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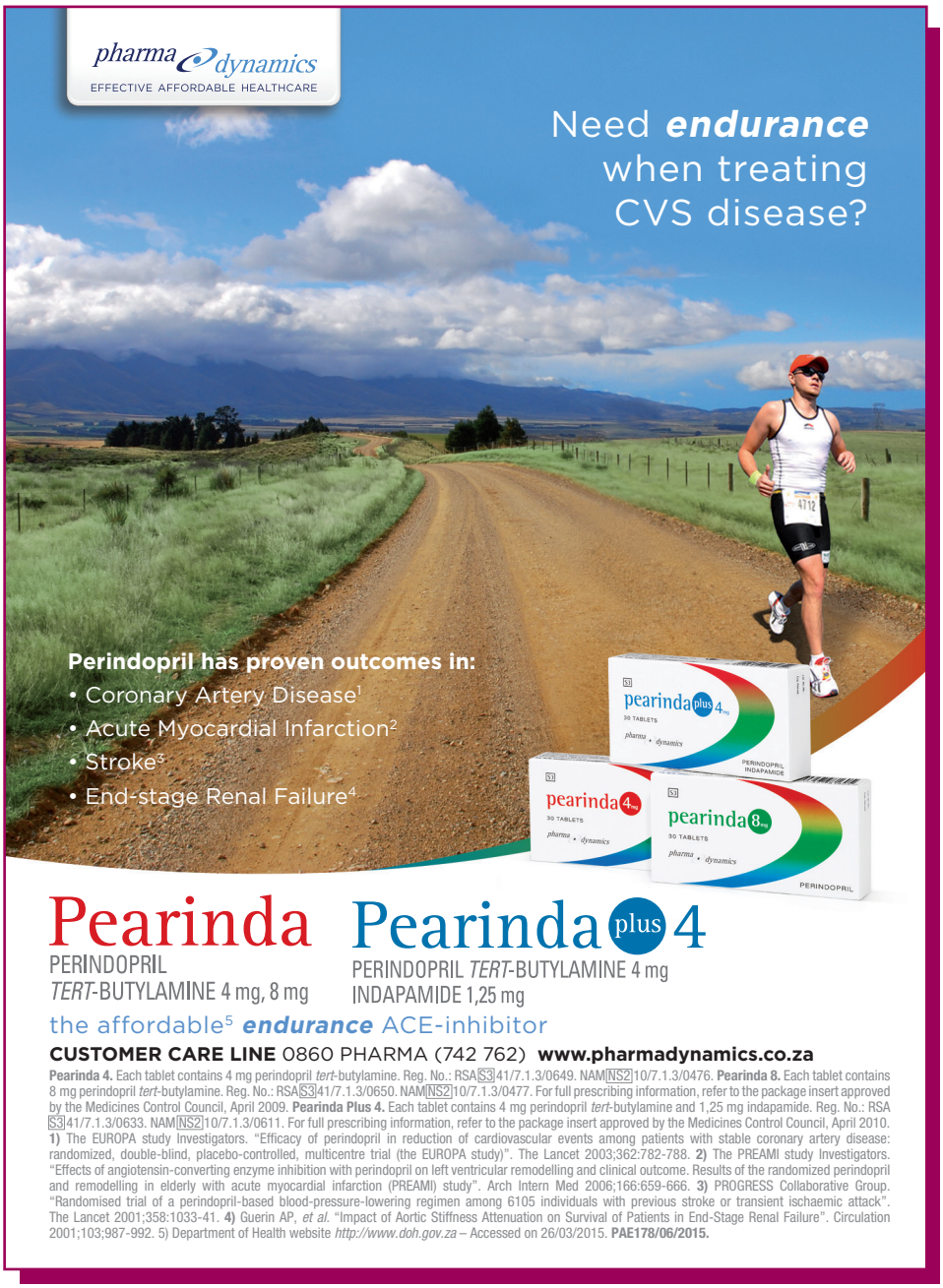

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From the Editor's Desk

There is probably no more important topic for discussion than the effect of lifestyle and diet on our health. The impact of lifestyle is hugely underestimated by the general public and is, in my view, by far the biggest contributor to non-communicable disease today. 'You are what you eat' is an old statement that still holds true.

We, the human race, have lost the plot when it comes to diet, and our over-consumption of high-calorie and nutritionally poor-quality food is endangering our health. The food industry, driven by profit not health, and supported by the advertising industry, has largely contributed to this problem.

This is very topical currently, and has recently been revived by Prof Tim Noakes. It was with excitement that I read the review by the legendary Prof Lionel Opie (page 5) on the topic of diet and cardiovascular disease. This is a must read for every healthcare professional who is questioned daily about the Banting and Noakes diets. The review gives a balanced and scientific opinion on the different diets and the best options.

Of concern to me is the large number of people who follow their own version of these diets, unsupervised, with potentially hazardous consequences. Strong evidence supports the association between low-density lipoprotein cholesterol (LDL-C) and cardiovascular disease, and if a diet increases LDL-C levels, it will very likely increase the risk of cardiovascular disease.

Prospective studies comparing these diets in a large number of people are urgently needed, to look at surrogate cardiovascular markers as well as cardiovascular endpoints. Such studies would be challenging because of people's inability to stick to a specific diet.

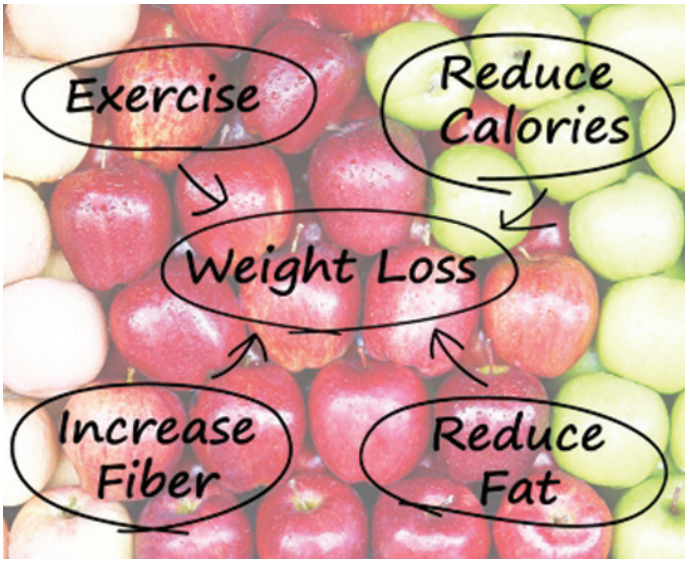
Pop and co-workers (page 8) discuss non-conventional cardiovascular risk factors that could be used to estimate cardiovascular risk in women. They highlight many options and discuss the validity of these risk factors.

A novel approach to peripheral neuropathy is discussed in a very interesting article by Seewoodhary and others (page 12), using stem cell transplants. This appears promising, but is unlikely to be a therapeutic option for diabetic neuropathy in the near future, and will probably be a very costly option. There are currently not enough human data to support this mode of therapy.

In a study called the Dyslipidaemia International Study (DYSIS), Raal and colleagues (page 21) assessed cholesterol therapy in South African patients in private practice. In total, 1 029 patients were included in the study, and it was shown that more than 50% of patients did not reach their LDL-C targets and over 70% were at high cardiovascular risk. Many patients in South Africa experience persistent dyslipidaemia despite statin treatment, and further measures are needed to reduce the burden of cardiovascular disease in our population.

In another observational study assessing lipid control on home soil, Rapeport and co-workers (page 30) also reported sub-optimal lipid control in 2 996 patients (52%) from the private and public sectors. They reported important gender and ethnic differences and reinforced control of risk factors.

Controlling lipid levels in high-risk patients is a fairly easy intervention and many potent statins are available at a fair price. However, drug side effects are sometimes a stumbling block to achieving our goals. We need to improve on the numbers of patients reaching target levels in order to lower cardiovascular risk in our patients, especially in the diabetic subgroup, where glucose targets are much more difficult to achieve. Target control of glucose levels is much lower than 50% in diabetic patients, but we know that lipid control gives much better cardiovascular protection and therefore this should be a priority, as it is achievable.



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The cardioprotective diet – carbohydrates versus fat

FJ RAAL

The global burdens of cardiovascular disease, obesity and type 2 diabetes mellitus continue to rise in both developed and developing countries.[1] Much of these burdens are preventable as they are the result of sub-optimal lifestyle, which includes poor diet, excess calorie intake, physical inactivity and cigarette smoking.[2]

As discussed by Lionel Opie (page 5), several diets, such as the new Adkins diet, the Noakes diet and the Dukan diet, which encourage the restriction of carbohydrates rather that the restriction of fat, has recently been introduced and many more are likely to follow.[3] Each claim to be better that the next at addressing this global health burden. However one has to consider what these diets are trying to achieve. Are they trying to achieve weight loss and prevention of the onset of type 2 diabetes, or are they trying to achieve cardiovascular protection?

It is correct that excessive carbohydrate intake, particularly refined carbohydrate as found in sugary drinks and energy snacks, is contributing to the global epidemic of obesity and type 2 diabetes mellitus but it is wrong to conclude that a high-carbohydrate intake is the major cause of atherosclerosis, the leading cause of cardiovascular disease worldwide. Atherosclerosis, particularly coronary artery disease, is not a disease of carbohydrate metabolism and there is little evidence to show that a low-carbohydrate diet will prevent atherosclerosis.

Restriction of refined carbohydrates, being our major energy source, will assist with weight reduction in the short term. However in terms of prevention of atherosclerosis and cardiovascular disease in the longer term, restriction of saturated fat is more important. It is therefore incorrect, and in fact it may be harmful, to advocate the substitution of refined carbohydrates with saturated fats.

Increasing the intake of saturated fats raises serum low-density lipoprotein (LDL) cholesterol levels.[4] Innumerable epidemiological studies have shown a positive relationship between serum LDL cholesterol levels and risk for cardiovascular disease, particularly coronary artery disease. In fact the link between LDL cholesterol and coronary artery disease is one of the most thoroughly researched in all of medicine.[5]

There is overwhelming evidence, accumulated over more than three decades, to show that the more you lower LDL cholesterol the lower your cardiovascular risk. For every 1 mmol/l reduction in LDL cholesterol using statins, there is approximately a 12% reduction in total mortality and a 21% reduction in major vascular events.[6] We have not yet identified a threshold below which LDL cholesterol reduction is no longer beneficial but harmful.[7]

So what should we be advising our patients at risk for cardiovascular disease? Obesity is not so much about diet but

about energy balance – calories consumed versus those expended. Appropriate restriction of calorie intake whether it be carbohydrate, protein or fat is important for weight maintenance and prevention of obesity and type 2 diabetes. However in terms of achieving cardiovascular protection or maintaining a low LDL level, cholesterol is pivotal.

As Lionel Opie emphasises, we need to encourage and promote a healthy lifestyle with regular exercise, non-smoking and a healthy diet consisting of moderate portions of all three of the major components of our diet, namely carbohydrate, protein and fat.[3] If LDL cholesterol levels remain elevated or if the individual has established cardiovascular disease or diabetes, or is considered at high cardiovascular risk, international guidelines worldwide recommend that statin therapy should be initiated.[8,9] This will be much more beneficial for long-term cardiovascular protection than the short-term benefit of weight reduction achieved with marked carbohydrate restriction.

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Lifestyle and diet

LIONEL H OPIE

Abstract

Currently, there is widespread interest in many different diets. The best-known diets include the New Atkins diet in the USA, the Dukan diet in France, and in South Africa the Noakes diet. Two different approaches have emerged, one focusing on a life-long healthy lifestyle and the other emphasising weight loss. These are in fact complementary aims, as will be reviewed and reconciled. Furthermore, besides the dietary approach, there is a valid case for added drug therapy for selected lipid disorders with the use of statins. In addition, new drugs are emerging that in the future might eventually considerably reduce the negative health impact of coronary artery disease.

Keywords: diet, cardiovascular risk, Noakes diet, Banting diet, Mediterranean diet

Lifestyle is life-long

Lifestyle is life-long in its health implications.[1] Although diet is only one of the five components of a healthy lifestyle,[2] diet has recently come to the fore.[3] When considering overall health, the most important are non-smoking and regular exercise, followed by body weight and diet, in order of importance (Table 1). These proposals are based on a series of important studies on over 100 000 US health professionals over 10 to 25 years, which defined the contribution to health of four major lifestyle factors, only one of which is diet (Table 1).[2,4,5]

While there are many diets to choose from, the majority focusing on weight loss, few diets have had scientifically solid outcome studies to prove that the diet in question actually improves health and increases life span. An exception is the Mediterranean diet, so called because of the very low incidence of heart attacks observed by Ancel Keys in the Mediterranean islands of Corfu and Crete, thus leading to the concept that the Mediterranean diet is an ideal diet,[1,6,7] also protecting against heart failure.[8]

Palaeolithic, the oldest diet

What is the paleolithic diet? Mankind evolved over hundreds of millions of years, therefore the paleolithic diet must have been the standard diet that also evolved over that time. Studies on the teeth of the paleolithic man, as found in East Africa (also in its congener from South Africa), showed that the dental bones and teeth had adapted to process large quantities of low-quality vegetation rather than hard objects.[9] The paleolithic diet is now recognised as a nutritional pattern based on the ancient diet of wild plants and animals that our ancestors consumed over 10 000 years ago.

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Table 1. The 'big-five' components of the healthy lifestyle, with contributions of the various components to give protection from risk of death, with and the proposed mechanisms of action. Note that the missing 21% is probably stress related. From Opie,[1] page 33.

Lifestyle: 'big five'	Reduced all-cause death risk (%)	Mechanism
Non-smoking	28	Protects arteries
Exercise 30 min or more daily	17	Slows the heart rate, lowers BP
Ideal weight	14	Less toxic chemicals released from fat cells
Ideal diet	13	High unsaturated fatty acids, high vegetables and fruit, low red meat
Modest alcohol	7	Red wine preferred, contains melatonin
All five	79	Remaining 21% may be stress related

In the Kitava dietary study on isolated tribes in Papua, New Guinea, who even recently ate a pre-Westernised diet of 55 to 65% animal foods and 35 to 45% plant foods, these societies had no incidence of stroke, heart disease, diabetes or hypertension.[10] The diet consisted mainly of fish, grass-fed pasture-raised meats, vegetables, fruits, roots, spices and nuts. There was no restriction on calories or on the foods to be cooked.

Although the Mediterranean diet overlaps with the palaeolithic diet in terms of fibre, antioxidants, saturated fat and mono-unsaturated fat, the paleolithic diet improved glucose tolerance more than did the Mediterranean diet.[10] Furthermore, this diet is more food satiating than a Mediterranean-like diet in persons with ischaemic heart disease.[11] Therefore the paleolithic diet both preceded the Mediterranean diet and was apparently better, so it may be that 'the simpler, the better'.

Diet and lipids

Moving on in history, it was the early Cape Town studies that made the link between fat in the diet and blood cholesterol values. Nearly 60 years ago, Professor John Brock and Brian Bronte-Stuart from Groote Schuur and the University of Cape Town Department of Medicine used their specialised metabolic unit to give a high-fat diet to subjects with an initially low blood cholesterol level (Fig. 1).[12,13] A butter load of 100 grams given daily increased blood cholesterol by proximately 40% within five days. The addition of large amounts of olive oil to the butter load restored cholesterol levels to their prior low levels (Fig. 1). Therefore the type of fat diet affected blood cholesterol levels.

The decisive further link between circulating cholesterol values and coronary heart disease came from the Framingham study, which found that higher blood cholesterol values were associated with increased cardiovascular and total mortality rates.[15] Over time,

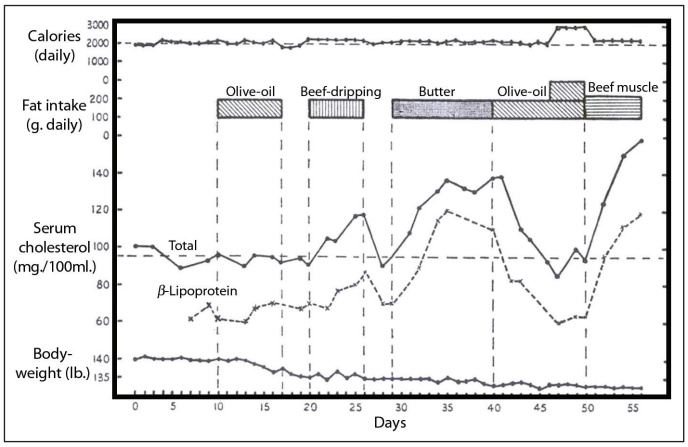

Fig 1. A historical study in Cape Town. The effect of dietary fats on blood lipid levels and their relation to ischaemic heart disease, neutralised by the effect of added olive oil. Note the rapid rise in serum cholesterol levels with the provision of the high-butter diet. All values were obtained in the Metabolic Unit, University of Cape Town, South Africa. From Bronte-Stewart.[14]

the emphasis on selection of drug therapy via statins has shifted to the blood level of low-density lipoprotein (LDL) cholesterol.[16]

In South Africa in 2000, high blood cholesterol levels have been estimated to have caused 24 144 deaths (95% CI: 22 404–25 286) or 4.6% of all deaths.[17] Studies in the Cape Peninsula and in the South African Indian population support links between lipid abnormalities and coronary heart diseases.[18,19] Severely obese South African white women have greatly reduced values for serum high-density lipoprotein (HDL) cholesterol or 'good' cholesterol, rather than high levels of LDL cholesterol.[20]

Lipids in diabetes: the role of statins

The ideal approach to nip diabetes in the bud is by testing HbA_{1c} values in those with the metabolic syndrome or obesity, and then to go for weight loss induced by combined diet and exercise. In those with established type 2 diabetes (DM2), a population study in Hong Kong suggested that statin therapy attenuated the associated increased cancer risk.[21] For diabetes, in a large study with 215 725 person-years of follow up, statin use before the diagnosis of diabetes reduced diabetic retinopathy (hazard ratio 0.60, 95% CI: 0.54–0.66; $p < 0.0001$), diabetic neuropathy (HR 0.66, 95% CI: 0.57–0.75; $p < 0{\cdot}0001$), and gangrene of the foot (HR 0·88, 95% CI: 0.80–0.97; $p = 0.010$).[22] Regarding the general adult population, statins are recommended as first-line therapy in those up to and including 75 years of age, who have clinical atherosclerotic cardiovascular disease (ASCVD) (Table 4 in Stone *et al*.[23]).

