

Insulin Effect on Morphological Fat Changes and Survival Rate Fat Graft in New Zealand White Rabbit

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ABSTRACT

Fat often used in soft tissue reconstruction procedures that require adding volume and contour to the defect. According to the International Society of Aesthetic Plastic Surgery, more than 1,000,000 procedures were performed in 2016. However, data shows that the fat graft survival rate is 20% – 90% depending on the method of taking the donor, the process, transplantation, and the condition of the recipient. Insulin has been shown to stimulate adipocyte cell proliferation, differentiation and trophic activity in vitro. This study aims to prove morphologically the effect of insulin administration on increasing fat weight and volume on the success of a significant fat graft survival rate. This research used experimental method using a randomized post-test only control group design. The experimental animals in this study are male New Zealand rabbits (*Oryctolagus cuniculus*). Separation is carried out after fat grafting process at the end each 2nd, 4th and 8th week from the animal population. The final weight and volume were measured for macroscopic evaluation, and for each sample was observed microscopically pre and post treatment using a microscope Nikon Eclipse Ci-E with digital camera DS Fi2 300 megapixel. Comparison of the means each group was tested using the analysis of Mann Whitney test, Wilcoxon test and sample T-test. Comparison of fat volumes in the control and treatment groups, at the 2nd week, 4th week and 8th week, a comparison test was carried out using the Paired T-test with $p < 0.05$ (0.014). Fat grafting volume in the control group 8 week, there was a significant difference between weight of the pre fat graft group compared to the post fat graft control group with a p -value of $p < 0.05$ (0.043). A comparison test using Wilcoxon test in the pre fat graft control group compared to the post fat graft control group at 4 weeks showed a significant difference with a p -value of $p < 0.05$ (0.043). Insulin administration has been observed to have an impact on the macroscopic alterations in the morphology of fat grafts, depending on whether weight or volume is maintained. These alterations contribute to the overall survival rate of the grafts. Additionally, the administration of insulin has been found to induce microscopic changes in the morphology of fat grafts, specifically in the form of fat cell hypertrophy. This further enhances the survival rate of the grafts.

Keywords: Fat Graft, Insulin, Survival Rate, Volume Maintenance, Weight Maintenance

INTRODUCTION

Fat cells are a form of fibroblasts that do not differ from mesenchymal cells, and under certain conditions, histologists believe that adipose cells are formed from undifferentiated embryonic stem cells and formed into preadipocytes, under the influence of periodic factor activation. Fat cells have the function of storing and synthesizing triglycerides, which produce adipokine hormones [1]. Fat is frequently utilized in reconstructive procedures for soft tissues that require additional volume and contour to address defects [2]. Research spanning several decades reveals that fat grafts are commonly employed in plastic surgery. According to the International Society of Aesthetic Plastic Surgery, over 1,000,000 procedures were performed in 2016, with breast augmentation, buttocks, and facial rejuvenation surgeries being the most prevalent [3]. This technique was initially introduced by Gustav Adolf Neuber in 1983, involving the injection of small fat globules into soft tissue areas to correct facial contour defects [4].

Soft, easily available fat structures can be harvested with minimal morbidity using direct excision and cannula aspiration techniques. Fat graft success is unpredictable and often unsuccessful [2]. However, data shows that the survival rate of fat grafts is 20% - 90% depending on the donor collection method, process, transplantation, and condition of the recipient. The theory of fat graft survival rate was first proposed by Peer [5] known as "Cell survival theory". Transplanted fat cells are able to survive because they get vascularization from the

recipient, some fat cells that do not get vascularized will degenerate [5].

Fat grafts are very sensitive to becoming ischemic, resulting in a delay in blood supply which adversely affects the viability of fat adipocytes. The lack of vascularization and blood flow in the recipient bed requires an immediate revascularization process in the transplanted tissue [6]. Insulin has been shown to stimulate adipocyte cell proliferation, differentiation, and trophic activity in vitro. One study showed that in vivo it had no effect on fat graft success [2]. Insulin has the effect of increasing the glucose pathway in fat and muscle cells by stimulating the translocation of Glucose transporter type-4 from intracellular to plasma membrane, as well as the effect of increasing triglyceride synthesis and storage of adipose pauci cells that transform into typical adipocyte cells [7].

Morphologically in adipose tissue there is a process of increasing cell size (hypertrophy) and increasing the number of cells (hyperplasia). Cell hypertrophy will occur if the plasticity process of adipocyte cells fails to form, resulting in additional volume or mass in the muscle [8]. Siswanto [9] suggested that significant results were obtained in the survival rate of fat grafts in rabbit groups conducted at the end of the second, fourth, and eighth weeks in insulin-administered fat, through assessment of fat weight and volume [9]. This is still not supported by accurate evidence based on morphology

and related examinations of fat weight and volume gain in the success of fat graft survival rates.

Research Objective

The objective of this study is to investigate and analyze the effects of administering insulin on the macroscopic and microscopic changes in the morphology of adipose tissue. By examining both the macroscopic and microscopic alterations in the morphology of adipose tissue, this study aims to provide a comprehensive understanding of the impact of insulin administration on fat grafts. The findings from this research can potentially inform clinical practices and improve the success rates of fat graft procedures, ultimately benefiting patients seeking aesthetic or reconstructive surgeries involving adipose tissue transplantation.

