

# *In Vivo* Visceral Fat Removal in Rats using 1205nm Diodes

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**Abstract:** Selective laser assisted visceral fat removal in rats prevents epididymal fat regrowth and compensatory fat increase, but does not affect glucose tolerance or insulin sensitivity.

**OCIS codes:** (170.1020) Ablation of tissue; (140.3510) Lasers, Fiber

Using 1205nm fiber coupled laser diodes tuned to an adipose absorption peak, we surgically remove visceral fat through a midline incision, and we monitor these animals for 12 weeks before sacrificing and measuring fat mass. The experiment involves 20 male rats, 10 having fat lased and removed from various fat pads and 10 undergoing a sham procedure that includes surgical laparotomy without laser treatment. While previous results indicate that surgically removed fat rapidly regrows [1], we find that removed epididymal fat (visceral fat connected to the testes) does not return even after 12 weeks, suggesting that laser ablation prevents regrowth of fat removed using this technique. Despite visceral fat removal, we do not find a change in metabolic parameters as measured through glucose tolerance tests and fasting blood insulin levels. Therefore, the laser removal of visceral fat does not exhibit the compensatory fat growth seen in other methods, nor an improvement in glucose tolerance or insulin sensitivity.

As obesity and its related co-morbidities are a growing concern worldwide, there is a burgeoning interest in removing fat and understanding how this may improve metabolism. We focus on visceral fat removal because studies have shown that its removal improves insulin sensitivity in rats [2]. Also, studies have found that fat grows quickly when surgically excised [1]. Contrary to these studies, we find that laser removal of fat seems to inhibit regrowth of epididymal fat, but does not lead to a statistically significant improvement in glucose tolerance or insulin sensitivity.

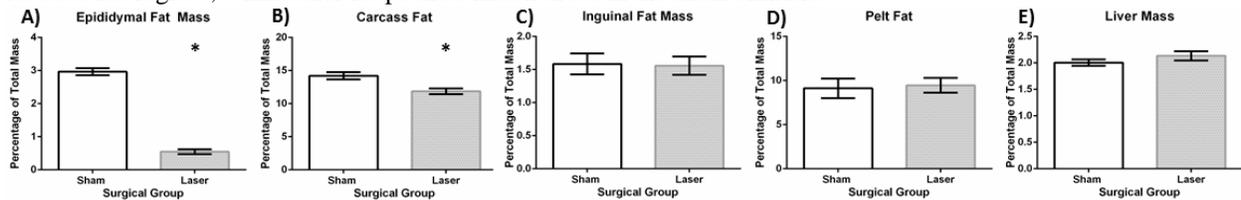
The laser experiments are conducted using a 1205nm fiber coupled laser diode (BrightLase 6012, QPC Lasers). We constructed a probe comprising of a lens tube with collimating and focusing optics to focus ~2.1W CW light into an 800 $\mu$ m spot 4cm away from the end of the probe. This corresponds to an intensity of 417W/cm<sup>2</sup> at the focus. 1205nm is selected because at this wavelength the adipose absorption exceeds water absorption, and the intensity is tuned to maximize the speed and safety of laser treatment. Previously, we demonstrated that by using another such wavelength (1708nm), we can damage a large volume of fat with only minimal damage to neighboring organs [3]. The 1205nm light from the laser diodes is used to selectively damage and remove visceral fat without damaging surrounding organs, such as the testes, GI tract, or important blood vessels.

Twenty male, Long-Evans rats are used in the animal studies because diet induced obese Long-Evans rats exhibit metabolic characteristics similar to those of obese humans. These rats are kept on a 40% high fat diet for ~12 weeks prior to experiments. One week prior to surgery they are placed into an NMR machine (EchoMRI) to confirm that they have at least 100g total fat mass and then separated into sham and laser fat removal groups. Sham animals undergo anesthesia (isoflurane) followed by the manipulation of fat pads through an abdominal incision, with no fat removal. For experimental animals, 1205nm light is focused onto the epididymal, perinephric, and retroperitoneal fat pads to weaken and melt these fat depots. Forceps are then used to remove this damaged adipose tissue. Furthermore, the light is focused onto the fat lining the mesentery until discoloration occurs (<1 second exposure time). Total surgical procedure time is ~2.5 hours per animal. Post-operative pain medication injections are issued for 3 days post procedure with daily monitoring for 7 days.

All animals are kept on high fat diet and monitored for 12 weeks post-surgery with periodic NMR scans to determine lean and fat masses. An intraperitoneal glucose tolerance test is performed one month after surgery. The animals are fasted overnight (16hrs) then injected with 1.5g glucose/kg lean mass. Glucose readings are taken at 0, 15, 30, 45, 60, and 120 min time points via the rat's tail vein. Blood samples are also collected to ascertain fasting insulin and leptin (protein secreted by fat cells) levels via ELISA assays. 12 weeks after surgery all animals are sacrificed and the liver, inguinal fat pads, and epididymal fat pads are removed and weighed. Furthermore, each animal is skinned, and the animal pelt and carcass are NMR'd separately to ascertain visceral and subcutaneous fat mass. All data is expressed as the mean value between animals in each group,  $\pm$  the standard error of the mean.