Exercise versus drugs

In studies on the secondary prevention of coronary heart disease and pre-diabetes, randomised trials on exercise interventions suggest that exercise and many drug interventions are often potentially similar in terms of their mortality benefits, rehabilitation after stroke, treatment of heart failure, and prevention of diabetes.[24] This important observation reinforces the essential role of exercise in any programme aimed at overall cardiovascular health (Table 1).

Banting first linked diet to mortality

Banting in his pamphlet[25] in 1869 emphasised the role of diet in weight loss, stating that: 'The dietary is the principle point in the treatment of corpulence.' The key points in the Banting diet were his method of reducing obesity by avoiding fat, starch and sugar in the food. Therefore the proposal that the Banting diet is similar to the Noakes high-fat diet[3] appears to need re-appraisal. Banting also made wider overall claims that the diet was 'a simple remedy to reduce and destroy superfluous fat; it may alleviate if not cure gout; prevent or eradicate carbuncles, boils, dyspepsia, makes life more enjoyable, and promotes longevity'. One interesting small but important point is that Banting took the fat off the gravy. For these reasons, it seems preferable to separate the Banting diet from the Noakes low-carbohydrate, high-fat diet.

Israeli study and new Atkins diet

The low-carbohydrate, high-fat diets that were introduced by Atkins and his successors[26] have had very wide influence. Some of the key features are as follows, with the relevant book pages given in brackets:

- Protein intake though high has recommend protein ranges (51).
- Fat intake though also high, has a desirable range (70).
- Vegetables including avocadoes are the basis of the permitted carbohydrate intake (102).

In a major landmark Israeli diet, the new Atkins diet was compared with others from the same Israeli population group in a dedicated communal restaurant where the food intake could be monitored.[27] In the group given the new Atkins diet, besides weight loss, the blood cholesterol pattern showed some favourable changes.

In the comparative group taking a calorie-limited Mediterranean diet, similar changes were found in weight loss and blood lipid levels. However, the Mediterranean diet was calorie limited whereas the Atkins group had a spontaneous loss of appetite. The molecular mechanism to explain the appetite loss is not clear. Reservations are that there was no placebo group and the study was too short to judge any clinical effects on cardiovascular events.

A broadly similar conclusion was reached in a meta-analysis of diets of varying carbohydrate and lipid composition, the new Atkins diet is one of several reduced-calorie diets that have all resulted in clinically meaningful weight loss, regardless of which macronutrients they emphasised.[28]

What about high-fat weight-losing diets?

The two potential problems with high-fat diets lie in their adverse effects on the blood lipoprotein pattern, and on the impairment of specific mental functions, as observed by Kieran Clarke in Oxford students. In the Oxford study, a short-term, high-fat, low-carbohydrate diet led to higher circulating free fatty acid (FFA) concentrations, impaired patterns of myocardial high-energy phosphate metabolism, and decreased cognition in healthy subjects.[29]

The site of these deleterious effects on the brain was the hippocampus. In the heart, sophisticated non-invasive nuclear imaging techniques measured levels of high-energy phosphate compounds, which were relatively low in those taking the high-fat diet. The proposal was that elevated circulating FFA levels were underlying the cognitive and cardiac abnormalities. Therefore Clarke and her associates conclude that high-fat, low-carbohydrate diets are potentially detrimental to human heart and brain.[29,30]

For these reasons, there are arguments to support the view that the diet overweight persons could best start is with a new Akins

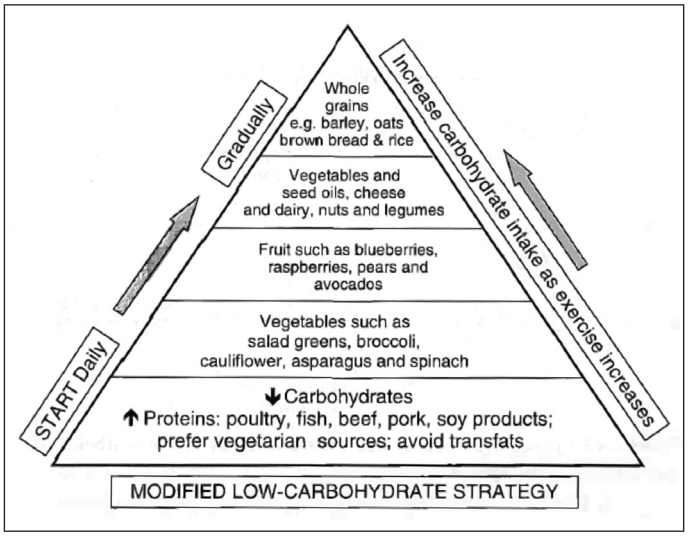

Fig. 2. This dietary pyramid starts at the bottom, with low carbohydrate intake of about 20 g per day, then as exercise increases, works up to 40 to 100 g of carbohydrates per day while maintaining weight loss, with the lifelong aim of maintaining the ideal weight. Note that poultry, fish and beef (free of visible fat) are allowed in the initiating phase. From Opie,[1] page 67.

type of diet for weight loss, coupled with an exercise programme, and then move onto the Mediterranean-type diet to achieve life-long health benefits, thereby avoiding the cognitive and cardiac changes of high-fat diets. Therefore starting a diet to lose weight, such as the new Atkins or Noakes diet, is complementary with a later switch to the long-term Mediterranean diet. As these diet types come in sequence, they are not competitive.

The future

A safe prediction is that there will be more editions of existing major books (Atkins in the USA, Dukan in Europe, Noakes in South Africa) besides new diet books. New lipid-lowering pharmaceutical agents are already being tested in large new outcomes-based studies on their preliminary promise.

The best self-help policy may well be to start with a dedicated programme for weight loss however achieved, whether by the new Atkins or Noakes diet, but associated with sufficient exercise. The next step would be to move on to the modified Mediterranean diet (Fig. 2) aimed at living longer and living better.

Looking to the far future, having both fish and meat in the daily diet of large populations would need substantial resources, which will be increasingly limited as the human race expands. Maybe the answer will lie in novel fresh nutritional sources such as algae-based diets.

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Novel cardiovascular risk markers in women with ischaemic heart disease

DANA POP, ALEXANDRA DĂDÂRLAT, D ZDRENGHEA

Abstract

The incidence of coronary heart disease in premenopausal women is lower than in men because of their hormonal protection. Angina pectoris occurs in women about 10 years later than in men. However, mortality from ischaemic heart disease remains higher in women than in men. Current studies are focusing on novel cardiovascular risk biomarkers because it seems that traditional cardiovascular risk factors and their assessment scores underestimate the risk in females. Increased plasma levels of these newly established biomarkers of risk have been found to worsen endothelial dysfunction and inflammation, both of which play a key role in the pathogenesis of microvascular angina, which is very common in women. These novel cardiovascular risk markers can be classified into three categories: inflammatory markers, markers of haemostasis, and other biomarkers.

Keywords: ischaemic heart disease, women, new cardiovascular risk factors

Cardiovascular disease (CVD) represents the leading cause of death among women in Europe. About 53% of female deaths are due to CVD, particularly coronary heart disease and stroke.[1-9] The incidence of coronary heart disease is significantly lower in premenopausal women, due to their hormonal protection, but there are reportedly more complex mechanisms involved. Angina pectoris and heart attack occur in women about 10 and 20 years, respectively, later than in men.[5]

There are significant gender-related differences concerning coronary heart disease. The particularities regarding women are: higher prevalence in women over 75 years, the first coronary event is 10 years later than in men, atypical symptoms, high incidence of non-Q-wave myocardial infarction, and the prevalence of coronary arteries without angiographic findings is twice as common as in men.[6]

Since 2004, guidelines have been emphasising the importance of recognising cardiovascular risk factors in women and also to classify women at high, intermediate or 'ideal' cardiovascular risk.[2-4] A high-risk status is given not only by the presence of coronary artery disease, cerebrovascular disease, chronic arterial occlusive disease, aortic aneurysm or a Framingham score over 10%, but also by the presence of chronic kidney disease or diabetes.[2]

Women who face the threat of cardiovascular disease present with one or more risk factors including: smoking, pro-atherogenic diet, obesity (especially central obesity), family history of cardiovascular disease at a young age, hypertension and dyslipidaemia. Furthermore, it seems that subclinical vascular disease (such as coronary calcification), the metabolic syndrome, a low effort capacity or an abnormal heart rate recovery after the exercise stress test creates a prominent cardiovascular risk among women.[2] Latest studies show that women diagnosed with collagen disease (auto-immune disease), a history of pre-eclampsia, gestational diabetes or pregnancy-induced hypertension require strict medical management due to their high predictive ability for the development of cardiovascular disease.[2]

Ideal cardiovascular health status is gained by women with blood pressure below 120/80 mmHg, total cholesterol level below 200 mg/dl, fasting plasma glucose below 100 mg/dl (without specific treatment), body mass index (BMI) below 25 kg/m^2 and, undoubtedly, by those who practice intense physical exercise at least 150 minutes per week, or moderate exercise for 75 minutes per week, and by non-smoking women.[2]

Review of the evidence reveals that compilation of traditional risk factors and cardiovascular risk scores underestimates the risk in women. Therefore, ongoing areas of research are focusing on novel markers of cardiovascular risk. These novel cardiovascular risk biomarkers have been selected because their increased plasma levels worsen endothelial dysfunction and inflammation, both being key players in the pathogenesis of microvascular angina, which is a common phenomenon in women.[1]

The Women's Health Initiative hormone trials showed that at least 18 new biomarkers are useful in estimating cardiovascular risk in postmenopausal women. These are lipoprotein (a), homocysteine, insulin, C-reactive protein (CRP), E-selectin, interleukin-6, matrix metalloproteinase-9, fibrin D-dimer, factor VIII, plasminogen activator inhibitor-1 antigen, prothrombin fragment 1.2, plasmin–antiplasmin complex, thrombin-activatable fibrinolysis inhibitor, von Willebrand factor, fibrinogen, haematocrit, leukocyte and platelet counts.[10] These novel biomarkers of cardiovascular risk are classified into three categories: inflammatory markers, haemostasis markers, and other biomarkers.

Inflammatory markers

High-sensitivity C-reactive protein (hs-CRP)

The latest European guidelines on CVD prevention in clinical practice (2012) recommend the determination of high-sensitivity CRP levels as part of the refined risk assessment in patients with an unusual or moderate CVD risk profile (class IIB, level B).[11] Normal values for this inflammatory factor are below 2 mg/dl.

CRP levels in women are higher than in men, especially during puberty.[2] The JUPITER trial reported that an hs-CRP value over 2 mg/dl in association with a low-density lipoprotein (LDL) cholesterol value below 130 mg/dl in women without cardiovascular pathology increases the risk of cardiovascular events.[12] Moreover, high levels of CRP in women without cardiovascular disease are important predictors for the development of fatal heart attack and stroke.[13]

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The greater the number of cardiovascular risk factors that apply to a woman, the higher her hs-CRP level.[13] Elevated CRP levels have been associated with the presence of the metabolic syndrome, diabetes and chronic heart failure. Furthermore, recent studies show that a high CRP value is correlated with an increased incidence and prevalence of auto-immune diseases in women, such as rheumatoid arthritis and lupus erythematosus.[3-6]

The Women's Health study demonstrated that the addition of hs-CRP to the Framingham score improved the predictive accuracy of cardiovascular risk, especially in women with a 5–20% risk in 10 years.[14] Evidence from the Women's Ischemia Syndrome Evaluation, a prospective study, reported that high levels of amyloid serum A, IL-6, sICAM1 and CRP had the highest predictive accuracy in 27 347 postmenopausal women apparently without cardiovascular disease.[15] The guidelines do not however recommend routine evaluation of this inflammatory biomarker, CRP.[2,11]

Fibrinogen

High levels of fibrinogen are associated with an increased risk of cardiovascular disease in both men and women, but there are still substantial gender-specific differences.[6,13] On one hand, plasma fibrinogen levels increase with menopause, but also during the use of oral contraceptives and pregnancy.[16,17] On the other hand, hormone replacement therapy lowers serum levels of fibrinogen.[16]

The latest European guidelines on cardiovascular disease prevention in clinical practice recommend the determination of fibrinogen levels as part of a refined risk assessment in patients with an unusual or moderate CVD risk profile (class IIB, level B).[11]

Interleukin-6 (IL-6)

IL-6 stimulates hepatic release of CRP and fibrinogen, both acute-phase reactants involved in the process of atherosclerosis and atherothrombosis. Unfortunately, there are contradictory data regarding the role of IL-6 in the development of coronary heart disease in women.[10,17]

Atherosclerosis reported from the British Women's Heart and Health study that the level of this cytokine was not directly associated with the risk of coronary heart disease.[18] Interestingly, the Women's Health Initiative showed a direct correlation between high levels of IL-6 and ischaemic heart disease.[10] Undoubtedly, cardiovascular risk was not assessed only by measuring the IL-6 plasma levels, but also by determining other cardiovascular risk factors.[10]

Matrix metalloproteinase-9 (MMP-9)

MMP-9, along with CRP, IL-6 and increased levels of leukocytes may provide accuracy in the prediction of developing coronary heart disease in women.[10,17,19]

E-selectin

Various studies impugn the relationship between E-selectin and cardiovascular risk.[17,19] On the other hand, there is evidence to support the predictive value of E-selectin for cardiovascular events.[17,19,20]

Haemostasis markers

There are sufficient data concerning the association of D-dimer, coagulation factor VII, von Willebrand factor and fibrinogen levels with the risk of coronary heart disease in women (after statistical adjustments for traditional risk factors).[21] Studies demonstrated the presence of high levels of coagulation factor VII in women suffering from angina or other cardiovascular diseases.[22-25] However, the most eloquent reports support the use of D-dimer in estimating prognosis of cardiovascular death and other events in women.[26]

Plasminogen activator inhibitor-1 (PAI-1)

Recent studies identified lower PAI-1 levels in premenopausal than postmenopausal women.[16,17] The concentration of PAI-1 was lower in women taking hormone replacement therapy, compared with non-users.[6,16]

Gene-specific differences and changes in PAI-1 values during the postmenopausal years may be related to PAI-1 gene polymorphism. The 4G/5G mutation was found more frequently among postmenopausal women with coronary heart disease than in premenopausal women.[16]

Lipoprotein (a) [Lp(a)]

As is well known, elevated levels of Lp(a) increase the risk of ischaemic heart disease in both men and women. Investigators demonstrated a clear association between Lp(a), LDL cholesterol, hypertension, hyperhomocysteinaemia and hyperfibrinogenaemia in men. Also in women an increase in Lp(a) levels with age has been reported.[6]

Notably, Lp(a) is an emerging cardiovascular risk factor in both pre- and postmenopausal women as it contributes to the formation of atherosclerosis. Sometimes high levels of Lp(a) are correlated with high CRP levels.[27]

High Lp(a) values together with abnormal blood lipid levels are risk factors for cardiovascular disease in women, even in those under 60 years.[16] New research on women offers strong evidence that heart attack risk increased as Lp(a) levels rose.[28]

Lipoprotein-associated phospholipase A2 (Lp-PLA2)

Recent data confirm the involvement of Lp-PLA2 in the development of atherosclerosis by modifying the affinity of LDL particles for extracellular matrix proteins.[30-32] Moreover, Lp-PLA2 favours lipid accumulation in arterial walls, lipid peroxidation, and hydrolysis of lysophospholipids and free fatty acids.[33,34] Lp-PLA2 may be identified as an independent risk factor for rupture of atheroma plaque and thrombo-embolic events.[12]

The latest European guidelines on cardiovascular disease prevention in clinical practice recommend the determination of Lp-PLA2 values as part of a refined risk assessment in patients at high risk of a recurrent acute atherothrombotic event (class IIB, level B).[11] The 2010 ACCF/AHA Guideline for the Assessment of Cardiovascular Risk in Asymptomatic Adults reported that calculation of Lp-PLA2 levels may be reasonable for cardiovascular risk assessment in intermediate-risk asymptomatic adults (class IIb level B).[35]

The recent Nurses' Health study showed that levels of Lp-PLA2 were significantly associated with the incidence of ischaemic heart disease in women.[36] According to some research results, women have higher levels of secretory phospholipase A2 (sPLA2) than men. It was reported that elevated sPLA2 levels were correlated with high CRP levels.[27,37]

Homocysteine

In general, women present with lower homocysteine values than men, but elevation occurs during the menopausal years.[16,38]

Also, a number of studies suggested a relationship between serum homocysteine levels and the presence of coronary artery disease in women, but not in men.[14] Therefore, it represents a stronger atherogenic factor in women than in men.[16] Other studies however did not identify homocysteine as a significant factor in predicting statistical risk of coronary heart disease after adjustment for traditional risk factors, even though they found a positive correlation between this biomarker and ischaemic heart disease.[38]

Nevertheless, the latest European guideline on cardiovascular disease prevention in clinical practice states that homocysteine may be measured as part of a refined risk assessment in patients with an unusual or moderate CVD risk profile (class IIB, level B).[11] The measurement of serum homocysteine levels is not part of the routine screening process for cardiovascular risk assessment.[11]

Other markers

Natriuretic peptides

The Framingham Offspring study showed that 10 elevated biomarkers, and high B-type natriuretic peptides (BNP) indicated cardiovascular risk.[39] On the other hand, the Swedish Malmö diet and cancer cohort showed that only BNP and mid-region pro-adenomedulin levels were associated with a doubled cardiovascular risk.[40]

The 2012 ESC guidelines for the management of heart failure revealed that BNP, N-terminal pro B-type natriuretic peptide (NT-proBNP) and mid-regional pro-atrial natriuretic peptide (MR-proANP) levels showed usefulness in detecting heart failure patients, a differential diagnosis of dyspneoa and risk stratification.[41]

The KORA study included 1 005 women and men aged between 25 and 75 years. The goal of this study was to determine the variation in the NT-proBNP and BNP levels in a 10-year period. They reported a strong correlation between gender, age and plasma levels of natriuretic peptides. Both NT-proBNP and BNP serum concentrations recorded an elevation during the follow-up period, especially in women.[42] However, it has been shown that a BNP value that exceeds 500 pg/ml represents a stronger predictor of death in women than men with heart failure.[43]

Growth-differentiation factor-15 (GDF-15) is a novel biomarker under investigation, which is synthesised in ischaemic myocytes. There is evidence that it strongly indicates an increased risk of cardiovascular death.[44]

Conclusion

Despite the use of these novel cardiovascular risk factors, the presence of hypertension, diabetes, physical inactivity and inflammatory markers remain the most potent cardiovascular risk factors in women, regardless of age. Novel cardiovascular risk factors may play a decisive role in the early diagnosis of ischaemic heart disease, especially in women with suspected myocardial ischaemia, but without electrocardiographic, echocardiographic or angiographic findings. However, their routine measurement is difficult to implement. The guidelines regarding coronary artery disease in women could suggest the determination/ evaluation of these novel cardiovascular risk factors when a differential diagnosis should be considered.