Methods

This type of research is experimental research using Randomized post- test only control group design. The experimental animals in this study are New Zealand male rabbits (*Oryctolagus cuniculus*) which will be divided into control groups and treatment groups. In each group using 5 (five) minimum samples for each group. In one rabbit has two ears (right and left) both can be used as control and treatment (30 subjects) at the same time, so it takes 15 New Zealand male rabbits (*Oryctolagus Cuniculus*), with three treatments at the end of week 2, 4 and 8. To reduce bias, the researchers determined the inclusion criteria, the experimental animals in this study were male rabbits with a weight range of 2000-3500 grams, with an age of 12 weeks, free from ear and mouth infections, kept in the same cage in a shaded room with room temperature and humidity maintained, kept clean, given animal feed in the form of pellets of the same type at the rate of 5 grams of food / 100 grams of body weight of experimental animals per day, drinks given clean water free from contamination ad libitum using a special bottle. Exclusion criteria in this study were rabbits with body weight < 2000 grams, and rabbits with high fat diet. Before harvesting and fat tissue transplantation, all experimental animals were anesthetized using ketamine injection (dose of 20mg/kg body weight) and xylazine injection (dose of 3mg/kg body weight) intramuscularly then weighed and recorded in grams. Experimental animals were positioned in the supine position. To prepare for harvesting (donor collection) and fat tissue transplantation, the skin of the right/left inguinal area and right/left ear dorsal side was shaved using an electric shaver. Disinfection of the operating area was performed using 10% Povidone iodine solution and the operating field was narrowed using disposable sterile doek, asepsis was maintained throughout the procedure. Incision design was performed on the right and left inguinal (donor) areas with a 3 cm long incision following the fold area on the rabbit inguinal. A skin-deep incision was made, and the inguinal area was dissected for superficial fat to be taken from the right inguinal as a donor fat graft and transplanted to the recipient on the dorsal side of the right ear as a control group. In the same process in one rabbit, the left inguinal fat donor was harvested as a fat graft donor and transplanted to the dorsal side recipient on the left ear as the control group. In the control group, fat donors were treated with washing using 0.9% NaCl infusion fluid until clean from debris and blood, fat samples were taken before fat grafting to be fixed using 10% neutral buffer formalin (NBF 10%), which was then continued for histopathological examination using Hematoxylin eosine painting and examined using a light microscope with 100x and 400x

magnification. Donor fat was measured by weight (grams) using a scale (ACIS BC-500) and volume (ml) using a 5 cc Terumo® syringe, after which it was recorded before the fat graft action was performed. Then after that, fat grafting was performed on the dorsal side of the right ear as a control group. In the treatment group, fat donors that had been taken from the inguinal were treated with washing using 0.9% NaCl infusion liquid until clean from debris and blood, then immersed in 1,000 IU short acting insulin (*Apidra Solostar®*) for 15 minutes. Fat samples were taken before fat grafting for fixation using 10% neutral buffer formalin (NBF 10%), which was then continued for histopathological examination using Hematoxylin eosine painting and examined using a light microscope with 100x and 400x magnification. Fat donors were then measured in weight (grams) using a scale (ACIS BC-500) and volume (ml) using a 5 cc Terumo® syringe, after which they were recorded before fat grafting on the dorsal side of the left ear as a treatment group. Each control and treatment group will be evaluated at the end of week 2, 4 and 8. Macroscopic evaluation was carried out by measuring weight (grams) using a scale (ACIS BC-500) and volume (ml) using a 5 cc Terumo® syringe, after which records were taken. Microscopic evaluation was carried out by taking post fat grafting fat samples for fixation using 10% neutral buffer formalin (NBF 10%) liquid, which was then continued for histopathological examination using Hematoxylin eosine painting and examined using a light microscope with 100x and 400x magnification with a calibrated Nikon Eclipse Ci-E brand, and equipped with a 300 mega pixel DS Fi2 digital camera and Nikon Image System image processing software. This examination aims to measure the area of fat cells (adipocyte cells) in fat tissue. Fat cell area data is the mean value of fat cell area (μm^2) evaluated in five different fields of view in each sample. The reason for evaluation up to the end of 8 weeks post fat grafting is because fat graft survival ends at 3 months and is followed by a chronic stabilization process that can occur up to 9 months. If the graft volume is small, fat absorption and adipocyte cell regeneration end at 3 months post-action, and there will be volume changes after 3 months [10]. All data were analyzed using SPSS 26 software and statistically significant values were set at $p < 0.05$. Data are presented as mean $\pm$ SD. Comparison of the means of each group was tested using Mann whitney test, Wilcoxon test and paired and unpaired samples t-test.

Findings and Discussion

Findings

Research Data

This study is an experimental study using a randomized post-test only control group design. This study was conducted from August to October 2023, with a total of 15 research samples, which were evaluated at the end of week 2, week 4, and week 8 after fat grafting with each group consisting of 5 experimental animals. This study has received a certificate of ethical eligibility from the Animal Care and Use Committee (ACUC) Airlangga University.

Fat Weight

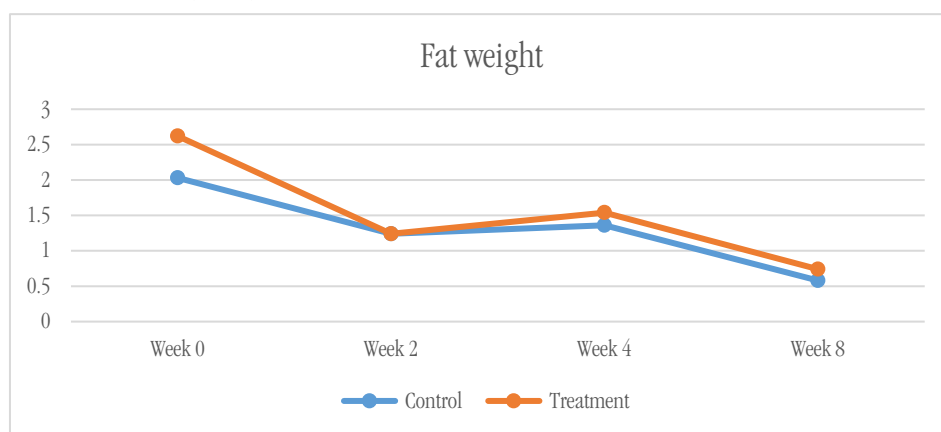
Thirty fat samples were taken from the dorsal ear of rabbits (fat graft recipients), with 15 fat samples taken from the dorsal right ear of rabbits (control) and 15 fat samples taken from the dorsal left ear of rabbits (treatment). The fat weight varied from 0.6-6.8 grams in the control group and 0.6-11.1 grams in the treatment group.

Table 1: Fat weight of control and treatment groups, before and after fat graft treatment.

