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Transport of Low-Density Lipoprotein Into the Blood Vessel Wall During Atherogenic Diet in the Isolated Rabbit Carotid Artery

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Background: Atherosclerosis is a chronic fibroproliferative disease that includes accumulation of cholesterol-rich lipids in the arterial wall. Though numerous studies have investigated atherosclerosis, not enough is known about the exact mechanisms of low-density lipoprotein (LDL) transport into the blood vessel wall. Therefore, we explored the ^{125}I-LDL transport into the arterial wall under constant perfusion flow and pressure as well as the influence of duration of atherogenic diet on ^{125}I-LDL transport and biomechanical properties of carotid artery.

Methods and Results: The isolated segment of rabbit carotid artery was used under constant perfusion flow and pressure-induced (0 mmHg and 140 mmHg) blood vessel distension, with the possibility to change and precisely calculate shear stress during the experiment. Obtained results indicate the influence of atherogenic diet duration and consequent variation of shear stress on ^{125}I-LDL transport into the blood vessel wall. ^{125}I-LDL transport into the blood vessel wall at low pressure-induced blood vessel distension decreases by the increase of the shear stress and in relation to the atherogenic diet duration. At high pressure-induced blood vessel distension, ^{125}I-LDL transport increases in relation to the atherogenic diet duration and the increase of shear stress.

Conclusions: The influence of shear stress is a more dominant parameter on LDL uptake at low pressure-induced blood vessel distension; however, the atherogenic diet duration has more of a dominant influence on LDL uptake at high pressure-induced vessel distension. (*Circ J* 2015; **79:** 1846–1852)

Key Words: Atherogenic diet; Blood vessel; Low-density lipoprotein; Shear stress; Transport

It is known that the leading cause of death worldwide is coronary artery disease (CAD), and special attention is given to atherosclerosis, mainly dyslipidemia, as an important risk factor for CAD.[1] Atherosclerosis, a chronic fibroproliferative disease of the arterial wall, occurs principally in large- and medium-sized elastic and muscular arteries and may induce ischemia of the heart, brain or extremities, resulting in infarction.[2–4] Because high plasma concentrations of cholesterol, particularly low-density lipoprotein (LDL) cholesterol, represent one of the principal risk factors for atherosclerosis,[5] the process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall.[6] By providing cholesterol to peripheral tissues, LDL is the key component in physiological cholesterol metabolism.[7,8] Furthermore, blood vessels are constantly exposed to various types of hemodynamic forces induced by the pulsatile blood flow and pressure, and the spatial distribution of these hemodynamic factors such as wall pressure, fluid velocity or wall shear stress may be involved in the transport of atherogenic substances and their subsequent redistribution within the vessel wall.[9–13] Compared to other mechanical forces, shear stress acts on a surface of the blood vessel wall (endothelium)[14] and appears to be particularly important. Studies showed endothelial cell dysfunction in areas of low shear stress, which also demonstrate increased uptake of lipoproteins.[9,15–19]

The goal of this work was to examine LDL uptake by the blood vessel wall in a very short period of time, taking into account shear stress variation and possible influence of cholesterol exposure to the blood vessel wall. All previous studies that examined LDL transport and accumulation into the blood vessel wall were performed either on isolated blood vessel strips[20] or segments.[21] These were done either without perfusion flow or in vitro, using cultured endothelial cell monolayers[22] or they were performed by using mathematical and computational models of blood vessels[18,23,24] based on experimental results, especially those of Meyer et al.[21] This study

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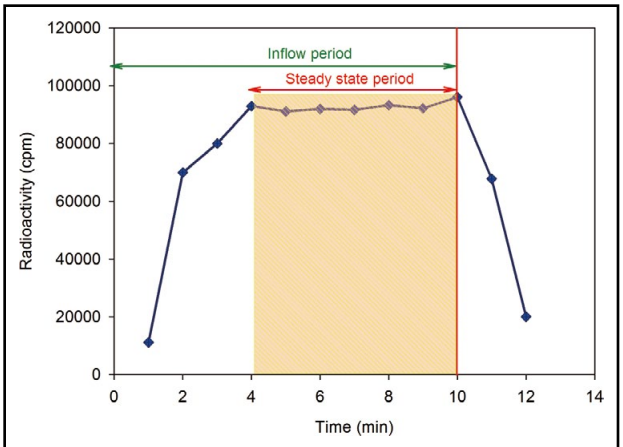

Figure 1. Experimentally obtained curve of recovered radioactivity during time. One representative experiment is shown.

