% of subjects demonstrated a decrease in waist circumference after two weeks. A similar percentage was observed for the hips and thighs. 75% of subjects had a reduction in circumference of their hips, while 81% of subjects had a decrease in left and right thigh circumference.

**Conclusion:** These preliminary data suggest that low-level laser therapy can reduce overall circumference of specifically treated regions.

This article published in Nature magazine in June of 2008 offers a concise look at fat cells and their nature.

Rodrigo Neira, MD, has been affiliated with Erchonia® Medical since 1999 when he met Steven Shanks, the company's president, at a conference. Together Dr. Neira and Mr. Shanks have worked to advance the role of low level laser in cosmetic and plastic processes. The articles below show both the time and depth of research that ultimately led to the development of ZERONA™.



# Education: Photochemistry

Excerpt from *BIOCHEMICAL MECHANISM OF LOW LEVEL LASER THERAPY FOR THE NON-INVASIVE REDUCTION OF SUBCUTANEOUS ADIPOSE TISSUE*. >>View Full Article

The question that immediately arises is, "How can light, when externally applied, be capable of inducing such a phenomenal effect at the cellular level?" Well, the answer is best explained using the basic principles of photochemistry. Photochemistry is a discipline of chemistry that studies the interaction between atoms, molecules, and light. According to quantum theory, light radiation energy is absorbed as discrete units called photons, and at the molecular level, it is this photon-induced chemistry that ultimately gives rise to the observable effect at the biological level. The first law of photochemistry states that the observable biological effects subsequent to LLLT can only transpire in the presence of a photoacceptor molecule, a molecule capable of absorbing the photonic energy being emitted. A molecule capable of photonic absorption usually contains a light-absorbing center, referred to as a chromophore. Light absorbing centers often house transition metals, elements that are readily identified by their incomplete subshell. Based on physicist Niels-Bohr's model, subshells of an atom identify the possible quantum states in which an individual electron can reside, depending on its energy level. Electrons are capable of undergoing quantum leaps, where an electron transitions between quantum states, shifting from one energy level to another following the absorption or emission of a photon. The shift from a lower energy state to a higher state is referred to as the excitation of an electron, the change from an occupied orbital to a given unoccupied orbital. Regarding transition elements, such as copper (Cu) or iron (Fe), these elements are more susceptible to an electron shift because of their unique electron configuration. The photoacceptor molecules responsible for the photobiological effects subsequent to laser irradiation contain transition metals. The photon absorption is followed by a rapid vibrational relaxation which causes the molecule to reach an equilibrium geometric configuration corresponding to its electronic excited state. This change may modulate the biological behavior of photoabsorbing molecules.

Studies have revealed that cytochrome c oxidase serves as a photoacceptor molecule. Cytochrome c oxidase is a multicomponent membrane protein that contains a binuclear copper center (CuA) along with a heme binuclear center (a3-CuB), both of which facilitate the transfer of electrons from water soluble cytochrome c oxidase to oxygen. Cytochrome c oxidase is a terminal enzyme of the electron transport chain and plays a vital role in the bioenergetics of a cell. Studies indicate that following laser irradiation at 633nm, the mitochondrial membrane potential and proton gradient increases, causing changes in mitochondria optical properties, increasing the rate of ADP/ATP exchange. It is suggested that laser irradiation increases the rate at which cytochrome c oxidase transfers electrons from cytochrome c to dioxygen. Moreover, it has been proposed that laser irradiation reduces (gain of electrons) the catalytic center of cytochrome c oxidase, making more electrons available for the reduction of dioxygen. The photoactivation of terminal enzymes, like cytochrome c oxidase, plays a vital role in the activation of the diverse

biological cascade observed subsequent to laser irradiation.

The peak absorption of cytochrome c oxidase is found in the red to near-infrared spectrum. Therefore, optimal biological stimulation can be achieved utilizing a device that emits light within the red spectrum. Furthermore, to ensure proper depth penetration and deep tissue stimulation, the use of a coherent laser source is absolutely vital. Biologically speaking, the difference between a light emitting diode (LED) and laser diode are negligible at extremely superficial surfaces. However, when attempting to target deep tissue, such as subcutaneous adipocytes, it is essential that a coherent laser source is administered.