Identity of the Animal	Fat Weight of Control Group (Gram) Pre-Fat Grafting	Fat Weight of Control Group (Gram) Post Fat Grafting			Fat Weight of Treatment Group (Gram) Pre-Fat Grafting	Fat Weight of Treatment Group (Gram) Post Fat Grafting		
		Week 2	Week 4	Week 8		Week 2	Week 4	Week 8
A	0,9	0,8			1,6	1,0		
B	0,9	0,9			1,5	0,7		
C	0,9	0,9			1,6	0,7		
D	1,7	2,4			2,4	2,5		
E	1,1	1,2			1,0	1,3		
F	2,5		2,8		3,0		3,1	
G	6,8		0,6		11,1		0,6	
H	2,5		2,8		3,0		3,1	
I	2,9		0,3		3,0		0,5	
J	2,9		0,3		3,0		0,4	
K	2,7			1,0	2,3			1,4
L	0,6			0,4	0,6			0,5
M	1,4			0,5	1,8			0,6
N	1,5			0,4	2,1			0,6
O	1,2			0,6	1,4			0,6
Average	2,03	1,24	1,36	0,58	2,62	1,24	1,54	0,74

Rabbits were randomly assigned to be evaluated at the end of week 2, end of week 4 and end of week 8 in the control and treatment groups. The average weight of fat in the control group before the fat graft action was gram. The average fat weight of rabbits in the post fat graft control group at week 2 was 1.24 grams, week 4 was 1.36 grams, week 8 was 0.58 grams (Table 1). The average weight of rabbit fat in the

treatment group before the fat graft action was gram. The average fat weight in the post fat graft treatment group at week 2 was 1.24 grams, week 4 was 1.54 grams, week 8 was 0.74 grams (Table 1). Shown in Figure 1 shows a graph of fat weight comparing the control and treatment groups which both decreased compared to week 0 (before action).

**Figure 1:** Fat Weight at the beginning and after fat grafting at the end of week 2, week 4, and week 8.

Fat Volume

The range of fat volume in the control group before fat graft treatment varies between 0.5-7 ml with an average volume of 2.04 ml. The range of fat volume in the treatment group before fat graft treatment varied between 0.6-11 ml with an average volume of 2.54 ml.

Similar to the weight of fat, the variation in volume is proportional to the size of body weight and also the anatomical structure of inguinal fat in rabbits that vary in this study. The diagram in Figure 2 shows that each control and treatment group experienced a decrease in volume at week 0 to week 2, then increased at week 4 and decreased again at week 8.

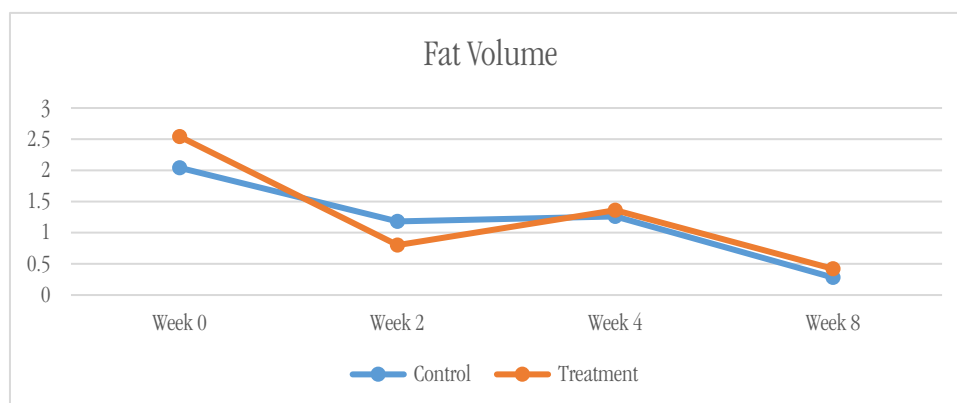
**Figure 2:** Fat Volume at baseline and after fat grafting at the end of week 2, week 4, and week 8.

Table 2: Fat volume of control and treatment groups, before and after fat graft treatment.

Identity of the Animal	Fat Volume Of Control Group (ml) Pre-Fat Grafting	Control Group Fat Volume (ml) Post Fat Grafting			Fat Volume of Treatment Group (ml) Pre-Fat Grafting			Fat Volume of Treatment Group (ml) Post Fat Grafting		
		Week 2	Week 4	Week 8				Week 2	Week 4	Week 8
A	0,9	1			1,1			0,4		
B	0,9	0,9			1,0			0,3		
C	0,9	0,9			1,1			0,3		
D	1,9	2,1			2,3			2		
E	0,8	1			1,1			1		
F	2,5		2,8		3,0				2,9	
G	7		0,5		11				0,5	
H	2,5		2,8		3,0				2,9	
I	2,9		0,1		3,0				0,3	
J	2,9		0,1		3,0				0,2	
K	2,5			0,4	2,3					0,8
L	0,5			0,2	0,6					0,3
M	1,5			0,3	1,8					0,4
N	1,5			0,2	2,1					0,3
O	1,4			0,3	1,7					0,3
Average	2,04	1,18	1,26	0,28	2,54			0,8	1,36	0,42

Post-treatment fat volume was recorded and measured at the end of week 2, week 4 and week 8, each of which assessed the average fat volume after fat grafting. In the control group at week 2, the average fat volume was 1.18 ml, the control group after the fat graft at week 4 had an average fat volume of 1.26 ml and the control group at week 8 had an average fat volume of 0.28 ml.

There was an increase in volume at week 2 to week 4, and a decrease in volume at week 8. In the post-fat graft treatment group, week 2 obtained an average fat volume of 0.8 ml, week 4 treatment group obtained an average fat volume of 1.36 ml and week 8 treatment group obtained an average fat volume of 0.42 ml. There was an increase in volume at week 2 to week 4, and a decrease in volume at week 8.

