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DYSLIPIDEMIA IS ASSOCIATED WITH SYMPATHETIC NERVOUS ACTIVATION AND IMPAIRED ENDOTHELIAL FUNCTION IN YOUNG FEMALES

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Abstract

Background: Dyslipidemia is one of the most well established risk factors for cardiovascular disease development. Moreover, hypercholesterolemia and plasma cholesterol level in the high normal range are established triggers for impairment in endothelial function. Evidence indicates that endothelial function is closely linked with sympathetic nervous activity in healthy individuals. We therefore investigated whether both endothelial and sympathetic functions may be impaired in young females with abnormal plasma cholesterol levels. **Methods:** Baseline endothelial function (digital pulse amplitude) and muscle sympathetic nervous activity (MSNA, microneurography) were retrospectively analysed in 14 young healthy females with dyslipidemia as indicated by any of the following: total cholesterol ≥ 197 mg/dL or HDL cholesterol ≤ 39 mg/dL or LDL >116 mg/dL, and in 13 females with lipids in the healthy range. **Results:** Subjects with dyslipidemia had significantly impaired endothelial function compared to those with a normal cholesterol profile (reactive hyperaemia index; 1.61 ± 0.10 vs 2.32 ± 0.14 , $P < 0.001$), increased MSNA (after adjusting for body mass and age, 36 ± 3 vs 27 ± 3 bursts per 100 heartbeats, $P = 0.049$) and elevated high-sensitive C-reactive protein (4.13 ± 0.77 vs 1.92 ± 0.61 mg/l, $P = 0.03$). **Discussion:** Our results indicate that young healthy females with dyslipidemia present with a strong impairment of endothelial function and increased sympathetic drive. The sympathetic activation observed in the subjects with an elevated cholesterol profile may play a role in the development of cardiovascular disease development.

Introduction

Epidemiological data support a strong association between cholesterol and cardiovascular disease (CVD) mortality. Individuals with high plasma concentrations of cholesterol, in particular those of low-density lipoprotein (LDL) cholesterol, are prone to develop atherosclerosis¹ and have been

shown to present with impaired endothelium-dependent vasodilation in the forearm ² and in the coronary vasculature ³. Endothelial dysfunction is now recognised as being the initial step in the development of atherosclerosis and subsequent CVD development ⁴. Indeed, the assessment of endothelial function by non-invasive peripheral arterial tonometry has recently been shown to predict late cardiovascular adverse events beyond the traditional risk scores such as arterial blood pressure and lipid profile ⁵. Impaired endothelial function and sympathetic nervous system activation often coexist in disease states such as heart failure, obesity and hypertension⁶. In healthy individuals we recently showed that there is a direct inverse relationship between sympathetic nervous system activity and endothelial function ⁷. While the causal relationship remains to be determined, experimental and clinical data support the idea that endothelial dysfunction contributes either directly or indirectly to impairment in the autonomic nervous system and vice versa ⁶.

Atherosclerosis in coronary and peripheral arteries occurs rarely in healthy premenopausal women; however the presence of subclinical carotid atherosclerosis is evident ⁸. The factors significantly associated with subclinical carotid atherosclerosis measured before menopause include lower HDL, and higher LDL, triglycerides, insulin, body weight, and systolic and diastolic blood pressures ⁸.

Recently, it was shown that cholesterol lowering statin drugs (HMG-CoA reductase inhibitor) significantly improved endothelial function in normocholesterolaemic patients with type-2 diabetes ⁹ and decreased muscle sympathetic nerve activity (MSNA) in hypertensive patients ¹⁰. Given the close link between cholesterol, endothelial function and the autonomic nervous system, we hypothesized that young females with dyslipidemia but who were otherwise healthy may present with disturbed endothelial and sympathetic nervous function.

