Changes in NMR lipoprotein subclass profile after manual lymphatic drainage

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ABSTRACT

During the last few years, many studies have described a close connection between blood and lymph with an exchange of fluids between both systems. For that reason, our study is set to determine whether there is a variation on plasma lipoprotein concentration before and after performing a lymphatic drainage massage (LDM). The study included 25 apparently healthy young men older than 18 years old. The volunteers were submitted to a blood extraction, the LDM procedure followed by a second blood extraction. The LDM started in the lower extremities, followed by the anterior part of the trunk and ended in the upper extremities. In total, the massage lasted for 35 minutes. Fasting venous blood samples were collected in EDTA tubes and centrifuged to obtain the plasma. To measure lipoprotein particle size, diffusion-ordered NMR spectroscopy (DOSY) was used. Results show significant changes on triglycerides but not in cholesterol concentrations. Individuals consistently display a significantly decrease in VLDL-related parameters (cholesterol, triglycerides and number of particles). The effect on VLDL is diameter-dependent as we have observed that the smaller the VLDL particle the larger the decrease associated with LDM. In summary, we have confirmed the hypothesis that LDM modulates circulating lipoprotein concentration, being this effect associated with TG-rich lipoproteins.

INTRODUCTION

During the last few years, many studies have described a close connection between blood and lymph with an exchange of fluids between both systems.[1] Lymphatic system comprises a network of lymphatic vessels that carry the lymph. The lymphatic system is involved in different processes such as trafficking of lymphocytes and antigen-presenting cells to regional lymph nodes, where the immune system operates[2]; clearing the interstitial fluid, draining different fluids from the tissues and transporting them to the lymph nodes[3]; and it also contributes to the intestinal absorption of lipids since chylomicrons synthetized from lipid digestion access the bloodstream through the lymphatic system.[4]

Apart from this, we know that it also takes part into the transport of lipoproteins. A recent study[1] has shown the role of the lymphatic system in cholesterol transport, which clearly confirms there is a relation between lipoproteins and the lymphatic system: disruption in the lymphatic vasculature provokes a decrease in cholesterol transport and as a result, arteriosclerosis. For instance, it was described in 2013 that the drainage by the lymphatic vessels is essential for the removal of cholesterol from peripheral tissues[5], so this emphasizes the passive exchange barrier that exists.

The transport of lipids and lipoproteins is critical for the development of atherosclerosis since the transport of cholesterol by LDL particles to the arteries and its accumulation in the artery walls causes its obstruction and increases the morbidity and mortality of incident cardiovascular diseases.[6] On the other hand, the reverse cholesterol transport (RCT) with the participation of the HDL particles is the process whereby excess of cholesterol from the tissues goes to the liver in order to be finally excreted.[7]

For all that, the aim of our study was to determine whether there is a variation in the concentration of lipoproteins in the blood after performing a manual lymphatic drainage massage. Therefore, we hypothesize that we can modify the concentrations of lipoproteins in the blood by acting on the lymphatic system.

MATERIALS AND METHODS

Volunteers

The study included 25 apparently healthy young men, older than 18 years old; these were the selection criteria in order to ensure the homogeneity of the sample. Exclusion criteria included, chronic diseases, lymphatic pathologies as well as having varicosities which may alter the functionality of the lymphatic vessels or having a tattoo since the lymphatic drainage is performed quite superficially and the skin may have suffered changes after having a tattoo.

The ethics committee of the hospital approved the protocol and each volunteer provided informed consent to participate in the study.

Participants came after an overnight fast of at least 8 hours. The volunteers were submitted to an initial blood extraction; after that, we performed a lymphatic drainage massage (LDM), and finally, they were submitted to a final blood extraction.

Manual lymphatic drainage massage

The LDM begun at the lower extremities, following to the anterior part of the trunk and ending in the upper extremities. In total, the massage lasted for 35 minutes.