Survival Rate

Survival rate is the survival rate of a population within a certain period of time. In fat graft surgery, the survival rate of the transplanted fat tissue is important so that the final result after fat graft surgery can last for a long time. There are several methods used to assess fat graft survival rate. The author uses weight maintenance and volume maintenance to assess fat graft survival rate. Weight maintenance is a comparison of the weight of fat tissue in a certain period of time, compared to the initial fat weight. Weight maintenance was measured at the end of week 2, end of week 4, end of week 8 after fat graft action, using the following formula

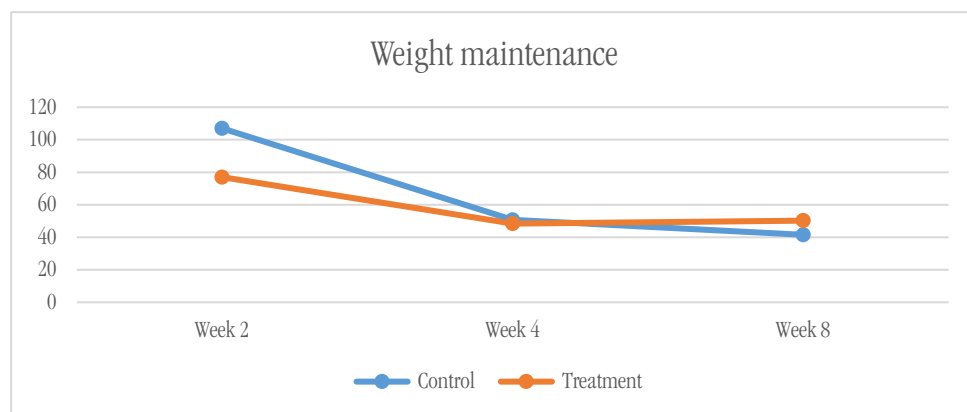
$$\text{Weight maintenance} = \frac{\text{Fat weight (grams) post fat graft}}{\text{Fat weight (grams) pre fat graft}} \times 100 \%$$

Table 3: Weight maintenance of fat in control and treatment groups.

Time	Average Weight Maintenance Control Group (%)	Average Weight Maintenance Treatment Group (%)
Week-2	107	77
Week-4	50,7	48,4
Week-8	41,52	50,24

In this study, weight maintenance in the control group decreased from week 4 from 50.7% to 41.52% at week 8. In the treatment group,

there was a decrease at week 4 to 48.4%, but it increased again at week 8 to 50.24% (Table 4).

**Figure 3:** Diagram of fat graft survival rate based on weight maintenance.

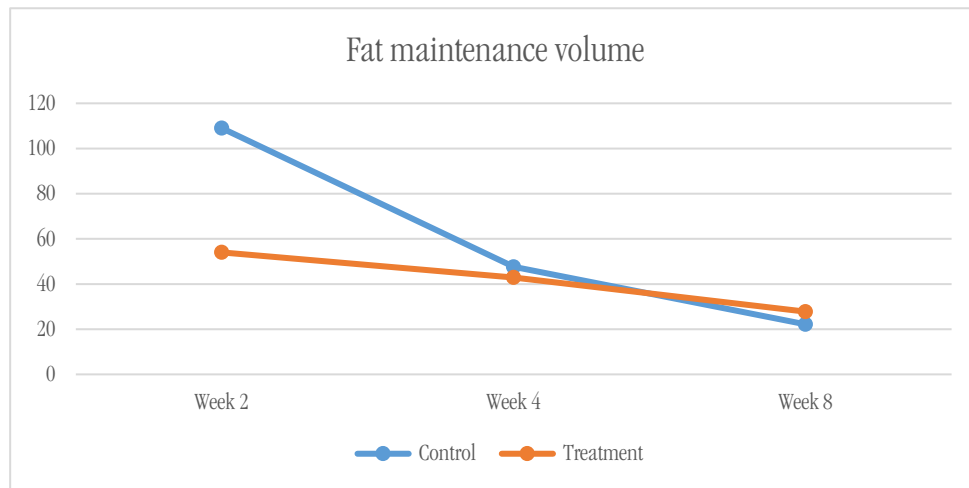
Fat graft survival rate is also reflected by the maintenance volume, which can give an idea of how much fat tissue remains in the recipient within a certain period of time. Maintenance volume is measured by comparing the volume of fat tissue after a certain period of time compared to the initial fat volume before action. In this study,

measurements were taken and then recorded at week 2, week 4 and week 8. Maintenance volume was measured with the following formula:

$$\text{Volume maintenance} = \frac{\text{Fat volume (grams) post fat graft}}{\text{Fat volume (grams) pre fat graft}} \times 100 \%$$

Table 4: Fat maintenance volume in control and treatment groups.

Time	Average Maintenance Volume Control Group (%)	Average Maintenance Volume Treatment Group (%)
Week-2	109	54
Week-4	47,60	42,90
Week-8	22,15	27,78

**Figure 4:** Fat graft survival rate diagram based on maintenance volume.

In this study, there was a decrease in maintenance volume in the control and treatment groups at week 4 with 47.60% and 42.90% respectively and week 8 with 22.15% and 27.78% respectively (Table 3 and table 4).

Fat Graft Morphology

The average area of normal fat cell obtained from 15 experimental rabbits (without treatment) was $1573.68 \mu\text{m}^2$, and the average area of fat cells that were treated with insulin 1000 IU for 15 minutes before transplantation was $1138.59 \mu\text{m}^2$. On average, a decrease in fat cell area was observed over 2 weeks, with a decrease of $269.652 \mu\text{m}^2$ in the control group. However, an increase was observed in the treatment group in the second week, amounting to $1015.08 \mu\text{m}^2$. In the fourth week, the average decrease in fat cell area was $724.82 \mu\text{m}^2$ in the control group and $352.99 \mu\text{m}^2$ in the treatment group (Figure 4). At

week 8, the mean decrease in fat cell area was $1044.2 \mu\text{m}^2$ in the control group and $183.59 \mu\text{m}^2$ in the treatment group (Table 5). Microscopic examination was carried out to calculate the area of each fat cell in the control and treatment groups in the pre fat grafting and post fat grafting fat. There were unilocular cells with varying sizes, cell nuclei at the edges, and there were also parts of lysed cells at weeks 4 and 8 (see Figure 5).

Statistical Analysis

Normality test was carried out on all variables using Shapiro wilk, obtained on the variable volume of pre fat grafting in the control and treatment groups with $p < 0.05$, it was concluded that the data distribution was not normal, and a comparison test was carried out with the Mann whitney test, the results of all variables showed $p > 0.05$ (Table 6).