Methods

Subject selection

A retrospective analysis of data from females who had participated in previous research investigations ^{7,11,12} was performed using the Human Neurotransmitters Laboratory database. We selected all females aged between 18 – 30 years and who fulfilled the following criteria: non-

smokers and not taking medication except for oral contraceptives and with no history of cardiovascular or cerebrovascular disease. Sample size calculation indicated that based on t-test comparison, a minimum of 25 subjects was required to demonstrate a significant difference in MSNA of 10 bursts per 100 heartbeats between the 2 groups (Power 0.9, α value of 0.05). Data was extracted from 27 subjects. The study protocols were approved by the Alfred Hospital Ethics Committee and all subjects gave written informed consent before participating in studies.

Clinical assessment

Demographic details of age, clinical status and blood pressure were obtained from standard measurements and questionnaires. A detailed history and physical examination was conducted to exclude cardiovascular disease. Supine blood pressure (BP) was measured after 5 minutes rest using a Dinamap monitor (Model 1846SX, Critikon Inc, Tampa, FL, USA) as the average of 3 consecutive measurements. Body weight was measured in light indoor clothes without shoes, using a digital scale. Waist circumference was measured at the midpoint between the lowest rib and iliac crest, and hip circumference at the level of the greater trochanters. Fat mass was assessed according to the formula described by Womersley¹³. In order to determine the menstrual cycle phase at the time of study, participants were required to provide the first day of their last period and the average length of their menstrual cycles. Their level of physical activity during the week was also recorded. All participants underwent ambulatory blood pressure monitoring over 24-26 hours using an oscillometric monitor (Model No. 90207, SpaceLabs Medical Inc, WA, USA) to measure brachial blood pressure every 30-minutes. Blood pressure values were averaged over the total period of the recording and averaged over both day and night periods.

Biochemistry

Fasting blood samples were drawn from a cannula placed in an antecubital vein for biochemical analysis of insulin, total cholesterol, triglycerides (TG), HDL, LDL cholesterol, glucose, high sensitivity C reactive protein (hs-CRP) and leptin. Dyslipidemia was defined as total cholesterol \geq

197 mg/dL or HDL cholesterol \leq 39 mg/dL or LDL $>$ 116 mg/dL according to the Third Report of the National Cholesterol Education Program ¹

Digital vascular function

Digital pulse amplitude was measured in the fasting state with a pulse amplitude tonometry (PAT) device placed on the tip of each index finger (Itamar Medical Ltd., Caesarea, Israel). Pulse amplitude tonometry was assessed in response to reactive hyperemia. Measurements were obtained for 5-10 minutes at baseline followed by 5 min of occlusion of one arm, with the cuff inflated on the upper arm to supra-systolic pressure (60 mmHg above systolic pressure or 200 mmHg) and then released to induce reactive flow-mediated hyperemia, measured for 5-10 min. The reactive hyperaemia index (RHI) was calculated as the ratio of the average amplitude of the PAT signal over 1 minute time interval starting one minute after cuff deflation divided by the average amplitude of the PAT signal over a 3.5 minute time period at baseline.

Muscle sympathetic nerve activity

Recordings of multi-unit postganglionic MSNA were made in the fasting state. A tungsten microelectrode (FHC, Bowdoinham, ME, USA) was inserted directly into the right peroneal nerve at the fibular head. A subcutaneous reference electrode was positioned 2 to 3 cm away from the recording site. The nerve signal was amplified (X50,000), filtered (bandpass, 700 to 2000Hz), and integrated. During MSNA recording, BP was measured continuously using the Finometer system (Finapres Medical System BV, Amsterdam, The Netherlands) and heart rate (HR) was extracted from 3-lead ECG. Blood pressure, ECG, and MSNA were digitized with a sampling frequency of 1000Hz (PowerLab recording system, model ML 785/8SP, ADI Instruments, NSW, Australia). Resting measurements were recorded over a 15-minute period and averaged. The MSNA was expressed as burst incidence (bursts/100 heartbeats).

Assessment of spontaneous arterial baroreflex control of MSNA

Over a 5 to 8 min resting period, diastolic blood pressures associated with individual heart beats were grouped in intervals of 2 mmHg and, for each interval, the percentage of diastoles associated

with a sympathetic burst was plotted against the mean of the pressure interval . Muscle sympathetic bursts were advanced by 1.3 sec to compensate for baroreflex delay ¹⁴. The sensitivity of the sympathetic baroreflex gain was defined as the slope of the regression line and was expressed as bursts per 100 heartbeats.mmHg⁻¹.