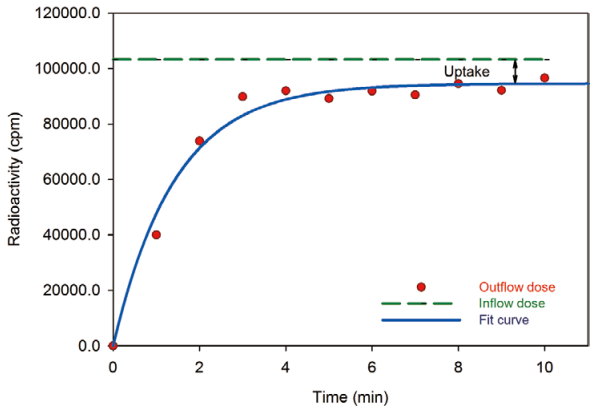

Figure 2. Experimentally obtained dilution profile curve within the inflow period, fitted using Eqn 1: $y=a(1-e^{-bx})$, where y represents the radioactivity (cpm) and x represents time units (min), the coefficient *a* has radioactivity units (cpm) and *b* has time units (min^{-1}). Represents ^{125}I-low-density lipoprotein Steady state uptake (Us) by the blood vessel wall during time.

presents new experimental research using radiolabeled ^{125}I-LDL on an ex vivo isolated segment of carotid artery of cholesterol-fed rabbits in order to research LDL uptake by the blood vessel at constant perfusion flow and pressure, and to estimate the influence of shear stress and atherogenic diet duration (prolonged hyperlipidemia) on the LDL uptake. Mathematical equations used in this work enabled us to precisely describe and predict some parameters in research.

Methods

Ex vivo blood vessel experiments of LDL transport were performed on the isolated rabbit's common carotid arteries. All research procedures were carried out in accordance with the European Council Directive (86/609/EEC) and principles of Good Laboratory Practice (2004/9/EC, 2004/10/EC) and approved by the Ethics Committee for the Welfare of Experimental Animals, Faculty of Medical Sciences University of Kragujevac, Serbia.

Materials

Krebs-Ringer physiological solution (KRS) contained (in mmol/L): NaCl 117, KCl 4.7, $NaHCO_3$ 24.8, $MgSO_4 \times 7H_2O$ 1.2, $CaCl_2 \times 2H_2O$ 2.5, KH_2PO_4 1.2 and D-glucose 11.1 (AppliChem GmbH, Darmstadt, Germany).

^{125}I-Low-density lipoprotein (Biomedical Technologies Inc, Stoughton, MA, USA); specific activity: 0.102 μCi/ml; quantity: 525 μCi/2 ml, radioiodinated [I-125] by the Iodine Monochloride Method. ^{125}I-LDL was purified on a 10 ml (dry bed) Sephadex G50. This removes ~99% of the free [I-125]. The resulting product was in 50 mmol/L Tris-HCl, 0.15 mol/L NaCl and 0.3 mmol/L EDTA at a pH of 7.4. It was membrane filtered and aseptically packaged. ^{125}I-LDL was isolated by density gradient centrifugation; the product is a mix of all LDL particle sizes that float at a density of 1.063 in KBr solutions and the LDL varies in MWs from 2.2 to 2.9 million Daltons and had a size range of 180–250 Angstroms. The final concentration of ^{125}I-LDL in the perfusion solution was 0.0013 mg/ml.

Ketamine® (Laboratorio Sanderson, Santiago, Chile) — 500 mg/10 ml, sterile solution for intramuscular and intravenous use.

Sodium-pentobarbital (Carbone Scientific Co, LTD, London, UK), 50 mg/1 ml, sterile solution for intramuscular and intravenous use.

New Zealand white rabbits of both sexes (n=20) were used. Five animals were used as a control and 15 animals were on atherogenic diet Energon chow (Energon, Austria),[25] enriched with 1% cholesterol, approximately 150 g/head every day for XII weeks. Water was given ad libitum. At the beginning of the diet, the weight of the animals was 1.7±0.2 kg and at the end in the 8th, 10th and 12th week of feeding, the weights were 3.04±0.12 kg, 3.66±0.17 kg and 4.03±0.32 kg, respectively. Each feeding group contained 5 animals.