It has been observed that in order to avoid a collapse of the lymphatic vessels the pressure at the time of performing the drainage massage cannot exceed 30-40 mmHg. This indicates that a gentle pressure must be exerted because the pressure outside the vessel cannot exceed the hydrostatic pressure (optimal pressure to keep the vessels open).[8]

Lymphatic drainage is based on two processes. First; the uptake, in which the lymphatic capillaries must absorb the content of the interstitial fluid. Second; the evacuation, in which the lymph is transported to the collectors.

For these processes to occur, it is necessary to perform specific movements. Taking all this into account, the technique is performed with the palm of the hand, beginning with the ulnar border of the 5th finger and ending with the radial edge of the index. During the maneuver, the arms make a movement of abduction and adduction of the elbow.

So executing these hand movements the technique consists on forming circles, made with the fingers, and pressures in the form of bracelet.

Biochemical analyses

Fasting venous blood samples were collected in EDTA tubes at the beginning and at the end of the procedure, and centrifuged immediately for 15min at 4°C for 1500 x g to obtain the plasma. The samples were then divided into aliquots and stored at -80°C until the determination of the analytical variables.

Standard laboratory methods were used to quantify glucose, total cholesterol, TG and HDLc. LDLc values were calculated using the Friedewald formula. Apolipoproteins were quantified using an immunoturbidimetric assay with specific antibodies for both apolipoprotein A1 (apo A1) and apolipoprotein B100 (apo B100). These analyses were adapted to the Cobas-Mira-Plus auto-analyzer (Roche Diagnosis, Spain).

The nuclear magnetic resonance (NMR) was used to measure lipoprotein particles sizes. The diffusion-ordered NMR spectroscopy (DOSY) was employed as alternative due to its robustness and simple sample manipulation.[9] DOSY has been extensively used to measure the size distribution of different materials, including lipid vesicles and gold nanoparticles.

DOSY is able to verify 35 variables: (a) total LDL cholesterol; large, medium and small LDL cholesterol (mg/dL); (b) total HDL cholesterol; large, medium and small HDL cholesterol (mg/dL); (c) total VLDL-particles; large, medium and small VLDL-particles (nmol/L); (d) total LDL-particles; large, medium and small LDL-particles (nmol/L); (e) total HDL-particles; large, medium and small HDL-particles (µmol/L); (f) mean particle sizes (VLDL, LDL and HDL – in Å); and the component of triglyceride in the fractions (g) total VLDL-TG (mg/dL); large, medium and small VLDL-TG (mg/dL) ; (h) total LDL-TG; large, medium and small LDL-TG (mg/dL) ; (i) total HDL-TG; large, medium and small HDL-TG (mg/dL).

Statistical analyses

Values are presented as mean (standard deviation) for variables with normal distribution or as median ±interquartile range for variables with non-normal distribution. We used the Kolmogorov-Smirnov to test the normality. The Wilcoxon Signed Rank test was used to compare samples before and after the manual lymphatic drainage. The SPSS package version 23.0 (IBM, Madrid, Spain) was used throughout.

RESULTS

In Table 1 we have summarized the baseline characteristics of the participants. We studied 25 subjects who were all men, with a mean age of 25 years and they were all apparently healthy with normoweight and normal lipid parameters. Only 6 subjects were active smokers.

Table 2 shows the lipid parameters obtained by nuclear magnetic resonance before and after the manual lymphatic drainage massage.

Individuals globally display a significantly decrease in VLDL-related parameters. There is a significant decrease by 2% in the total VLDL particle concentration in plasma, and this was accompanied by a 27% decrease in VLDL-cholesterol content and a 2% decrease in VLDL-triglyceride content. The effect on VLDL is diameter-dependent as we have observed that the smaller the VLDL particle the larger the decrease associated with LDM and the more consistent the effects are on most of the participants. Therefore, after the massage, there is a slight increase by 2% in the mean concentration of the large VLDL particles, however when we take a look at the individual data (Figure 1) we can see that the majority (64% of the subjects) present a decrease in large VLDL particles after the massage. For the medium and the small VLDL particles, the mean effect is a significant decrease in the number of circulating particles (8% decrease for the medium and 4% for the small VLDLs), and individually we can see in figures 2 and 3 that the decreasing effects on the smallest particles are more consistent among the participants, since it almost affect all the participants (80% of the subjects).