Assessment of spontaneous cardiac baroreflex function

The sequence method of estimation of baroreflex sensitivity has been described by Parati *et al* ¹⁵. This procedure identifies the "spontaneous" sequences of three or more consecutive beats in which systolic BP progressively rose and cardiac interval progressively lengthened (type 1 sequences), or systolic BP progressively fell and cardiac interval progressively shortened (type 2 sequences), with a lag of one beat. For each sequence, the linear correlation coefficient between cardiac interval and systolic BP was computed and the sequence validated when $r > 0.85$. The slope between cardiac interval and systolic BP was calculated for each validated sequence and expressed as msec.mmHg⁻¹

Metabolic measurements

After resting sympathetic neural measurements were completed, a standard 75-g oral glucose tolerance test (OGTT) was performed. A blood sample was withdrawn from a cannula placed in an antecubital vein 120 min post glucose administration (Glucaid, Fronine PTY, LTD, Australia). Insulin resistance was calculated from OGTT parameters according to the homeostatic model assessment (HOMA) method.

Statistics

Data is reported a mean±SEM. Statistical analysis was performed using the Student's *t*-test. MSNA has been shown to increase with increasing age and BMI ¹⁶ hence all MSNA variables measured were adjusted by both age and BMI using the following formula:

$$Y_o = y_i - b_1(x_{01} - \bar{x}_{i1}) - b_2(x_{02} - \bar{x}_{i2}) - \dots - b_p(x_{0p} - \bar{x}_{ip})$$

where y_i and x_{01} are data points of the original linear regression with slope b_1 , and where \bar{x} is the mean of variable x and Y_o represents the adjusted value for variable y.

Results

Anthropometric, hemodynamic and biological characteristics of the participants

Participants were divided according to their plasma cholesterol profile. Fourteen (52%) were found to have abnormal values of TC, HDL or LDL cholesterol. Six subjects had elevated plasma TC and LDL, one had elevated TC and low HDL, 3 subjects had elevated TC, 3 had low HDL and 1 had high LDL. There were none with raised TG (>150 mg/dL). There were 5 females taking the oral contraceptive pill in the normal group and 4 in the abnormal cholesterol group. Based on questioning during medical check-up, it was estimated that 6 females in the control group and 7 in the dyslipidemia group were in the follicular phase of their menstrual cycle while the remaining 2 and 3 in each group were in the luteal phase. There was no difference in age, height, weight and BMI between the 2 groups, however females in the dyslipidemia group tended to have an increased waist circumference (P=0.051) (Table 1). None of the females presented with abnormal fasting plasma glucose or insulin. OGTT was normal in all individuals and did not differ between the 2 groups. Likewise, the HOMA index was similar between the 2 groups of subjects. Twenty-four hour BP and HR measurements were similar between the 2 groups.

Females with dyslipidemia presented with significantly elevated plasma leptin concentration (P<0.010) and elevated hs-CRP (P=0.03).

Endothelial, sympathetic and baroreflex function

Endothelial function, as assessed by RHI, was significantly impaired in the dyslipidemia group compared with the normal group (RHI; 1.61 ± 0.10 vs 2.32 ± 0.14 , P<0.001, Figure 1). Sympathetic activity assessed in the skeletal muscle was significantly increased in the dyslipidemia group compared with the normal group (37 ± 3 vs 27 ± 3 bursts per 100 heartbeats, P=0.049, Figure 2). Sympathetic baroreflex function tended to be impaired in the elevated cholesterol group (-4.68 ± 1.10 vs -8.75 ± 1.82 bursts per 100 heartbeats.mmHg⁻¹, P=0.07). There was no difference in cardiac baroreflex function between the normal and the dyslipidemia groups (20 ± 3 vs 25 ± 3 msec.mmHg⁻¹ respectively, P=0.29)

Discussion

Impaired endothelial function is considered to precede the clinical manifestation of atherosclerosis⁴ and is a strong predictor of subsequent adverse cardiovascular events⁵. Steinberg and colleagues demonstrated that among healthy individuals, those with higher plasma cholesterol levels, even when still in the normal range, presented with impaired endothelial function compared to those with the lowest cholesterol levels¹⁷. This strongly indicated that cholesterol plays an important role in initiating endothelial damage. In line with this view, the present study indicates that in young healthy women with dyslipidemia, there is impairment of endothelial function. Moreover, this degree of endothelial dysfunction is accompanied by increased sympathetic neural drive, which may exert downstream effects initiating end organ dysfunction and predispose to cardiovascular disease development.