The initial physical and/or chemical changes of cytochrome c oxidase have been shown to alter the intracellular redox state. It has been proposed that the redox state of a cell regulates cellular signaling pathways that control gene expression. Modulation of the cellular redox state can activate or inhibit signaling pathways such as redox-sensitive transcription factors and/or phospholipase A2. Two well defined transcription factors, nuclear factor Kappa B (NF- $\kappa$ B) and activator protein-1 (AP-1), are regulated by the intracellular redox state; moreover, NF- $\kappa$ B and AP-1 become activated following an intracellular redox shift to a more alkalized state.<sup>32,33</sup> Subsequent to laser irradiation, a gradual shift towards a more oxidized (alkalized) state has been observed; more importantly, the activation of redox-sensitive transcription factors and subsequent gene expression has been demonstrated.

Based on its ability to modulate cellular metabolism and alter the transcription factors responsible for gene expression, low level laser therapy (LLLT) has been found to alter gene expression<sup>36</sup>, cellular proliferation, intra-cellular pH balance, mitochondrial membrane potential, generation of transient reactive oxygen species and calcium ion level, proton gradient, and consumption of oxygen. Moreover, the proliferation of keratinocytes and fibroblasts has been reported in the literature for extremely low doses of laser irradiation.

The modulation of transcription factors has become a common therapeutic strategy to prevent or provoke the expression of specific genes, and the approach could potentially provide a means to treat a wide assortment of medical disorders. Jackson and coworkers (2002) identified more than 20 transcription factors that are regulated by the intracellular redox state. It is proposed that laser therapy, because it has been identified to alter the intracellular redox state, could affect the function of transcription factors associated with the formation and maintenance of adipocyte membranes. To support this claim, further studies are highly warranted. However, there is enough evidence to support that laser irradiation within the red spectrum does play a unique role in the expression of specific genes, and is plausible that the transitory pore observed following LLLT could result from the alteration in gene expression.

3.) Niera, R., Arroyave, Ramirez, H., et al. Fat liquefaction: Effect of low-level laser energy on adipose tissue. *Plast. Reconstr. Surg.* (2002): 110; 912-22.

32.) Sun, Y. and Oberley L.W. Redox regulation of transcriptional activators. *Free Radic. Biol. Med.* (1996): 21; 335-348.

33.) Haddad, J.J. Oxygen-sensing mechanisms and the regulation of redox responsive transcription factors in development and pathophysiology. *Respir. Res.* (2002): 3;26-53.

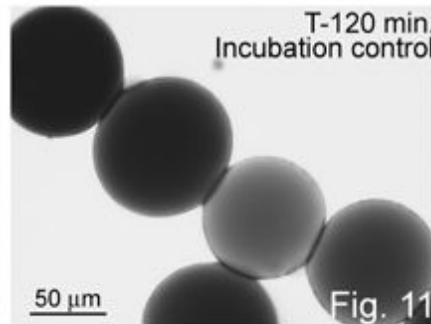
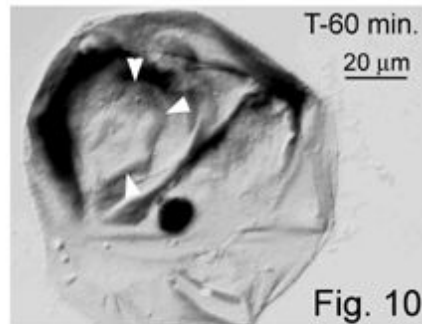
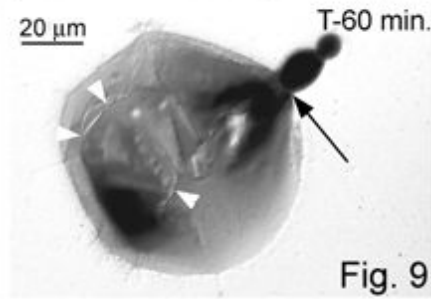
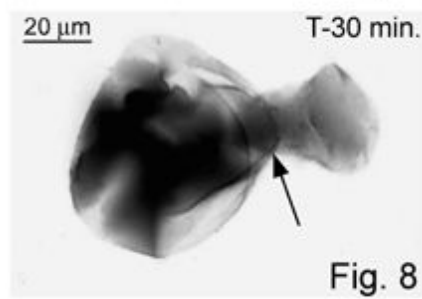
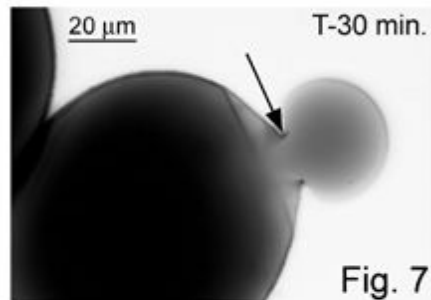
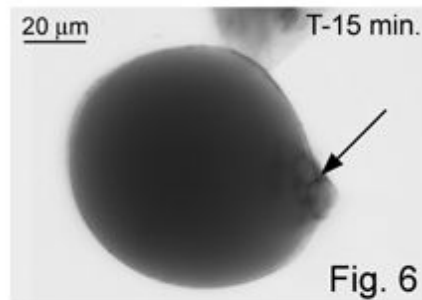
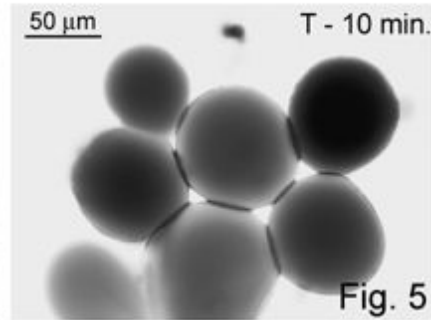
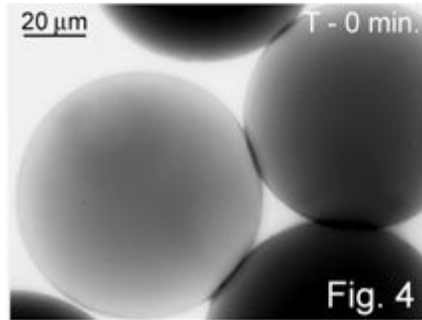
36.) Byrnes KR, Wu X, Waynant RW, Ilev IK, Anders JJ. Low power laser irradiation alters gene expression of olfactory ensheathing cells in vitro. *Lasers Surg. Med.* 2005;37:161-171

