




The Influence of Low- and High-Negative-Pressure Liposuction and Different Harvesting Sites on the Viability and Yield of Adipocytes and Other Nucleated Cells

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Abstract

Background The volume effect of fat grafting is highly dependent on the presence of viable adipocytes and other nucleated cells within the lipoaspirate. We suspected that one of the crucial factors influencing cell viability is the negative pressure applied during the fat graft harvesting and the suitability of various harvest sites when compared to others. Despite much discussion, there is no consensus on the optimal negative pressure or the best site for harvesting so we designed an experiment to test this.

Methods Fat graft taken under low negative pressure (− 200 mmHg) or high negative pressure (− 700 mmHg) from the thigh or abdominal regions from 21 healthy human donors was evaluated. The principal variables studied were: a) total number and viability of nucleated cells, b) liposuction duration and c) blood admixture. Other variables studied were body mass index, the impact of age and enzymatic digestion.

Results The absolute number and viability of nucleated cells and the blood admixture did not differ significantly

between lipoaspirates obtained under different vacuum conditions or from different regions. The time taken to acquire the same volume of lipoaspirate was significantly increased using low negative pressure. The time taken to collect cells in the thigh region significantly increased with increasing BMI but this correlation was not found when harvesting in the abdominal region. The BMI and age did not impact the results in any of the measured variables. The enzymatic digestion rate was independent of the negative pressure used to harvest.

Conclusion Our results indicate that neither the negative pressure used nor the area chosen has any significant influence on the viability and yield of harvested cells. The time taken to obtain lipoaspirate using low pressure is significantly longer than when using high pressure. No significant difference was found in the value of blood admixture using different vacuum pressures, and no correlation exists between the body mass index and the cell viability or age of the patients and the time of liposuction.

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Introduction

Since the revolutionary and comprehensive work on fat grafting done by Coleman, fat grafting became an accepted and routinely used procedure not only in plastic surgery but also in other medical specialities [1–8]. Currently adipose

tissue is used as a filler with a potential regenerative effect. However, considerable differences in methodology and also in success levels are reported. Moreover different fat graft success is reported even when implementing the same method. The absorption rate of the applied fat graft over time was found to be between 25 and 70% [9–12].

Multiple factors are involved in the fat grafting procedure and all of them affect the fat graft success and the final result of the whole procedure. Even after excluding the patient-related factors, we still have to consider the factors associated with the fat graft harvesting process (*i.e.* tumescent solution, syringe aspiration or excision or use of a pump machine, the negative pressure setting, the cannula length and calibre, etc.). Then the graft processing (*i.e.* decantation, filtration or centrifugation, addition of stem cells or other agents) and the graft application (*i.e.* cannula length and calibre, applied positive pressure, the technique of the fat insertion, the amount of applied graft, etc.) [9, 13–18].

Mature adipocytes are fragile cells with minimal resistance to any trauma while adipose tissue derived stem cells (ASCs) are more tolerant [19, 20]. The volume effect of fat grafting is more dependent on adipocytes while the regenerative potential depends more on ASCs, but both cell types are important for the fat graft success. One presumes that gentle handling of the fat graft in all steps is important. The value of the negative pressure setting for harvesting fat tissue using any technique can be one of the dominant differentiating factors potentially affecting the viability and function of adipocytes and ASCs. We therefore concluded that it is important to understand the effect of negative pressure on these cell types. In addition, one must recognise that lipoaspirate contains not only adipocytes and ASCs but also other nucleated cells originating from the stromal vascular fraction (SVF) such as preadipocytes, fibroblasts, endothelial cells and haematopoietic cells.

The current literature that deals with the influence of negative pressure on adipocytes and/or ASCs is very inconsistent in methodology and reported results [19, 21–39]. All the studies that we found were of low evidence-based medical value (Level IV.) [40, 41], and though not a single study suggested better results could be obtained using higher-negative-pressure harvesting, the large variability in their findings prevents a statistically significant conclusion to be drawn [42].

This led us to design this experimental study in which we aimed to scientifically evaluate the differences in the viability and total count of adipocytes, ASCs and other nucleated cells in lipoaspirate harvested using various negative pressures and also to see whether the choice of fat harvesting sites had an influence. All other variables of the harvesting process (cannula type and size, fat-collecting containers, tubes, etc.) were kept constant. We also studied

the impact of various negative pressures and anatomic sites on the time of liposuction and on the resulting blood admixture. Finally we evaluated the relationship of these with the body mass index (BMI) and age of the donors. We consider that this controlled prospective cohort study better clarifies the relationships among these variables and especially the influence of various negative pressures on the quality of lipoaspirate.

Materials and Methods

The Cohort of Patients

A prospective cohort study was performed on samples of subcutaneous adipose tissue harvested from 21 healthy donors after written informed consent was obtained at the Department of Plastic Surgery, First Faculty of Medicine Charles University and Na Bulovce University Hospital in Prague. The study was performed in compliance with the Declaration of Helsinki on experiments involving human tissues and it was approved by the Ethical Committee of the Na Bulovce University Hospital.

The group of females ($n=20$) and one male ($n=1$) patients underwent tumescent liposuction under general anaesthesia. All patients were generally healthy with no allergies. The exclusion criteria for patient's selection were tobacco use, hypertension and diabetes mellitus. No age, BMI or gender exclusion criteria were applied. All patients without exclusion criteria and undergoing liposuction of the abdomen or/and thigh regions during the defined time periods were included in this cohort study. The adipose tissue from the thigh region ($n=12$) and from the abdominal region ($n=15$) was harvested. Individual numbers of samples used for the following analyses are specified in paragraphs concerning the methodology of the analyses. Demographic and clinical characteristics of the donors are summarised in Table 1.

Liposuction Procedure

The wet technique liposuction was performed with the tumescent solution containing 1000 mL of physiological solution with 1 mL of epinephrine (1 mg/ml) and 20 mL of 8.4% bicarbonate. In order to eliminate all other possible cytotoxic effects, no local anaesthetics were used. A liposuction machine (MEDELA dominant 50, Medela AG, Switzerland) was used with the continuous negative pressure mode setting. For the purpose of this study, the pump machine was freshly calibrated. The low negative pressure (LP) was set at -200 mmHg, and the high negative pressure (HP) was set at -700 mmHg. A Coleman Style blunt cannula with 4 holes and an internal diameter of 3

Table 1 Demographic and clinical characteristics of the donors¹

	All (<i>n</i> =27)	Thigh (<i>n</i> =12)	Abdomen (<i>n</i> =15)
Age (years) ² (<i>p</i> = 0.133) ³	37.96 ± 9.63	34.80 ± 8.49	40.50 ± 10.01
BMI (kg/m ²) ² (<i>p</i> = 0.319) ³	23.50 ± 3.53	22.80 ± 3.77	24.20 ± 3.32
Sex (female/male)	26/1	12/0	14/1
Tobacco user (yes/no)	0/27	0/12	0/15
High blood pressure (yes/no)	0/27	0/12	0/15
Diabetes mellitus (yes/no)	0/27	0/12	0/15

¹Samples of adipose tissue (*n*=27) were collected from 21 donors either from the thigh region (*n*=12) or from the abdominal region (*n*=15). The samples were always obtained both under low negative pressure (i.e. – 200 mmHg) and under high negative pressure (i.e. – 700 mmHg) in the same patient. The number of samples used for individual analyses is specified in each analysis description. No statistically significant difference was found between the abdominal and thigh groups when considering age (*p*=0.133) and BMI (*p*=0.319)

²Data are presented as mean ± SD

³*p* value is given for comparison between the thigh and the abdominal regions performed by unpaired t-test

mm was used in all cases. The schema of the fat harvesting set is shown in Fig. 1.

In accordance with fluid dynamics, we expected some negative pressure decrease in the system; thus, we performed a set of experimental measurements in advance that showed about 11.5% negative pressure loss on average (Table 2). This means that the real negative pressure at the end of the liposuction cannula was about – 177 mmHg for LP and about – 620 mmHg for HP harvesting. Though the

real negative pressure was about 11.5% lower than pressure set on pump machine (– 200 mmHg vs – 177 mmHg and – 700 mmHg vs – 620 mmHg) we decided that to prevent confusion in presenting the results, in the discussion and the conclusion we will continue to refer LP as – 200 mmHg and HP as – 700 mmHg.

Both LP and HP were used in each donor: LP was used on one side of the patient's body, and HP was used on the opposite side. A different cannula, tube and sterile fat-

Fig. 1 Schema of the fat harvesting set. A cannula with hand piece (15 cm long, internal diameter 5 mm) was connected to a sterile fat-collecting container of 500 mL using a 3 m long tube with internal diameter of 7 mm. This container was connected to a pump machine safety container of 2000 mL using the same tube and this container was connected to a negative pressure generator using a 0.5 m tube with 7 mm internal diameter

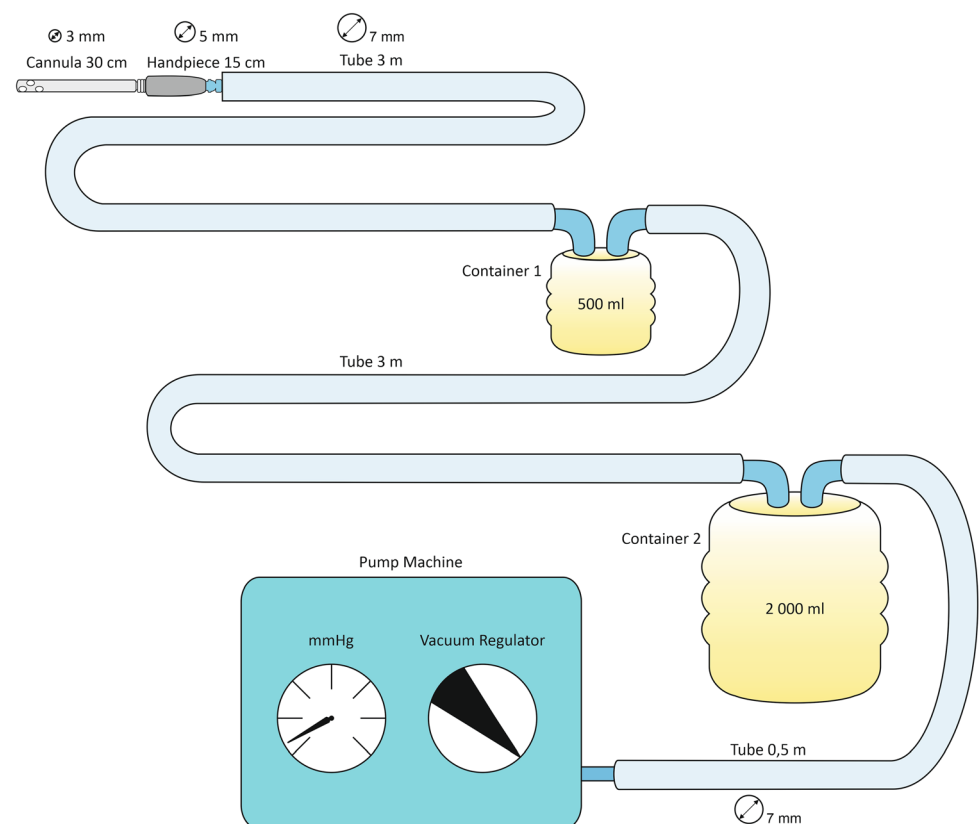


Table 2 Percentage loss of negative pressure in the fat grafting liposuction set

Measurement # ID	Machine negative pressure setting (mmHg)	Manometer measurement (mmHg)	Percentage of real negative pressure vs setting on manometer	Percentage of pressure loss
1	200	180	90.0	10.0
2	200	174	87.0	13.0
3	260	230	88.5	11.5
4	370	330	89.2	10.8
5	410	360	87.8	12.2
6	450	400	88.9	11.1
7	530	465	87.7	12.3
8	560	500	89.3	10.7
9	700	622	88.9	11.1
10	700	620	88.6	11.4
Average			88.6	11.4

A set of randomly chosen 10 measurements was taken using different negative pressures from – 200 mmHg up to – 700 mmHg. Measurements were taken using standard manometer connected to the end of tube that is originally connected to the hand piece of the liposuction cannula when performing liposuction. The differences in negative pressure are presented in absolute numbers and as a percentage of achieved negative pressure and as a percentage of negative pressure loss

collecting container were used for LP and HP harvesting in order to prevent contamination of the LP material with the HP material and *vice versa*. To minimise any other influences and variability, the samples for research were taken at the beginning of the liposuction procedure so the least disturbed adipose tissue was evaluated.