This demonstration of activation of the sympathetic nervous system in young women with dyslipidemia is novel. Increased neural drive has been associated with many conditions associated with cardiovascular risk such as hypertension¹⁸, obesity¹⁹ and heart failure²⁰, conditions which have also been associated with impaired endothelial function²¹⁻²³. However, the women in the present study, apart from presenting with a slightly abnormal cholesterol profile, were healthy and the two groups were matched for age, BMI and insulin sensitivity. Whether sympathetic and endothelial processes influence each other, or are both altered independently is unknown. There is some evidence in healthy individuals, by using systemic inhibition of nitric oxide (NO) synthase by N^G -monomethyl-L-arginine infusion, that NO, in addition to its direct vasodilator action, plays a role in the neural regulation of vascular tone²⁴. Experimental data support the idea that impaired endothelial function may influence sympathetic activity through alterations in neurotransmitter release, reuptake, or receptor sensitivity⁶. It seems that while endothelial function may impact on sympathetic tone, the reverse may also be true. Indeed, lower body negative pressure induced increased MSNA was shown to reduce endothelium-dependent flow mediated vasodilation

suggesting a specific inhibitory effect of sympathetic activation on shear-mediated NO release²⁵. Given the dual relationship between endothelial function and sympathetic tone, it is possible that elevated MSNA may contribute to or reinforce endothelial dysfunction in women with an abnormal plasma cholesterol profile or vice versa.

Cardiac baroreflex function was intact in the women with dyslipidemia and, while the baroreflex control of MSNA tended to be impaired, it did not reach significance thereby suggesting that sympathetic activation seen in young women with an abnormal cholesterol profile may not depend on reduced reflex restraint of baroreceptors on sympathetic tone.

While the two groups did not differ in body mass or body fat content, plasma leptin concentration was surprisingly elevated in the group of women with dyslipidemia. The cause of increased plasma leptin concentration in this group of women is uncertain. They tended to have increased waist circumference which is usually used as a surrogate marker of visceral fat²⁶. In women however, leptin is expressed and secreted more prominently in subcutaneous fat compared with visceral fat²⁷ so waist circumference may not be the best index for differences in plasma leptin concentration. Elevated cholesterol may not be a direct cause of increased leptin because it has been suggested that serum leptin concentration was independent of lipid metabolism²⁸. In vitro studies have suggested that leptin may cause endothelial dysfunction as it induced oxidative stress in cultured endothelial cells by increasing the generation of reactive oxygen species²⁹. Leptin also has been shown to stimulate the secretion of proinflammatory cytokines such as tumor necrosis factor alpha and interleukin-6 (IL-6) which are known to negatively affect endothelial function³⁰. Furthermore, leptin has also been shown to activate the sympathetic nervous system, most likely due to a central neural action of this hormone³¹, therefore we cannot exclude the possibility that sympathetic activation in women with elevated cholesterol levels may also occur in part through leptin's action. Women with dyslipidemia also presented with increased plasma hs-CRP. C-reactive protein is an acute phase reactant synthesized in the liver in response to IL-6 and is a marker of low grade inflammation. A study reported an independent association between waist girth and hs-CRP levels, most likely because the abdominal fat depot is a source of IL-6 that potently stimulates CRP

synthesis in the liver³². Waist circumference tended to be higher in the group of women with dyslipidemia, which may explain the increased hs-CRP. However a recent study indicates that CRP levels are significantly related to higher leptin levels in young women regardless of adiposity, insulin sensitivity, or physical activity³³. On the other hand it has also been suggested that autonomic function may play a role in regulating plasma hs-CRP³⁴. Previous studies have shown that CRP was able to induce pro-oxidative effects and inhibit endothelial NO synthase and therefore appears to be a key molecule able to accentuate endothelial dysfunction³⁵. Elevated hs-CRP is particularly important in this context because its levels have been shown to be predictive of the development of hypertension in prehypertensive and normotensive individuals³⁶.