Apart from the effect on the VLDL particles, we have also found that after the lymphatic drainage massage there is a decrease in the content of TG in other lipoprotein particles. Therefore, there is a significant decrease of 5% in the TG content in the IDL particles, and a 9% decrease in the TG content in LDL particles.

DISCUSSION

We hypothesized that after performing a lymphatic drainage massage, we would be able to detect a variation on plasma lipid and lipoprotein concentrations, and in fact, our results show significant changes on triglycerides but not in cholesterol concentrations.

Lipids are fats that are either absorbed from dietary food or synthesized by the liver. All lipids are hydrophobic and mostly insoluble in blood so, for their transport in blood, they require hydrophilic and spherical structures called lipoproteins. The lipoproteins can be distinguished by their size and their density. In the surface of the lipoproteins, there are the apolipoproteins, which are cofactors and ligands for lipid-processing enzymes and for the interaction with cellular receptors.

The digestion of dietary fats ends up with the intestinal production of the lipoproteins called chylomicrons, which are very rich in TG but they can also transport some cholesterol. The chylomicrons are formed in the intestine and reach the bloodstream through the lymphatic system. Once in the blood, the chylomicrons interact with the lipoprotein lipase (LPL) that is bound to the endothelial cells, and hydrolyse the TG into glycerol and fatty acids. The fatty acids can now be used as energy source for the muscle cells, or can be stored in the adipocytes. While in circulation, the chylomicrons lose their content and the derived particles are finally removed from circulation in the liver.

Under fasting conditions, the liver is the organ responsible of the synthesis of endogenous TG, and they reach the bloodstream forming the VLDLs (very low-density lipoproteins). Similarly to the chylomicrons, the VLDLs mainly transport TG, but they can also transport some cholesterol, to the peripheral tissues, and in circulation they interact with the LPL. The product of the LPL processing of the VLDL is the IDL (intermediate density lipoproteins). The IDL are smaller and with less TG content compared to the former VLDLs. The IDL can be removed from circulation in the liver, or it can be metabolized to LDL (low-density lipoproteins), which are the most cholesterol-rich of all the lipoproteins. The LDLs transport cholesterol to the peripheral tissues. Finally, the HDLs (high-density lipoproteins) are responsible of the uptake of excess cholesterol from the tissues and it transports the cholesterol back to the liver for its degradation. [10]

Therefore, the appropriate transport and control of circulating lipids and lipoproteins is essential for the cardiovascular health.

We have described that acting on the lymphatic system in our participants on a fasting state, we can modulate the concentration and the lipid content of the VLDL particles,

and consequently we can modulate the concentration of plasmatic TG. We have found that both the content of lipids and the number of VLDL particles are decreased after forty minutes of manual lymphatic drainage massage performed in the trunk of healthy and young men. The physiological mechanism underlying this effect is unknown.

This is the first study describing this effect. The role of lymphatic system on circulating lipoproteins has been previously evidenced mostly in animal models, probably since the lymph, or the lymphatic system, is harder-to-access compared to the blood.

Early in the 70s it was described that the human lymph contains cholesterol that was derived from plasma[11], but the evidences showing the transport of lipoproteins through the lymphatic system are obtained from cultured cells or animal models.[12] For example, it is clear now that HDL particles can enter the lymphatic vessels interacting with SR-BI receptors promoting the reverse cholesterol transport[5] and it is also clear that the discontinuous “button-like” junctions, typical from lymphatic capillaries, are optimal for fluid and large molecules uptake, allowing the process of absorption by the lymphatic capillaries.[13]

We have considered that stimulating the lymphatic system we could have induced a dilution of the blood by promoting the transport of fluids from the interstitial space into the circulatory system. If that would be the case, one would expect to find that all the lipidic parameters were equally diluted, whereas the effect has been detected only on some specific lipoproteins and affecting only TG levels. For that, we firmly believe that the described effects are not caused by a dilution, however further studies are needed to confirm this.