From perioperative medication, only antibiotic prophylaxis in three doses (at the beginning of anaesthesia, 8 and 16 hours postoperatively) was administered using intravenous cefalosporine—cefazolin (Vulmizolin 1 gr, BB Pharma a.s., Prague, CZ).

Measurements and Observations During the Liposuction Procedure

Liposuction Time

During the liposuction, the time taken for a standardised volume (50 mL) was recorded. The measurement was taken with a standard stopwatch. Samples obtained either under LP or under HP from the thigh region ($n=12$) and from the abdominal region ($n=13$) were investigated.

The Evaluation of Blood Admixture During Liposuction

The visual estimation of blood contamination of harvested fat tissue was performed immediately after liposuction before sedimentation and two hours after harvesting and sedimentation. The harvested fat tissue was stored in closed sterile containers, where the samples were kept at room

temperature and were sent immediately (within 2 hours) to the laboratory for further processing and analysis.

Biological Analyses of Freshly Harvested Lipoaspirate

The freshly harvested lipoaspirates were analysed within 2 hours after the liposuction procedure. The lipoaspirates were minimally manipulated to preserve constant conditions for assessment. The visual schema of lipoaspirate analyses is presented in Fig. 2.

Analysis of Blood Admixture in Freshly Harvested Lipoaspirate

The initial evaluation of blood admixture to lipoaspirate immediately after liposuction with minimal sedimentation was done visually (Fig. 2a). In order to precisely analyse the admixture of blood elements in the lipoaspirate, 7 mL of the lower part of the lipoaspirate suspension (Fig. 2b) was aspirated and transferred to 15-mL conical plastic tubes (in triplicate for each sample from each donor). The plastic tubes were stored overnight at 4 °C to allow blood elements to sediment. Subsequently, the supernatant was aspirated and the pellet, composed of blood elements, was resuspended in 2 mL of phosphate-buffered saline (PBS; Sigma-Aldrich). This suspension was pipetted into a 96-well plate and the blood admixture was estimated on the basis of the absorbance measurements at $\lambda=540$ nm, *i.e.* at the absorption peak for haemoglobin. Samples obtained under LP or HP from the thigh region ($n=8$) and from the abdominal region ($n=10$) were analysed.

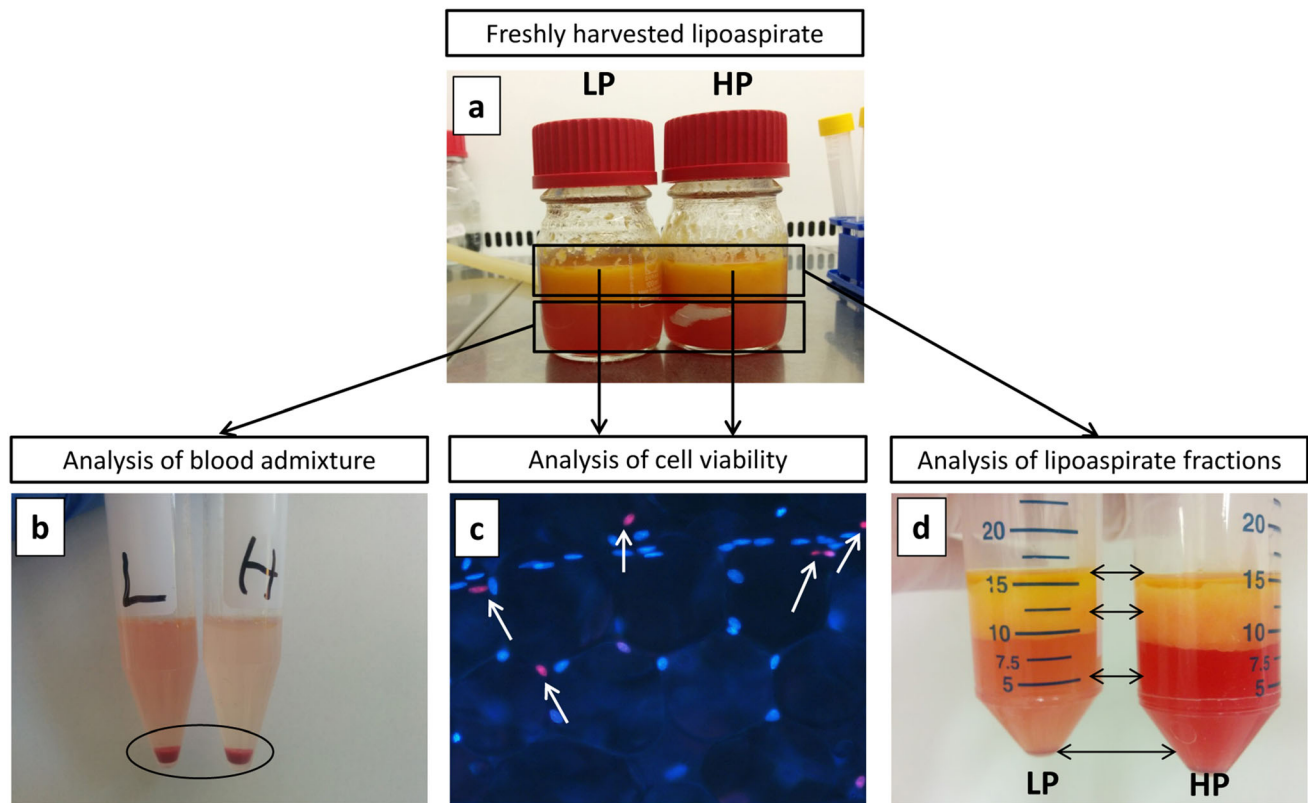


Fig. 2 Schema of the biological analyses performed on the fresh lipoaspirates. **a** Macroscopic evaluation of the blood admixture, **b** processed analysis of blood admixture, **c** analysis of cell counting

Viability and Yield of Nucleated Cells in Freshly Harvested Lipoaspirate

For the analysis of viability and number of nucleated cells, the solid upper part of the lipoaspirate (Fig. 2c) was washed 3 times with PBS to remove blood elements. Then 1 mL of native lipoaspirate was aspirated and was incubated with Hoechst 33342 (10 $\mu\text{g}/\text{mL}$ in PBS; Cat. No. B2261, Sigma-Aldrich) and with ethidium homodimer-1 (3 $\mu\text{g}/\text{mL}$ in PBS; Cat. No. E1169, ThermoFisher Scientific) dyes for 15 minutes (at 37 $^{\circ}\text{C}$). Subsequently, from 28 to 51 microphotographs of the stained lipoaspirate were taken for each evaluated sample under an epifluorescence Olympus IX71 microscope, equipped with a DP71 digital camera using an objective magnification of $\times 10$ and $\times 20$. The number of dead nucleated cells (*i.e.* the nuclei of red or purple colour, positively stained with ethidium homodimer-1 and Hoechst 33342, respectively) and the number of viable nucleated cells (*i.e.* the nuclei of blue colour positively stained only with Hoechst 33342) were manually counted from the microphotographs. The viability of all nucleated cells within the lipoaspirate was then calculated as the ratio of viable cells *vs.* all cells. The cell density, *i.e.* the absolute number of viable cells for each sample, was

and viability and **d** observed lipoaspirate fractions volume. Lipoaspirate from the same donor was harvested under low negative pressure (LP – 200 mmHg) or high negative pressure (HP – 700 mmHg)

related to a microscopic field under objective magnification $\times 10$. Samples obtained under LP or HP from the thigh region ($n=6$) and from the abdominal region ($n=7$) were analysed.

Comparison of the Ratio of Specific Lipoaspirate Fractions

Samples of lipoaspirate obtained under LP and HP from 5 donors were studied to visually compare the volume of specific lipoaspirate fractions after enzymatic digestion of the tissue (Fig. 2d). The lipoaspirate was washed several times with PBS. Then, 8 mL of lipoaspirate was mixed with 8 mL of type I collagenase solution. This solution contained 1% (wt/vol) bovine serum albumin (Sigma-Aldrich) and 0.1% (wt/vol) type I collagenase (Worthington). The digestion was performed for 1 hour at a temperature of 37 $^{\circ}\text{C}$. Then, the digested tissue was centrifuged for 5 min at 300 g. Subsequently, the ratio of particular fractions was visually compared with respect to the LP and HP used during the liposuction procedure.

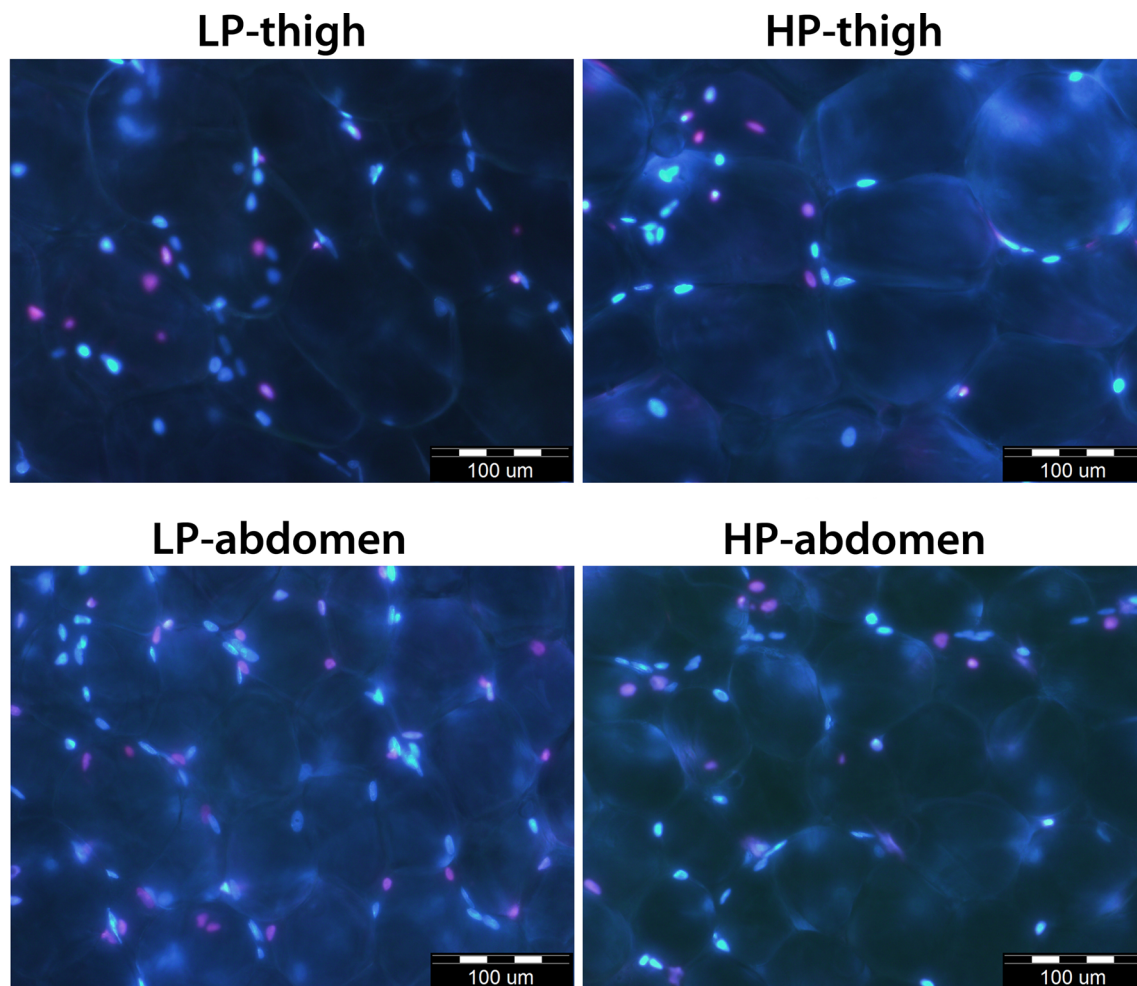


Fig. 3 Microphotographs of native lipoaspirate fluorescence staining. The lipoaspirate was obtained from the same donor from the thigh region and from the abdominal region under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP – 700 mmHg). All cell nuclei were stained with Hoechst 33342 (blue), and at the same time, the nuclei of dead cells were stained with