Blood Vessel Preparation

New Zealand white rabbits of both sexes (n=20) were anesthetized using sodium pentobarbital (0.5 mg/kg of body weight, intravenously) and ketamine (0.5 mg/kg of body weight intravenously).[26] Blood vessels were excised and placed into a water bath. The length and the outer diameter of isolated blood vessel segments were measured using a digital camera and the originally developed software at the applied pressure of 0 mmHg and 140±10 mmHg. Cannulas with equally matched tip diameters (2 mm) were mounted at proximal (cardiac) and distal (cranial) ends of the blood vessel, and the lumen was perfused with the KRS using a peristaltic pump at 1 ml/min. The perfusate was continuously bubbled with 95% O_2 and 5% CO_2, with the pH adjusted to 7.4 at 37°C. The blood vessel was stretched to its approximate in vivo length. The distal cannula was connected to the Resistance changing device and perfusion pressure was measured using a pressure transducer. The blood vessel was considered to be viable if it contracted when 25 mmol/L KCl was added to the bath, and also if the presence of functional endothelium was verified by dilation with Ach (1 μmol/L) at the end of experiment. The blood vessel wall thickness was measured at the beginning of each experiment using a light microscope and a microscopically graduated plate.

Steady State Method

In order to examine ^{125}I-LDL uptake by the blood vessel wall, we used initially the Rapid dual-isotope dilution method.[27–29] The results obtained using this method showed that the blood vessel wall uptake of ^{125}I-LDL had very low capacity and slow dynamics, hereof the protocol was changed and the continuous 10-min perfusion of the ^{125}I-LDL was applied (Steady state

Table. Steady State of ^{125}I-LDL Uptake Obtained From Experimental Data at Low Pressure-Induced Blood Vessel Distension and High Pressure-Induced Blood Vessel Distension

	Week of diet (experimental group)			
	0 (control)	VIII (group 1)	X (group 2)	XII (group 3)
Low pressure-induced distension (0 mmHg)				
Shear stress (dyn/cm^2)	0.109±0.0019	0.1778±0.0071	0.436±0.041	0.783±0.141
LDL uptake (%)	Not detectable	3.54±0.34	2.55±0.307	0.486±0.095
High pressure-induced distension (140±10 mmHg)				
Shear stress (dyn/cm^2)	0.0046±0.0005	0.0079±0.0008	0.0107±0.001	0.014±0.0016
LDL uptake (%)	3.524±0.263	8.01±0.38	11.72±0.42	12.04±0.51

The values are represented as mean ± SE (standard error). LDL, low-density lipoprotein.

method). To verify the Steady state method, a series of technical experiments were performed using a plastic tube instead of a blood vessel under the experimental conditions that would be applied in the experiments with the blood vessels. The obtained results showed full recovery of the ^{125}I-LDL injected into the plastic tube (instead of the blood vessel) in each time interval (no uptake for ^{125}I-LDL). Then, the pilot experiments were done using blood vessels, and retention of the injected ^{125}I-LDL dose appeared in the perfusion effluent samples. This difference between inflow dose of ^{125}I-LDL and the outflow dose (in samples) within the period of the Steady state indicates the ^{125}I-LDL uptake by the blood vessel (see below in the section **Mathematical Analysis of Dilution Profiles**).

The isolated blood vessel was placed into the water bath with physiological buffer. After the equilibration period (20–30 min) at a constant perfusion flow of 1 ml/min and perfusion pressure of 0 mmHg, a resistance changing device was applied to achieve a constant perfusion pressure (140±10 mmHg). When target pressure was achieved, the period of equilibration (15–30 min) was allowed. Then, the ^{125}I-LDL standard solution (100μl/min) was continuously injected into the blood vessel (inflow dose) using a rapid infusion pump for 10 min. The first 12 samples (cumulative 1 min samples of perfusion effluent) and 6 cumulative 3 min samples were continuously collected. The radioactivity in each sample represented the outflow dose in the corresponding time period.

^{125}I-LDL Dilution Profiles

The ^{125}I-LDL specific activity in each sample was measured with a Gamma counter (Wallac Wizard 1470 Automatic; Wallac Oy, 2005; Finland). The ^{125}I-LDL specific activity in each sample was used to obtain radioactivity-time curves; that is, dilution profiles for ^{125}I-LDL during its flow through the blood vessel at constant perfusion pressure. The ^{125}I-LDL specific activity in the first 10 samples during the inflow period was used for the creation of the dilution profile curves (**Figures 1**,**2**).