In summary, we have described that LDM modulates circulating lipoprotein concentration, being this effect associated with TG-rich lipoproteins.

For that, we have postulated that the manual lymphatic drainage may somehow affect the activity of the LPL which is the most important enzyme involved in TG metabolism.

If this were the case, our results would be of main clinical interest, mostly for a group of subjects presenting with severe hypertriglyceridemia. The severe hypertriglyceridemia is genetic disorder that presents with circulating TG levels above 1000mg/dL and causes repeated pancreatitis and abdominal pain. Nowadays it has no treatment, except for following a very strict diet. We think that it would be of tremendous interest if the manual lymphatic drainage would be some sort of treatment helping lowering the TG levels in these patients.

TABLES

Table 1. Baseline characteristics of all participants

	All participants, n=25
Age, years	25.72 (5.62)
BMI, kg/m^2	23.53 (2.21)
Active smokers, n	6 (24.00)
Total cholesterol, mg/dL	169.76 (23.11)
Triglycerides, mg/dL	80.53 ±37.17
LDL cholesterol, mg/dL	91.70 (22.09)
HDL cholesterol, mg/dL	57.92 ±16.22
Apo A1, mg/dL	140.04 (18.46)
Apo B100, mg/dL	80.00 (17.09)
Apo CIII, mg/dL	7.83 (3.25)

Values are presented as mean (sd) for variables with normal distribution, median ±interquartile range for variables with non-normal distribution and number (%) for qualitative variables. BMI: body mass index; LDL: low density lipoprotein; HDL: high density lipoprotein; Apo: apolipoprotein.

Table 2. Lipid profile before and after the lymphatic drainage massage

	Pre massage (Median ± IQR)	Post massage (Median ± IQR)	p-value
VLDL-P, nm/L	24.10 ± 12.10	23.60 ± 7.50	0.001
VLDL-C, mg/dL	4.72 ± 4.42	3.44 ± 3.75	0.008
VLDL-TG, mg/dL	40.00 ± 19.50	39.10 ± 12.50	0.002
Large VLDL, nm/L	0.78 ± 0.41	0.8 ± 0.40	0.042
Medium VLDL, nm/L	4.05 ± 1.97	3.70 ± 1.57	0.002
Small VLDL, nm/L	19.50 ± 8.90	18.80 ± 6.00	0.002
VLDL- size, nm	42.50 ± 0.40	42.50 ± 0.50	0.443
IDL-C, mg/dL	3.97 ± 2.82	3.71 ± 3.01	0.040
IDL-TG, mg/dL	5.83 ± 1.92	5.55 ± 1.86	<0.001
LDL-P, nm/L	618.0 ± 134.0	624.0 ± 134.0	0.339
LDL-C, mg/dL	91.0 ± 20.0	92.40 ± 19.60	0.397
LDL-TG, mg/dL	7.50 ± 2.20	6.80 ± 2.70	0.001
Large LDL, nm/L	87.0 ± 10.0	85.0 ± 12.0	0.174
Medium LDL, nm/L	223.0 ± 70.0	221.0 ± 75.0	0.326
Small LDL, nm/L	309.0 ± 66.0	313.0 ± 67.0	0.968
LDL- size, nm	21.13 ± 0.12	21.10 ± 0.14	0.757
HDL-P, µm/L	29.0 ± 5.50	29.60 ± 4.90	0.581
HDL-C, mg/dL	62.80 ± 11.0	63.20 ± 9.60	0.989
HDL-TG, mg/dL	6.80 ± 2.50	6.80 ± 2.10	0.638
Large HDL, µm/L	0.14 ± 0.09	0.13 ± 0.05	0.042
Medium HDL, µm/L	9.11 ± 1.85	9.28 ± 1.89	0.861
Small HDL, µm/L	20.10 ± 3.30	20.10 ± 3.10	0.443
HDL- size, nm	8.21 ± 0.03	8.22 ± 0.01	0.989

Values are presented as median ±interquartile range (IQR). P-value denotes significance between pre and post massage groups. VLDL: very low density lipoproteins: IDL: intermediate density lipoproteins; LDL: low density lipoproteins; HDL: high density lipoproteins; -P: total particle concentration; -C: cholesterol content; -TG: triglyceride content.