ethidium homodimer-1 (red). The living cells are therefore recognizable as having blue nuclei and dead cells are recognizable as having purple nuclei (combination of blue and red) observed with an Olympus IX71, camera DP71, objective magnification $\times 20$, scale bar 100 μm

Statistical Analyses

Statistical analysis was performed to test the significance of the results. The paired *t*-test was used for data having a normal distribution. The Wilcoxon matched-pairs signed-rank test was used for data not having a normal distribution. Associations between BMI/age and liposuction time/cell viability were described by Pearson's correlation coefficient with a calculation of linear regression and a 95% confidence interval. The statistical analysis and graphs were performed by GraphPad Prism 8 (GraphPad Software, Inc.). Results with $p < 0.05$ were considered significant.

The detailed demographic and clinical characteristics of donors with a summary of analyses performed are described in Supplementum (Table S1).

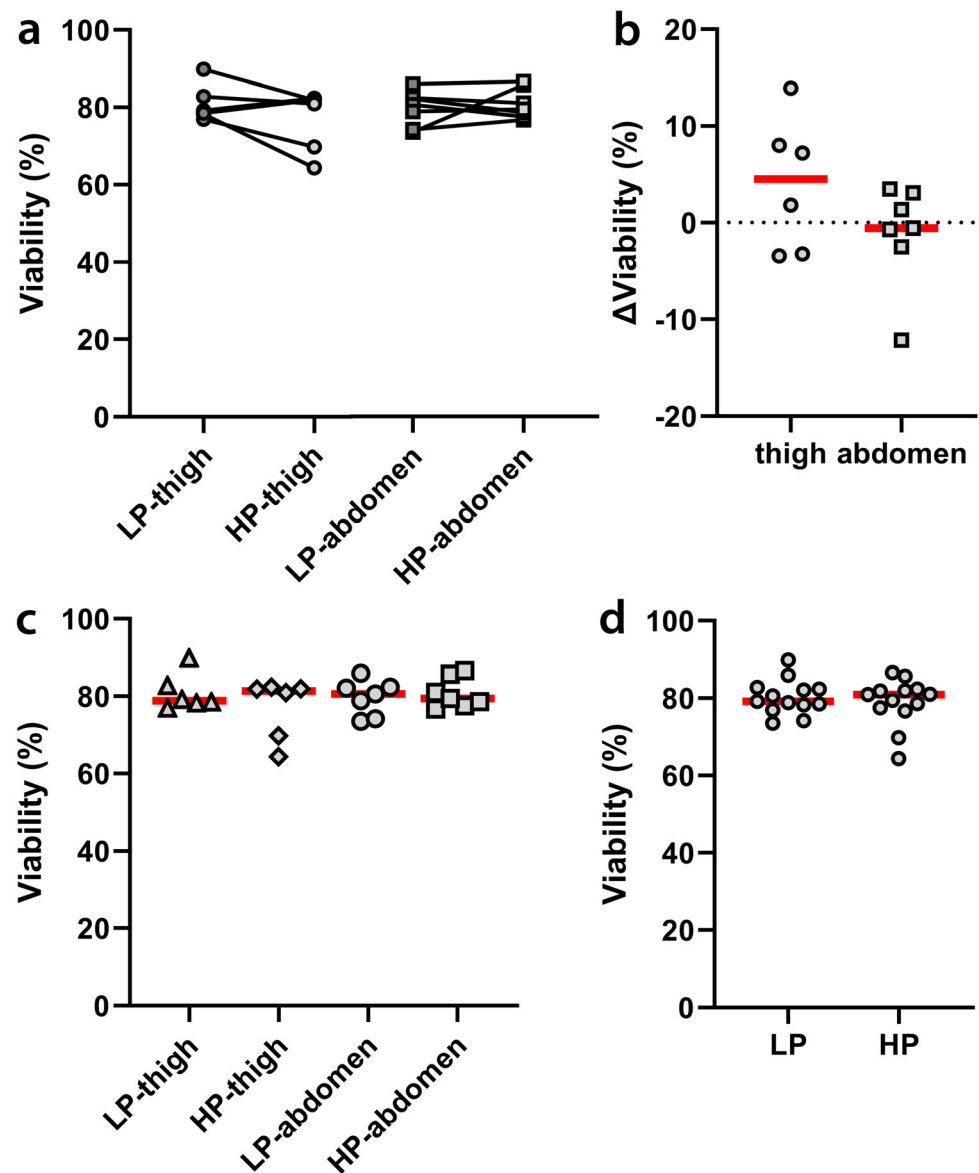
Results

Viability and Number of Nucleated Cells in Freshly Harvested Lipoaspirate

Representative images of the staining used for cell viability evaluation (Hoechst 33342 and ethidium homodimer-1) are shown on Fig. 3.

The relationship between LP and HP value for each patient is detailed in Fig. 4. In the thigh region, the viability of all nucleated cells was slightly higher under LP than under HP. However, in the abdominal region, the viability was similar in both lipoaspirate groups (Fig. 4a). These differences were quantified by Δ viability, *i.e.* the LP value minus HP value for each donor (Fig. 4b). Nevertheless, the absolute median values of the cell viability were similar for

Fig. 4 Relationship between cells viability and LP and HP value for each patient. **a** Viability of all nucleated cells in the native lipoaspirate from the thigh region and from the abdominal region harvested under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP – 700 mmHg). The lipoaspirate was obtained from each donor under both pressures; thus, the line between the symbols represents one donor. The figure **b** represents the difference in cell viability (Δ Viability) counted as LP value minus HP value for each donor. The figure **c** represents comparison of LP and HP values with a respect to the anatomic region of lipoaspirate origin. The figure **d** represents the comparison between LP and HP pressure values together from the thigh and from the abdominal region. The median values are marked red. No significant difference ($p < 0.05$) between LP and HP was observed



all groups studied, *i.e.* from 78.9% (LP thigh) to 81.4% (HP thigh) (Fig. 4c). No significant difference was observed when the all LP values were compared to all HP values irrespective of the anatomic region (Fig. 4d).

The clear dependence of the negative pressure used during the liposuction procedure on the absolute numbers of viable nucleated cells was not observed (Fig. 5a). Based on Δ Cell number (*i.e.* LP value minus HP value for each donor), the median value was slightly higher for LP thigh than for HP thigh. Contrarily, the median value of Δ Cell number was slightly lower for LP abdomen than for HP abdomen (Fig. 5b). Similarly to the cell viability, the absolute numbers of viable nucleated cells per microscopic field were similar for all studied groups, *i.e.* from 113 cells/field (HP thigh) and 123 cells/field (HP abdomen) (Fig. 5c). No significant difference was observed when the

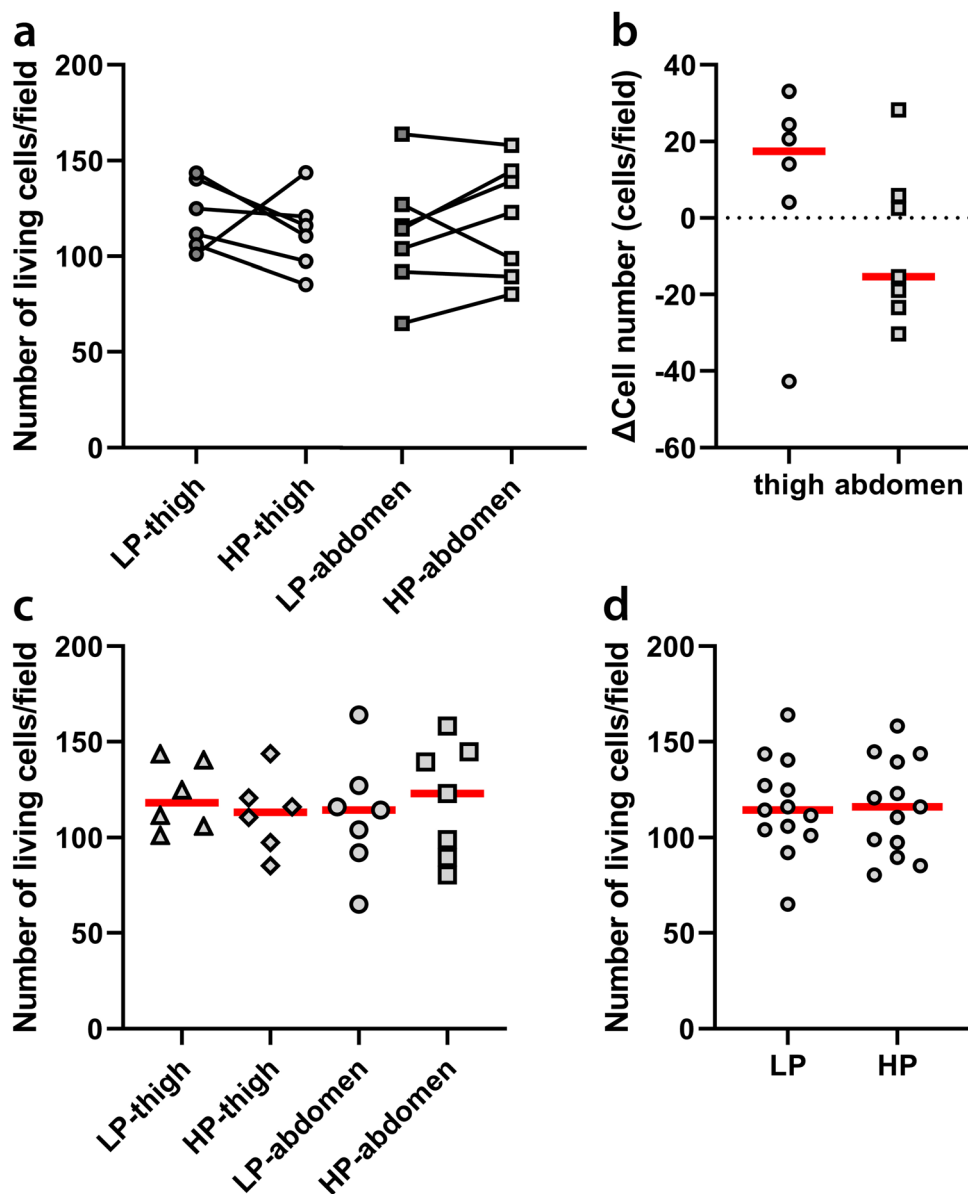
all LP values were compared to all HP values irrespectively of the anatomic region (Fig. 5d).