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In Memoriam: Cephas Musabayane, 1948–2015

It is with sadness that we report the death of Prof Cephas Musabayane, professor of physiology at the University of KwaZulu-Natal, and leader of a team of researchers who discovered a new method of administering insulin into the bloodstream via a skin patch. The discovery could eventually pave the way for diabetic patients to control their insulin levels in a pain-free manner with reduced negative side effects.

Musabayane was born and schooled in Zimbabwe, obtained his BSc from Hertfordshire University in the UK, and then returned to the University of Zimbabwe where he obtained his MSc and PhD. He joined the University of KwaZulu-Natal as professor of human physiology in 2003, and served as head of the School of Medical Sciences for four years.

Musabayane was an academic who was passionate about teaching and research. He inspired his students and motivated them to achieve success. His areas of research included diabetes, malaria and renal physiology, and he obtained national and international recognition for his work. He was a life fellow of the Physiology Society of Southern Africa and a member of the US and UK physiological societies.

Stem cell therapies for neuropathic pain

JASON SEEWOODHARY, JOHN N HARVEY

Abstract

Neuropathic pain is a large-scale epidemiological problem effecting 13–26% of the diabetic population. The complex aetiology and pathophysiology coupled with the lack of a diagnostic test for the underlying cause renders the assessment of neuropathic pain subjective and the treatment difficult, especially as current licensed treatments are limited in their application towards the attainment of palliation.

Cell therapies offer a novel curative therapeutic dimension for neuropathic pain. This is based on replacing damaged neuronal tissue, protecting against progressive nerve damage, and releasing soluble factors that act in a paracrine or endocrine manner, which facilitate repair and reversal of the pathology that underlies the genesis and propagation of damage within the somatosensory system. Cell therapies with potential utility for the treatment of neuropathic pain include embryonic stem cells, adult stem cells and induced pluripotent stem cells.

Keywords: stem cell, neuropathic pain, cell therapy, regeneration, analgesia, diabetes

Introduction

Current licensed therapies for the treatment of neuropathic pain are limited in their application towards the attainment of palliation. They are not disease modifying or neuroprotective, being incapable of reversing or repairing the pathology that underlies the genesis and propagation of damage within the somatosensory system. Stem cells may offer a novel curative regenerative therapeutic dimension. This review will discuss the potential utility of stem cells for the treatment of neuropathic pain.

Neuropathic pain

Neuropathic pain is a subtype of pain defined by IASP as "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system". The predominant symptom is paroxysmal episodes of stabbing or shooting pain arising in an area of hyperexcitability or numbness. Other symptoms include: spontaneous pain, hyperalgesia and allodynia; dyaesthesias; abnormal thermal sensations; and deep-seated gnawing pain.

Epidemiology

Neuropathic pain is a large-scale problem; epidemiological data estimate neuropathic pain affects 13–26% of diabetic patients.[1] However, due to the complex aetiology of neuropathic pain coupled with the lack of a diagnostic test and standardised measurement methods, exact data are deficient, which renders the overall prevalence difficult to quantify. Accordingly the health economic costs of neuropathic pain to society are undetermined.

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Aetiology and pathophysiology

The aetiology of neuropathic pain can be categorised morphologically into four groups: peripheral nervous system and multi-focal lesions e.g. diabetic mononeuropathy; peripheral nervous system generalised polyneuropathies e.g. alcohol-related neuropathy; central nervous system lesions e.g. spinal cord injury; and complex neuropathic disorders, such as complex regional pain syndrome types I and II.[2] However, this classification system is not universally accepted.

The pathophysiology of neuropathic pain is complex and results from several processes, which cumulatively lead to peripheral and central sensitisation associated with ectopic activity and hyperexcitability in pain pathways.[3] This is associated with histopathological changes within affected nerves characterised by: Wallerian degeneration; sprouting; formation of end-neuromas and neuromas-in-continuity; and compression induced atrophy.[4] Peripheral mechanisms act on nociceptors. The hyperactivity in nociceptors induces hyperexcitability in the spinal cord and brain, referred to as central sensitisation. A schematic depicting the various alterations in nerve function leading to neuropathic pain is illustrated in Fig. 1.

Diagnosis and treatment

Neuropathic pain is diagnosed clinically. Screening methods are based on questionnaires such as the Leeds Assessment of Neuropathic Symptoms and Signs or the pain DETECT questionnaire.

The Neuropathic Pain Special Interest Group of the IASP has developed evidence-based guidelines for the pharmacological treatment of neuropathic pain.[5] Based on the results of randomised controlled trials, first-line agents include: tricyclic anti-depressants, selective serotonin and noradrenaline reuptake inhibitors, voltage-gated Ca^{2}+ channel $\alpha 2$-δ ligands, and topical local anaesthetics. Second-line medicines include opioid analgesics and tramadol. Third-line drugs include valproate, topiramate, mexiletine and topical capsaicin.[6]

Non-pharmacological treatments include: surgical and chemical sympathectomy, which are limited in their application by a sparse evidence base and considerable complications;[7] neurodestructive procedures, which are hindered by a risk of exacerbating symptomatology; neurostimulation e.g. transcutaneous electrical nerve stimulation, although this is not based on conclusive evidence;[8] spinal cord stimulation; acupuncture; and cognitive behavioural therapy.

Limitations of current treatment

Therefore, the management of neuropathic pain is challenging with a poor prognosis. Treatment is hindered for a number of reasons: there is no definitive corroboration between published

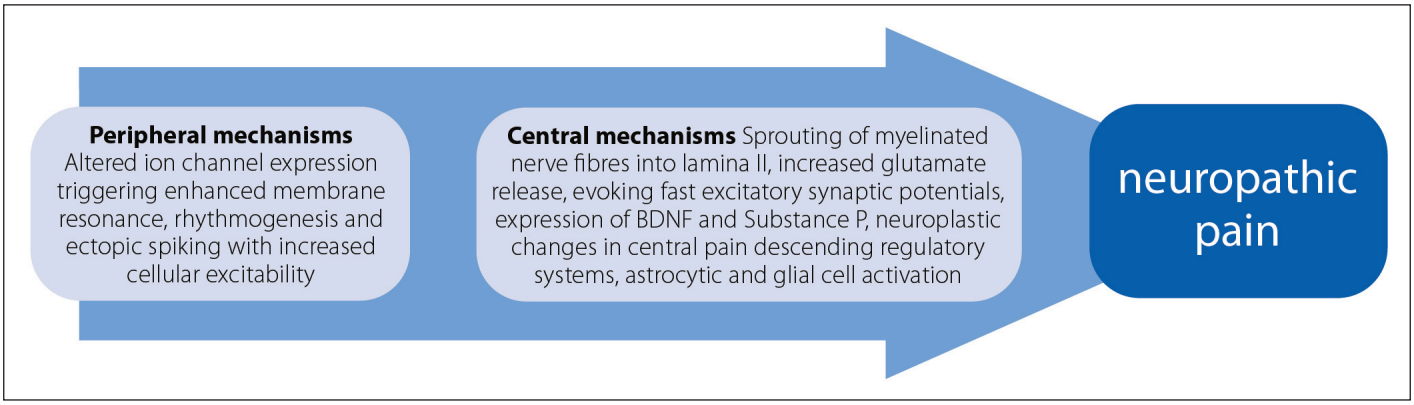

Fig. 1. An overview summary of the peripheral and sensory mechanisms leading to neuropathic pain.

guidelines regarding the assessment of first-, second-, and third-line pharmacotherapy; the evidence underlying published algorithms is biased in favour of peripheral neuropathic pain disorders as opposed to central neuropathic pain; evidence from trials is biased in favour of monotherapy over combination therapy; guidelines and algorithms are sourced from appraisals of independent heterogeneous controlled trials rather than head-to-head comparative studies; there is a high number of negative clinical trials with equivocal data; and the short duration of most trials provides limited data on chronic neuropathic pain. Additionally, the utility of current licensed drugs is further hindered by dose-limiting side effects. Emerging evidence suggests the multifactorial challenges associated with the treatment of neuropathic pain may be surmountable by regenerative approaches based on the utility of cell therapies.

Stem cells

Stem cells are undifferentiated cells capable of unlimited proliferation and self-renewal while retaining the potential towards differentiation into any cell type of endodermal, ectodermal or mesodermal origin. There are three main types of stem cells: embryonic stem cells, adult stem cells, and induced pluripotent stem cells.

The main advantages of stem cells hone in on their potential use for regenerative therapies, with the overall aim of repairing or replacing diseased tissues and organs. Stem cell technology provides a potentially limitless purified population of patient- and disease-specific cells, which confers a range of clinical benefits. These include: understanding the pathogenesis of disease; facilitating drug discovery; and generating cells for transplantation.

Embryonic stem (ES) cells

ES cells are pluripotent cells derived from the inner cell mass of the developing blastocyst.[9] ES cells confer the advantage of being: renewable; accessible to genetic modifications; and expandable *in vitro* for lengthy periods. Thus ES cells can be yielded in very high purified quantities for potential regenerative purposes. Disadvantages of ES cells include: a relatively high tumorigenic potential; transplant rejection; and ethical concerns relating to disaggregating the developing blastocyst.[10]

Adult stem cells

Adult stem cells are multipotent undifferentiated cells. They are derived from specific tissues within the embryo, foetus or adult e.g. the SVZ situated throughout the lateral walls of the lateral ventricles, which contains NSCs, or the bone marrow, which contains two types of adult stem cells, namely, MSCs and haematopoietic stem cells. The amniotic membrane is also a plentiful source of non-immunogenic MSCs, which are easily and non-invasively harvested. Amniotic membrane MSCs have demonstrable anti-inflammatory properties, which have been used clinically in pain relief and wound healing.[11,12]

Advantages of adult stem cells include: self-renewability; fewer ethical issues relative to ES cells; and the potential to be harvested from easily accessible organs and expanded. Furthermore, adult stem cells have a superior safety profile with a lower tumorigenic potential relative to ES cells. Disadvantages include: a lower degree of plasticity, expandability, and renewability, coupled with a greater susceptibility to senescence compared to ES cells; and invasive harvesting methods e.g. bone marrow trephine and biopsy to obtain MSCs. Furthermore, in contrast to ES cells, adult stem cells are rarer in number in mature tissues. This is significant as large numbers of cells are needed for stem cell replacement therapies.

Induced pluripotent stem (iPS) cells

iPS cells are derived from non-pluripotent somatic cells such as dermal fibroblasts, which have been transformed and genetically 'reprogrammed' into a pluripotent state akin to ES cells. This is achieved by transfection with transcription factors such as Oct-3/4, Sox 2 and Nanog, which are core transcription factors that repress the expression profile of differentiated cells and activate an array of genes involved in pluripotency.[13] Other key transcription factors include Klf-4, Lin28 and c-Myc. Similarities to ES cells include: the expression of certain stem cell genes and proteins; viable chimera formation; chromatin methylation patterns; doubling times; teratoma formation; embryoid body formation; and potency and differentiability.[14]

Four traditional strategies are available to reprogramme somatic cells to an iPS cell state: viral transduction; nuclear transfer; cell fusion; and cell explantation. Reprogramming is commonly achieved with viral vectors, which can be either integrating e.g. retroviral or lentiviral vectors, or non-integrating such as adenoviral vectors.

Limitations of the transcription factor approach to make iPS cells include: a low throughput; mutations being inserted into the target cell's genome; tumours, especially with c-Myc; and incomplete reprogramming. These limitations can be overcome by novel techniques to make iPS cells, which include: ES cell-specific miRNA to prompt iPS cell reprogramming;[15] using biomimicry with

recombinant proteins injected into cells via polyarginine anchors, which has coined the nomenclature 'piPSCs' – protein-induced pluripotent stem cells;[16] and small-compound mimicking, which raises reprogramming efficiency[17]

iPS cells offer the advantage of being: easily and non-invasively harvested; useful tools for drug development; models for disease processes *in vitro*; and a source of autologous cells for transplantation due to a lower risk of immunorejection. Disadvantages include a propensity towards tumorigenesis and a lack of long-term data on stability and safety.[18]

Peripheral and central injury models

Progress on the utility of cell-based therapies for neuropathic pain research is dependent upon the application of appropriate experimental animal models of peripheral and central nerve lesions.

There are two broad groups of experimental animal models of neuropathic pain: those that localise the lesion e.g. dorsal root ganglion lesion, peripheral nerve lesion, spinal cord lesion, and dorsal and ventral root lesion; and those that describe the type of lesion e.g. transection, tumour cell invasion or laser radiation, cryoneurolysis, crush, stimulation of perineuronal inflammation, and tight or loose ligature.[19] However, no single animal model entirely recaptures the full range of neuropathic pain mechanisms.

In animal models the assessment and quantification of neuropathic pain by direct evaluation is not feasible. Rather subjectively, most data obtained using animal models have relied on the use of evoked pain-related behaviours such as withdrawal responses as surrogate markers for neuropathic pain.[20] Leading on from this, complexities regarding assessing neuropathic pain in animal models are further exemplified by attempts to extrapolate and identify relevant markers for spontaneous pain. This is particularly problematic for patients with neuropathic pain but rather difficult to measure in rodents. Surrogate indicators include: changes in general innate behaviours such as locomotion, burrowing, digging, excessive grooming and nesting;[21] and more complicated paradigms using Pavlovian conditioning methods such as conditioned place preference and aversion.[22] Notwithstanding these methods, laboratory tools for objectively assessing neuropathic pain in animal models are available and utilise two surrogate markers, namely: thermal hyperalgesia using the acetone test; and mechano-allodynia using the von Frey test.[19]

Evidence on the utility of ES cells for the treatment of neuropathic pain

ES cells have been used to treat neuropathic pain by regenerating GABAergic interneurons with restoration of the inhibitory tone in the dorsal horn of the spinal cord, the lack of which would otherwise contribute to the hyperexcitability that underlies allodynia and hyperalgesia. Evidence in support of this used mouse ES cell-derived MGE cortical inhibitory precursor cells, which were transplanted into a mouse model of peripheral sciatic nerve injury.[23] Using ES cell-derived MGE cells that expressed GFP under the control of the Gad1 promoter, it was demonstrated that the ES cell-derived MGE grafts adapted and thrived in the novel spinal cord environment and migrated throughout the ipsilateral dorsal and ventral horns. Within two weeks of transplantation the grafted cells showed immunocytochemical evidence of differentiation towards a neuronal phenotype (NeuN+) and likewise demonstrated immunoreactivity for markers of cortical GABAergic interneurons, namely GABA, neuropeptide Y, parvalbumin and somatostatin. Furthermore, the transplants structurally integrated into host spinal cord circuitry as evidenced by neurite outgrowth, sprouting, path-finding andsynapse formation with host primary afferent and post-synaptic neurons.

The grafts targeted and influenced a range of spinal cord neurons including projection neurons of lamina I that normally receive nociceptive stimuli. There was a close temporal relationship between the improvement in mechanical allodynia and integration of the grafted cells, which indicated the latter was instrumental for recovery. There was no correlation between the number of transplanted cells and observed anti-nociceptive effects, which suggests there may be a threshold above which the number of grafted cells is of less significance in attaining analgesia. There was a differential effect observed for ES cell-derived MGE transplants with efficacy demonstrated specifically for neuropathic pain, which was not matched in a model of inflammatory pain in response to formalin-induced tissue injury. This suggests MGE grafts are disease rather than symptom modifying.

The strength of Braz *et al.*'s study is based on the novel observation that ES cell-derived MGE grafts restored anti-nociceptive inhibitory GABAergic neurotransmission by structural integration into host spinal cord neuronal circuits. This contrasts with previous simplistic mechanisms of achieving the same by studies that merely focused on releasing GABA using adenoviral and HSV vectors expressing the GABA synthesising enzyme GAD65 in neuropathic models of trigeminal neuralgia[24] and spinal nerve ligation.[25] Leading on from this, the conclusion of Braz *et al.*'s study has been corroborated by other groups, which increases its reliability.[26]

Braz *et al.*'s study failed to account for the anti-nociceptive summative effects of other co-existent endogenous inhibitory pathways, which may have confounded the cause and effect relationship between the independent and dependent variables. It was presumed the anti-nociceptive effects observed post ES cell-derived MGE transplantation was GABA mediated, as evidenced by normalisation of GAD65 mRNA levels in the peripheral nerve injury model, which is normally associated with low GAD65 mRNA levels. However, the inhibitory neurotransmitters glycine and serotonin, which co-exist in some spinal cord GABAergic neurons, may have accounted for the inhibition and anti-allodynic effects recorded.[27] Additionally, evidence suggests that following nerve injury, activation of microglia results in a BDNF-mediated shift in the chloride gradient of projection neurons in lamina I and deep in the dorsal horn, which leads to GABAergic inputs becoming somewhat paradoxically excitatory and pro-nociceptive.[28,29] The results from Mackie, De Koninck and Price's studies do not corroborate the functional integrative mechanism postulated by Braz *et al.*'s study.