FIGURES

Figure 1. Individual representation of the effect of the lymphatic drainage massage on the circulating concentration of large VLDL particles.

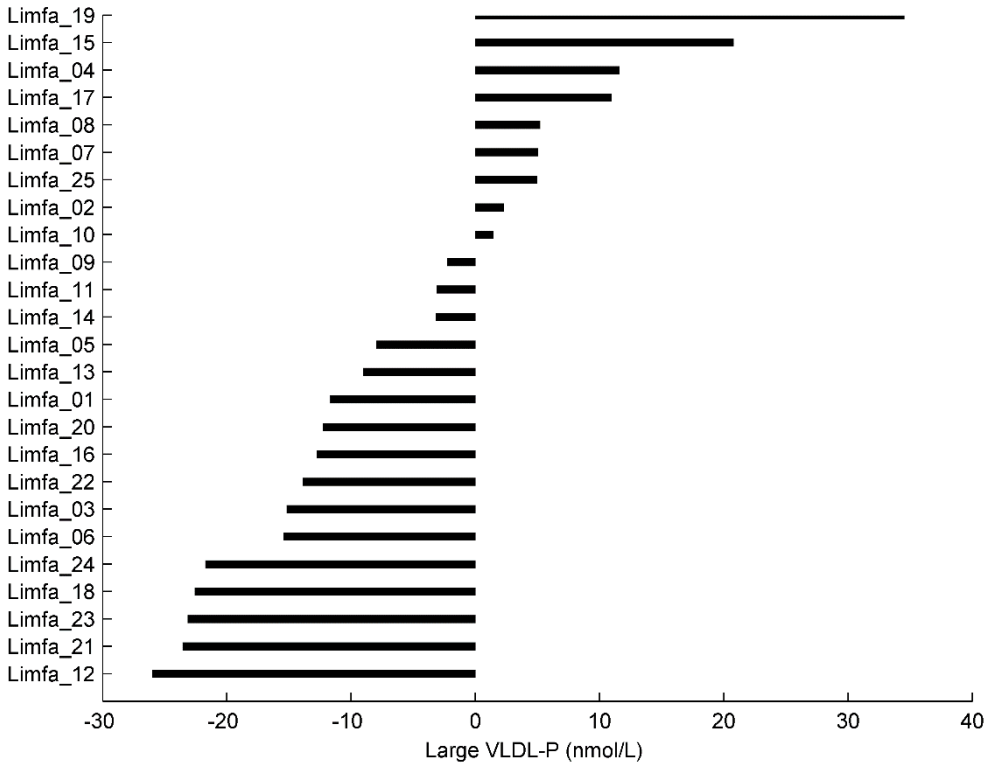


Figure 2. Individual representation of the effect of the lymphatic drainage massage on the circulating concentration of medium VLDL particles.