Liposuction Time

For most of the donors, the liposuction procedure under LP took longer than when using HP. These results were obtained both for the thigh region and for the abdominal region. The measurements are summarised in Table 3.

The relationship between the LP and HP value for each patient is demonstrated in Fig. 6a. In order to quantify the differences, Δ Time duration (*i.e.* the LP value minus the HP value for each donor) was calculated. The median value of Δ Time duration showed that LP liposuction lasted longer than HP liposuction by 21 seconds (thigh region) and by 31 seconds (abdominal region) (Fig. 6b). In general,

Fig. 5 Absolute numbers of viable nucleated cells. **a** Absolute number of viable nucleated cells per microscopic field (objective magnification x10) in the native lipoaspirate from the thigh region and from the abdominal region harvested under low negative pressure (LP – 200 mmHg) and under high negative pressure (HP – 700 mmHg). The lipoaspirate was obtained from each donor under both pressures; thus, the line between the symbols represents one donor. The figure **b** represents the difference in cell number (Δ Cell number) counted as LP value minus HP value for each donor. The figure **c** represents comparison of LP and HP values with a respect to the anatomic region of lipoaspirate origin. The figure **d** represents the comparison between LP and HP pressure values together from the thigh and from the abdominal region. The median values are marked red. No significant difference ($p < 0.05$) between LP and HP was observed



the median values showed that harvesting 50 mL of lipoaspirate did not exceed 90 s. Specifically, the median values were as follows: 45 s (HP abdomen), 54 s (HP thigh), 68 s (LP thigh) and 87 s (LP abdomen) (Fig. 6c). Even stronger statistical significance between LP vs. HP harvest time values was observed when values from both regions (thigh and abdominal) were evaluated together (Fig. 6d).

Analysis of Blood Admixture in Harvested Lipoaspirate

The results of visual assessments of blood admixture to lipoaspirates immediately after harvesting and two hours later are presented in Table 4.

The measured values did not show a clear dependence on varying negative pressures (LP vs. HP) in individual donors (Fig. 7a). No significant differences were observed among the absolute median values of the studied groups. Nevertheless, the LP regions (*i.e.* LP thigh and abdomen) reached slightly higher median values than the HP regions (*i.e.* HP thigh and abdomen) (Fig. 7b and c). No significant difference was observed when all LP values were compared to all HP values irrespectively of the anatomic region (Fig. 7d).

Correlation of Other Specific Values

Figure 8 shows the correlation between BMI and liposuction time. Interestingly, in our group of donors, we

Table 3 Liposuction time for harvesting of 50 mL of lipoaspirate

Patient # ID	Region	LP liposuction time (sec)	HP liposuction time (sec)
1	Thigh	66	47
	Abdomen	113	105
2	Thigh	154	86
3	Thigh	35	44
	Abdomen	103	52
4	Thigh	70	94
	Abdomen	35	40
5	Abdomen	72	24
6	Abdomen	95	25
7	Thigh	45	45
	Abdomen	85	98
8	Abdomen	60	41
9	Abdomen	80	40
10	Thigh	115	48
	Abdomen	95	34
11	Thigh	98	76
12	Thigh	158	125
13	Abdomen	–	–
14	Abdomen	120	95
15	Thigh	66	60
16	Abdomen	62	45
17	Abdomen	–	–
18	Thigh	66	35
	Abdomen	120	53
19	Thigh	55	40
20	Abdomen	87	56
21	Thigh	128	65

Liposuction performed under low negative pressure (LP – 200 mmHg) and high negative pressure (HP – 700 mmHg). Liposuction time was measured in seconds using a standard stopwatch

observed significant correlation between BMI and liposuction time in the thigh region (*i.e.* the higher BMI was, the longer harvesting 50 mL of lipoaspirate took). However, the reverse seemed to be true for the abdominal region.

Almost no correlation was observed between the age of the donors and time of the liposuction procedure (Fig. 9).

Similarly, the correlation coefficient was not found to be significant in the case of BMI compared to cell viability (Fig. 10).

Comparison of the Ratio of Specific Lipoaspirate Fractions

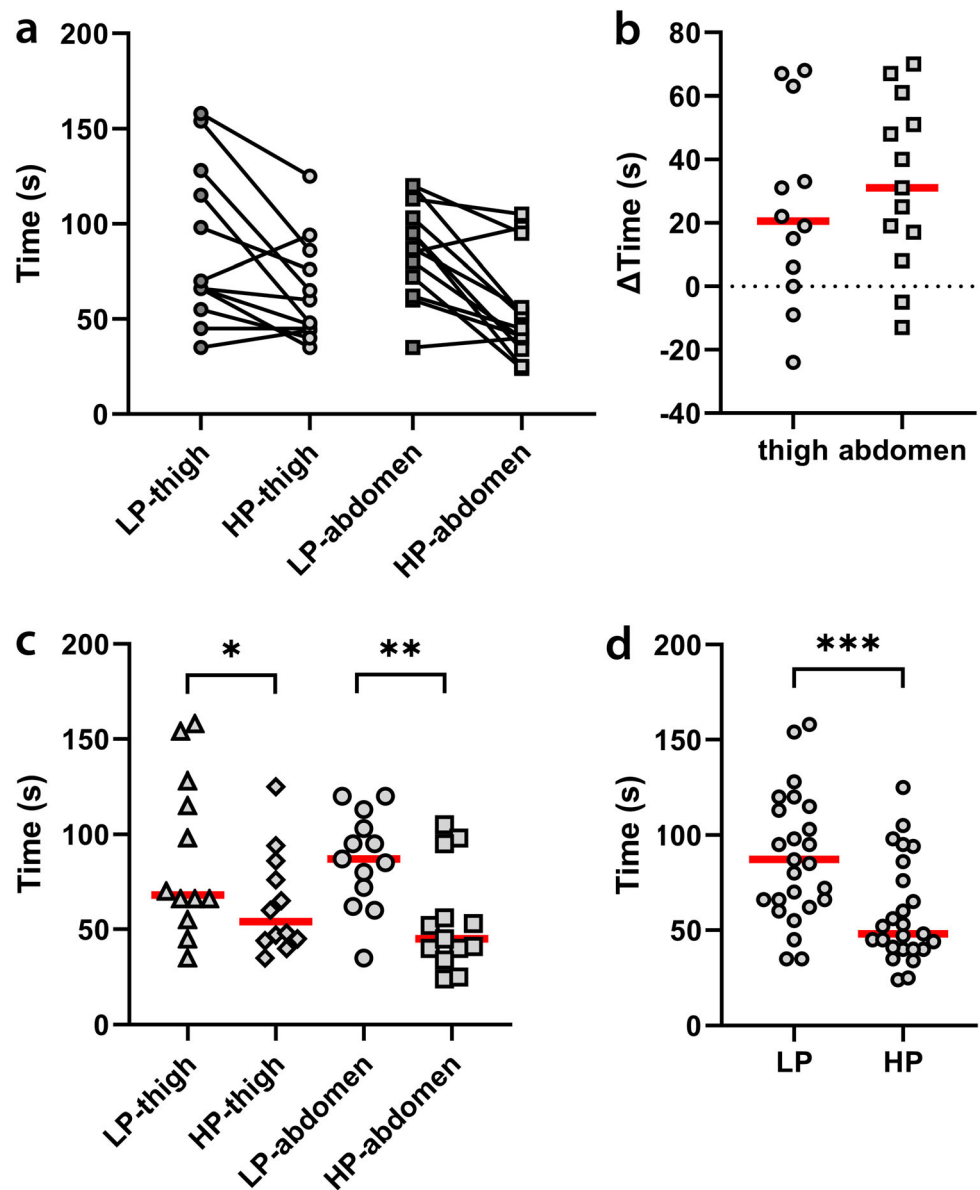
Visual observation was used to compare the amount of specific lipoaspirate fractions in lipoaspirates obtained under LP and HP from 5 donors. Figure 11 shows the separated fractions after collagenase digestion and centrifugation.

Based on the visual observation, the ratios are also given in Table 5. Although the ratios of mainly the oily part and lipoaspirate part differed among the donors, the values obtained within the single donor both under LP and HP were similar (rounded to the nearest 0.5 mL).

Discussion

It has been postulated that negative pressure can be one of the crucial factors that can affect the condition of harvested cells especially adipocytes as they are very fragile and because of their size they are more prone to mechanical damage [19, 20]. Reviewing the studies on the potential influence of negative pressure during harvesting fat grafts on the viability and function of adipocytes and ASCs, we discovered that the reported results were inconsistent and often controversial [19, 21–39]. This inconsistency is most probably caused by the large variability in methodologies

Fig. 6 Duration of the liposuction procedure. Time required for obtaining 50 mL of lipoaspirate from the thigh region and from the abdomen under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP – 700 mmHg). Graph **a** represents the lipoaspirates obtained from each donor under both pressures; thus, the line between the symbols represents one donor. Graph **b** represents Δ Time calculated as LP value minus HP value for each donor. Graph **c** represents a comparison of LP and HP values with respect to the anatomic region of the lipoaspirate origin. Graph **d** represents a comparison between LP and HP pressure values combined from the thigh and from the abdominal region. The median values are marked red. Paired *t*-test (for data passing the normality test) or Wilcoxon matched-pairs signed-rank test (for data failing the normality test) were used. Significant differences between LP and HP are illustrated as follows: * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$)



used (different harvesting techniques, harvesting instruments, value of negative pressure, fat processing, viability assessment methods, etc.) [42].

Nevertheless, it is important to ensure whether the quality of the harvested fat graft is significantly affected by the value of negative pressure used during harvesting. For this reason, we conducted a prospective cohort study and we evaluated our crucial variables—the viability and number of nucleated cells, in the lipoaspirate samples that were only minimally manipulated. This way we eliminated all other possible factors that could potentially mask the influence of negative pressure on the lipoaspirate. Other variables that we studied were the blood admixture to the lipoaspirate, the difference between lipoaspirate from two

anatomic sites (abdomen vs. thighs), the liposuction time, the BMI and donor age.