Table 5: Fat cell area of control and treatment groups at the end of week 2, week 4, and week 8.

Identity of the Animal	Fat Area Control Group (mm ²) Pre-Fat Grafting Microscopy	Fat Area of Control Group (mm ²) Post Fat Grafting Microscopic			Fat Area Treatment Group (mm ²) Pre-Fat Grafting Microscopic	Fat Area of Treatment Group (Mm ²) Post Fat Grafting Microscopic		
		Week 2	Week 4	Week 8		Week 2	Week 4	Week 8
A	1734,40	2088,5			436,93	2512,78		
B	1730,25	2294,1			505,51	2504,62		
C	1732,30	409,57			550,91	1417,09		
D	1408,28	464,80			1759,45	1062,57		
E	881,04	1408,51			675,23	1506,4		
F	2595,04		1299		2495,07		2048	
G	553,79		539,09		189,52		701,66	
H	2590,1		488,65		2490,1		823,24	
I	1086,28		464,8		1371,92		1062,57	
J	1055,20		409,57		1270,91		1417,09	
K	2867,40			478,14	1020,05			771,89
L	649,54			586,08	401,89			705,46
M	2078,75			629,44	1821,59			1093,58
N	1789,01			480,43	1251,12			713,18
O	853,89			844,38	838,7			1131,26
Average	1573,68	1333,09	640,22	603,69	1138,59	1800,69	1210,51	883,074

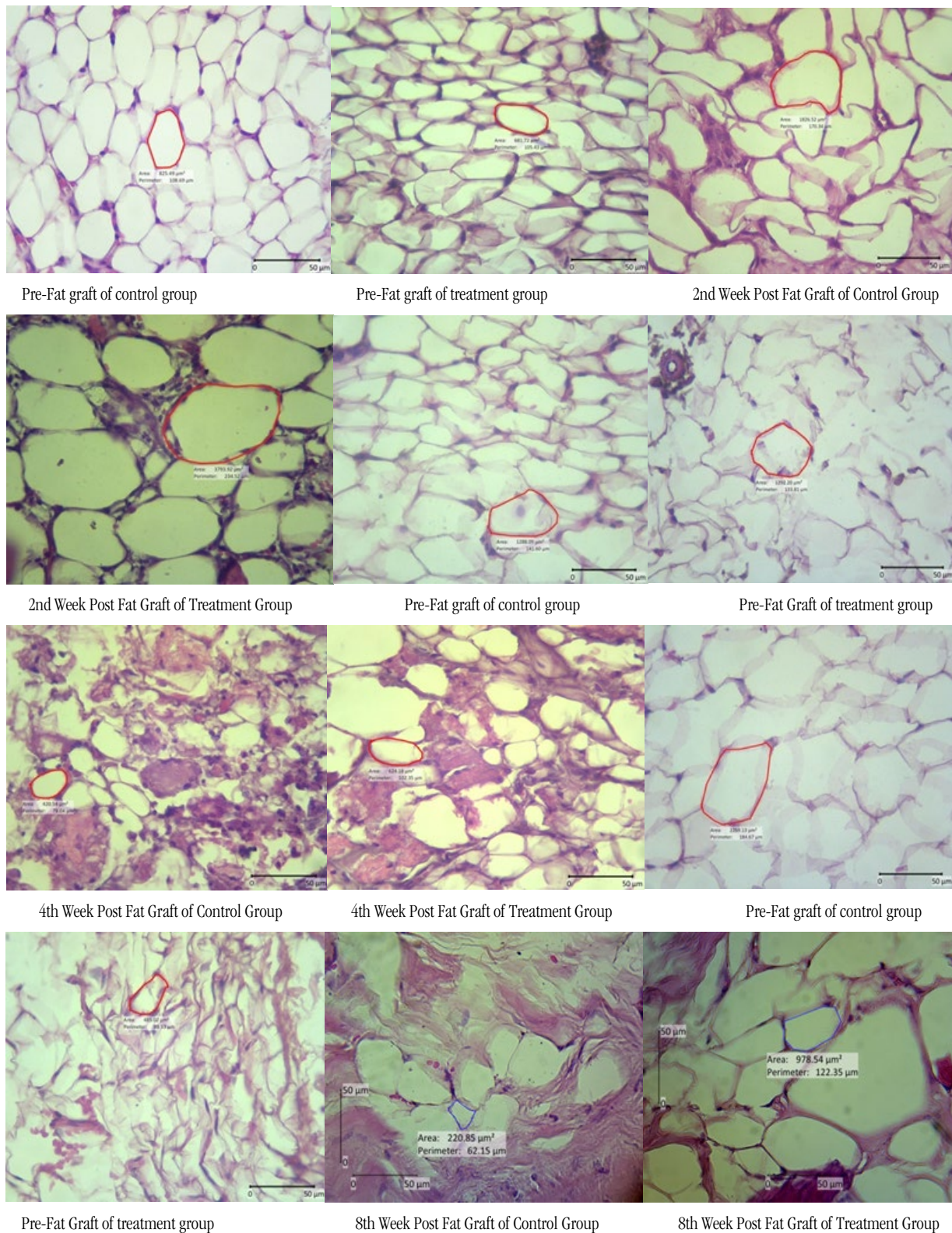


FIGURE 5: Histopathologic examination using a 400x magnification light microscope, a calibrated Nikon Eclipse Ci-E light microscope, equipped with a 300-megapixel DS Fi2 digital camera and Nikon Image System image processing software.

TABLE 6: Pre-fat grafting volume.

Variable	Total (n)	Fat Volume Mean $\pm$ SD	Median (Min - Max)	P-Value *Mann Whitney Test
Control Group Compared to Treatment	15	2,29 $\pm$ 2,06	1,85 (0,5-11)	0,349
Control Group Compared to Treatment in the 2nd Week	5	0,99 $\pm$ 0,62	0,95 (0,3-2,1)	0,242
Control Group Compared to Treatment in the 4th Week	5	1,31 $\pm$ 1,33	0,5 (0,1-2,9)	0,398
Control Group Compared to Treatment in the 8th Week	5	0,35 $\pm$ 0,17	0,3 (0,2-0,8)	0,178

In the examination of normality test data on the volume comparison in the control and treatment groups, in the 2nd week, 4th week, and 8th week, all data distributions were found to be non-normal. The comparison test results yielded non-significant results, except for the pre-fat graft in the control group compared to post-fat graft in the control group in the 8th week.