Mathematical Analysis of Dilution Profiles

Experimentally obtained dilution profile curves within the inflow period were fitted using the following exponential equation:[30]

$$y=a(1-e^{-bx}) \quad \text{(Eqn 1)}$$

where y represents the specific radioactivity (cpm), x represents time units (min), and a and b are the coefficients of this relation where a has radioactivity units (cpm) and b has time units (min^{-1}).

This function is shown in **Figure 2** as the radioactivity vs. time curve. The constant a represents the maximum developed radioactivity (the radioactivity corresponding to the Steady state period). The Steady state uptake (Us) was calculated by measuring the difference between inflow dose of ^{125}I-LDL and the outflow dose (in samples) within the period of the Steady state.

Assuming that the blood vessel wall was incompressible, we were able to determine the wall thickness (δ_t) for the current mean radius, R_{mt}:

$$\delta_t = R_{mt}-\sqrt{R^2_{mt}-(V_0/\pi L)} \quad \text{(Eqn 2)}$$

where L is the length of the isolated blood vessel, and V_0 is the original volume of the blood vessel wall, with the lower index "0" indicating the original values:[31]

$$V_0=\pi(R_0-\delta_0)\delta_0 L_0 \quad \text{(Eqn 3)}$$

We also calculated the wall shear stress (dyn/cm^2) with the following equation:[32,33]

$$\tau = \frac{4\mu Q}{\pi R^3} \quad \text{(Eqn 4)}$$

where τ is the shear stress (dyn/cm^2), μ is the viscosity (poise), Q is the flow (ml/s), and R is the blood vessel internal diameter (cm).

During the experiment, the blood vessel internal diameter was calculated from the measured value of blood vessel external diameter and the calculated thickness of the blood vessel wall, which was obtained using Eqn 2 and Eqn 3.

Statistical Analysis

A Student's t-test, independent t-test, ANOVA test and Pearson's coefficient of correlation were used for statistical comparisons. P values <0.05 were considered as statistically significant. All statistical calculations were made with the SPSS computer program, version 22.0 (SPSS Inc, Chicago, IL, USA). Data are presented as means ± standard error (SE).

Results

The length of the isolated blood vessel segment was 2.26±0.14 cm. The blood vessel external diameter was measured using a digital camera, as previously described, and it depends on the applied pressure under the constant flow conditions. The blood vessel external diameter in all our experiments increased from 1.55±0.095 mm at lower pressure conditions, to 2.81±0.14 mm at higher pressure. The inner (intraluminal) diameter was 0.95±0.09 mm at lower pressure and 2.67±0.14 mm at higher pressure.

The experimental data were analyzed using the method described in the previous section, and results are shown in **Table**.

In the control group of experimental animals, detectable

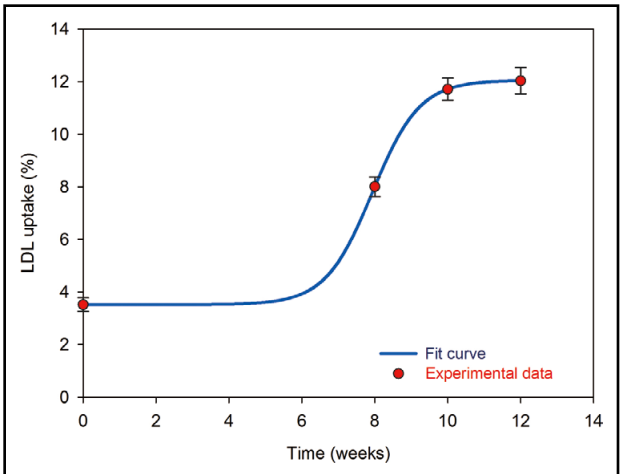

Figure 3. Effects of the atherogenic diet duration on the ^{125}I-low-density lipoprotein (LDL) uptake at a perfusion pressure of 140±10 mmHg. Obtained results were fitted by Eqn 5, with the coefficients: a=8.494, b=0.635, x_0=7.93 and y_0=3.52.