The lipoaspirate is composed of many cells types—adipocytes, preadipocytes, ASCs, pericytes, fibroblasts, endothelial cells and haematopoietic cells. ASCs are one of the important cellular components of the lipoaspirate. In the systematic review focused on fat enrichment strategies, it was concluded that in most studies ASCs positively influenced the success of a fat graft [43]. Therefore, in our previous study we investigated the influence of negative pressure and of the harvesting site on the viability and characteristics of ASCs. Although the study showed approximately twofold higher initial numbers of attached ASCs harvested from the outer thigh region than from the abdominal region, the subsequent cell analyses did not

Table 4 Visual assessment of blood contamination of the lipoaspirate immediately and 2 hours after liposuction

Patient # ID	Region	LP directly after suction	HP directly after suction	LP 2 hours after suction	HP 2 hours after suction
1	Thigh	Less	More	–	–
	Abdomen	Less	More	–	–
2	Thigh	Equal	Equal	–	–
3	Thigh	Less	More	–	–
	Abdomen	Less	More	–	–
4	Thigh	More	Less	More	Less
	Abdomen	More	Less	More	Less
5	Abdomen	More	Less	–	–
6	Abdomen	Equal	Equal	–	–
7	Thigh	Equal	Equal	–	–
	Abdomen	Equal	Equal	–	–
8	Abdomen	Less	More	Equal	Equal
9	Abdomen	Equal	Equal	Less	More
10	Thigh	Equal	Equal	Less	More
	Abdomen	Equal	Equal	More	Less
11	Thigh	More	Less	Equal	Equal
12	Thigh	Equal	Equal	Less	More
13	Abdomen	–	–	More	Less
14	Abdomen	Less	More	More	Less
15	Thigh	Less	More	More	Less
16	Abdomen	Equal	Equal	Less	More
17	Abdomen	–	–	Less	More
18	Thigh	More	Less	More	Less
	Abdomen	More	Less	More	Less
19	Thigh	–	–	More	Less
20	Abdomen	–	–	More	Less
21	Thigh	Equal	Equal	More	Less

Fat was harvested under low negative pressure (LP – 200 mmHg) and high negative pressure (HP – 700 mmHg). Less/equal/more are internal comparisons of each donor sample not between donors

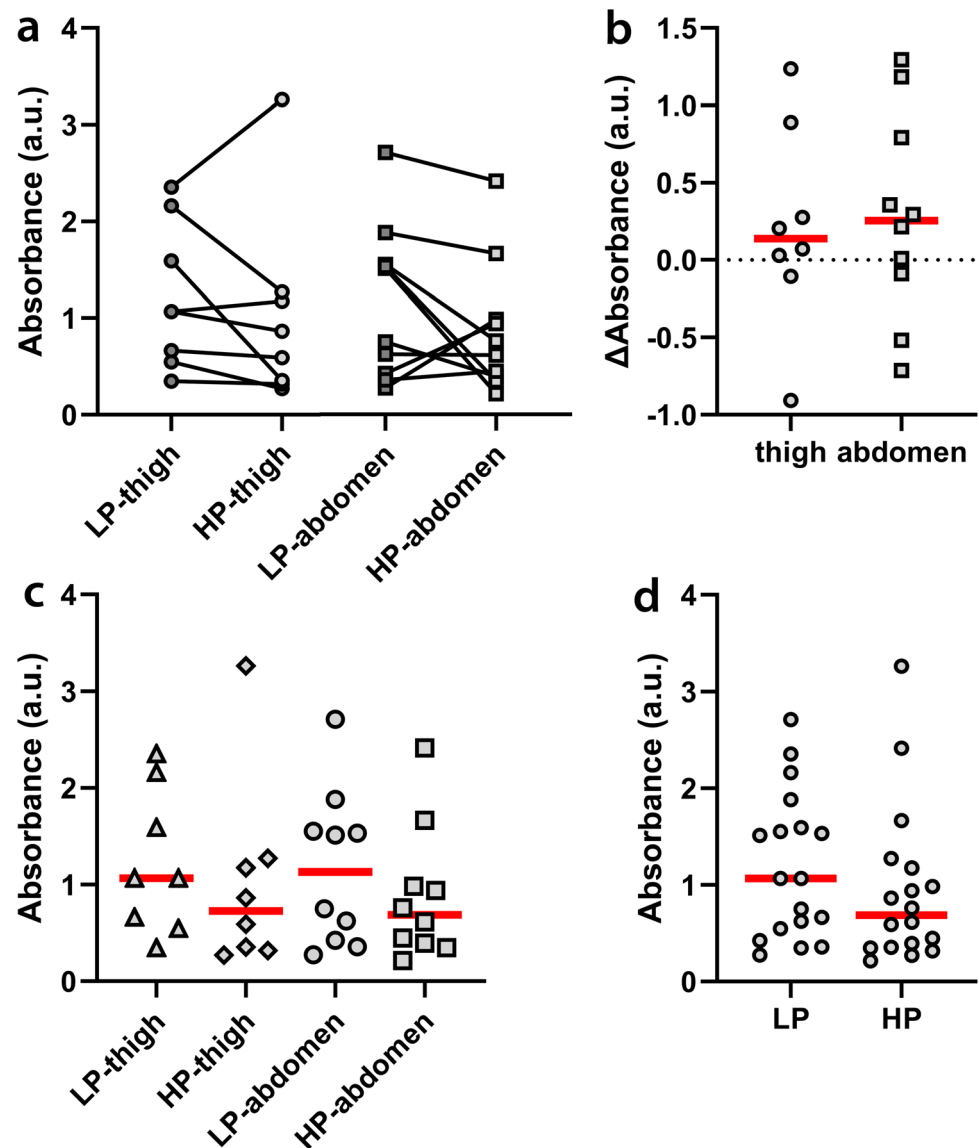
reveal pronounced differences among the cell groups. All cells showed similar proliferation, viability and phenotypic characteristics [39]. In this study, we focused not only on ASCs but on all nucleated cells, predominantly adipocytes.

The Yield and Viability of Nucleated Cells

The level of negative pressure was reported to influence the yield of adipocytes and ASCs in several studies. The absolute number of cells yielded under different LP (*i.e.* – 220, – 250, – 225 and – 350 mmHg) was reported to be higher (in some reports with statistical significance) than when using HP (*i.e.* – 720, – 750, – 410 and – 700 mmHg) [30, 32, 38, 44]. Also significantly more preadipocytes were derived using LP (*i.e.* – 537 mmHg)

comparing to HP (*i.e.* – 643 mmHg) [24]. In contradiction, Charles-de-Sá *et al.* in their study, on a larger patient cohort (*i.e.* $n=15$ vs. $n=3, 10, 3$ and 5), found no significantly different counts of adipocytes per mL and no significant viability difference of ASCs no matter what negative pressure (*i.e.* – 275, – 350, – 394, – 550 and – 700 mmHg) was used [37]. The findings of Charles-de-Sá are more in accordance with our results. Our study did not show significant differences in viability or in absolute numbers/yield of all nucleated cells between LP and HP groups (– 200 mmHg vs. – 700 mmHg). Our current results are in accordance with our previous study focused on ASCs, where no significant influence of negative pressure on the number or on the ASCs viability was found [39].

Fig. 7 Absorbance measurement of haemoglobin in the native lipoaspirate. **a** Lipoaspirate from the thigh region and from the abdominal region harvested under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP – 700 mmHg). The lipoaspirate was obtained from each donor under both pressures; thus, the line between the symbols represents one donor. The higher the absorbance value is, the higher the blood admixture is. Graph **b** represents Δ Absorbance counted as LP value minus HP value for each donor. Graph **c** represents comparison of LP and HP values with a respect to anatomic region of lipoaspirate origin. Graph **d** represents the comparison between LP and HP pressure values combined from the thigh and from the abdominal region. The median values are marked red. Paired *t*-test (for data passing the normality test) or Wilcoxon matched-pairs signed-rank test (for data failing the normality test) were used. No significant difference ($p < 0.05$) between LP and HP was observed



The Influence of Different Harvesting Sites

The influence of the harvesting site on the amount and quality of cells in lipoaspirate was considered to be important in previous studies. Padoin *et al.* reported significantly higher concentration of ASCs taken from the lower abdomen and the inner thigh than other sites (*i.e.* upper abdomen, trochanteric region, knees and flanks) [45]. Qu *et al.* reported that cryopreserved ASCs from the abdominal and thigh regions maintained significantly higher proliferation, ability, migration and differential potential compared with ASCs harvested from the upper limbs and flanks [46]. On the other hand, several studies contradicted this. Rohrich *et al.* found no statistical difference in the means of adipocytes viability between fat harvested from abdominal, thigh, flank or knee regions [47]. Ullman *et al.* concluded no significant difference in

fat graft success among fat harvested from one donor, from the breast, the abdomen or the thigh and implanted into a mouse subcutaneous region [48]. Similar research was done by Li *et al.* In that study, the fat taken from six donors from the upper and lower abdomen flanks, inner and outer thighs was injected subcutaneously into mice. After 12 weeks, no significant difference was found in graft success nor in the characteristics of SVF cells [49]. In our study, we did not find a statistical difference between two different regions (abdomen vs. thigh) when considering the nucleated cell count and viability. This was also our finding when using both negative pressures: LP (– 200 mmHg) or HP (– 700 mmHg).

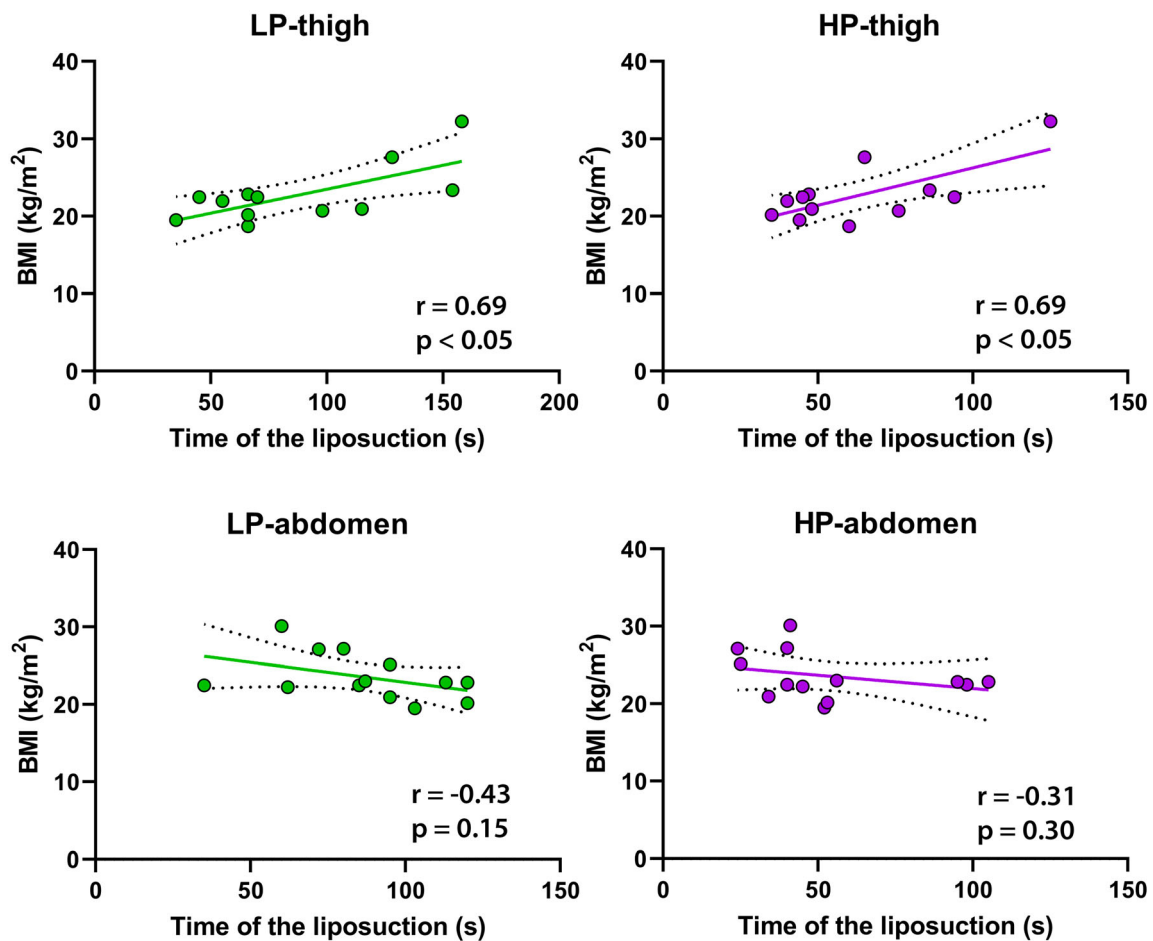


Fig. 8 Correlation between the BMI of donors and the liposuction time when harvesting 50 mL of lipoaspirate. Liposuction from the thigh region or from the abdominal region under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP

– 700 mmHg). Pearson's correlation with a calculation of linear regression and a 95% confidence interval was performed. The p value has been found to be significant ($p < 0.05$) in the case of the thigh region but not the abdominal region ($p > 0.05$)

Liposuction Time

Elam *et al.* in his study reported that using LP (*i.e.* – 380 mmHg) produced a graft with surprisingly lower manual effort and in shorter time than when using HP (*i.e.* – 760 mmHg) [50]. We found, however, that for harvesting the same amount of lipoaspirate, the liposuction procedure performed under HP (– 700 mmHg) was significantly faster than the liposuction procedure performed under LP (– 200 mmHg) from both the thigh and abdominal region. It has been suggested that time duration within one operation zone can play an important role in obtaining a fat graft with more viable cells. The aspiration periods longer than 2 minutes within a particular operation zone are prone to produce a lipoaspirate with a decreased adipocyte viability due to accelerated tissue injury [16]. If this is the case, we can speculate that HP may be more beneficial as the desired amount of adipose tissue can be

harvested significantly faster than when using LP. However, our time frames were shorter and we did not see this.