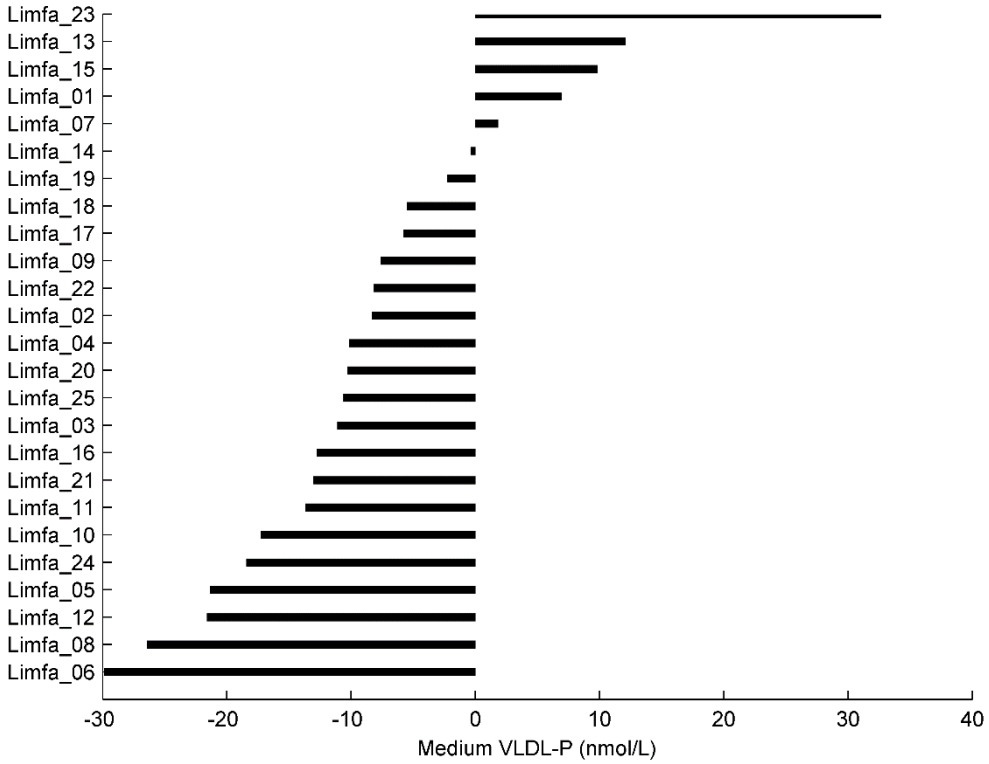
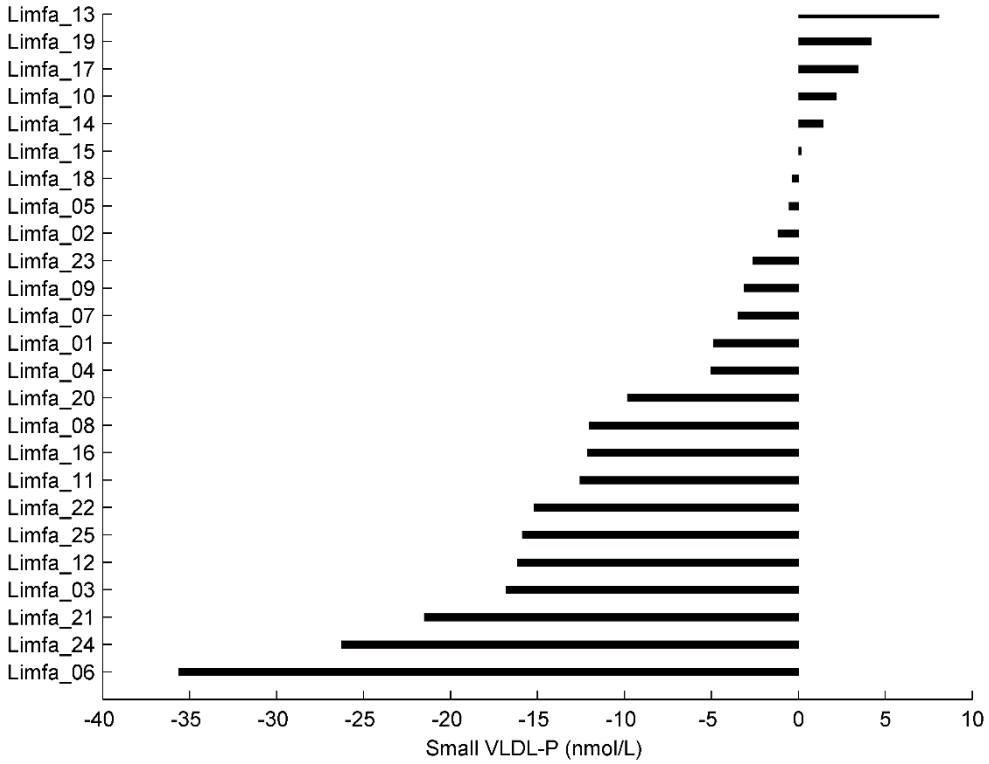


Figure 3. Individual representation of the effect of the lymphatic drainage massage on the circulating concentration of small VLDL particles.



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