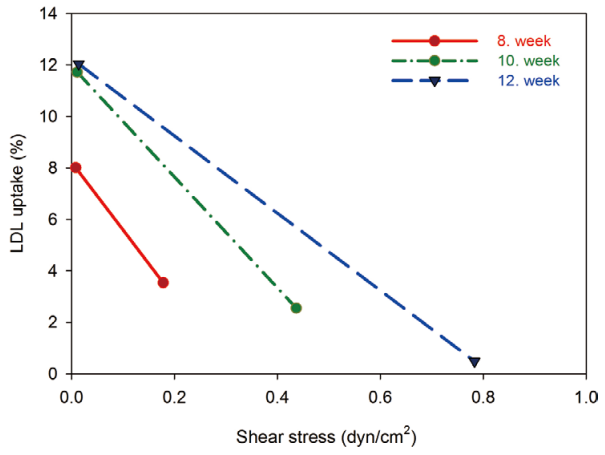

Figure 4. Effects of shear stress on ^{125}I-low-density lipoprotein (LDL) uptake in VIII, X and XII week of atherogenic diet. A significant decrease of ^{125}I-LDL uptake can be seen when the shear stress increases.

^{125}I-LDL uptake was not found (it was in a range of SE) at low pressure-induced blood vessel distension, in respect to 3.524±0.263% at high pressure-induced blood vessel distension.

The ^{125}I-LDL uptake by the blood vessel wall during time is presented in **Figure 2**.

Duration of Atherogenic Diet and LDL Uptake

Experiments were performed in weeks VIII, X and XII of the atherogenic diet at low and high pressure-induced blood vessel distension. The results (**Table**) showed decreased ^{125}I-LDL uptake in relation to the duration of the atherogenic diet at low pressure-induced blood vessel distension. A significant difference was observed between week VIII and XII ($P<0.05$). At high pressure-induced blood vessel distension, an increased ^{125}I-LDL uptake was observed in relation to the duration of the atherogenic diet between weeks VIII and X, as well as between weeks VIII and XII ($P<0.05$).

The effects of the duration of the atherogenic diet on ^{125}I-LDL uptake at high pressure-induced blood vessel distension are presented in **Figure 3**. These results were fitted by the following equation:

$$y = y_0 + \frac{a}{1+e^{-1\left(\frac{x-x_0}{b}\right)}} \qquad \text{(Eqn 5)}$$

Results obtained from these experiments indicate a significant increase of ^{125}I-LDL uptake, which occurs in VI-VII weeks after the atherogenic diet outset, and reaches its maximum within week X-XII at high pressure-induced blood vessel distension.

Shear Stress and ^{125}I-LDL Uptake

These study results showed (**Table**; **Figure 4**) that mean shear stress values (obtained at 0 mmHg and 140±10 mmHg) were significantly different ($P<0.05$), and that shear stress values obtained at higher pressure, regardless of the week of diet, were lower than values obtained at lower pressure ($P<0.05$). Moreover, shear stress values increase with increasing duration of the atherogenic diet (at lower pressure, the significant differences were between weeks VIII and X, as well as between weeks VIII and XII ($P<0.05$), and at higher pressure, the significant difference was between weeks VIII and XII ($P<0.05$).

In order to describe the relationship between the values of shear stress and ^{125}I-LDL uptake, experiments were performed on separate group of animals, in XII week of the atherogenic diet, by changing perfusion flow from 1 to 4 ml/min and perfusion pressure from 0 to 195 mmHg in order to obtain various shear stress conditions. These results (**Figure 5**) strongly suggest a decrease of ^{125}I-LDL uptake by increase of shear stress. The decreased trend line (fitted curve) may generally be described by the following equation:

$$y=ae^{-bx} \qquad \text{(Eqn 6)}$$

where y represents Us (%) and x represents shear stress (dyn/cm^2).

Previously described results (**Table**) indicate the increase of shear stress during the atherogenic diet. This increase has a sigmoid form (**Figure 6**), and may also be described by the Eqn 5, the same form of equation by which we can mathematically express the increase of ^{125}I-LDL uptake in relation to the duration of the atherogenic diet (see above). Again, a significant increase of the shear stress occurs VI-VII weeks after the atherogenic diet outset; however, according to results obtained with a fitted curve, it can be presumed that the shear stress reaches its maximum approximately at 20th week of atherogenic diet (**Figure 6**), after maximal ^{125}I-LDL uptake has been achieved (**Figure 3**).