The Blood Admixture

The effect of various negative pressures on the quantity of red blood cells in lipoaspirates was studied by several authors. Chen *et al.* visually observed less erythrocytes in samples harvested under LP (*i.e.* – 225 mmHg) than HP (*i.e.* – 410 mmHg) [38]. However, the study did not outline whether the same results were obtained for all patients or whether these differences were somehow quantified. A similar finding was reported by Elam *et al.*. The authors estimated macroscopically the differences between LP (*i.e.* – 380 mmHg) and HP (*i.e.* – 760 mmHg) liposuction and they found that LP liposuction produced a graft with lower blood contamination [50].

Usually a tumescent solution with epinephrine is used for infiltration of the fat tissue before liposuction to

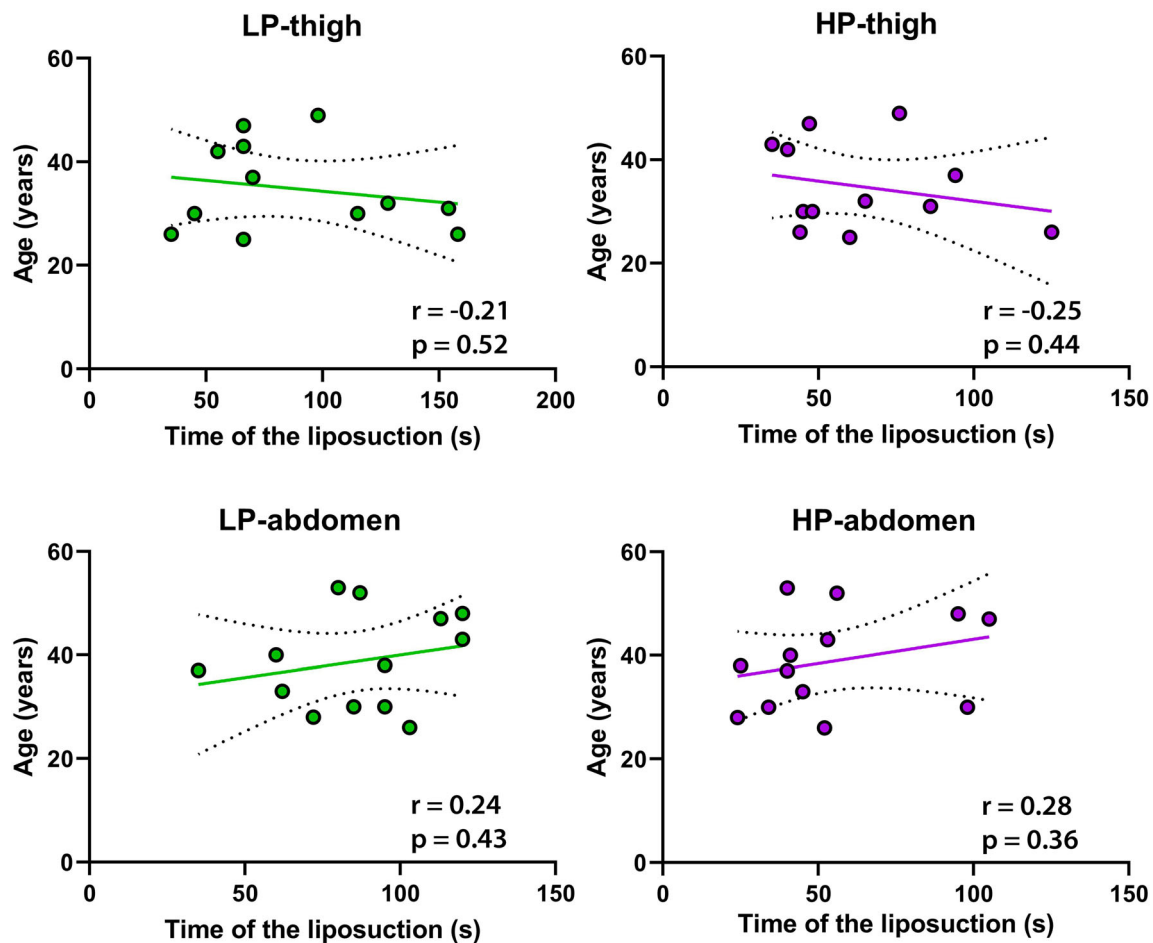


Fig. 9 Correlation between the age of donors and liposuction time when harvesting 50 mL of lipoaspirate. Liposuction from the thigh region or from the abdominal region under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP – 700 mmHg).

Pearson's correlation with a calculation of linear regression and a 95% confidence interval was performed. The p value has not been found to be significant ($p < 0.05$)

minimise the overall blood loss and blood contamination of lipoaspirate that is used for lipografting. It was reported that more blood contamination can impair the viability of harvested adipocytes and ASCs, so minimisation of blood admixture to lipoaspirate seems to be important [51]. However, it was also reported that using a solution with epinephrine can decrease the volume of fat graft success in the long term [52]. Even here there is controversy, nevertheless, epinephrine is a generally used component of tumescent solution as it seems that the benefits outweigh the drawbacks. Our tumescent solution used a standard dose of 1 mL of epinephrine (1 mg/ml) per 1 L of tumescent solution.

In our study, we noticed several discrepancies between visual estimation of blood contamination of lipoaspirates immediately after harvesting and two hours later. This may be due to the fact that directly after liposuction, the lipoaspirate is not decanted and it is a mixture of rich fat with more or less tumescent solution and air bubbles. It is

therefore more demanding to assess the blood contamination from this dense mixture, and consequently, it is fraught with more inaccuracy. Two hours later, the air bubbles are released and the decanting process divides the fat tissue from the tumescent solution containing blood so the colours of components are more distinguishable and visual estimation of blood contamination is more precise.

Our observations immediately after harvesting revealed 7 samples having a higher blood contamination for LP, 10 samples having equal contamination for LP and HP and 6 samples having a higher blood contamination for HP. These results provided variable and unclear findings concerning the influence of negative pressure on blood contamination. The quantitative evaluation performed two hours after harvesting showed slightly lower blood contamination in most of the HP samples (11 out of 18) than in the LP samples. This difference was not significant. We have to point out, however, that the admixture of blood cells to lipoaspirate can be significantly affected by

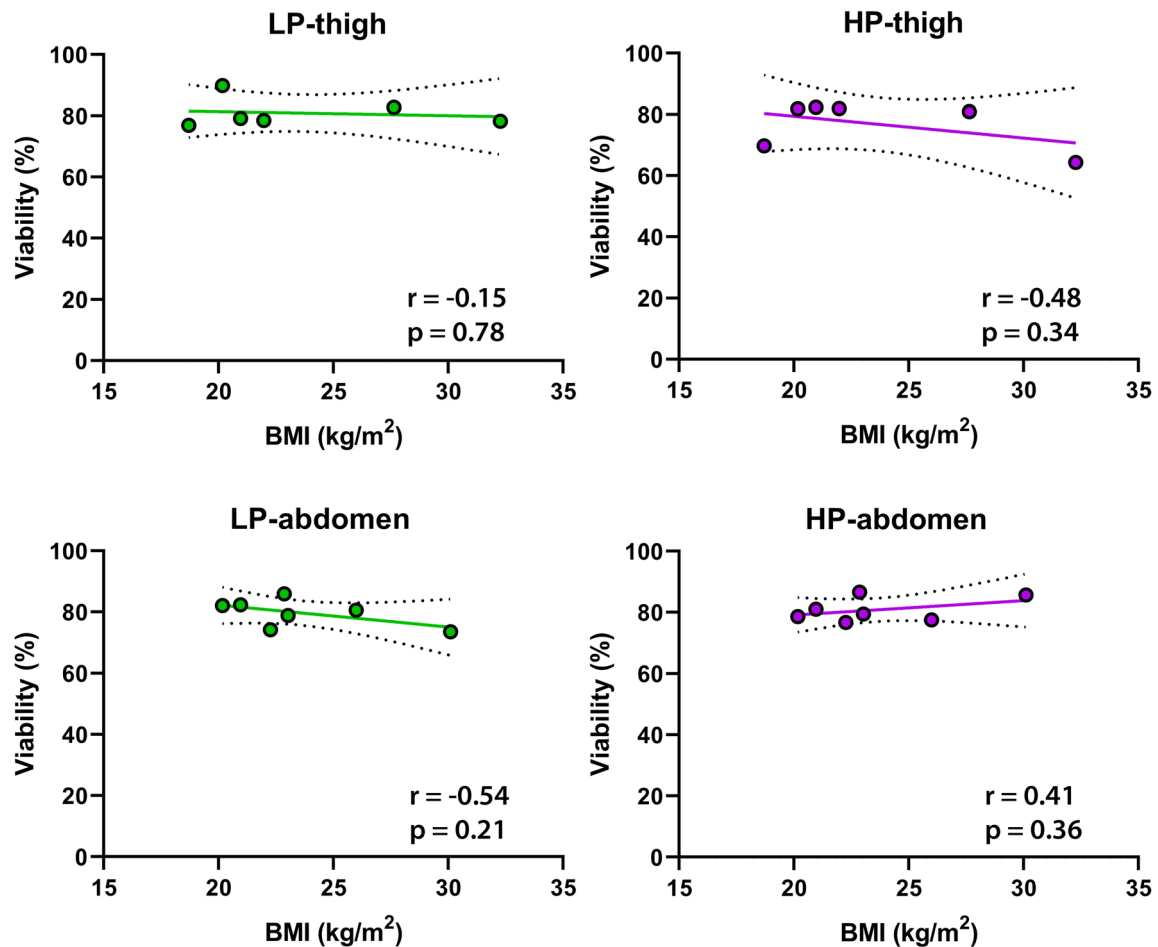


Fig. 10 Correlation between the BMI of donors and the viability of nucleated cells in the harvested adipose tissue. Liposuction from the thigh region and from the abdominal region under low negative pressure (LP – 200 mmHg) or high negative pressure (HP

– 700 mmHg). Pearson’s correlation with a calculation of linear regression and a 95% confidence interval was performed. The p value has not been found to be significant ($p < 0.05$)

accidental damage of a large vessel in subcutaneous tissue and this is completely out of the surgeon’s control. This is independent of the makeup of the tumescent solution or the suction pressure used.