Perspectives

Increased adrenergic tone is particularly relevant in disease states such as hypertension and heart failure, where enhanced sympathetic outflow has been linked with target organ damage and increased cardiovascular mortality^{18,20}, and in metabolic disorders¹⁹ where sympathetic activation is linked with insulin resistance³⁷. While it may be premature to state that the finding of increased sympathetic outflow in young women with dyslipidemia has a direct clinical implication we recently demonstrated that in young healthy individuals, increased MSNA positively correlated with left ventricular mass index and was negatively associated with renal function, independently of body mass and blood pressure⁷. Importantly, there exists data indicating that sympathetic activation may precede the development of hypertension and hyperinsulinemia³⁸. Altogether, this suggests that despite no apparent sign of current clinical manifestation of cardiovascular disease, women with dyslipidemia may be more predisposed to progression towards cardiovascular disease development. Whether this group of individuals would benefit from cholesterol lowering interventions such as statins remains to be seen but, given that statins exert a beneficial effect on sympathetic tone beyond its effect on endothelial function¹⁰, such an approach may hold promise.

Limitations

A number of limitations should be noted. This study was a retrospective analysis rather than a prospective trial. Additionally, cholesterol and endothelial function may be influenced by many factors such as obesity, insulin resistance, diabetes mellitus, hypertension, age, menstrual cycle and physical activity. Our groups were matched for body weight, fat mass, fasting glucose and insulin concentration, insulin sensitivity, blood pressure and physical activity thereby excluding these as possible confounding factors. Menstrual phase has been shown to influence endothelial function and sympathetic tone in young women. Indeed, a fall in endothelial function is diminished soon after ovulation which then rises again during the luteal phase of the menstrual cycle³⁹, and higher MSNA was observed in the luteal compared with the follicular phase⁴⁰. In the present study, females were at various stage of the menstrual cycle and some were taking oral contraceptives. While this may be a limitation of the study there were an equal number of subjects on the contraceptive pill or in the luteal or follicular phase in each group. Secondly, fat content and distribution were not determined using a robust technique such as DEXA scan so we cannot exclude that subtle changes in visceral adiposity may have played a role in influencing MSNA and endothelial function. Third, plasma cholesterol was measured on one occasion only. Finally, our measure of endothelial function relied on the measurement in the digits rather than in the coronary circulation or in the brachial artery. Nevertheless, endothelial function assessed in the fingers has been shown to provide a high degree of sensitivity and specificity when compared to the assessment of coronary artery endothelial function⁴¹.

Conclusion

This study demonstrates that young females with dyslipidemia present with endothelial dysfunction and elevated inflammatory markers which was accompanied by a marked increase in sympathetic neural tone. Whether sympathetic activation contributes to the initiation of cardiovascular disease in this setting remains to be elucidated.

Disclosure

Professor Esler serves on scientific advisory boards of Servier Australia, Abbott (formerly Solvay) Pharmaceuticals and ARDIAN Inc. Professor Schlaich serves on scientific advisory boards for Abbott (formerly Solvay) Pharmaceuticals and Novartis Pharmaceuticals. The laboratories of Professors Esler, Schlaich and Lambert currently receive research funding from ARDIAN Inc. and Allergan. The funding organizations played no role in: design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. The investigators report no conflicts of interest with regards to this manuscript.

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Legend to the figures

Figure 1

Endothelial function as assessed by reactive hyperaemia index (RHI) in females with normal and elevated plasma cholesterol (Ch) levels. * $P < 0.001$

Figure 2

Muscle sympathetic nerve activity (MSNA) in females with normal and elevated plasma cholesterol (Ch) levels. * $P < 0.05$. (MSNA is corrected for body mass index and age)