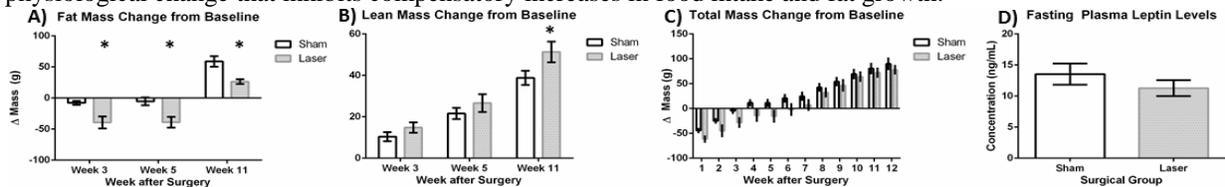
Twelve weeks post-surgery animals are sacrificed and fat mass data is obtained (Fig. 1). The epididymal fat mass is significantly lower in the laser treated group versus the sham group (Fig. 1A). This 2.4% difference in mass matches the 2.4% decrease seen in the skinned carcass fat mass (Fig 1B). Furthermore, there is no discernible difference in the

inguinal fat mass, subcutaneous fat found in the pelt, or in the liver mass (Fig 1C-1E). We conclude that the epididymal fat does not regrow, while other fat pockets match those in the sham animals.



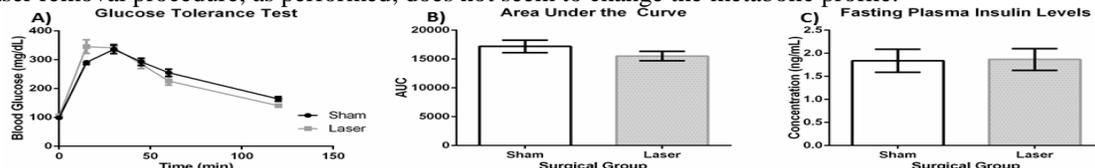
**Figure 1: Fat and liver mass percentages 12 weeks after surgery. \* Indicates significance  $P < 0.05$ . Data depicted as mean  $\pm$  SEM**

Although the rats begin with comparable masses and body compositions, after surgery there is a difference in fat and lean mass between surgical groups. Total fat mass is reduced by 39g in the laser treated group (n=6) and remains consistently 32g below sham animals (n=9) (Fig. 2A), while lean mass (Fig. 2B) increases in the laser treated group, normalizing total mass (Fig. 2C) between the groups. Additionally, both surgical groups consume a weekly average of 133g high fat diet corresponding to the comparable fasting blood leptin levels between groups (Fig. 2D). This suggests that the suppression of energy expenditure associated with surgical fat removal does not occur when fat is ablated with a laser rather than by using a traditional scalpel [4]. Therefore, laser surgery seems to facilitate a physiological change that inhibits compensatory increases in food intake and fat growth.



**Figure 2: Fat mass remains 32 grams lower in laser treated animals. Leptin levels not significantly different.**

To test whether visceral fat removal results in metabolic changes, we run glucose tolerance tests (Fig. 3A, 3B) and measure fasting insulin levels (Fig. 3C). However, we do not find any statistically meaningful changes in either. Our laser removal procedure, as performed, does not seem to change the metabolic profile.



**Figure 3: Neither the glucose tolerance test nor the area under its curve were significantly different. Fasting Insulin levels also matched.**

Contrary to expectations based on the literature, our experiments do not show compensatory fat regrowth or metabolic improvements when we use the 1205nm laser for visceral fat removal. Typically, in excision based removal, untouched fat pads compensate for the decrease in fat mass by increasing in size within a month after surgery [5]. Several studies have shown that the removal of visceral fat in rats and mice can reverse the onset of diabetes for a short period of time after surgery, but an increase in lipogenesis eventually diminishes this effect [1]. Because Fig. 1A shows that only the epididymal fat pad was different between groups and Fig. 2A shows a consistent difference in fat mass, we can conclude that normal fat mass compensation did not occur. This suggests that laser assisted lipectomy is fundamentally different from typical surgical resection. We hypothesize that by thermally damaging the fat cells, we inhibit the adipose tissue's ability to rapidly increase in size.

In summary, by using a laser wavelength where adipose absorption exceeds water absorption, we remove visceral fat in rats and find that the epididymal fat pads do not regrow. The fat removed with the laser stays off, even after 12 weeks of growth on high fat diet. We find that laser surgery seems to facilitate a physiological change that inhibits compensatory increases in food intake and fat growth. However, we do not find the expected change in metabolism, as evidenced by the sham and laser group rats exhibiting similar results in the glucose tolerance test and insulin baseline levels.

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