Using the Shapiro-Wilk test, a normal data distribution with a p-value > 0.05 was obtained, and a comparison test was conducted with the Paired T-test with $p < 0.05$ (0.014), indicating a significant difference between the fat volume pre-fat grafting and the fat volume in the control group post-fat grafting in the 8th week. Based on the mean values (1.48 ± 0.70 and 0.28 ± 0.86), the pre-fat graft in the

control group and post-fat graft in the control group in the 8th week were smaller compared to pre-fat graft, indicating a significant decrease in volume in the control group (Table 7).

Supporting this, in the treatment group, comparing pre-fat graft to post-fat graft in the 8th week, a non-normal data distribution was found. However, in the comparison test using the Wilcoxon test, $p < 0.005$ (0.042) was obtained, indicating a significant difference between the volumes in the treatment group. Based on the mean values (1.7 ± 0.65 and 0.42 ± 0.21), the pre-fat graft in the treatment group and post-fat graft in the treatment group in the 8th week were smaller compared to pre-fat graft, indicating a significant decrease in volume in the treatment group (Table 7).

Table 7: Comparison of fat volume in control and treatment groups, at week 2, week 4, and week 8.

Variable	Total (n)	Fat Volume of Pre-Fat Grafting Mean $\pm$ SD	Median (Min-Max) Pre-Fat Grafting	Fat Volume of (Post-Fat Grafting) Mean $\pm$ SD	Median (Min-Max) Post Fat Grafting	P-Value *Wilcoxon Test
Comparison of Pre-Fat Graft in the Control Group to Post-Fat Graft in the Control Group in the 2nd Week.	5	$1,08 \pm 0,46$	0,9 (0,8-1,9)	$1,18 \pm 0,51$	1,0 (0,9-2,1)	0,102
Comparison of Pre-Fat Graft in the Treatment Group to Post-Fat Graft in the Treatment Group in the 2nd Week.	5	$1,32 \pm 0,54$	1,1 (1,0-2,3)	$1,32 \pm 0,73$	0,4 (0,3-2,0)	0,42
Comparison of Pre-Fat Graft in the Control Group to Post-Fat Graft in the Control Group in the 4th Week.	5	$3,56 \pm 1,93$	2,9 (2,5-7,0)	$1,26 \pm 1,41$	0,5 (0,1-2,8)	0,221
Comparison of Pre-Fat Graft in the Treatment Group to Post-Fat Graft in the Treatment Group in the 4th Week.	5	$4,6 \pm 3,57$	3,0 (3-11)	$1,36 \pm 1,4$	0,5 (0,2-2,9)	0,042
Comparison of Pre-Fat Graft in the Control Group to Post-Fat Graft in the Control Group in the 8th Week.	5	$1,48 \pm 0,70$	1,5 (0,5-2,5)	$0,28 \pm 0,86$	0,3 (0,2-0,4)	0,014 *Paired T-Test
Comparison of Pre-Fat Graft in the Treatment Group to Post-Fat Graft in the Treatment Group In the 8th Week.	5	$1,7 \pm 0,65$	1,8 (0,6-2,3)	$0,42 \pm 0,21$	0,3 (0,3-0,8)	0,042

Based on the normality test results using the Shapiro-Wilk test on the fat weight of the control group compared to the treatment group in the 2nd week, 4th week, and 8th week pre-fat grafting, all data were

found not to be normally distributed with a p-value > 0.05 . The comparison test results using the Mann-Whitney test did not yield any significant or meaningful differences (Table 8).

Table 8: Pre-Fat Grafting Weight.

Variable	Total (n)	Fat Weight Mean $\pm$ sd	Median (Min-Max)	P- Value *Mann Whitney Test
Control Group Compared to Treatment	15	$2,3 \pm 2,04$	1,75 (0,6-11,1)	0,228
Control Group Compared to Treatment in the 2nd Week	5	$1,24 \pm 0,66$	0,95 (0,7-2,5)	0,916
Control Group Compared to Treatment in the 4th Week	5	$1,45 \pm 1,29$	0,6 (0,3-3,1)	0,398
Control Group Compared to Treatment in the 8th Week	5	$0,66 \pm 0,30$	0,6 (0,4-1,4)	0,233

Based on the normality test using the Shapiro-Wilk test on the fat weight of the treatment group in the 2nd week, a normal data distribution was obtained. The comparison of data was continued using the paired T-test, and a p-value of 0.194 ($p > 0.005$) was obtained, indicating no significant difference between the fat weight in the treatment group pre-fat graft compared to post-fat graft in the 2nd week. In the normality test using the Shapiro-Wilk test on the fat

weight of the control group pre-fat graft compared to post-fat graft in the 8th week, a normal data distribution was obtained ($p > 0.005$). The comparison test yielded significant results with a p-value of 0.023 ($p < 0.005$). A significant difference was found between the fat weight pre-fat graft in the treatment group compared to post-fat graft in the treatment group in the 8th week with a p-value of 0.043 ($p < 0.05$) (Table 9).

Table 9: Comparison of weight in control and treatment groups, at week 2, week 4, and week 8.