## Technology

The following is another example of scientific proof of the Erchonia lasers effect on adipocytes. All of the SEM, TEM, MRI and all other research

of this website was done using an Erchonia laser.

The research represented below was done in the US using an Erchonia laser and shows time released photography of one fat cell and its contents being emptied. Please note the cell is still alive. Erchonia only uses its own research to prove its patent pending applications.



## Clinical Data

# EML Laser Body Contouring Pilot Study Results

October, 2006

Presented at the ASLMS 2008

**Table 1:** Average inches by measurement time point: n=16

<i>Average inches</i>	Pre-Treatment	End of Week 2
<b>Waist</b>	44.14	42.86
<b>Hips</b>	51.28	49.94
<b>Right Thigh</b>	27.46	25.75
<b>Left Thigh</b>	26.93	25.43

**Table 2:** Change in circumference measurements from pre-treatment to end of week 2: n=16

	<b>Waist</b>	<b>Hips</b>	<b>Right Thigh</b>	<b>Left Thigh</b>
<b>inches</b>	44.14 to 42.86 in.	51.28 to 49.94 in.	27.48 to 25.75 in.	26.93 to 25.43 in.
<b>Change in ins.</b>	- 1.28 in	- 1.34 in	- 1.73 in	- 1.50 in
<b>% change</b>	-2.85%	-2.40%	-4.82%	-3.88%

**Table 3:** Pre-treatment body area measurements in inches: n=16

<b>Subject</b>	<b>Waist</b>	<b>Hip</b>	<b>Right Thigh</b>	<b>Left Thigh</b>
1	40	49	31	29

2	33.5	38.5	24	23
3	35	39.5	22	20.25
4	98	106	57	57
5	79	97	57	56
6	46.5	54.3	26	24.6
7	33.3	39	21.5	20.1
8	37.6	46.5	23.2	24
9	31.3	37	19.7	19.7
10	33.5	41.7	23.6	23.6
11	38	43.3	23.2	23.2
12	32.7	39	19.3	19.3
13	35.8	42.5	21.3	21.3
14	34.1	40.6	19.7	19.3
15	50.4	54.1	26.8	26.2
16	47.6	52.4	24	24.4