Among values of shear stress and ^{125}I-LDL uptake, a linear correlation was observed depending on the applied perfusion pressure, taking into account the atherogenic diet duration; however, the correlations were not very strong. At low pressure-induced blood vessel distension (0 mmHg), Pearson's coefficient was –0.511 with regard to +0.416 at high pressure-induced blood vessel distension (140±10 mmHg).

Discussion

Until now, many studies have tried to explain LDL transport within the blood vessel wall and its accumulation in the wall due to its essential role in the development of atherosclerosis. These studies were performed under different pressure conditions without perfusion flow either on isolated blood vessel

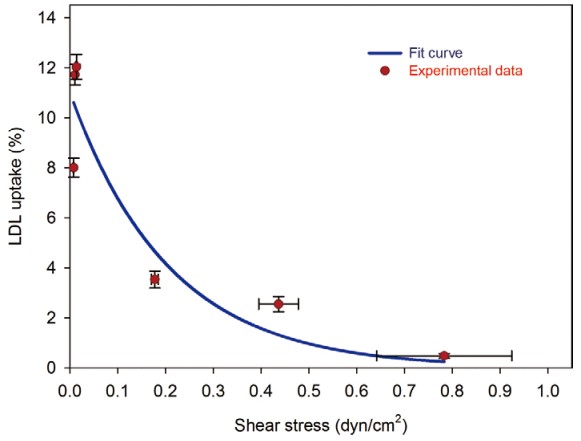

Figure 5. Effects of shear stress on ^{125}I-low-density lipoprotein (LDL) uptake. Obtained results were fitted by Eqn 6, where y is the Steady state uptake (Us; %) and x represents shear stress (dyn/cm^2); the coefficients are a=11.019 and b=4.85.

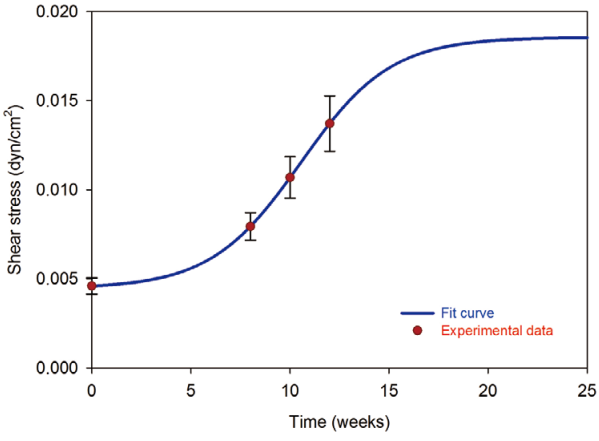

Figure 6. Effects of the atherogenic diet duration on shear stress at a pressure of 140 mmHg. The results indicate that the increase of shear stress during an atherogenic diet has a sigmoid form (fitted by the Eqn 5), where the coefficients are a=0.014, b=2.268, x_0=10.53 and y_0=0.0045.

strips incubated in a tissue holding device[20] or by using incubated blood vessel segments,[21] or by using in vitro cultured endothelial cell mono-layers without changing pressure conditions.[22] Some studies presented mathematical and computational models of blood vessels based on known experimental results.[18,23,24] However, none of these studies were performed with perfusion flow. That was the main reason why we decided to perform a research on the isolated blood vessel segment with constant perfusion flow and pressure in order to examine LDL uptake by the blood vessel wall in a very short period of time. Another advantage of our design is its possibility to control and precisely change the experimental parameters such as pressure, flow, shear stress, the blood vessel diameter to investigate their affects on LDL uptake.