The Negative Pressure and Rate of Enzymatic Digestion

It was previously reported that enzymatic digestion is more successful in lipoaspirates obtained under LP (*i.e.* – 225 mmHg) than under HP (*i.e.* – 410 mmHg) [38]. In our study, we visually analysed and volumetrically measured 5 samples obtained under LP (– 200 mmHg) and HP (– 700 mmHg). Samples were digested using collagenase type I and centrifuged. We found no significant differences in enzymatic digestion depending on the value of the negative pressure. We only observed slight inter-donor variabilities. The volumes of particular fractions (*i.e.* oily

part, digested adipose tissue, digesting solution with blood and SVF) were almost identical within same donor.

Other Evaluated Specific Parameters

A significant correlation was observed between BMI and liposuction time in the thigh region (*i.e.* the higher BMI, the longer the time was required for harvesting of 50 mL of lipoaspirate). We cannot find any logical anatomic explanation for this finding. On the contrary, the abdominal region showed the opposite tendency (*i.e.* the higher BMI was, the shorter harvesting time). However, we point out that the trend in the abdominal region did not reach statistical significance. We postulate that the opposite trends observed between these two regions could potentially stem from more fibrous tissue present in the abdominal subcutaneous fat (*i.e.* Scarpa’s fascia, Camper’s fascia). It may be that in obese patients the amount of fat highly overlaps the fibrous tissue and harvesting of fat is easier and quicker.

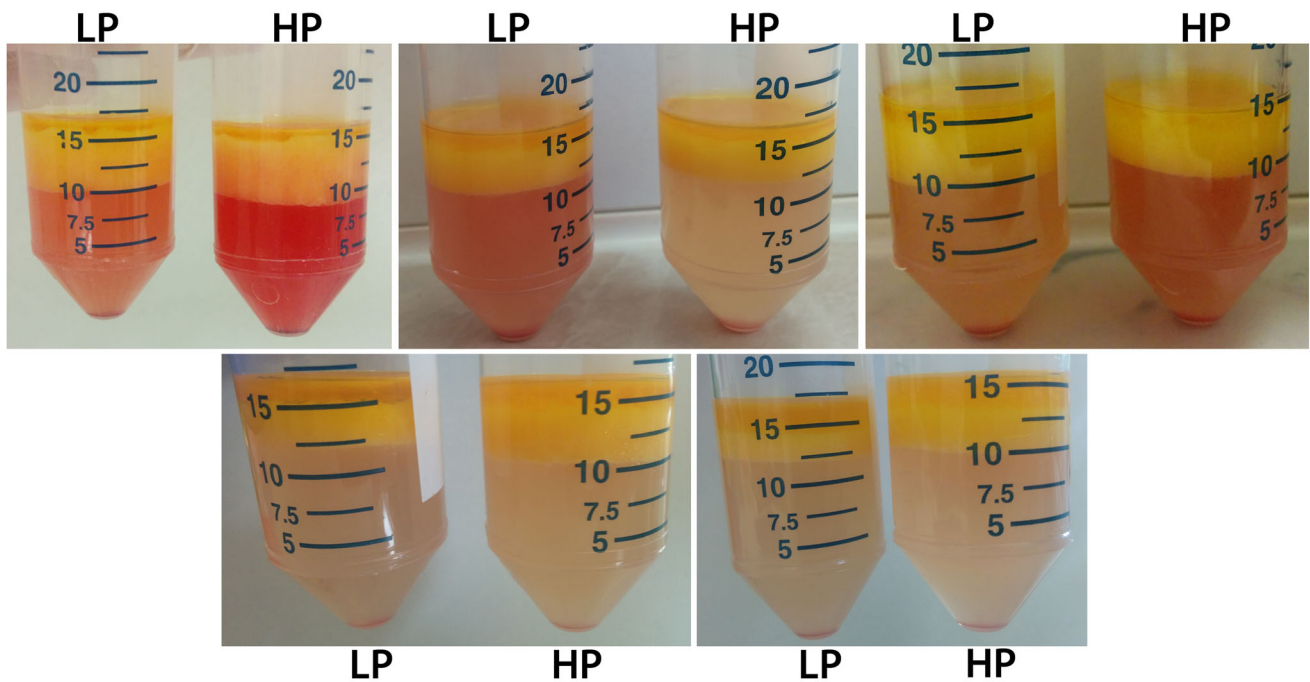


Fig. 11 Ratio of specific lipoaspirate fractions after digesting. Digestion using type I collagenase (1 hour at 37 °C) and centrifuging (5 min at 300 g). Each photograph represents the same amount of the lipoaspirate from one donor harvested under low negative pressure (LP – 200 mmHg) or under high negative pressure (HP

– 700 mmHg). The specific lipoaspirate fractions are represented by the uppermost oily part, by the layer of digested adipose tissue with adipocytes, by the infranatant layer composed of blood and digesting solution and by the lowest layer composed of stromal vascular fraction

Table 5 Ratio of lipoaspirate fractions in 5 donors

Patient # ID	Pressure	Oily p. [mL]	Lipoasp. [mL]	Liquid p. [mL]
1	LP	0.5	5.5	10
	HP	0.5	5.5	10
2	LP	1	4	11
	HP	1	4	11
3	LP	0.5	5.5	10
	HP	0.5	5.5	10
4	LP	1	4	11
	HP	1	4	11
5	LP	2.5	2.5	11
	HP	2.5	2.5	11

Fat tissue harvested under low negative pressure (LP – 200 mmHg) and high negative pressure (HP – 700 mmHg). The specific lipoaspirate fractions are represented by the uppermost oily part (Oily p.), by the layer of digested adipose tissue with adipocytes (Lipoasp.) and by the infranatant layer composed of blood and of digesting solution (Liquid p.). The lowest layer composed of stromal vascular fraction is not included here as it produces only insignificant minimal layer at the bottom of the tube, and it also contains other tissues that may obscure its real volume. The values are rounded to the nearest 0.5 mL

The BMI did not significantly influence the viability of nucleated cells collected from the lipoaspirates irrespective of the negative pressure (LP – 200 mmHg or HP – 700 mmHg). Neither did the age of patients influenced the liposuction time when using different negative pressure (LP – 200 mmHg vs. HP – 700 mmHg) nor from different regions (abdomen vs. thigh).

Limitations of the Study

Despite care in the design of our study, we need to point out some potential shortcomings of our methodology.

In an effort to minimally manipulate the lipoaspirate before measurement of our variables, for the evaluation of viability and the yield of cells Hoechst 33342/ethidium

homodimer-1 staining was selected. This staining provides fast and simple distinction between living cells *vs.* dead cells. However, a limitation of this selected method may lie in the fact that this stains all nucleated cells not only adipocytes and ASCs. The lipoaspirate contains many cell types: adipocytes, preadipocytes, ASCs, pericytes, fibroblasts, endothelial cells and hematopoietic cells. The precise percentage of cell fractions is difficult to estimate, as it is highly dependent on multiple factors (*i.e.* BMI, age, technique of harvesting, region of liposuction, etc.) [53]. While adipocytes seem to be the most dominant cell fraction of the lipoaspirate, it is estimated that adipocytes produce about 90% of the healthy fat tissue volume but only 50% of overall cell numbers. The other 10% of the volume and 50% of the cell number is made by supportive cells from SVF [54, 55]. With only several clean-up steps performed before staining, all these cell types would be involved in our analysis of cell viability and of the total number of cells. Our analysis therefore did not specifically focus on the influence of negative pressure on the quality and quantity of particular cell types (adipocytes and ASCs). From the practical point of view, however, we suggest this not be a preventative drawback as all cell types included in lipoaspirate are important for graft success, so their viability and function is still important. The analysis of particular cell types can be obtained after enzymatic digestion. However, digestion adds another layer of probable influence, with a more intense manipulation which could obscure the initial differences in cell characteristics and skew the findings.

Another limitation of our study is that not all samples underwent all measurements and analyses. The detailed list of which measurement and analysis were done with which sample is given in Table S1—Supplementary material. From this table, one can see that viability and number of nucleated cells was studied on 13 out of 27 samples. Time of liposuction was measured in 25 out of 27 samples. Visual blood admixture was evaluated in 23 out of 27 samples, and blood admixture analysis was done on 18 out of 27 samples. This seeming inconsistency is caused by the fact that the study was designed as a two-step study; it started as a clinical study and laboratory analyses were added as a second step for the second half of specimens. The ratio of lipoaspirate fraction after digestion was measured in 5 out of 27 samples (see Table 5). This analysis was added to the results just as an interesting finding that was tested on a limited number of samples and this information is therefore not included in the conclusion.

Another considered limitation may be that no randomisation was performed when selecting the patient for including in to the study and also no randomisation was done for selecting which analysis was performed on which sample. However, this study was designed as a prospective

cohort study so each patient without the exclusion criteria but undertaking the liposuction procedure during the defined time periods was included in the study. The second half of cohort samples underwent laboratory analyses according to the study design. This cohort study is not based on randomisation but on including the individuals on the basis of their exposure to some influence, in our case liposuction.

Conclusion

The volume effect of fat grafting is highly dependent on the presence of viable, undamaged and unspoiled cells within the lipoaspirate. One of the crucial factors influencing cell viability and function was considered to be the negative pressure applied during fat graft harvesting.

Our study scientifically evaluated several variables using two different negative pressures (– 200 mmHg *vs.* – 700 mmHg) applied during the liposuction using pump machine within two harvesting sites (abdomen *vs.* thighs). The most crucial evaluated variables were: a) the time duration of harvesting 50 mL of lipoaspirate, b) the blood admixture and c) the viability plus the total number of viable nucleated cells present in the lipoaspirate.

Our precisely managed standardised and controlled prospective cohort study proved that:

1. No significant difference was found in nucleated cells viability between LP and HP groups.
2. No significant difference was found in the absolute number of viable nucleated cells harvested using LP or HP liposuction.
3. No significant difference was observed in nucleated cells viability between treated regions—thighs or abdomen.
4. The liposuction time in harvesting 50 mL of lipoaspirate using LP is significantly longer than when using HP.
5. No significant difference was found pertaining to the blood admixture to lipoaspirate using LP or HP liposuction.
6. No significant correlation was found between BMI and cell viability.
7. Significant correlation was found between BMI and liposuction time in the thigh region—the higher BMI, the longer time. This correlation was not apparent in abdominal region.
8. No significant correlation was observed between the age of patients and the liposuction time.

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Compliance with Ethical Standards

Conflict of interest All authors declare no conflict of interest concerning the research, authorship and publication of this article. Financial funding of research has not any influence on the results of the research in any form.

Human or Animal Rights The study was approved by institutional Ethical Committee (Na Bulovce University Hospital, Prague). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki declaration and its later amendments.

Informed consent Written informed consent was obtained from all human participants included in the study. Informed consent was approved by institutional Ethical Committee Na Bulovce University Hospital, Prague.