The findings of Braz *et al.*'s study are further limited in their long-term application; they only provide data on the utility of ES cell-derived MGE grafts for the treatment of neuropathic pain for 28 days post-transplantation. Accordingly the study was unable to determine whether: the anti-nociceptive effects observed were sustained; if tolerance developed; and if the experimental models showed delayed anti-allodynic effects or developed an analgesic phenotype characterised by mechanical thresholds greater than the baseline.

A separate study used a modified retinoic acid protocol to induce differentiation of mouse ES cells into neural and glial precursors. These were used for transplantation into a mouse model of central neuropathic pain induced secondary to excitotoxic spinal

cord injury.[30] Following transplantation a significant attenuation in mechanical and thermal allodynia with associated pain-induced behaviour, marked by excessive grooming, was observed and reduced to pre-injury levels.

In contrast to Braz *et al.'s* study, which only assessed outcome measures for 28 days post-transplantation, Hendricks *et al.'s* study observed a sustained anti-nociceptive effect for up to 60 days post-transplantation with immunohistochemical evidence of sustained graft viability. Furthermore, in contrast to Braz *et al.'s* study, ES cell-derived neural and glial grafts exhibited, in addition to anti-neuropathic effects, significant analgesic effects in a formalin-induced inflammatory hyperalgesia model suggesting they may be symptom rather than disease specific.

In a follow-up study Hendricks *et al.'s* group in 2012 anecdotally reported that predifferentiated ES cell grafts rescued a neuropathic phenotype in a mouse model. The ES cell-derived grafts exerted neuro-modulatory effects characterised by an increase in neurotrophic factors and cAMP and decreased levels of pro-inflammatory cytokines. This contrasts with the functional integrative mechanism in the Braz *et al* study.

The discrepancies between Braz *et al.'s* study and Hendricks *et al.'s* study may, in part, be explained by the different models of neuropathic pain used; Braz *et al.* used a peripheral model of neuropathic pain (sciatic nerve injury) whereas Hendricks *et al.* used a central model.

A separate study reported that engraftment of predifferentiated ES cells which tonically secrete serotonin and BDNF into the lumbar region of rodent models of central neuropathic pain significantly reduced mechanical allodynia and thermal hyperalgesia for up to four weeks post-transplantation.[31] The anti-nociceptive effects of serotonin were augmented by the administration of the serotonin antagonist methysergide and the serotonin re-uptake inhibitor fluvoxamine. In contrast to Braz and Hendricks *et al.'s* studies, in this case the putative anti-nociceptive mechanism was the regeneration of interrupted descending inhibitory serotonin neuronal inputs.

In summary, the evidence on the utility of ES cells for the treatment of neuropathic pain is in its infancy. Preclinical research has focused on the role of ES cells in restoring the inhibitory effects of GABAergic and serotonergic neurotransmission and, to a lesser extent, modulation of the hostile pro-inflammatory environment in central neuropathic pain. The utility of ES cells to regenerate or modulate other pathophysiological mechanisms of neuropathic pain such as glutamate release, C-fibre hyperexcitability, altered ion channel and NMDA receptor expression, and astrocytic and glial cell activation remains unexplored. Further research is required to determine the potential utility of ES cells to treat neuropathic pain.

Evidence on the utility of adult stem cells for the treatment of neuropathic pain

There is a larger evidence base for the efficacy of adult stem cells for the treatment of neuropathic pain relative to ES cells. Evidence using an adult NSC line, sourced from the rodent SVZ, showed that NSCs attenuated neuropathic pain and promoted nerve regeneration in a rodent chronic constriction injury model.[32] NSCs were administered via intravenous injection and preferentially homed towards the ipsilaterally lesioned nerve; evidence suggests this pattern of homing may be related to myelin modifications induced by nerve injury.[33] Analgesic effects measured by a reduction in mechanical allodynia and thermal hyperalgesia were observed within three days following NSC administration, which correlated with histological evidence of NSC presence at the nerve injury site. The persistence of the analgesic effect between seven and 14 days following grafting correlated with perilesional migration of a high density of fibroblasts, Schwann cells and macrophages, which facilitated regeneration, neurite outgrowth, sprouting, and an improvement in nerve morphology. Evidence suggests this is due to grafted NSCs exhibiting trophic and reparative effects.[34] In support of this, the correlation between NSC administration and anti-nociceptive effects were associated with: a rapid decrease in Fos expression in laminae I–VI (high levels are normally associated with neuronal activity following noxious stimulation);[35] a decrease in immunoreactivity for substance P in the same region (substance P has been associated with increased neuropathic pain in rodents);[36] and a reduction in mRNA levels of the pro-inflammatory pro-algesic cytokines IL-1 and IL-6, coupled with a rise in mRNA levels of the anti-inflammatory cytokine IL-10. These findings suggest that NSCs act as local modifying agents transforming the hostile pro-inflammatory neurochemical environment associated with nerve injury into a more permissive milieu. This facilitates nerve regeneration and analgesia. However, the pro-inflammatory micro-environment may not be entirely harmful to regeneration (see later).

The strength of Franchi *et al.'s* study is based on new first evidence that intravenous administration of NSCs has bidirectional effects on the immune response – decreasing the injurious pro-inflammatory cytokine cascade and activating the neuroprotective anti-inflammatory cytokine response. Interestingly, the analgesic effect of NSCs preceded the morphological signs of nerve repair and was sustained after NSCs disappeared from the lesion site.

Franchi *et al.'s* findings are corroborated by a study that found MSCs transplanted into neuronal tissue ameliorated peripheral neuropathic pain.[37] Similar to the immunomodulatory mechanisms from Franchi *et al.'s* study, in this case the recorded anti-nociceptive effects were secondary to the prevention of injury-induced changes in galanin, neuropeptide Y, and neuropeptide Y Y1-receptor expression in a single ligature nerve constriction rodent model. This may be explained by the bi-directional effects of galanin on neuropathic pain. Galanin is upregulated following nerve injury; however, the functional significance of this is dependent on the type and location of the GAL receptor stimulated. This may result in either: pro-nociceptive effects via activation of pre-synaptic GAL2 receptors on primary afferents; or anti-nociceptive effects via stimulation of GAL1 receptors on dorsal horn neurones.[38] In Coronel *et al.'s* study it is likely that the grafted MSCs exerted anti-nociceptive effects by either preventing injury-induced galanin upregulation with stimulation of pre-synaptic GAL2 receptors on primary afferents, or alternatively, stimulating GAL1 receptors. Other evidence supportive of the reparative immunological mechanisms in Franchi *et al.'s* study have shown that: the efficacy of transplanted NSCs in the treatment of neuropathic pain is mediated via neuroprotective and immunomodulatory mechanisms;[39] and that in a mouse model of spared nerve injury, intra-ventricular injection of human MSCs decreased mRNA levels of the pro-inflammatory IL-1 gene and suppressed activation of astrocytes and microglia, which was associated with a reduction in pain-like behaviours.[40]

The utility of intravenous systemic NSC administration in the treatment of neuropathic pain is further corroborated by evidence on the physiological mechanisms underlying NSC migration, namely, that NSCs cross the blood–brain barrier and enter the CNS where they modulate pain.[41] Leading on from this, the intravenous route of NSC administration has a more transferable putative clinical

application relative to other invasive methods of delivery such as intrathecal or intraventricular injection.

However, Franchi *et al.'s* results are hindered by a number of limitations. For example, other studies have found contradictory results, namely, that NSC transplantation does not affect neuropathic pain[42] and may somewhat paradoxically exacerbate nociceptive symptoms.[43] Furthermore, the analgesic effects were only demonstrated acutely for 28 days post-grafting, which provides no information on: the utility of NSCs in the treatment of chronic neuropathic pain; whether tolerance developed; and whether the experimental models showed delayed anti-allodynic effects. Leading on from this, during the study period repeated NSC injections were required to sustain the analgesic effect, which could be a potential hindrance to putative clinical translation in relation to patient compliance with a multi-dose regime.

The pro-inflammatory milieu associated with neuropathic pain may not be entirely harmful; in Franchi *et al.'s* study, anti-nociceptive effects on pain-like behaviour were observed three days post-NSC grafting. This coincides with the time to recruit macrophages to the injury site to phagocytose myelin debris, which would otherwise have contributed to neuropathic pain.

Franchi *et al.* reported that NSCs specifically homed towards neuronal lesions. However, this has not been supported by other studies on the biodistribution of NSCs following intravenous administration.[44]

Finally, Franchi *et al.'s* study results are restricted to the chronic constriction injury model of peripheral neuropathic pain and cannot be extrapolated to other neuropathic pain models.

In a separate study, NSCs were found to reduce allodynia in a central neuropathic pain model of spinal cord injury if they preferentially differentiated into oligodendrocytes rather than astrocytes.[45] Normally when NSCs are transplanted into the brain or spinal cord they tend to differentiate into astrocytes.[46] However, NSCs derived from the spinal cord that were virally transfected to co-express the transcription factor neurogenin-2 and the marker GFP, differentiated predominantly into oligodendrocytes post-transplantation. This was somewhat unexpected; *in vitro* neurogenin-2 normally promotes neuronal differentiation. In comparison, the naïve GFP-NSCs predictably differentiated into astrocytes.[47] In a spinal cord injury model, transplanted neurogenin-2 NSCs generated more oligodendrocytes and significantly reduced allodynia relative to the naïve GFP-NSC group. The neurogenin-2 NSC-grafted animals showed significantly greater white matter area relative to the naïve GFP-NSC group, which suggested the observed analgesic effects may be secondary to increased remyelination of injured axons. Interestingly, in the naïve GFP-NSC group an increased nociceptive effect was recorded. This may be explained by a higher density of naïve GFP-NSC-derived astrocytes; astrocytes in neuropathic injury models secrete trophic factors such as NGF, which promote neurite outgrowth and cell survival that facilitates locomotor and sensory recovery.[48] However, the neurite outgrowth with associated nociceptive fibre sprouting into inappropriate regions of the dorsal horn may account for the pro-algesic effects observed.[43]

The strengths of these findings are twofold, namely: the importance of differentiating NSCs towards an oligodendrocytic lineage prior to transplantation; and specifically targeting NSC migration to precise regions of the spinal cord. This may reduce pro-nociceptive effects associated with astrocyte-derived neurotrophic factor induced neuronal sprouting into the dorsal horn.

However, in Klein *et al.'s* study no control group containing animals without neuronal lesions that received astrocyte grafts were used for comparison. This would have enabled the observers to assess whether 'healthy' control animals developed a neuropathic phenotype. Therefore, the observation that NSC-derived astrocytes exacerbate neuropathic pain cannot be reliably concluded.

hMSCs have also demonstrated efficacy for the treatment of neuropathic pain. Evidence suggests hMSCs may have the best potential results for treating neuropathic pain[49] and, in contrast to NSCs isolated from the SVZ, hMSCs are more readily accessible and harvested. Evidence supporting this is derived from a study that injected bone marrow derived hMSCs into the rodent tail vein of the spared nerve injury model four days after sciatic nerve surgery by which time neuropathic pain was firmly established.[50]

The strength of Siniscalco *et al.'s* results is the novel observation that hMSCs attenuate neuropathic pain through an anti-inflammatory restorative mechanism based on two components, namely: a cell-to-cell contact activation mechanism – hMSCs drive macrophages towards an anti-inflammatory neuroprotective M2 phenotype; and through down-regulation of pro-inflammatory cytokines. These findings are supported by other studies.[51]

No safety concerns were associated with the use of hMSCs. This coupled with the non-invasive intravenous route of administration provides for potentially favourable clinical translation. Furthermore, the inherently strong anti-inflammatory immunomodulatory properties of hMSCs, which would negate the need for pharmacological immunosuppression, adds weight to their clinical appeal. However, somewhat paradoxically, exploiting pro-inflammatory chemotaxis may also facilitate clinical translation; the chemokine driven homing potential of hMSCs towards pro-nociceptive lesions could be exploited as a bioactive site-specific delivery system, which would avoid the dose-limiting adverse effects associated with systemic administration of current licensed drugs.

However, the effects of hMSCs in Siniscalco *et al.'s* study are limited in their application by methodological flaws. For example, during the progression of neuropathic pain, time-course tracking of intravenous hMSCs was not performed, thus homing of hMSCs towards areas involved in neuropathic pain modulation cannot be reliably elucidated. Leading on from this, there is contradictory evidence on the homing capabilities of hMSCs towards sites of neuropathy, for example, evidence has shown that hMSCs transplanted into the mouse tail vein are predominantly sequestrated in the lung.[52] Furthermore, the utility of hMSCs for the treatment of neuropathic pain was only assessed for 90 days, thus providing no information in relation to attenuating chronic neuropathic pain or reducing associated complications such as deconditioning e.g. reduced mobility, muscle atrophy and contractures.

In summary, there is an emerging body of preclinical data based on exploiting the multipotency, self-renewing capacity, high expansion potential and genetic stability of adult stem cells that supports their utility for the treatment of neuropathic pain. The mainstay of evidence has honed in on the immunomodulatory and trophic effects of adult stem cells. The progression on to clinical trials would be the next stage in defining the potential utility of adult stem cells for the treatment of neuropathic pain.

Evidence on the utility of iPS cells for the treatment of neuropathic pain

There are no published data on the utility of iPS cells for the treatment of neuropathic pain; however, there has been a lot of

interest in the potential utility of iPS cells in this regard. Potential uses of iPS cells include: modelling neuropathic disease processes *in vitro*; developing and screening candidate drugs that selectively target diseased neuronal cells with particular genetic profiles; and offering a novel paradigm of cell replacement therapy to support neuronal regeneration.

The enormous potential utility of iPS cells for the treatment of neuropathic pain is in its infancy and remains unsupported by an evidence base. In addition to the limitations of iPS cells already mentioned, a number of other problems would need to be surmounted. For example, the retention of epigenetic profiles from senescent cells may cause iPS cells, used for neuropathic pain disease modelling or therapy, to lose their differentiated properties.

Conclusions

The evidence on the potential utility of cell therapies for the treatment of neuropathic pain is predominantly based on research in animal models on their efficacy and safety. The evidence suggests that *prima facie* cell therapies reduce neuropathic pain and may modify some of the cellular and molecular neuropathic pain mechanisms. However, critical appraisal of the evidence thus far reveals it to be far from conclusive and future research geared towards progression on to clinical trials would need to address a number of issues. Firstly, the preclinical evidence reported to date suggests that *in vivo* cell therapies have a relatively short survival, which limits their clinical utility in the treatment of chronic neuropathic pain. In this regard future research on long-term graft viability is required.

Furthermore, prior to grafting, stem cells require expansion *in vitro* and with increasing passaging time the stability of the cells changes, which decreases the probability of them differentiating into neurons.[53] Accordingly, future research on the stability of cell therapies intended for transplantation is required. The need for future research on the issue of long-term stability and safety of cell therapy is brought into even sharper focus by the observation that following transplantation stem cell-derived grafts maintain a high proliferative potential, which carries a significant oncogenic risk.[54] This was highlighted by the first case report of a donor-derived brain tumour following NSC transplantation.[55]

The evidence thus far on the potential disease-modifying regenerative effects of cell therapies for the treatment of neuropathic pain is limited to neuropathic conditions characterised by focal nerve damage. Indeed, the mainstay of preclinical evidence has used experimental animal models with limited focal nerve damage. Future research would need to assess the potential utility of cell therapies for more diffuse and widespread nerve damage, for example in chemotherapy-induced polyneuropathy or diabetic neuropathy, which are more common than focal neuropathies.

There may be a fourth dimension on the potential utility of cell therapies for the treatment of neuropathic pain based on stem cell-derived microvesicles. Research on the utility of stem cell-derived microvesicles that carry miRNA, chemo-attractant, anti-apoptotic, and anti-scarring factors are under investigation and early results have demonstrated non-inferiority relative to cell therapies.[56] However, the evidence on stem cell-derived microvesicles is sparse; future research on the role of stem cell-derived microvesicles is required.

In summary, cell therapies offer a novel curative therapeutic dimension for the treatment of neuropathic pain. This is based on replacing damaged neuronal tissue, protecting against progressive nerve damage, and releasing paracrine and endocrine factors, which repair the pathology that underlies the genesis and propagation of damage within the somatosensory system.

Key messages

- Cell therapies may offer palliative and curative potential in diabetic neuropathy
- Stem cell treatments for neuropathic pain reverse and repair the pathology that underlies the genesis and propagation of damage within the somatosensory system
- Stem cell therapies can replace damaged neuronal tissue, protect against progressive nerve damage, and release soluble factors to facilitate neuronal repair

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Diabetes mellitus and male reproductive function: where do we stand?

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Diabetes mellitus (DM) is a metabolic disorder caused by absolute (type 1 diabetes) or relative (type 2 diabetes) deficiency of insulin and is associated with alterations in carbohydrate, lipid and protein metabolism.[1] The disease has been closely related with a wide range of long-term systemic complications and co-morbidities, such as renal failure or hypertension.[2] Therefore, the study of DM implications in human health is a challenge to experts in any field of research.