Variable	Total (N)	Fat Weight of Pre-Fat Grafting Mean $\pm$ SD	Median (Min-Max) Pre-Fat Grafting	Fat Weight of Post Fat Grafting) Mean $\pm$ SD	Median (Min-Max) Post Fat Grafting	P- Value *Wilcoxon Test
Comparison of Pre-Fat Graft in the Control Group to Post-Fat Graft in the Control Group in the 2nd Week.	5	$1,1 \pm 0,34$	0,9 (0,9-1,7)	$1,24 \pm 0,66$	0,9 (0,8-2,4)	0,414
Comparison of Pre-Fat Graft in the Treatment Group to Post-Fat Graft in the Treatment Group in the 2nd Week.	5	$1,62 \pm 0,5$	1,6 (1,0-2,4)	$1,24 \pm 0,74$	1,0 (0,7-2,5)	0,194 *Paired T-Test
Comparison of Pre-Fat Graft in the Control Group to Post-Fat Graft in the Control Group in the 4th Week.	5	$3,52 \pm 1,84$	2,9 (2,5-6,8)	$1,36 \pm 1,32$	0,6 (0,3-2,8)	0,221
Comparison of Pre-Fat Graft in the Treatment Group to Post-Fat Graft in the Treatment Group In the 4th Week.	5	$4,62 \pm 3,62$	3,0 (3,0-11,1)	$1,54 \pm 1,42$	0,6 (0,4-3,1)	0,223
Comparison of Pre-Fat Graft in the Control Group to Post-Fat Graft in the Control Group in the 8th Week.	5	$1,48 \pm 0,76$	1,4 (0,6-2,7)	$0,58 \pm 0,24$	0,5 (0,4-1,0)	0,02 *Paired T-Test
Comparison of Pre-Fat Graft in the Treatment Group to Post-Fat Graft in the Treatment Group in the 8th Week.	5	$1,64 \pm 0,67$	1,8 (0,6-2,3)	$0,74 \pm 0,37$	0,6 (0,5-1,4)	0,04

In the comparison of the results of the fat cell area of the control group compared to the treatment of pre fat grafting week 8, the normality test results were obtained with normal data distribution ($p > 0.05$). Comparison test with paired T-test test was obtained with a p-value of 0.042 ($p < 0.05$).

It can be concluded that statistically there is a meaningful or significant difference based on the mean value in the treatment group (883.07 ± 211.3) which is greater than the control group (586.08 ± 149.8), the change in fat cell area in the pre-fat grafting control group at week 8 was $296.99 \mu\text{m}^2$. (Table 10).

Table 10: Comparison of microscopic pre fat graft fat cell area results.

Variable	Total (N)	Fat Volume Mean $\pm$ SD	Median (Min-Max)	P- Value *Paired T-Test
Control group compared to treatment	15	1356 $\pm$ 756,04	1261,01 (189,52-2867,4)	0,117
Control group compared to treatment in the 2nd week	5	1566,89 $\pm$ 777,06	1461,74 (409,57-2512,78)	0,372
Control group compared to treatment in the 4th week	5	925,36 $\pm$ 531, 15	762,45 (409,57-2048)	0,047 *Mann Whitney test
Control group compared to treatment in the 8th week	5	743,38 $\pm$ 226,97	709,32 (478,14-1131,26)	0,042

From the results of the normality test using the Shapiro-Wilk test, the entire distribution of microscopic data on the area of pre-fat graft adipocytes in the control and treatment groups in the 2nd and 4th weeks was not normal. However, in the normality test of the microscopic data on the area of pre-fat graft adipocytes in the control and treatment groups in the 8th week, a statistically normal distribution was obtained, with a $p > 0.005$.

A comparison test using the Wilcoxon test on the pre-fat graft control group compared to the post-fat graft control group in the 4th week revealed a significant difference with a p-value of $p < 0.005$ (0.043). There was a significant decrease in the area of adipocytes, as seen from the significant decrease in mean adipocyte area from pre-control (1576.08 ± 951.68) to post-control (640.22 ± 371.20), with a decrease of $935.86 \mu\text{m}^2$.

Table 11: Comparison of microscopic results of pre fat graft fat cell area in control and treatment groups, week 2, week 4, and week 8.

Variable	Total (n)	Fat Cell Area Pre-Fat Grafting Mean $\pm$ SD	Median (Min-Max) Pre-Fat Grafting	Fat Cell Area Post Fat Grafting Mean $\pm$ SD	Median (Min-Max) Post Fat Grafting	P- Value *Paired T-Test
Comparison of Pre-Fat Graft Control Versus Post-Fat Graft Control in the 2nd Week.	5	1497,25 $\pm$ 371,95	1730,25 (881,04-1734,4)	1333,09 $\pm$ 881,29	1408,51 (409,57-2294,1)	0,686
Comparison of Pre-Fat Graft Treatment Versus Post-Fat Graft Treatment in the 2nd Week.	5	785,6 $\pm$ 551,28	550,91 (436,93-1759,45)	1800,69 $\pm$ 667,3	1506, 40 (1062,57-2512,78)	0,08 *Wilcoxon test
Comparison of Pre-Fat Graft Control Versus Post-Fat Graft Control in the 4th Week.	5	1576,08 $\pm$ 951,68	1086,28 (553,79-2595,04)	640,22 $\pm$ 371,2	488,65 (409,57-1299)	0,043
Comparison of Pre-Fat Graft Treatment Versus Post-Fat Graft Treatment in the 4th Week.	5	1563,5 $\pm$ 966,5	1371,92 (189,52-2495,07)	1210,51 $\pm$ 541,95	1062,57 (701,66-2048)	0,394
Comparison of Pre-Fat Graft Control Versus Post-Fat Graft Control in the 8th Week.	5	1647,71 $\pm$ 911,02	1789,01 (649,54-409,57)	640,2 $\pm$ 371,2	488,65 (409,57-1299)	0,082
Comparison of Pre-Fat Graft Treatment Versus Post-Fat Graft Treatment in the 8th Week.	5	1066,67 $\pm$ 524,4	1020,05 (401,89-1821,59)	1210 $\pm$ 541,95	1062,57 (701,66-2048)	0,433