Before the investigation of ^{125}I-LDL uptake into the blood vessel wall at low (0 mmHg) and high (140 mmHg) pressure conditions, a series of pilot experiments have been performed in order to allow standardization of the experimental setting and experimental protocol. In our pilot experiments, the resistance changing device (see Methods section) was applied to allow the changes in the perfusion pressure (70±10 mmHg and 140±10 mmHg) under constant flow conditions. When a pressure of 70±10 mmHg was applied, the calculated Steady state uptake (Us) of ^{125}I-LDL was within the value of SE (±1.3%) and not detectable (data not shown). According to these results, in our experimental conditions, ^{125}I-LDL uptake by the blood vessel wall occurred at extremely low shear stress, which is below the physiological values. In addition, Meyer et al[21] have found that when the blood vessel distension is totally prevented using an external rigid wrap, the uptake of LDL is not changed significantly, despite the increase in the intraluminal pressure from 70 to 160 mmHg. Having in mind that pressure-induced distension of the blood vessel wall is a major determinant of LDL transport across the arterial wall,[21] and that research mentioned above was done without perfusion flow, we wanted to research LDL uptake by the blood vessel at constant perfusion flow and to estimate the influence of shear stress on the LDL uptake (the existence of flow and consequently shear-stress are the features of in vivo conditions). As the parameter that most affects the value of shear stress is the diameter of the blood vessel, and considering the results of our pilot experiments, we decided to significantly increase the differences of shear stress via considerable changes in pressure-induced blood vessel distension (0 mmHg and 140 mmHg).

In order to investigate the impact of the duration of the atherogenic diet on the ^{125}I-LDL uptake, we performed experiments at low and high pressure-induced blood vessel distension, using rabbits fed VIII, X and XII weeks with atherogenic diet. Results obtained in experiments during weeks VIII, X and XII of the atherogenic diet at low and high pressure-induced blood vessel distension (**Table**) showed slight differences. Namely, under low pressure-induced (0 mmHg) wall distension (when shear stress is higher) conditions, ^{125}I-LDL uptake decreased in relation to the atherogenic diet duration. A significant difference was observed between weeks VIII and XII ($P<0.05$). At the same time, the values of shear stress increased with duration of the atherogenic diet, with significant differences between weeks VIII and X, as well as between weeks VIII and XII ($P<0.05$). Under high pressure-induced (140 mmHg) wall distension (when shear stress is low) conditions, ^{125}I-LDL uptake increased in relation to atherogenic diet duration, with significant difference between weeks VIII and X, as well as between weeks VIII and XII ($P<0.05$), although the shear stress was also increased between weeks VIII and XII ($P<0.05$).

In order to closely describe the relationship between ^{125}I-LDL uptake and duration of atherogenic diet, Eqn 5 was used to fit the results that are presented in **Figure 3**. These results, presented as the S-shape curve, indicate that the significant increase of ^{125}I-LDL uptake occurs VI-VII weeks after the atherogenic diet outset, and reaches its maximum within weeks X-XII of the diet. This is in accordance with the results found by Kamimura et al,[34] who showed that duration of atherogenic diet leads to histological alterations within the blood vessel wall in week V of exposure already, with progression over time reaching its maximum distribution in the vascular system in week XV of the diet. Bearing this in mind, it is possible that the formula (Eqn 5) may be used as a biological rule for better prediction of atherogenic diet-uptake relation.

The results further showed (**Table**; **Figure 4**) that mean values of shear stress (obtained at 0 mmHg and 140±10 mmHg)

are significantly different ($P<0.05$) as expected, because shear stress is indirectly dependent upon the diameter of the blood vessel and the diameter depends on the applied pressure.[32,33] The results showed that values of shear stress obtained at high pressure-induced blood vessel distension, regardless of the week of diet, were approximately 22-, 41- and 56-fold lower than values obtained at low pressure-induced blood vessel distension (**Table**). Also, shear stress values increased with increasing duration of the atherogenic diet (**Table**).

In order to describe the relationship between shear stress and ^{125}I-LDL uptake, we performed experiments on separate groups of animals in week XII of the diet. Perfusion flow was changed from 1 to 4 ml/min and perfusion pressure from 0 to 195 mmHg in order to obtain various shear stress conditions. These results (**Figure 5**) suggest a significant decrease of ^{125}I-LDL uptake by the increase of shear stress. This decreased trend line (fitted curve) may be generally described by Eqn 6 and, as well as it may mathematically express and predict uptake of macromolecules such as LDL. Studies performed so far agree that lipids accumulate at endothelial surfaces where the blood velocity and the shear stress are low and where the permeability of the endothelium is enhanced.[9,12,15–18] It was also demonstrated that endothelial cells become stiffer in response to higher shear stress[10] and that stiffness may be a useful marker of the extent of atherosclerosis in the aorta.[35] Furthermore, the increase of shear stress may modulate endothelial cell connections in a chemical and structural manner; that is, endothelial cell connections are getting stronger with increasing shear stress,[36,37] which inhibits macromolecule transport.[10]