According to the latest fact sheets from World Health Organisation (WHO), DM is one of the most rapidly growing threats to public health in modern societies. Over the past 20 years, the global prevalence of DM has increased approximately six-fold and nearly 350 million people worldwide suffer with the disease. The WHO estimated that, in 2004, over three million people died from consequences of high blood sugar levels and estimates that DM-related deaths will increase by two-thirds between 2008 and 2030.[1,3] Nonetheless, the existing statistical data can be underestimated since the factors known to be responsible for the disease progression, such as obesity and lifestyle habits, may aggravate these numbers.[2]

When we take a close look at fertility rates in modern societies, we observe that the increased incidence of DM is concurrent with the falling birth rates and decreased fertility.[4,5] This fact is partly due to the alarming increase in the number of men developing DM during the reproductive age. Indeed, the great majority of patients with type 1 diabetes (T1D) are diagnosed before the age of 30,[6] and there is a worrying number of children and adolescents with type 2 diabetes (T2D).[7] Moreover, Western lifestyle habits, together with the increasing obesity among young individuals, strongly contribute to the high incidence of T2D in youth.[2] DM is responsible for several biochemical and homeostasis alterations that may result in male subfertility and or infertility, yet the real impact of DM on male reproductive health remains undisclosed.

Although there is some controversy on the subject, diabetic individuals are frequently described as possessing some sexual neuropathies, such as reduction in sexual appetite,[8] which are explained as lethargy and tiredness related to their hyperglycaemic state. Other disorders such as erectile dysfunction (ED)[9] or retrograde ejaculation[10,11] are also well known to occur in male diabetics.

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Nonetheless, when examining sperm parameters and sperm quality markers, the literature shows some conflicting results. There are several studies since the 1970s comparing young or adult diabetics with control individuals. While some studies report that diabetic men present lower sperm counts and significant differences in sperm motility and morphology,[12] as well as in sperm volume and count,[13] others report only a slight, non-significant, decrease in sperm counts, although sperm volume and motility are frequently lower.[14] Others have reported that sperm count and concentration were increased in the ejaculatant of diabetic individuals, although sperm motility and semen volume were decreased. Noteably, in these last studies, sperm morphology and motility were described to remain unaffected.[15]

Another more recent study reported no correlations between sperm motility and age, age of onset of T1D and duration. The same study reported that several sperm motility parameters such as track speed, path velocity, progressive velocity, and lateral head displa cement remained unchanged, while others, such as linearity and linear index (which reveal the straightness of sperm swimming), were increased in diabetic men.[16] This study evidenced that T1D effects in male fertility may be related to the disease complications and not the disease itself.[16]

Interestingly, the sperm of diabetic individuals is reported to present high fructose and glucose content,[14] but the relationship between an ineffective metabolic control and the observed alterations in the semen was never established and therefore should deserve a special focus in the next years.

An extensive study of spermatozoa cryopreservation from patients with various pathologies reported that only sperm from diabetic men presented significant differences in sperm parameters,[17] while a recent study reported no alterations in semen parameters from T1D and T2D individuals.[6] Nonetheless, these authors reported that sperm from diabetic men presented a higher level of damage in sperm nuclear and mitochondrial DNA.[6]

Although most of the studies have focused on analysis of sperm parameters, there is an important study from 1985, performed using testicular biopsies from impotent men with DM that reported ultrastructural lesions in the cytoplasm of Sertoli cells (SCs) and morphological changes in the interstitial compartment of diabetic men's testes.[18] These anatomical, structural and morphological alterations suggested that diabetic men may suffer from disruption of the spermatogenic event, resulting in the subfertility and/or infertility often associated with DM.

Even though there are apparent contradictory results concerning sperm parameters and the real impact of DM in male reproductive function, it is not consensual that DM effects are only reflected in sperm or in the ejaculatant. Moreover, apart from the direct studies of sperm, new important findings have been reported using *in vitro* strategies. For instance, diabetic individuals are known to have fluctuations in sex hormone concentrations.[19] Recent *in vitro* studies in rat[20] and human[21] SCs showed that sex hormones are able to modulate these cells' metabolism. This is significant since

SC metabolism is crucial for successful spermatogenesis. These cells are known as 'nurse cells' since one of their main functions is to metabolise glucose into lactate, which is then consumed by the developing germ cells.[22] Therefore the hormonal control of SC metabolism has a direct effect on spermatogenesis[23] and should deserve special attention when studying metabolic diseases that are also related with hormonal (de)regulation.

Moreover, diabetic individuals have severe insulin deregulations that should be taken in consideration when discussing the effects of DM. Euglycaemia is difficult to maintain in diabetic patients and hypoglycaemia/hyperinsulinaemia as well as hyperinsulinaemia/hypoglycaemia are common events that diabetic individuals may face daily. Therefore, insulin can have a major role in the male sexual dysfunction associated with DM.

In fact, recent reports show that only a few hours of insulin deprivation can alter not only glucose metabolism in SCs[24] but also completely suppresses *in vitro* acetate production.[25] The regulation performed by insulin in these crucial processes for a normal spermatogenesis is clear evidence that the molecular mechanisms by which DM affects the male reproductive function may also be linked to insulin fluctuations and not only to glucose concentrations.

There is an urgent need for clarification whether DM can alter sperm parameters and overall male reproductive function. Furthermore, there is also a lack of consensus concerning sperm analysis, and it has been recently discussed that conventional sperm analysis is very limited and needs standardisation before it can give definite answers to the fertility status of individuals.[26] Besides, when assessing the effect of DM, there are several factors that are very difficult to control, such as the duration of the disease, glycaemic levels, type of treatment, as well as all the co-morbidities associated, which may obscure the real impact of DM in male fertility.

It is evident that not all diabetic men are infertile and sperm analysis is not able to give an absolute answer to the question. Nonetheless, the molecular mechanisms of spermatogenesis and sperm maturation might be altered even when conventional sperm parameters appear normal. Therefore it is imperative to focus not only on the mechanisms that have a direct effect in natural and assisted conception, such as DNA integrity and oxidative stress, but also in the molecular basis of the disease that may affect testicular cells, spermatogenesis, sperm production and sperm maturation. These molecular studies may not only open new insights on the DM effects in male reproductive function, but also point toward possible therapeutic sites for intervention to decrease DM-related male subfertility and/or infertility.

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Prevalence of dyslipidaemia in statin-treated patients in South Africa: results of the DYSlipidaemia International Study (DYSIS)

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Abstract

Introduction and objectives: **Cardiovascular disease (CVD) is the leading cause of mortality worldwide and increased levels of low-density lipoprotein cholesterol (LDL-C) are an important modifiable risk factor. Statins lower LDL-C levels and have been shown to reduce CVD risk. Despite the widespread availability of statins, many patients do not reach the lipid targets recommended by guidelines. We evaluated lipid goal attainment in statin-treated patients in South Africa and analysed variables contributing to poor goal attainment as part of the DYSlipidaemia International Study (DYSIS).**

Methods: **This cross-sectional, observational study enrolled 1 029 consecutive South African patients consulting office-based physicians. Patients were at least 45 years old, had to be treated with a stable dose of statins for at least three months and had been fasting for 12 hours. We evaluated lipid goal attainment and examined variables associated with residual dyslipidaemia [abnormal levels of LDL-C, high-density lipoprotein cholesterol (HDL-C) and/or triglycerides (TG)].**

Results: **We found that 50.3% of the patients overall did not achieve target LDL-C levels and 73.5% of patients were at very high cardiovascular risk. In addition, 33.7% had low levels of HDL-C, while 45.3% had elevated TG levels despite statin therapy. Asian and mixed-ancestry patients but not black (vs Caucasian ethnicity), as well as obese individuals in South Africa were more likely to still have dyslipidaemia involving all three lipid fractions.**

Conclusions: **We observed that many patients in South Africa experienced persistent dyslipidaemia despite statin treatment, supporting the concept that there is a need for more intensive statin therapy or the development of novel treatment strategies. Measures aimed at combating obesity and other lifestyle-related risk factors are also vital for effectively controlling dyslipidaemia and reducing the burden of CVD.**

Keywords: cardiovascular disease (CVD), dyslipidaemia, lipid abnormalities, statins, low-density lipoprotein cholesterol (LDL-C)

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Cardiovascular disease (CVD) is the leading cause of mortality worldwide. In 2008, World Health Organisation (WHO) estimates suggested that 30% (17.3 million) of all deaths worldwide could be attributed to CVD.[1] ("Cardiovascular diseases (CVDs) Fact sheet no. 317," September 2012) In 2008 and 2009, the two most recent years for which South African data are available, CVD was responsible for 13.7 and 14.0% of total deaths, respectively.[2-4] However, CVD mortality rates are expected to rise in South Africa as unhealthy lifestyle trends associated with urbanisation spread to the countryside, and the population of people surviving life-threatening infections continues to grow.[5,6]

Well-known risk factors for CVD include age, gender, dyslipidaemia, tobacco smoking, high blood pressure and diabetes mellitus (DM). Other lifestyle behaviours such as excessive alcohol consumption, sedentary lifestyle and poor diet with resultant obesity further contribute to CVD risk.[7,8] The WHO 2008 estimates indicated that the prevalence of obesity, tobacco smoking and physical inactivity in South Africa were 31.3 (≥ 20 years old), 14 and 51.1%, respectively.[9] Furthermore, in 2010 the prevalence of DM was 4.5% for individuals ≥ 15 years old,[10,11] and the WHO estimated the rate of high blood pressure at 42.2% in 2008.[9] As the prevalence of these risk factors rise in South Africa,[5] so will the rate of CVD.

The main effect of statins is to lower LDL-C levels and they are used extensively in both primary and secondary prevention of CVD.[12-14] Importantly, several large clinical trials have indicated that for every 1-mmol/l reduction in LDL-C levels there is a 23% reduction in CVD risk.[15-18] In a further meta-analysis of studies comparing high and low statin doses, more intensive lowering of LDL-C (0.51 mmol/l additional reduction) in the high-dose statin arm was associated with a further 15% reduction in CVD risk.[19] In the most recently published statin cardiovascular outcomes trial (JUPITER study: men and women free of overt cardiovascular disease over the ages of 50 and 60 years, respectively; baseline LDL-C < 3.37 mmol/l and high-sensitivity C-reactive protein of 2 mg/l or more; randomised to rosuvastatin 20 mg/day or placebo), statin treatment was associated with a 39% reduction in primary endpoints (myocardial infarction, stroke, admission to hospital for unstable angina, arterial revascularisation or CV death) in patients with at least one risk factor for DM.[20]

The results of these and other studies have resulted in treatment guidelines recommending progressively lower LDL-C targets.[21-23] However, studies from all over the world have demonstrated that many patients on lipid-lowering therapy do not reach their recommended lipid targets.[24-26] The South African Heart Association (SA Heart) together with the Lipid and Atherosclerosis Society of Southern Africa (LASSA) therefore recently emphasised that intensive management of dyslipidaemia could significantly reduce the South African CVD health burden.[21]

The DYSlipidaemia International Study (DYSIS) is a cross-sectional, observational study that has examined the efficacy of lipid-lowering therapies in patients from various regions of the world, including Canada and Europe (11 countries), in order to better characterise predictive factors for dyslipidaemia and CVD.[24,25] Here, as part of DYSIS, we have analysed residual dyslipidaemia in statin-treated South African patients.

Methods

As part of DYSIS, this epidemiological, observational, cross-sectional study was conducted in South Africa between 1 November and 9 December 2011. Data for the study were collected in the South African private healthcare sector by 16 physicians; 50% were primary-care physicians and 50% were specialised office-based physicians (e.g. cardiologists).

Prior to study initiation, the relevant local ethical review committees approved the study protocol and all patients gave written informed consent before enrolling in the study. Key eligibility criteria were: (1) age of at least 45 years, (2) receiving stable statin therapy for at least 3 months, and (3) fasting for at least 12 hours at the time of visit while on statin therapy. Participating physicians were instructed to include all eligible and consenting patients consecutively.

Patient demographic, lifestyle and clinical characteristics were documented. Lipid levels (total cholesterol, LDL-C, HDL-C and triglycerides) were measured using the CardioChek® device (http://www.cardiocheck.com) at the time of patient enrollment to reliably collect lipid measurements uniformly at all sites. The LDL-C test strip provided measures LDL-C directly across a range of 1.29–5.18 mmol/l in about two minutes.

Additionally, the lipid-lowering regimen at the time of the most recent blood sample was recorded for each patient (in particular, statin type and daily dose) as well as any information regarding other lipid-modifying therapies. The potency of different types of statins was normalised using a calculation that allows benchmarking against six different simvastatin dose levels (5, 10, 20, 40, 80

Table 1. Patient characteristics, risk categories and lipid parameters in different ethnic groups

	All patients (*n* = 1 029)	Caucasian (*n* = 582; 56.6%)	Black (*n* = 226; 22.0%)	Asian (*n* = 99; 9.6%)	Mixed ancestry (*n* = 122; 11.9%)
Age (years) (mean ± SD)	65.4 ± 10.8	69.0 ± 11.0	60.0 ± 8.9	61.8 ± 9.0	60.9 ± 7.4
Family history of premature CHD (%)	26.7	34.0	1.8	44.4	23.0
Current smokers (%)	10.7	11.2	5.3	11.1	18.0
Hypertension (%)	76.8	69.8	93.3	64.6	89.3
Systolic BP (mmHg) (mean ± SD)	134.4 ± 20.0	134.9 ± 20.4	135.2 ± 19.4	129.0 ± 17.1	134.9 ± 20.7
Diastolic BP (mmHg) (mean ± SD)	79.7 ± 11.0	79.6 ± 11.1	79.6 ± 11.5	78.3 ± 9.6	81.2 ± 10.5
Waist circumference (cm) (mean ± SD)	100.7 ± 15.1	99.5 ± 16.5	105.0 ± 13.4	96.1 ± 9.6	101.8 ± 12.5
BMI (kg/m^2) (mean ± SD)	29.6 ± 6.4	28.6 ± 6.4	32.8 ± 6.5	27.0 ± 4.5	30.4 ± 5.6
BMI > 30 kg/m^2 (%)	42.2	36.8	61.9	22.2	47.5
CVD (%)	36.2	41.1	9.7	51.5	49.2
Diabetes mellitus (%)	40.4	25.6	71.2	44.4	50.8
Metabolic syndrome (IDF) (%)	67.2	59.8	83.2	59.8	78.7
ESC risk level (2011)*					
Very high-risk patient (%)	73.5	69.9	77.9	73.7	82.0
High-risk patient (%)	8.9	11.2	4.0	11.1	5.7
Moderate-risk patient (%)	13.5	15.6	11.5	9.1	10.7
Low-risk patient (%)	4.1	3.3	6.6	6.1	1.6
South African guidelines					
Very high-risk patient (%)	68.6	61.2	77.9	73.5	82.8
High-risk patient (%)	9.2	11.7	6.2	8.2	3.3
Moderate-risk patient (%)	21.6	26.6	15.9	15.3	13.9
Low-risk patient (%)	0.6	0.5	0.0	3.1	0.0
Lipids (mmol/l) (mean ± SD)					
LDL-C	2.3 ± 1.1	2.2 ± 1.0	2.1 ± 1.0	2.6 ± 1.2	2.7 ± 1.1
HDL-C	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4	1.3 ± 0.5
Total cholesterol	4.4 ± 1.3	4.4 ± 1.2	4.4 ± 1.4	4.7 ± 1.6	4.7 ± 1.3
Triglycerides [median (IQR)]	1.6 (1.1–2.3)	1.5 (1.1–2.2)	1.7 (1.2–2.4)	1.7 (1.2–2.7)	1.5 (1.1–2.4)
Blood glucose					
FBG (mmol/l) [median (IQR)]	4.9 (4.3–6.4)	4.6 (4.2–5.4)	6.2 (4.7–9.0)	5.3 (4.2–7.0)	5.6 (4.7–7.2)
HbA_{1c} (%) diabetics [median (IQR)]	7.4 (6.6–8.8)	7.1 (6.0–8.0)	8.2 (6.8–9.9)	7.8 (7.0–8.7)	7.4 (7.0–8.8)

CHD, coronary heart disease; BP, blood pressure; BMI, body mass index; CVD, cardiovascular disease; DM, diabetes mellitus; IDF, International Diabetes Federation.

and 160 mg/day), with potency scores ranging from 1 (5 mg/day simvastatin) to 6 (160 mg/day simvastatin).[23,27]

The 2011 ESC guidelines were used to classify CV risk, LDL-C level treatment goals, and sub-optimal HDL-C and triglyceride levels.[21,28] Variables independently associated with dyslipidaemia were evaluated with logistic regression modelling using the following variables: age (≥ 70 years), female gender, family history of premature coronary heart disease (CHD), current tobacco smoker, sedentary lifestyle, alcohol consumption (> 2 units/week), body mass index (BMI) ≥ 30 kg/m^2, large waist circumference (> 102 cm in men, > 88 cm in women[29]), hypertension, DM, coronary heart disease, cerebrovascular disease, heart failure, peripheral artery disease, systolic/diastolic blood pressure ≥ 140/90 mmHg, simvastatin equivalent dose of either 20 to 40 versus 10 mg/day, or > 40 mg versus 10 mg/day, ezetimibe use, and physician's specialty (cardiologist, endocrinologist, diabetologist, internal medicine or other).

Statistical analysis

To estimate the sample size needed for South Africa we assumed a prevalence of residual lipid abnormalities between 20 and 60% in patients fulfilling the entry criteria for this study and a design effect of 20% (variance inflation due to cluster sampling design). We calculated that, within this range, a sample size of 1 000 would be sufficient to estimate the prevalence of residual dyslipidaemia with a given precision of ± 3.4% (range of 95% confidence interval: 6.8%). Furthermore we determined that this size guaranteed enough information for estimating the prevalence in smaller subgroups (representing one-quarter or more of the population) with a precision of ± 6.8% (95% CI: 13.6%).