References

- Coleman SR (2001) Structural fat grafts: the ideal filler? *Clin Plast Surg* 28:111–119
- Coleman SR (2002) Hand rejuvenation with structural fat grafting. *Plast Reconstr Surg* 110:1731–1744
- Coleman SR (1995) Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg* 19:421–425
- Coleman SR (1997) Facial recontouring with liposuction. *Clin Plast Surg* 24:347–367
- Bank J, Fuller SM, Henry GI, Zachary LS (2014) Fat grafting to the hand in patients with Raynaud phenomenon. *Plast Reconstr Surg* 133(5):1109–1118
- Kakagia D, Pallua N (2014) Autologous fat grafting: in search of the optimal technique. *Surg Innov* 21:327–336
- Khouri RK Jr, Khouri RK (2017) Current clinical applications of fat grafting. *Plast Reconstr Surg* 140(3):466e–486e
- Tuncel U, Kurt A, Gumus M, Ayodogdu O, Guzei N, Demir O (2017) Preliminary results with non-centrifuged autologous fat graft and percutaneous aponeurotomy for treating Dupuytren's disease. *Hand Surg Rehabil* 36(5):350–354
- Condé-Green A, Gontijo de Amorim NF, Pitanguy I (2010) Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: A comparative study. *J Plast Reconstr Aesthet Surg* 63:1375–1381
- Peer LA (1950) Loss of weight and volume in human fat graft, with postulation of a cell survival theory. *Plast Reconstr Surg* 5:217–221
- Peer LA (1955) Cell survival theory versus replacement theory. *Plast Reconstr Surg* 16:161–168
- Tremolada C, Palmieri G, Ricordi C (2010) Adipocyte transplantation and stem cells: plastic surgery meets regenerative medicine. *Cell Transplant* 19:1217–1223
- Gutowski KA (2009) ASPS fat graft task force. current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. *Plast Reconstr Surg* 124:272–280
- Salinas HM, Broelsch GF, Fernandes JR, McCormac MC, Meppelink AM, Randolph MA, Colwell AS AWG Jr (2014) Comparative analysis of processing methods in fat grafting. *Plast Reconstr Surg* 134:675–683
- Gabriel A, Champaneria MC, Maxwell GP (2015) Fat grafting and breast reconstruction: tips for ensuring predictability. *Gland Surg* 4(3):232–243
- Ince B, Oltulu P, Yildirim MEC, Ismayilzade M, Dadaci M (2019) Effects of aspiration time on immediate viability of adipocyte cell in ultrasound-assisted liposuction (UAL) and in traditional suction-assisted lipectomy (SAL). *J Plast Surg Hand Surg* 53(1):14–19
- Özkaya O, Egemen O, Barutca SA, Akan M (2013) Long-term clinical outcomes of fat grafting by low-pressure aspiration and slow centrifugation (Lopasce technique) for different indications. *J Plast Surg Hand Surg* 47(5):394–398
- Alharbi Z, Opländer C, Almakadi S, Fritz A, Vogt M, Pallua N (2013) Conventional vs. micro-fat harvesting: How fat harvesting technique affects tissue-engineering approaches using adipose tissue-derived stem/stromal cells. *J Plast Reconstr Aesthetic Surg* 66:1271–1278
- Von Heimburg D, Hemmrich K, Haydarlioglu S, Staiger H, Pallua N (2004) Comparison of viable cell yield from excised versus aspirated adipose tissue. *Cells Tissues Organs* 178(2):87–92
- Fraser J, Wulur I, Alfonso Z, Zhu M, Wheeler E (2007) Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots. *Cytotherapy* 9(5):459–467
- Lee JH, Kirkham JC, McCormack MC, Nicholls AM, Randolph MA, Austen WG (2013) The effect of pressure and shear on autologous fat grafting. *Plast Reconstr Surg* 131:1125–1136
- Nguyen A, Pasyk KA, Bouvier TN, Hasset CA, Argenta LC (1990) Comparative study of survival of autologous adipose tissue taken and transplanted by different techniques. *Plast Reconstr Surg* 85(3):378–386
- Kononas TC, Bucky LP, Hurley C, May JW (1993) The fate of suctioned and surgically removed fat after reimplantation for soft-tissue augmentation: a volumetric and histologic study in the rabbit. *Plast Reconstr Surg* 91(5):763–768
- Gonzalez AM, Loboeki C, Kelly CP, Jackson IT (2007) An alternative method for harvest and processing fat grafts: an in vitro study of cell viability and survival. *Plast Reconstr Surg* 120(1):285–294
- Noaves F, dos Reis N, Baroudi R (1998) Counting of live fat cells used in lipoinjection procedures. *Aesth Plast Surg* 22:12–15
- Shiffman MA, Mirrafati S (2001) Fat transfer techniques: the effect of harvest and transfer methods on adipocyte viability and review of the literature. *Dermatol Surg* 27:819–826
- Pu LLQ, Cui X, Fink BF, Cibull ML, Gao D (2005) The viability of fatty tissues within adipose aspirates after conventional liposuction: a comprehensive study. *Annals Plast Surg* 54(3):288–292
- Smith P, Adams WP, Lipschitz AH, Chau B, Sorokin E, Rohrich RJ, Brown SA (2006) Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte graft survival. *Plast Reconstr Surg* 117(6):1836–1844
- Tambasco D, Arena V, Grussu F, Cervelli D (2013) Adipocyte damage in relation to different pressures generated during manual liposuction with syringe. *Plast Reconstr Surg* 131(4):645e–646e
- Cheriyian T, Kao HK, Qiao X, Guo L (2014) Low harvest pressure enhances autologous fat graft viability. *Plast Reconstr Surg* 133(6):1365–1368
- Leong DT, Huttmacher DW, Chew FT, Lim TC (2005) Viability and adipogenic potential of human adipose tissue processed cell population obtained from pump-assisted and syringe-assisted liposuction. *J Dermatol Sci* 37:169–176

32. Mojallal A, Auxenfans C, Lequeux C, Braye F, Damour O (2008) Influence of negative pressure when harvesting adipose tissue on cell yield of the stromal-vascular fraction. *Bio Med Mater Eng* 18(4–5):193–197
33. Keck M, Kober J, Riedl O, Kitzninger HB, Wolf S, Stulnig TM, Zayda M, Gugerell A (2014) Power assisted liposuction to obtain adipose-derived stem cells: impact on viability and differentiation to adipocytes in comparison to manual aspiration. *J Plast Reconstr Aesthetic Surg* 67(1):e1-8
34. Barzelay A, Levy R, Kohn E, Sella M, Shani N, Meilik B, Entin-Meer M, Gur E, Loewenstein A, Barak A (2015) Power-assisted liposuction versus tissue resection for the isolation of adipose tissue-derived mesenchymal stem cells: phenotype, senescence and multipotency at advanced passages. *Aesthet Surg J*. 35(7): NP230–NP240
35. Bony C, Cren M, Domergue S, Toupet K, Jorgensen C, Noel D (2016) Adipose mesenchymal stem cells isolated after manual or water-jet-assisted liposuction display similar properties. *Front Immunol*. doi:<https://doi.org/10.3389/fimmu.2015.00655>
36. Duscher D, Luan A, Rennert RC, Atashroo D, Maan ZN, Brett EA, Whittam AJ, Ho N, Lin M, Hu MS, Walmsley GG, Wenny R, Schmidt M, Schilling AF, Machens HG, Huemer GM, Wan DC, Longaker MT, Guntner GC (2016) Suction assisted liposuction does not impair the regenerative potential of adipose derived stem cells. *J Transl Med* 14(1):126–138
37. Charles-de-Sá L, Gontijo de Amorim NF, Dantas D, Han JV, Amable P, Teixeira MVT, Luiz de Ajauro P, Link W, Borojevich R, Rigotti G (2015) Influence of negative pressure on the viability of adipocytes and mesenchymal stem cell, considering the device method used to harvest fat tissue. *Aesthet Surg J* 35(3):334–344
38. Chen YW, Wang JR, Liao X, Li SH, Xiao LL, Cheng B, Xie GH, Song JX, Liu HW (2017) Effect of suction pressures on cell yield and functionality of the adipose-derived stromal vascular fraction. *J Plast Reconstr Aesthet Surg*.
39. Travnickova M, Pajorova J, Zarubova J, Krocilova N, Molitor M, Bacakova L (2020) The influence of negative pressure and of the harvesting site on the characteristics of human adipose tissue-derived stromal cells from lipoaspirates. *Stem Cells International*. doi.org/<https://doi.org/10.1155/2020/1016231>
40. ASPS evidence-based clinical practice guideline methodology, December 2016. <https://www.plasticsurgery.org/documents/medical-professionals/quality-resources/ASPS-Evidence%E2%80%90Based-Clinical-Practice-Guideline-Methodology.pdf>, accessed 12.3.2021
41. Burns PB, Rohrich RJ, Chung KC (2011) The levels of evidence and their role in evidence-based medicine. *Plast Reconstr Surg* 128(1):305–310
42. Molitor M, Trávníčková M, Měšťák O, Christodoulou P, Sedlář A, Bačáková L, Lucchina S (2021) The influence of high and low negative pressure liposuction and various harvesting techniques on the viability and function of harvested cells – a systematic review of animal and human studies. *Aesthetic Plast Surg*. Apr 19. doi:<https://doi.org/10.1007/s00266-021-02249-9>. Online ahead of print.
43. Vyas KS, Vascones HC, Morrison S, Mogni B, Linton S, Hockensmith L, Kabir T, Zielins E, Najor A, Bakri K, Mardini S (2020) Fat graft enrichment strategies: a systematic review. *Plast Reconstr Surg* 145(3):827–841
44. Cucchiani R, Corrales L (2016) The effects of fat harvesting and preparation, air exposure, obesity and stem cell enrichment on adipocyte viability prior to graft transplantation. *Aesth Surg J* 36(10):1164–1173
45. Padoin AV, Braga-Silva J, Martins P, Rezende K, Rezende ARR, Grechi B, Gehlen D, Machado DC (2008) Sources of processed lipoaspirate cells: influence of donor site on cell concentration. *Plast Reconstr Surg* 122:614–618
46. Qu Y, Mu D, Wang Q, Li Z, Liu T, Fu S, Luan J (2020) Effect of harvest sites on cryopreserved adipose-derived stem cells and asc-enriched fat grafts. *Aesth Plast Surg* 44:2286–2296
47. Rohrich RJ, Sorokin ES, Brown SA (2004) In search of improved fat transfer viability: a quantitative analysis of the role of centrifugation and harvest site. *Plast Reconstr Surg*. 113:391–395
48. Ullmann Y, Shoshani O, Fodor A, Ramon Y, Carmi N, Eldor L, Gilhar A (2005) Searching for the favourable donor site for fat injection: in vivo study using the nude mice model. *Dermatol Surg* 31:1304–1307
49. Li K, Gao J, Zhang Z, Li J, Cha P, Liao Y, Wang G, Lu F (2013) Selection of donor site for fat grafting and cell isolation. *Aesthetic Plast Surg* 37:153–158
50. Elam MW, Packer D, Schwab J (1997) Reduced negative pressure liposuction (RNPL) could less be more? *Int J Aesthet Restor Surg*. 5(2):101–104
51. Jin S, Yang Z, Han X, Li F (2021) Blood impairs viability of fat grafts and adipose stem cells: importance of washing in fat processing. *Aesthet Surg J* 41(1):86–97
52. Sirinoglu H, Yesiloglu N, Kaya OT, Ercan F, Filinte GT (2017) Effect of tumescent solution on fat survival. *Facial Plast Surg* 33(3):339–346
53. Carpaneda CA (1996) Study of aspirated adipose tissue. *Aesth. Plast. Surg* 20:399–402
54. Khouri RK, Rigotti G, Cardoso E, Khouri RK Jr, Biggs TM (2014) Megavolume autologous fat transfer: part I. Theory and principles. *Plast Reconstr Surg* 133:550–557
55. Khouri RK, Rigotti G, Cardoso E, Khouri RK Jr, Biggs TM (2014) Megavolume autologous fat transfer: part II. Practice and techniques. *Plast Reconstr Surg* 133:1369–1377

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