Discussion

Fat graft is a modality in tissue reconstruction, with the advantages of techniques that are not too difficult, donors can be easily obtained in large numbers, producing good outcomes for donors and recipients [11]. Besides its advantages, fat grafting has the disadvantage of a fairly high fat graft absorption rate of up to 40-60% (low fat graft survival rate) so that fat graft actions need to be repeated to get the desired results and microcalcification and cyst formation caused by fat necrosis [12]. The results of weight maintenance were obtained in the control group with a percentage of 107% (week 2), 50.7% (week 4), 41.52% (week 8) and the treatment group with a percentage of 77% (week 2), 48.4% (week 4), 50.24% (week 8), it can be concluded that there is a difference in weight in each group but in the treatment group the results are better and statistically significant in reducing fat weight in the control and treatment groups at week 8. This is comparable to the results of maintenance volume which also decreased in the control group in week 2 by 109%, week 4 by 47.60%, week 8 by 22.15% and in the treatment group in week 2 by 54%, week 4 by 42.9%, and week 8 by 27.79%, it can be concluded that the decrease in maintenance volume in the treatment group obtained

better results which were not more than 50% but statistically significant results were not obtained. This study aims to prove the effect of insulin administration on changes in fat graft morphology that support fat graft survival rate. Performed by measuring the weight, volume, and microscopic measurement of fat cell area in the control and treatment groups, before and after the fat grafting process. In the control group, the fat was washed using 0.9% NaCl infusion until clean, this is important to clean the fat from unwanted tissues such as blood, debris, free lipids and broken adipocytes while maintaining viable adipose tissue [13]. Adipose tissue is a connective tissue that functions for the main storage of fat in the form of triglycerides, when the body experiences excess energy intake it will be stored, if the body is in a state of starvation, it will be released in the form of free fatty acids. In adipose tissue, cells are polyhedral and white fat cells have a diameter of $50-150 \mu\text{m}^2$ where in the preparation the cell appears pushed to the edge by lipid droplets with the edge of the cytoplasm surrounding the pleated droplet containing fine endoplasmic reticulum cisternae and several pinocytosis vesicles [14]. Each adipose cell is surrounded by the lamina basalis, and partitioned by connective tissue rich in blood vessels and nerves [12]. In vitro, adipogenesis can be triggered by the administration of insulin, dexamethasone, and

isobutyl methylxanthine. After several rounds of cell division, the cells finally undergo further differentiation around 7 days, on the 7th and 8th days the cells become rounded, de novo synthesis of long-chain fatty acids and esterification of triacylglycerol increases, and large lipid droplet accumulation occurs [12]. An extracellular factor that affects adipogenesis is the hormone insulin, which is an anabolic hormone that has the potential to stimulate fat accumulation in adipose tissue and may also play a role in growth factors. Insulin supports lipid accumulation through stimulation of glucose uptake, increased enzyme activity, lipoprotein lipase and through inhibition of lipolysis [12]. This is in line with the research findings in the control and treatment groups post fat grafting, which revealed an increase in the adipocyte size. The average increase in adipocyte area was 467.59 μm^2 at the end of the 2nd week, 570.29 μm^2 at the end of the 4th week, and 279.38 μm^2 at the end of the 8th week. The maximum adipocyte area reached 2512.78 μm^2 , with the minimum adipocyte area in the control group at 409.57 μm^2 during the 2nd and 4th weeks post fat grafting. When adipose tissue is grafted, it is transplanted in an avascular form and must undergo vascularization for the graft to remain viable. The process of revascularization in fat grafts is supported by adipose-derived stem cells (ASCs) that release angiogenic factors and respond to ischemia.

To achieve adequate revascularization results in each droplet, interaction with the recipient capillaries should occur at a ratio of 1:1, resulting in a successful recipient fat graft. Having more fat droplets compared to blood capillaries in the recipient may lead to insufficient neo angiogenesis, causing fat resorption and necrosis. Ideally, to ensure the survival of the fat graft, it should receive good vascularization and oxygenation in the recipient site [10]. In this study, it was found that the treatment group's pre-fat grafting sample had the largest fat volume at 11 ml, which then reduced to 0.5 ml within 4 weeks and further decreased to the smallest volume of 0.3 ml within 8 weeks. This aligns with the theory presented by Shih et al [10] in the graft survival theory, indicating that a smaller graft volume corresponds to a higher survival rate. This is attributed to the ability to achieve complete diffusion and perfusion processes, facilitating the prompt formation of neovascularization and preventing the occurrence of necrosis and death of adipocytes. Sung Ju Hong et al. [14] demonstrated an increase in fat graft viability by analyzing differences in weight maintenance after fat grafting. The viability of fat grafts decreased overall in the entire group, with no significant differences in viability rates observed across the groups six months after

the procedure, with results showing $p > 0.005$. However, significant differences were found between the control and treatment groups. In the treatment group, which involved the use of insulin and β -fibroblast growth factor post autologous fat grafting, viability was significantly higher after 12 months compared to the control group [14]. In this study, no significant differences were found with $p > 0.005$ in the statistical test between the pre-fat grafting volume groups of the control and treatment groups during the 2nd, 4th, and 8th weeks. Based on the mean values of pre-fat graft volume in the treatment group and post-fat graft volume in the 8th week, with a smaller fat volume compared to pre-fat grafting, a significant decrease in volume was observed in the group receiving 1000 IU of insulin in the 8th week. This finding contrasts with the results presented in Sung Ju Hong et al. [14] since this study did not use growth factors, which are often involved as stimuli for interstitial cell proliferation [7].

Conclusion

The research findings lead to the following conclusions. Firstly, the effect of insulin demonstrates a notable impact on the macroscopic morphological changes observed in fat grafts. This influence is evident through the maintenance of weight and volume, ultimately contributing to an enhanced survival rate of the fat grafts. Secondly, the microscopic morphological alterations in fat grafts are also influenced by insulin administration, further reinforcing the favorable survival rate of the grafts. Several recommendations arise from the study's limitations and observations.

Firstly, due to constraints in time and sample size, it is advisable to pursue additional research with an expanded sample pool and an extended duration. Qualitative evaluations, encompassing factors like inflammatory response, necrotic processes, fibrosis, macrophages, and collagen, should be incorporated before translating the research findings to human applications. Secondly, for more standardized results, the sampling of fat should be homogenized, with uniform micro-particle sizes. Precise determination and marking of the recipient site are essential to streamline sample collection at the study's conclusion. Thirdly, further investigations could explore the integration of additional therapeutic modalities, such as growth factors, affecting microscopic morphological changes in adipocytes. Lastly, post-treatment evaluations should include assessing blood glucose levels in experimental rabbits, supporting the prolonged survival rate of these animals throughout the study

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