Following data collection, patient information was entered into a central web-based database housed and managed at the Institut für Herzinfarktforschung, Ludwigshafen, Germany. Real-time quality control (internal logic checks) occurred during web-based data entry. Continuous variables are presented as means with standard deviations or medians with 25th and 75th percentiles [interquartile range (IQR)] as indicated, and categorical variables are reported as absolute numbers and percentages.

Kernel density estimation was used to analyse the distribution of total cholesterol, LDL-C, HDL-C and triglyceride levels. The value of a kernel density and its slope at the lipid value equal to the ESC goal provides a crude indicator of the change in the proportions of patients meeting the goal from a small improvement or deterioration in lipid level starting from the ESC goal. This approach thus provides a sensitivity analysis for either changes in the ESC goals or changes in lipid levels for people whose levels are near the goals.

Multiple logistic regression analyses with backward selection ($\alpha = 0.05$) were used to identify variables independently associated with LDL-C, HDL-C and triglyceride irregularities. Two-tailed statistical comparisons were used ($p < 0.05$ was significant) and patients lacking the appropriate lipid parameters were not included within the analyses. All analyses were performed using SAS v 9.1 (SAS Institute Inc, USA).

Results

Patient characteristics, risk categories and lipid parameters are presented in Table 1. The study enrolled 1 029 patients (429 men, 600 women). The mean age of patients was 65.4 years, and 58.3% were female. The study population was of mixed ethnic (multi-racial) origin, including Caucasians (56.6%), blacks (22.0%), Asians (9.5%) and patients of mixed ancestry (12.0%).

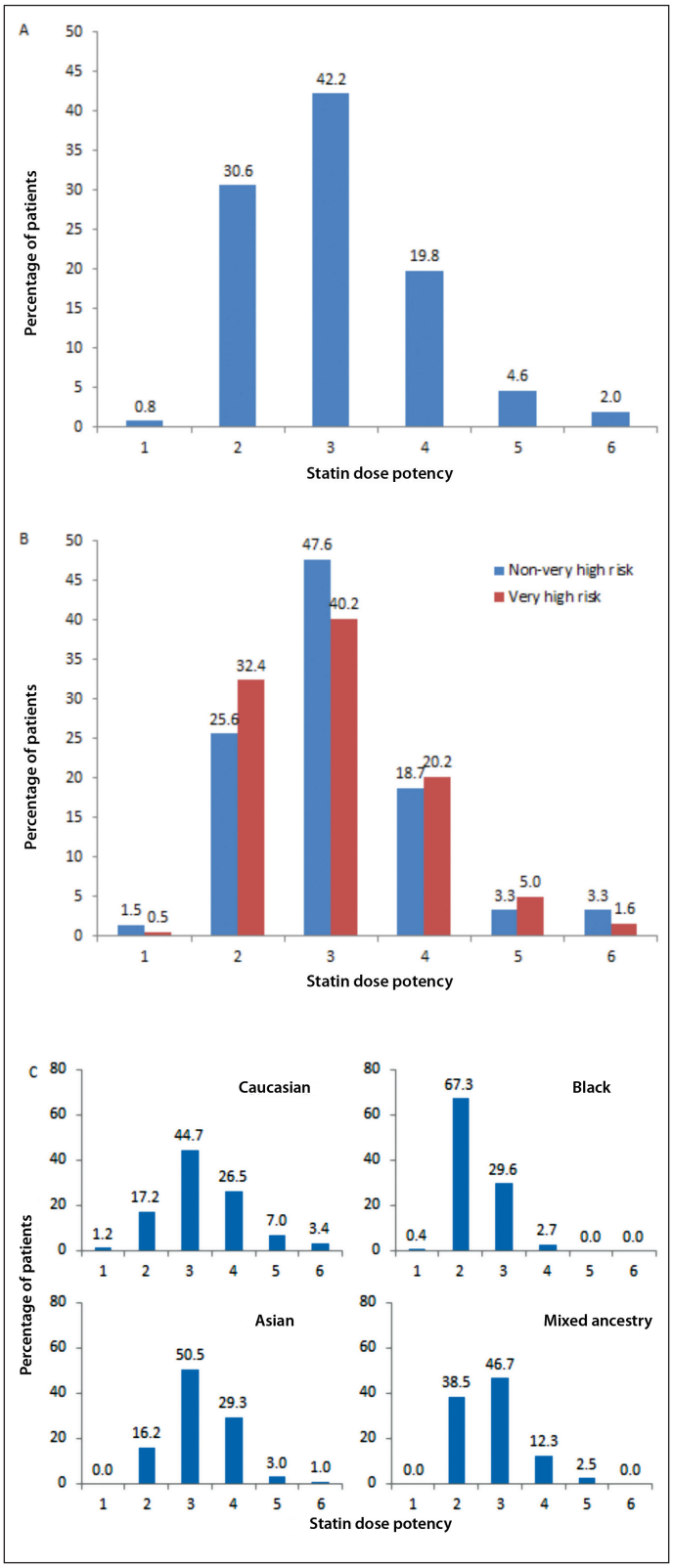


Fig. 1. Statin dose potency overall (A), according to patients' risk status (B), and by ethnicity (C) calculated according to references 22, 23. *Statin dose potency 1 is equivalent to simvastatin 5 mg/day, potency 2 is equivalent to simvastatin 10 mg/day, potency 3 is equivalent to simvastatin 20 mg/day, potency 4 is equivalent to simvastatin 40 mg/day, potency 5 is equivalent to simvastatin 80 mg/day, and potency 6 is equivalent to simvastatin ≥ 160 mg/day.

Table 2. Lipid abnormalities according to ESC guidelines (2011) on risk stratification

	All patients (*n* = 1 029)	Very high risk* (*n* = 756)	High risk (*n* = 92)	Moderate risk (*n* = 139)	Low risk (*n* = 42)
LDL-C not at target (%)†+	50.3	60.1	33.3	24.1	
Low HDL-C [< 1.0 (men)/1.2 (women) mmol/l) (%)‡	33.7	36.0	25.0	26.3	34.1
Elevated TG (> 1.7 mmol/l) (%)§	45.3	45.8	46.7	40.1	50.0

*Very high risk = CVD, diabetes, and/or SCORE risk ≥ 10% (chronic kidney disease was not documented in DYSIS)
†LDL-C ≥ 3.0 mmol/l in patients with SCORE risk 1–4%, LDL-C ≥ 2.5 mmol/l in patients with SCORE risk 5–9%, LDL-C ≥ 1.8 mmol/l in patients with CVD, DM, and/or SCORE risk ≥ 10%; LDL-C ≥ 1.8 mmol/l
+Data on 987 patients were available
‡Data on 1 025 patients were available
§Data on 1 027 patients were available
In the ESC 2011 guidelines, no LDL-C goal was specified for the low-risk group.

Patient characteristics and cardiovascular risk profile differed by ethnic group. A family history of premature CVD was reported by 34% of Caucasian patients while the diabetes prevalence of 25.6% was the lowest of all the ethnic groups studied. Hypertension was found in 69.8% and CVD in 41.1% of Caucasian patients. Black patients were least likely (1.8%) to report a family history of premature CHD and had the lowest (5.3%) smoking rates.

However, hypertension was almost universal (93.3%) and diabetes and obesity were highly prevalent at 71.2 and 61.9%, respectively. Despite the high prevalence of hypertension and obesity, only 9.7% of black patients had clinically overt CVD. Asian patients had the highest rates of CVD (51.5%) among all ethnic groups studied and also the highest reported rate of a family history of premature CVD (44%). Diabetes was highly prevalent at 44.4% while the hypertension prevalence of 64.6% was similar to that observed in Caucasian patients. Mixed-ancestry patients had the highest smoking rates (18%) while the diabetes and hypertension prevalences were 50.8 and 89.3%, respectively. CVD was documented in 49.2% of mixed-ancestry patients.

CVD was almost twice as common in men (49.9%) than women (26.3%). DM was more common in men than women (45.7% vs 36.7%), while obesity was more frequent in women (46.8% compared with 35.7%). Additionally, using the 2011 ESC criteria, 73.5% of patients (83.9% men and 66.0% of women) were classified as very high risk for CV complications [defined as having CVD, DM and/or an ESC systematic coronary risk evaluation (SCORE) risk of ≥ 10% on chronic statin therapy].

Lipid-modifying regimens and statin potency

Prior to enrollment in DYSIS, patients had been treated with various lipid-lowering therapies. The most commonly prescribed statin was simvastatin (64.6%), followed by atorvastatin (22.2%), rosuvastatin (10.9%), pravastatin (1.6%), fluvastatin (0.6%) and lovastatin (0.2%). Other lipid-lowering agents were used by only 2% of patients, including ezetimibe (1.2%), fibrates (0.9%) and bile acid sequestrants (0.2%).

The most frequently used statin dose potency was 3 (equivalent to 20 mg simvastatin per day) for both very high-risk patients (40.2%) and non-very high-risk patients (47.6%), while the second most-frequent dose potency was 2 (equivalent to 10 mg simvastatin per day) in 32.4 and 25.6% of very high-risk patients and non-very high-risk patients, respectively (Fig. 1). While statin dose potency 3 was most frequently used in Caucasian, Asian and mixed-ethnicity patients, a dose potency of 2 was most common in black patients.

Lipid abnormalities

Data on the frequency of lipid abnormalities, including sub-analyses by CVD risk level, are provided in Tables 2 and 3. Among all patients (*n* = 1,029), 50.3% had LDL-C levels not at goal. We defined 'not at LDL-C goal' as LDL-C ≥ 1.8 mmol/l and LDL-C reduction of < 50% for patients with CVD, DM and/or a SCORE risk of ≥ 10% (very high risk), and as ≥ 2.5 mmol/l and ≥ 3 mmol/l for patients with a SCORE risk of 5 to 9% (high risk) and 1 to 4% (moderate risk), respectively. Elevated TG levels (defined as > 1.7 mmol/l) were seen in 45.3% of patients, and 33.7% had low HDL-C levels (defined as < 1.0 mmol/l for men and < 1.2 mmol/l for women).

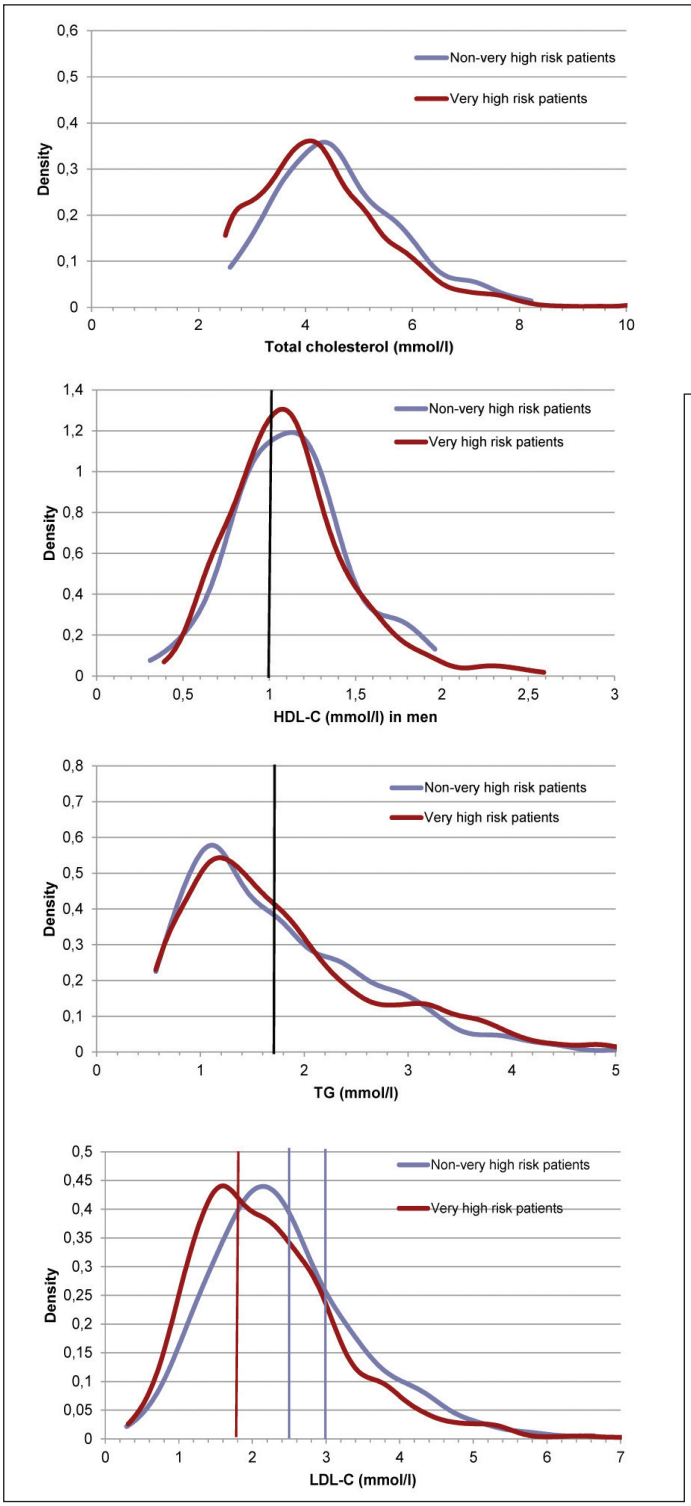
The most prevalent lipid disorder (either alone or in combination) in very high-risk patients was above-target LDL-C levels (60.1%), followed by elevated TG levels (45.8%), and low HDL-C levels (36.0%). By contrast, in both high- and moderate-risk patients, elevated TG levels were observed more frequently (46.7 and 40.1%, respectively in the two risk groups) than above-target LDL-C levels (33.3 and 24.1%, respectively) and low HDL-C levels (25.0 and 26.3% for both risk groups, respectively).

We next performed a sub-analysis of lipid abnormalities for only very high-risk patients (756 of all patients, Table 3), which we stratified as indicated. Of those with CVD and DM, 57.9%

Table 3. Lipid abnormalities according to ESC guidelines (2011) in very high-risk patients

	CVD + DM (*n* = 131)	CVD (w/o DM) (*n* = 241)	DM (w/o CVD) (*n* = 285)	SCORE ≥ 10% (*n* = 99)
LDL-C ≥ 1.8 mmol/l and LDL-C reduction < 50% (%)*	57.9	68.0	53.8	61.9
Low HDL-C [< 1.0 (men)/1.2 (women) mmol/l] (%)†	39.7	33.8	37.9	31.3
Elevated TG (> 1.7 mmol/l) (%)‡	54.2	38.2	51.9	35.4

*Data on 722 of a total of 756 high-risk patients were available
†Data on 755 patients were available
‡Data on 756 patients were available.



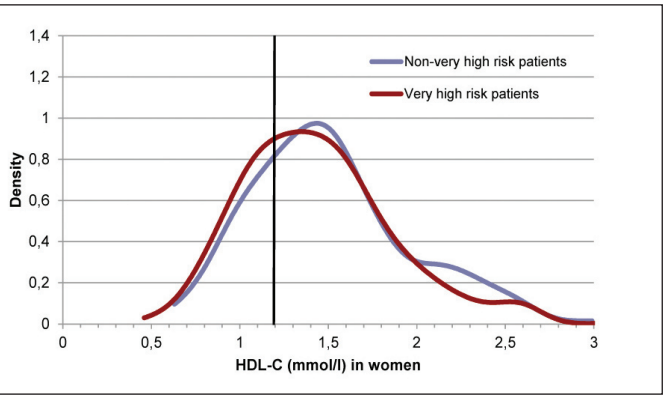


Fig. 2. Kernel density curves of lipids. Density curves were unimodal and positively skewed throughout. The data indicate that the very high-risk group (red line, upper right panel) showed slightly lower overall LDL-C levels than non-very high-risk patients (blue line). Moreover, we observed that women (right panel in the middle) maintained higher overall HDL-C levels than men (left panel in the middle) in both the very-high and non-very high-risk groups, while triglyceride levels were similar between the two risk groups (lower panel). Density curves for total cholesterol were mostly overlapping (upper left panel). Vertical lines mark the cut-off point of ESC guidelines (2011); LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

displayed off-target LDL-C levels (≥ 1.8 mmol/l and a decrease in LDL-C levels of < 50%), 39.7% showed low HDL-C levels, and 54.2% had elevated TG levels. In comparison, patients in the CVD without DM group showed a higher rate of LDL-C not at target (68.0%), decreased rates of low HDL-C (33.8%), and elevated TG levels (38.2%). Interestingly, the ESC SCORE group with risk of ≥ 10% showed a lower proportion of patients with low HDL-C and elevated TG levels. Overall, we found that LDL-C not at goal was the most common lipid abnormality observed in each of the four sub-sets.

Additionally, we analysed patient lipid abnormalities using kernel density curves for the empirical distributions of very high-risk and non-very high-risk patient groups with regard to total cholesterol, LDL-C, HDL-C (separately for men and women), and TG levels (ESC guidelines indicated as superimposed vertical lines) (Fig. 2). Overall, we found that the density curves were unimodal and positively skewed, and the data indicated that the very high-risk group showed slightly lower overall LDL-C levels than non-very high-risk patients. Moreover, we observed that women maintained higher overall HDL-C levels than men in both the very high and non-very high-risk groups, while TG levels were similar between the two risk groups.

Distributions of lipid abnormalities

Distributions of single and multiple combined lipid abnormalities for our study are shown in Figs 3–5. Here, we present the joint distribution of lipid abnormalities for the entire sample and then for sub-samples of very high-risk and non-very high-risk patients. Additionally, joint distributions that either include or exclude patients with no lipid abnormalities are provided for each patient group.

Fig. 3 shows that in 39.4% of patients with a total lipid profile, there was only one single-lipid abnormality, 32.8% had two abnormalities, and the remaining 7.3% had abnormalities in all three assessed components of the lipid profile. Among statin-treated patients, the most common abnormality was high LDL-C levels (18.8% of all cases), accounting for 47.7% of all single-lipid abnormalities. Among the 983 patients, 20.4% had no lipid abnormalities.