**Table 4:** End of week 2 body area measurements in inches: n=16

<b>Subject</b>	<b>Waist</b>	<b>Hip</b>	<b>Right Thigh</b>	<b>Left Thigh</b>
1	38.5	47.5	29	28
2	32.5	39.5	22	22
3	35.5	40	22.5	21.5
4	93	103	53	53
5	76	93	44.5	44
6	45.1	52.8	25.8	24.8
7	32.3	36.6	21.7	21.3
8	35.4	40.9	23.6	23.2
9	29.9	36.2	19.3	19.3
10	31.9	41.7	21.3	21.3
11	37.8	43.5	22.8	23.2
12	31.1	38.8	18.5	18.1
13	35.2	41.1	20.5	20.7
14	31.7	39.4	18.5	18.1
15	49.2	53	25.2	24.2
16	50.6	52	23.8	24.2

**Table 5:** Individual subject **waist** circumference: Pre-treatment to end of week 2: n=16

<b>Subject</b>	<b>Pre-treatment</b>	<b>End of week 2</b>	<b>Change in ins.</b>	<b>% Change</b>
1	40	38.5	-1.5	-3.90%
2	33.5	32.5	-1	-3.08%
3	35	35.5	0.5	1.41%
4	98	93	-5	-5.38%
5	79	76	-3	-3.95%
6	46.5	45.1	-1.4	-3.10%
7	33.3	32.3	-1	-3.10%
8	37.6	35.4	-2.2	-6.21%
9	31.3	29.9	-1.4	-4.68%
10	33.5	31.9	-1.6	-5.02%
11	38	37.8	-0.2	-0.53%
12	32.7	31.1	-1.6	-5.14%
13	35.8	35.2	-0.6	-1.70%
14	34.1	31.7	-2.4	-7.57%
15	50.4	49.2	-1.2	-2.44%

16	47.6	50.6	3	5.93%
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**Table 6:** Individual subject hip circumference: Pre-treatment to end of week 2: n=16

Subject	Pre-treatment	End of week 2	Change in ins.	% Change
1	49	47.5	-1.5	-3.16%
2	38.5	39.5	1	2.53%
3	39.5	40	0.5	1.25%
4	106	103	-3	-2.91%
5	97	93	-4	-4.30%
6	54.3	52.8	-1.5	-2.84%
7	39	36.6	-2.4	-6.56%
8	46.5	40.9	-5.6	-13.69%
9	37	36.2	-0.8	-2.21%
10	41.7	41.7	0	0.00%
11	43.3	43.5	0.2	0.46%
12	39	38.8	-0.2	-0.52%
13	42.5	41.1	-1.4	-3.41%

14	40.6	39.4	-1.2	-3.05%
15	54.1	53	-1.1	-2.08%
16	52.4	52	-0.4	-0.77%

**Table 7:** Individual subject **right thigh** circumference: Pre-treatment to end of week 2: n=16

<b>Subject</b>	<b>Pre-treatment</b>	<b>End of week 2</b>	<b>Change in ins.</b>	<b>% Change</b>
1	31	29	-2	-6.90%
2	24	22	-2	-9.09%
3	22	22.5	0.5	2.22%
4	57	53	-4	-7.55%
5	57	44.5	-12.5	-28.09%
6	26	25.8	-0.2	-0.78%
7	21.5	21.7	0.2	0.92%
8	23.2	23.6	0.4	1.69%
9	19.7	19.3	-0.4	-2.07%
10	23.6	21.3	-2.3	-10.80%
11	23.2	22.8	-0.4	-1.75%

12	19.3	18.5	-0.8	-4.32%
13	21.3	20.5	-0.8	-3.90%
14	19.7	18.5	-1.2	-6.49%
15	26.8	25.2	-1.6	-6.35%
16	24	23.8	-0.2	-0.84%

**Table 8:** Individual subject **left thigh** circumference: Pre-treatment to end of week 2: n=16

<b>Subject</b>	<b>Pre-treatment</b>	<b>End of week 2</b>	<b>Change in ins.</b>	<b>% Change</b>
1	29	28	-1	-3.57%
2	23	22	-1	-4.55%
3	20.25	21.5	1.25	5.81%
4	57	53	-4	-7.55%
5	56	44	-12	-27.27%
6	24.6	24.8	0.2	0.81%
7	20.1	21.3	1.2	5.63%
8	24	23.2	-0.8	-3.45%
9	19.7	19.3	-0.4	-2.07%

10	23.6	21.3	-2.3	-10.80%
11	23.2	23.2	0	0.00%
12	19.3	18.1	-1.2	-6.63%
13	21.3	20.7	-0.6	-2.90%
14	19.3	18.1	-1.2	-6.63%
15	26.2	24.2	-2	-8.26%
16	24.4	24.2	-0.2	-0.83%