Taking into account all previously mentioned, possible explanations why the increase of ^{125}I-LDL uptake is induced by a decrease of shear stress are:

(1) When the shear stress is lower, increased uptake of ^{125}I-LDL might occur because a smaller tangential force allows molecules to stay longer near the blood vessel wall and thus more molecules enter into the wall;[38]
(2) When high perfusion pressure is applied, the intraluminal diameter increases (shear stress is lower), and endothelial cells are wider, allowing molecules the larger contact surface and/or leaky junctions are more available for LDL molecules to pass through them. These reasons could explain the results of Meyer et al,[21] which suggest a major role of vessel wall distension, and not the applied pressure, on the increased LDL uptake;
(3) The chemical and structural modulations of endothelial cells and their connections that are caused by different values of shear stress.[39–41]

Furthermore, our results, which are described in **Table**, indicate the increase of shear stress during the atherogenic diet (**Figure 6**) and may be described by the Eqn 5, with the same form of equation but with different coefficients, by which we are able to mathematically express the increase of ^{125}I-LDL uptake in relation to the diet duration. Closer examination of these results indicates a significant increase of shear stress in weeks VI-VII after the diet outset. However, according to our results obtained with fitted curve, the shear stress probably reaches its maximum later, after 12 weeks of the diet, probably in 20th week, almost twice longer than our feeding period was (**Figure 6**), after maximal ^{125}I-LDL uptake had been achieved (**Figure 3**).

Taking into account previous results, we propose 3 phases of the disease:

(1) Prodromal phase — (the first 5 weeks, mechanisms are still unknown)
(2) Acceleration phase — (from weeks VI-X)
(3) Circulus vitiosus — (after week XI; with maximal ^{125}I-LDL within the wall)

Bearing all this in mind, it is considered that structural and functional changes of the blood vessel wall caused, during the diet, the increase of the rigidity of the blood vessel wall further leading to the increase of shear stress. On the basis of the histological data from our experiments, the computational model of *Three-dimensional numerical simulation of plaque formation and development in the arteries* was made and published.[42] These results showed an increased vessel-wall volume of the carotid artery in relation to the atherogenic diet duration,[42] which reduces the intraluminal diameter of the blood vessel and consequently increases shear stress. When the experiments were performed only in one week of diet under variable shear stress conditions, it was found that Us values increase with decreasing shear stress, which is in accordance with data from the literature. However, the results obtained at high pressure-induced blood vessel distension, with respect to the diet duration, showed that Us increases even if shear stress increases. It seems that in conditions at low pressure-induced wall distension, the influence of shear stress is far more dominant on LDL uptake, but the situation is quite the opposite for high pressure-induced wall distension when the atherogenic diet duration has more dominant influence on uptake, from so far unknown mechanisms. It may only be assumed that one of the reasons might be the disturbed flow field as it happens in the large arteries, while in the small diameter arteries, the dominant flow is laminar.[43] Xie et al[38] showed that the flow field disturbances as a consequence of vascular geometry changes lead to lipoprotein concentration polarization and increased LDL accumulation in the blood vessels and promote the development of atherosclerosis. These changes of the vessel wall induced by an atherosclerotic diet[42] lead to flow field disturbances and consequently increased LDL uptake at high pressure-induced distension (140 mmHg). This theory is also in accordance with results found by Van den Berg et al,[44] who showed that the disturbed flow impaired glycocalyx barrier properties and this led to enhanced intimal LDL accumulation at the carotid artery bifurcation in mice.

Conclusions

Taking into account our previously described results, we consider that prolonged exposure to an atherogenic diet and consequent variation of shear stress has an influence on the LDL uptake. The results of our study showed that in conditions at low pressure-induced wall distension, the influence of shear stress are far more dominant on LDL uptake; however, the duration of an atherogenic diet has more dominant influence on LDL uptake at high pressure-induced wall distension. Obviously, many biological mechanisms and factors that are involved in the initiation and development of atherosclerosis are still not sufficiently clarified and further research is needed in order to explore it. We consider that described experimental design and applied mathematical procedures might be a powerful tool in further investigations of atherosclerosis and the influences of other substances such as statins,[45] HDL,[1,46] Evacetrapib,[1] and Homocysteine, etc.[47,48]

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Disclosures

There are no conflicts of interest to declare.

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