Figs 4 and 5 present the joint distribution for non-very high-risk and very high-risk patients, respectively, and indicate different patterns of prevalence for these sub-groups. For the 261 non-very high-risk patients with at least one abnormality depicted in Fig. 4, 37.2% had only one lipid abnormality, 21.5% had two lipid abnormalities and the remaining 4.2% had all three lipid abnormalities.

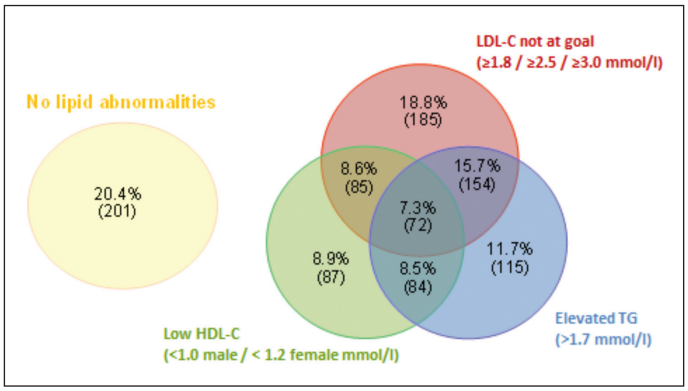


Fig. 3. Distribution of no, single and multiple combined lipid abnormalities for the total study population. TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; proportions add up to 99.9% because of rounding; thresholds for LDL-C are based on the ESC guidelines (2011): SCORE risk 1–4%: LDL-C ≥ 3.0 mmol/l; patients with SCORE risk 5–9%: LDL-C ≥ 2.5 mmol/l; patients with CVD, DM, and/or SCORE risk ≥ 10%: LDL-C ≥ 1.8 mmol/l.

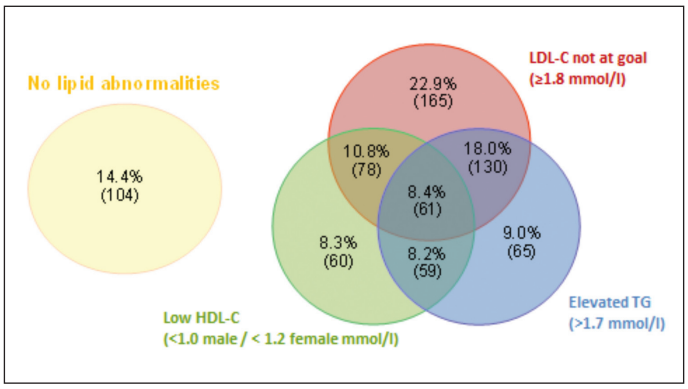


Fig. 4. Distribution of no, single and multiple combined lipid abnormalities in non-very high-risk patients (ESC 2011, SCORE < 10%). TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; thresholds for LDL-C are based on the ESC guidelines (2011): SCORE risk 1–4%: LDL-C ≥ 3.0 mmol/l; patients with SCORE risk 5–9%: LDL-C ≥ 2.5 mmol/l; patients with CVD, DM, and/or SCORE risk ≥ 10%: LDL-C ≥ 1.8 mmol/l.

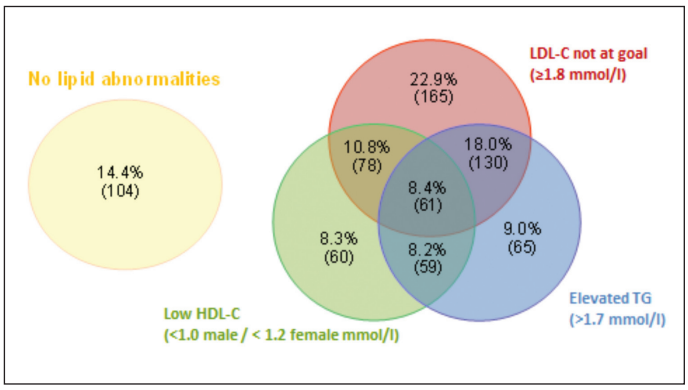


Fig. 5. Distribution of no, single and multiple combined lipid abnormalities in very high-risk patients (ESC 2011, SCORE ≥ 10%). TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; proportions add up to 100.1% because of rounding; thresholds for LDL-C are based on the ESC guidelines (2011): SCORE risk 1–4%: LDL-C ≥ 3.0 mmol/l; patients with SCORE risk 5–9%: LDL-C ≥ 2.5 mmol/l; patients with CVD, DM, and/or SCORE risk ≥ 10%: LDL-C ≥ 1.8 mmol/l.

By contrast, for the 826 very high-risk patients depicted in Fig. 5, the majority, 45.4%, had two or more lipid abnormalities (40.2% had one, 37.0% had two, and the remaining 8.4% had all three). For non-very high-risk patients, elevated triglycerides were the largest single abnormality present, appearing in 42.2% of all non-very high-risk patients. By contrast, among very high-risk patients, high LDL-C level was the most frequent abnormality, at 60.1% of all very high-risk patients.

Variables independently associated with dyslipidaemia

Multivariate logistic regression analyses indicated that among the 19 risk factors incorporated into the model, mixed ancestry, along with history of hypertension, DM and cerebrovascular disease were among the risk factors strongly, positively and independently associated with LDL-C levels not being at goal. Having low HDL-C levels was negatively associated with female gender and increased alcohol consumption, but positively associated with being treated by a specialist, increased waist circumference, and presence of DM. Having elevated triglyceride levels was negatively associated with age above 70 years, but positively associated with female gender, obesity, history of DM and peripheral artery disease. The three variables independently associated with having all three lipid abnormalities were Asian and mixed-ancestry ethnicity versus Caucasian ethnicity, and obesity, all of which were positively associated with not reaching goal (Table 4).

Discussion

In the DYSIS South Africa study we observed marked ethnic differences in cardiovascular risk profiles and the primary indication for statin therapy. While about half of Asian and mixed-ancestry patients had clinically overt CVD, the rate in black patients was less than 10%. The major indication for statin therapy in black patients was diabetes, which was present in 71.2% of patients. A family history of premature CVD was very uncommon (1.8%) in black patients.

These data are reflective of the epidemiological transition, which the South African black population is currently undergoing,[6] with increasing urbanisation and transition to a Westernised lifestyle. Hypertension, obesity and diabetes are highly prevalent in black patients while CVD, which results from prolonged exposure to cardiovascular risk factors, is still relatively uncommon. With further progression of the epidemiological transition, CVD rates in black patients are likely to rise and may well match or exceed those observed in the other ethnic groups if cardiovascular risk factors are not addressed intensively, both on a population and an individual level.

The DYSIS South Africa study identified a group of patients at high cardiovascular risk, with 73.5% of statin-treated patients assessed to be at very high risk for CVD. Within this very high-risk group, despite statin therapy, 85.6% had at least one lipid abnormality, of which a majority had two or more lipid abnormalities. The most common lipid abnormality was high LDL-C levels, which was diagnosed in 60.1% of all very high-risk patients.

Moreover, for all patients in the study, 50.5% had LDL-C levels not at goal, which is comparable with the findings from the recently published CEPHEUS-SA study and the Canadian/European cohort of the DYSIS study, and below the levels found in the Middle Eastern cohort (62%).[24,26,30] Not surprisingly, the metabolic syndrome was present in 67.2% of the sample, since its components also contribute to elevated CVD risk.

Statistically significant factors associated with high LDL-C levels included ethnicity, hypertension, DM, and the presence of coronary and cerebrovascular heart disease. Factors associated with low HDL-C levels were a high waist circumference, DM and being treated by a specialist. Elevated TG was associated with female gender, obesity, DM and peripheral artery disease. However, the only statistically significant factors independently associated with the presence of all three lipid abnormalities were obesity and Asian as well as mixed-ancestry ethnicity.

Based on the current data, it is unclear whether the findings in regard to ethnicity are biologically or sociologically determined. Even though this study was conducted exclusively in the private healthcare sector in South Africa, Asian or mixed-ancestry ethnicity most likely still correlates partially with social deprivation, which has been shown to be a risk factor for cardiovascular disease. Social deprivation may also affect access to medical care, with less access to specialist care and a bias towards less aggressive treatment. Studies from other countries have shown that ethnic minorities or immigrants often receive less aggressive cardiovascular care,[31] as also observed in this study, with black patients receiving lower-dose potency of statins, despite the majority of patients being at high risk.

Socio-economic status has also been associated with statin adherence,[32] as has ethnicity.[33] In the South African context, lower socio-economic status would, for instance, often correlate with membership of a medical scheme option that restricts lipid-lowering treatment to less-potent (and less-costly) options. Lower income may also influence the willingness and ability to pay 'co-payments' that are often required to access more potent lipid-lowering therapy. However, factors such as provider bias, access to treatment and differential adherence do not completely explain the observed ethnic differences, as black patients generally still experience the highest level of socio-economic deprivation as a legacy of South Africa's past history.

Lesser goal attainment may also in part be due to differences in baseline lipids. In the Heart of Soweto study, there were significant differences in untreated lipid profiles by ethnicity in patients presenting for cardiovascular care[34] at a tertiary referral centre. The odds ratio (compared to black patients) for elevated LDL-C levels in Asian and mixed-ancestry patients was 4.66 and 2.44 mmol/l, respectively. Indian and mixed-ancestry patients also had higher median TG levels (1.8 and 1.4 mmol/l, respectively) than black patients (1.1 mmol/l).

In addition to identifying factors that are associated with dyslipidaemia in statin-treated patients, DYSIS in South Africa (along with previous DYSIS studies) also highlights the deficiencies of lipid-lowering therapy in clinical practice. Other researchers analysing the efficacy of lipid-lowering therapies have supported this conclusion,[35,36] including another recent study analysing statin-treated South African patients.[26] Together, these findings suggest that there is a need to improve upon existing treatment strategies (e.g. combination of current therapies for optimal patient efficacy, utilisation of more-potent statins, improving adherence) while also developing novel therapeutic approaches.

Combination therapies were evaluated in the Austrian Cholesterol screening and Treatment (ACT) II study, which evaluated the effect of lipid-lowering therapies in high-risk, statin-treated patients with elevated LDL-C levels. Interestingly, combination therapy consisting of simvastatin and ezetimibe (used for 73% of patients in the ACT II study) resulted in 40.3% of patients meeting their LDL-C goals, with a decline in LDL-C levels from a baseline of 31.3% following 12 months of intensified therapy.[37]

High-dose statins are another option to achieve LDL-C targets in high-risk patients.[38,39] Improving adherence is a challenge that physicians face every day, and some strategies that have shown promise include regular phone calls by a practice nurse, regular review by a community pharmacist and providing a medication

Table 4. Factors independently associated with LDL-C, HDL-C and TG abnormalities: results from multiple regression analyses (or, 95% CI)

	LDL-C not at target*† (≥ 1.8/2.5/3.0 mmol/l)	Low HDL-C* [< 1.0 (m)/1.2 (w) mmol/l]	Elevated TG* (>1.7 mmol/l)	LDL-C not at target, low HDL-C, elevated TG*
Age ≥ 70 years	ns	ns	0.57 (0.43–0.77)	ns
Female	ns	0.43 (0.32–0.58)	1.33 (1.02–1.74)	ns
Asian vs Caucasian	ns	ns	ns	2.48 (1.19–5.16)
Black vs Caucasian	ns	ns	ns	ns
Mixed ancestry vs Caucasian	2.12 (1.36–3.32)	ns	ns	2.78 (1.50–5.19)
Alcohol consumption > 2 units/week	ns	0.50 (0.31–0.79)	ns	ns
BMI ≥ 30 kg/m² (obesity)	ns	ns	1.74 (1.33–2.29)	2.11 (1.27–3.50)
WC > 102 (m)/> 88 cm (w)	ns	1.71 (1.26–2.32)	ns	ns
Hypertension	1.55 (1.12–2.13)	ns	ns	ns
Diabetes mellitus	1.36 (1.01–1.82)	1.58 (1.17–2.15)	1.49 (1.12–1.98)	ns
Cerebrovascular disease	1.89 (1.39–2.57)	ns	ns	ns
Peripheral artery disease	ns	ns	2.35 (1.09–5.07)	ns
Specialist (Card/Endo/Dia/Int/Oth)	ns	2.01 (1.46–2.76)	ns	ns

*Models contained the following variables: age, gender, ethnicity, 1st-grade family history of premature CVD, current smoker, sedentary lifestyle, alcohol consumption > 2 units/week, BMI ≥ 30 kg/m² (obesity), waist circumference > 102 cm in men/> 88 cm in women, hypertension, diabetes mellitus, coronary heart disease, cerebrovascular disease, heart failure, peripheral artery disease, RR ≥ 140/90 mmHg (systolic/diastolic), 20–40 vs 10 mg/day simvastatin equivalent, ≥ 80 vs 10 mg/day simvastatin equivalent, ezetimibe.
Backward selection (alpha = 0.05) was done.
†Patients with SCORE risk 1–4%: LDL-C ≥ 3.0 mmol/l; patients with SCORE risk 5–9%: LDL-C ≥ 2.5 mmol/l; patients with CVD, DM, and/or SCORE risk ≥ 10%: LDL-C ≥ 1.8 mmol/l
Card = cardiologist, Endo = endocrinologist, Dia = diabetologist, Int = internist, Oth = other speciality, ns = not significant ($p > 0.05$), OR = odds ratio, CI = confidence interval.

calendar when patients filled their first prescription.[40] There is likely no single strategy that will work for all patients but studies show that adherent patients have much better cardiovascular outcomes than non-adherent patients, although some of the improvement may also be ascribed to the correlation between adherence and other healthy behaviours.[41-44]

According to a mathematical model of statin use in a population, increasing statin adherence from 50 to 75% at five years would prevent more events than lowering the risk threshold for prescribing statins.[45] Lastly, novel LDL-C-lowering therapies may be necessary for patients with very high baseline LDL-C levels, such as is seen in familial hypercholesterolaemia, and when patients are unable to tolerate adequate doses of potent statins.[46]

In South Africa, the modal statin dose potency prescribed to patients was 3, which was prescribed to 42.2% of the individuals. Interestingly, although the very high-risk patients had a disproportionately high share of the statin prescriptions with a potency of 4 and 5, they also had a disproportionately high amount of prescriptions with a potency of 2, and a disproportionately low share of the prescriptions with a potency of 6. In addition, although combination therapies may have the potential to benefit some patients,[37] we found that the use of combination treatment with lipid-lowering therapies was rare in South Africa. Only seven patients were co-prescribed statins and ezetimibe in this study.

DYSIS-South Africa had several limitations, including its cross-sectional design, which did not permit follow up to assess the effects of statins over time in either reducing CVD risk factors or their ultimate effects in reducing CVD. In addition, the cross-sectional nature of the study precludes us from drawing conclusions of temporality based on observed associations. The study was also only conducted in the private sector and does not therefore provide any information on the care provided in the public sector, which accounts for about 80% of patients in South Africa. As this study was conducted in the private sector, the ethnic make-up of the DYSIS study cohort is not representative of the South African population at large.

Furthermore physicians were aware of the study purpose, possibly making the results prone to a selection bias towards patients with better-than-average lipid goal attainment. DYSIS by its design is also unable to provide data on the important public health question on what proportion of patients with an indication for lipid-lowering therapy is actually being treated. Analysing patients that return for follow-up consultation and are still taking statins is not reflective of the entire statin treatment experience, as patients discontinuing early and defaulting on follow up are not captured. However, in spite of these potential limitations, the data obtained during this cross-sectional, observational study of South Africa has furthered our knowledge of CV risk and the factors that contribute to persistent dyslipidaemia in statin-treated patients.

Conclusions

The DYSIS study for South Africa, like the DYSIS studies in other countries and regions, indicates that large proportions of statin-treated patients have persisting lipid abnormalities, which place them at ongoing risk for CVD. While some observations with regard to co-morbid conditions and demographics associated with lipid goal attainment were expected, observations also demonstrate a decreased likelihood of obtaining lipid goals among two ethnic minority groups, independent of treatment, demographics and other co-morbidities. These findings deserve further attention. As statins remain among the most effective agents for preventing CVD, the findings of this study emphasise the necessity for more aggressive therapy in order to achieve recommended lipid targets, so as to reduce the burden of cardiovascular disease, which is on the increase not only in South Africa but worldwide.

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Gender and ethnic differences in the control of hyperlipidaemia and other vascular risk factors: insights from the CEntralised Pan-South African survey on tHE Undertreatment of hypercholeSterolaemia (CEPHEUS SA) study

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Abstract

Aim: **The aim of the CEntralised Pan-South African survey on tHE Under-treatment of hypercholeSterolaemia (CEPHEUS SA) was to evaluate the current use and efficacy of lipid-lowering drugs (LLDs) in urban patients of different ethnicity with hyperlipidaemia, and to identify possible patient characteristics associated with failure to achieve low-density lipoprotein cholesterol (LDL-C) targets. There is little published data on LDL-C attainment from developing countries.**

Methods: **The survey was conducted in 69 study centres in South Africa and recruited consecutive patients who had been prescribed LLDs for at least three months with no dose adjustment for six weeks. All patients provided written consent. One visit was scheduled for data collection, including fasting lipid and glucose, and HbA1c levels.**

Results: **Of the 3 001 patients recruited, 2 996 were included in the final analyses; 1 385 subjects were of Caucasian origin (818 male), 510 of African ancestry (168 male), 481 of mixed ancestry (222 male) and 620 of Asian origin (364 male). Only 60.5% of patients on LLDs for at least three months achieved the LDL-C targets recommended by the NCEP ATP III/2004 updated NCEP ATP III guidelines and 52.3% the fourth JETF/South African guidelines. African females were on average younger than females of other ethnic origins, and had the lowest smoking rates but the highest prevalence of obesity, hypertension, the metabolic syndrome and diabetes mellitus (DM), with the worst glycaemic control. Although women were less likely than men to reach goal [OR 0.65 (CI 0.54–0.77), p < 0.001 for NCEP ATP III guidelines and OR 0.76 (CI 0.64–0.91), p < 0.003 for fourth JETF guidelines], women of African ancestry were just as likely not to reach goal as their Caucasian counterparts.**

Conclusion: **The results of this survey highlight the sub-optimal lipid control achieved in many South African patients, and profile important gender and ethnic differences. Control of cardiovascular disease risk factors across gender and ethnic groups remains poor.**

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Elevated serum lipid levels have been identified as one of the modifiable risk factors in the aetiology of cardiovascular disease (CVD). The INTERHEART study established that elevated lipid levels was the greatest contributor to the development of myocardial infarction worldwide.[1] Multiple studies have evaluated the control of serum lipid levels in clinical practice but these studies originate almost exclusively from the developed nations of North America and Europe.[2-8] Cardiovascular risk-factor control is poorly studied in developing nations, and in particular, knowledge of the control of lipid levels is largely unknown. In the limited published data, there is no specific information on gender and/or ethnic differences.[9]

Different authorities, such as the Joint European Task Force (JETF) and the United States National Cholesterol Educational Program Adult Treatment Panel III (NCEP ATP III) have developed clinical guidelines for the management of CVD risk, and there are extensive data showing that modification of risk factors can delay the development of CVD or prevent recurrent events in those with CVD at baseline.[10-13] Over time, these guidelines have proposed progressively lower targets for CVD risk factors on the basis of evidence from clinical studies demonstrating that cardiovascular risk is further reduced by more rigorous risk-factor control. Aggressive low-density lipoprotein cholesterol (LDL-C) lowering remains the cornerstone of lipid management.

The CEntralised Pan-South African survey on tHE Undertreatment of hypercholeSterolaemia (CEPHEUS SA) was initiated to detect and quantify the degree of under-treatment of hypercholesterolaemia in South Africa, and the full overall results have been published.[14] This study afforded us an opportunity to study both gender and ethnic differences in the prevalence of these CVD risk factors in a developing world population on drug treatment for elevated serum lipid levels, and forms the basis of this article.

Methods

CEPHEUS was a non-interventional study conducted in South Africa between November 2009 and April 2010. To be representative of the varied population demographics in the country, 101 investigators in 69 urban study centres were involved in recruiting patients; 67 were general/primary healthcare practitioners, 13 were cardiologists, seven endocrinologists, and 14 internists/specialist physicians. Investigators were drawn from both the public and private sectors. Public sector sites were located in tertiary level referral hospitals.

Subjects 18 years or older who had been receiving lipid-lowering drugs (LLDs) for at least three months (without dose adjustments for at least six weeks) were eligible. Consecutive patients who came

for their regular scheduled visit to the doctor/clinic were invited to participate in the survey. Patients who agreed to participate provided informed written consent.

CEPHEUS was a single-visit non-interventional study. Each patient's record form documented patient demographics, current LLD treatment, smoking status, known diabetes mellitus (DM), family history of premature vascular disease, known arterial hypertension (HT) and cardiovascular medical history. Physical examination by the investigator was limited to measurement of height, weight, waist circumference and blood pressure. A fasting blood sample was drawn to evaluate the serum lipid profile [total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), triglycerides and apolipoprotein (apo) AI and apo B], fasting blood glucose (FG) and glycosylated haemoglobin (HbA_{1c}) levels.

The primary endpoint was the percentage of patients who achieved the LDL-C goals according to either the NCEP ATP III/2004 updated NCEP ATP III guidelines or the fourth JETF/South African guidelines, which were current in South Africa at the time the CEPHEUS SA study was conducted. Secondary endpoints included achievement of LDL-C goals in patients with and without features of the metabolic syndrome, and primary versus secondary prevention.

The parent study (CEPHEUS-Europe) has been registered with the US National Institutes of Health (ClinicalTrials.gov), number NCT00542867. The CEPHEUS study was sponsored by AstraZeneca. The sponsor oversaw data collection and monitored study sites. The authors had full access to the study database and all analyses reported here were performed independently of the sponsor.

Statistical analysis

We subdivided the cohort by gender and ethnicity for the purposes of this analysis. The four major ethnic groups in South Africa were black Africans, Caucasians, Asians (including patients of Indian descent) and patients with mixed ancestry. The risk category was determined for each patient and we calculated a dichotomous variable for each patient indicating whether their LDL-C had reached the guideline mandated target level.

We generated descriptive statistics for all clinical and laboratory parameters, following subdivision by gender only, and then following subdivision by both ethnicity and gender. We analysed the effect of ethnicity and gender on goal attainment using logistic regression with the logit function in a model that incorporated ethnicity and gender simultaneously.

We calculated odds ratios and 95% confidence intervals for the probability of not attaining LDL-C goal. The probability of not attaining LDL-C goal was referenced against Caucasian ethnicity and male gender, for which the odds ratio was set as 1. All *p*-values are two-sided and we regarded $p < 0.05$ as statistically significant. All analyses were performed with Statistica [StatSoft Inc (2011), STATISTICA (data analysis software system) version 10, www.statsoft.com].

Results

A total of 3 001 patients consented to participate in the survey. Full data sets were available from 2 996 patients and form the basis of this report. About two-thirds of patients were recruited from the private healthcare sector, with the remaining one-third coming from public sector institutions.

Demographic, anthropometric and clinical data are shown in Table 1. Of the total group, 47.1% had known DM but 2.4% of patients who did not give a history of DM had FG levels that would qualify for the diagnosis of DM. Glycaemic control in patients who gave a history of DM was generally poor, with a mean HbA_{1c} level of 8.33%.

The prevalence of a history of HT in this study was 71.6%. The mean systolic blood pressure in the entire study cohort was 133.2 mmHg, with a diastolic pressure of 80.2 mmHg. In those subjects with a history of HT (Table 2), the mean systolic blood pressure was 136.1 mmHg, with a diastolic pressure of 81.3 mmHg. African-ancestry males had the highest systolic blood pressure and females of mixed ancestry the highest diastolic blood pressure but the inter-ethnic differences were small.

More Caucasian patients were receiving LLDs for primary prevention compared to those of African ancestry, few of whom were on treatment for primary prevention. The majority were receiving treatment for the CVD risk equivalent of DM.

The percentage of African patients who were on LLDs for coronary artery disease (CAD) was lower than that seen in the other ethnic groups. The prevalence of CAD was higher in males than in females in all ethnic groups. Among male participants, CAD rates were highest in men of Caucasian, Asian and mixed ancestry. Among the women, the highest prevalence of CAD was in women of mixed and Asian ancestry. Few African patients gave a family history of CAD and the percentage of smokers was also lowest among the African patients. Most patients (95.9%) were on LLD monotherapy, with this being almost exclusively (98.9%) statin based.

Table 1. Baseline characteristics

	Entire study		Caucasian		African		Mixed ancestry		Asian	
Characteristics	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Number	1572	1424	818	567	168	342	222	259	364	256
Mean age (years)	59.2	59.6	60.9	62.0	57.4	57.4	58.1	59.0	56.8	58.0
Current smoker (%)	18.6	10.8	15.9	14.3	15.6	2.6	25.1	19.7	21.4	5.1
Family history of vascular disease (%)	27.0	29.5	29.0	36.3	4.1	11.1	25.2	30.9	39.2	37.5
Mean body mass index (kg/m²)	29.2	30.8	30.0	29.4	29.3	34.2	29.0	31.2	27.6	29.4
Mean waist circumference (cm)	101.0	101.0	105.7	95.6	101.5	102.2	101.7	100.4	99.4	97.6
Known diabetes mellitus (%)	45.8	48.5	34.4	26.8	70.6	74.9	54.1	54.5	38.7	55.1
Known systemic hypertension (%)	68.8	74.6	64.7	64.7	84.6	88.9	76.6	84.2	65.8	67.9
History of coronary heart disease (%)	45.8	23.9	46.3	19.6	19.0	14.3	58.1	38.2	49.4	31.6
History of cerebrovascular disease (%)	5.8	4.8	5.3	4.4	5.9	6.1	6.3	4.2	6.3	4.2
History of peripheral arterial disease (%)	6.2	3.5	7.8	3.2	3.0	2.6	6.8	6.2	3.3	2.7

Table 2. Laboratory results

Laboratory parameters	Entire study		Caucasian		African		Mixed ancestry		Asian	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Total cholesterol (mmol/l)	4.72	5.06	4.75	5.26	4.44	4.57	4.78	5.20	4.75	5.13
LDL cholesterol (mmol/l)	2.63	2.85	2.62	2.93	2.44	2.55	2.65	3.01	2.72	2.91
HDL cholesterol (mmol/l)	1.21	1.41	1.25	1.53	1.19	1.31	1.15	1.36	1.19	1.33
Triglycerides (mmol/l)	2.01	1.79	2.03	1.80	1.83	1.55	2.31	1.83	1.88	1.99
Non-HDL cholesterol (mmol/l)	3.51	3.65	3.51	3.73	3.25	3.26	3.63	3.84	3.56	3.80
Glucose (mmol/l)	6.52	6.76	6.0	5.8	7.2	8.1	6.8	6.7	7.1	7.0
Glycosylated haemoglobin (%)		7.34	638	6.31		8.74		7.48		7.91

The on-treatment lipid and FG values are listed in Table 3. Overall, patients of African ancestry had lower TC, LDL-C, and non-HDL-C levels and higher FG levels than subjects of other ethnic groups. In the cohort with DM (Table 2), the African-ancestry patients had the highest levels of HbA_{1c}, both for males and females.

The primary outcome or percentage of patients reaching LDL-C targets is given in Table 4. Overall 60.5% of patients reached goal as per the NCEP ATP II guidelines and 52.3% according to the JETF guidelines. Differences in attainment of goal were noted. Patients of mixed ancestry were less likely to get to either of the two goals, with the exception of mixed-ancestry males, who had similar not-at-goal percentages as the male patients of Asian ancestry. Females subjects were less likely to get to goal, both for the NCEP ATP III [OR 0.65 (CI 0.54–0.77), $p < 0.001$] and JETF [OR 0.76 (CI 0.64–0.91), $p < 0.003$] guidelines. This difference was maintained across the various ethnic groups.

The secondary outcomes or percentages of patients receiving LLDs with the metabolic syndrome, and the breakdown of those receiving LLDs for primary versus secondary prevention is given in Table 5.

Discussion

The World Health Organisation has indicated that cardiovascular disease will be the number one cause of mortality in the developing world by 2020.[15] Subjects with cardiovascular disease in underdeveloped countries tend to exhibit mortality 10 or more

Table 3. Control of diabetes and hypertension

Condition	Entire study		Caucasian		African		Mixed ancestry		Asian	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Diabetic patients										
Number	718	690	279	152	118	256	120	139	200	141
Glucose (mmol/l)	7.93	8.44	7.46	8.08	8.12	9.12	8.17	7.86	8.24	8.19
HbA_{1c} (%)	7.94	8.73	7.33	7.64	8.66	9.60	8.19	8.52	8.26	8.57
Hypertensive patients										
Number	1081	1063	529	367	142	304	170	218	240	174
Systolic blood pressure (mmHg)	134.9	137.3	135.6	135.6	137.3	137.1	136.6	141.9	130.6	135.3
Diastolic blood pressure (mmHg)	81.7	80.8	82.2	79.3	83.8	81.5	81.3	81.2	79.8	80.0

Table 4. Attainment of primary goal (%)

Guidelines	Entire study		Caucasian		African		Mixed ancestry		Asian	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
NCEP ATP III	63.4	56.8	67.3	61.5	68.4	60.8	61.0	41.7	58.7	52.7
EAS/ESC	55.0	49.3	57.5	50.1	62.3	55.8	44.3	42.0	51.6	48.0

Table 5. Secondary outcome variables

Variables	Entire study		Caucasian		African		Mixed ancestry		Asian	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Metabolic syndrome (%)	65.2	71.5	62.8	55.7	72.6	88.0	71.2	77.6	63.7	78.1
Primary prevention (%)	40.3	48.7	47.4	66.7	32.7	24.9	32.4	44.0	34.1	45.7
Secondary prevention (including DM) (%)	59.7	51.3	52.6	33.3	67.3	75.1	67.6	56.0	65.9	54.3

years before their counterparts from the developed nations.[16] These factors indicate that it is imperative to address all cardiovascular risk factors aggressively if there is to be any curtailment of the looming epidemic.

This survey, representing patients in a developing country, indicates that a large proportion of patients on LLDs are not reaching the accepted LDL-C goals. While these percentages are not dissimilar to those from other studies in Europe, they are below what is currently achieved in North America. The percent of patients at goal in North America has risen over the course of six years, from the NHANES survey of 1999/2000 and the follow up conducted in 2005/2006, indicating that increased awareness and education can result in a greater percentage of patients reaching LDL-C goals, despite the targets becoming more stringent.

The current study indicates both ethnic and gender variances in cardiovascular risk-factor distribution and control in South Africa. Smoking was less prevalent in those of African ancestry and very few black females were smokers. In general black subjects used fewer cigarettes than their Caucasian counterparts (possibly due to economic constraints). The South African Heart of Soweto study confirms that African patients have the lowest smoking prevalence, with patients of mixed ancestry twice as likely, and Caucasian patients three-fold more likely to be current smokers.[17] In the current study of patient on LLDs, patients of mixed ancestry had the highest prevalence of smoking among both males and females, followed by Asian males.

The majority of black subjects did not have a family history of premature heart disease, which probably reflects the evolution of the epidemiological transition in an urbanising population, compared to Caucasians, Asians and patients of mixed ethnicity. The Heart of Soweto study also noted that African patients were least likely to be diagnosed with CAD, and showed similar data to CEPHEUS SA for the Asian patients, who had the highest prevalence of a family history of vascular disease.

Control of DM was particularly poor in both male and female African subjects compared with their ethnic counterparts. This may have been due to differences in access to guideline-based management protocols. Despite a high prevalence of the metabolic syndrome in African females, with poor control of DM, their TG levels were the lowest of all subjects – male and female.

The Heart of Soweto study noted that patients of African descent had significantly lower total cholesterol (TC), LDL-C and triglyceride (TG) levels compared to other ethnicities.[17] These patients were not receiving LLDs. In CEPHEUS SA, in the African-ancestry group, the TGs were not elevated despite a high prevalence of DM with poor control. This would lend credence to the finding that this population group may inherently have low TG levels, and the influence of DM on TGs may be muted.

The Heart of Soweto study confirmed a high prevalence of obesity in patients of African ancestry (43% of the patients having a body mass index greater than 30 kg/m^2). This substantial burden of obesity among African subjects points to an elevated risk for the future development of DM. Given that DM in this group is poorly controlled, the ameliorating influence of lipid-lowering therapy on future cardiovascular risk could potentially be undermined, or at the very least minimised.

The number of African-ancestry patients who had CAD was low in proportion to the other ethnic groups; however these percentages reflect a change in the prevalence of a disease that was previously considered to be rare in this population. Other studies from South Africa have indicated a prevalence of CAD of less than 10% in the African population.[18] The prevalence of CAD in African subjects receiving LLDs in the current study was 15.9%.

The INTERHEART Africa study noted that patients of African ancestry presented with myocardial infarction a mean of 3.8 years earlier than patients from the overall INTERHEART study, and also found no inter-ethnic or gender differences.[1,19] Although data for the INTERHEART Africa study were drawn from patients from sub-Saharan countries, more than 80% of subjects were from South Africa, indicating that the data may be comparable to the current CEPHEUS SA study.

Limitations

This study had the same limitations that apply to many surveys that differ fundamentally from formal prospective studies. The study population was drawn from those already on LLDs and cannot be extrapolated to the general population. Although attempts were made to sample patients from as wide a spectrum as possible, potential selection bias may still have occurred. All centres were located in urban areas, and the applicability to patients of rural origin cannot be assumed.

The public sector provides healthcare to about 80% of the South African population but it made up only about one-third of the sample. Similarly, the study population does not strictly reflect the ratios of the different ethnic groups residing in South Africa. However all previous studies on lipid-lowering therapy in South Africa were predominantly Caucasian based. Private sector patients were recruited from a wide variety of both specialist and non-

specialist practices. The public sector patients were predominantly recruited from tertiary-care lipid and diabetes clinics. The majority of African patients came from the public sector.

Several private-sector centres had practitioners who dealt predominantly with patients with DM, and this could have further swayed the emphasis of the results on the diabetic cohort. DM is often associated with an increase in body mass index and other anthropometric measures of obesity, and data from a cohort with a high prevalence of DM may therefore not be reflective of the general population.

As the veracity of the patient questionnaires was not tested, the validity of the CVD history may have been inaccurate. Measured clinical parameters (such as blood pressure) were from a single visit and methods of measurement were not standardised or checked, and therefore inaccuracies could have arisen. Causal correlations were not established, and relationships should therefore be interpreted with caution.

Conclusion

Management of lipid-lowering treatment in South Africa is sub-optimal, and in general lags behind control achieved in the more developed nations. Furthermore, other cardiovascular risk factors are not receiving due attention and their prevalence in this population remains high. For any serious impact to be made on the looming epidemic of cardiovascular disease in the underdeveloped world, more attention needs to be focused on more aggressive treatment of dyslipidaemia as well as the other cardiovascular risk factors and, in particular, diabetes mellitus and obesity.

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Prevalence of the metabolic syndrome and determination of optimal cut-off values of waist circumference in university employees from Angola

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Abstract

Background: **Estimates of the prevalence of the metabolic syndrome in Africans may be inconsistent due to lack of African-specific cut-off values of waist circumference (WC). This study determined the prevalence of the metabolic syndrome and defined optimal values of WC in Africans.**

Methods: **This cross-sectional study collected demographic, anthropometric and clinical data of 615 Universitary employees, in Luanda, Angola. The metabolic syndrome was defined using the third report of the National Cholesterol Education Program Adult Treatment Panel (ATPIII) and the Joint Interim Statement (JIS) criteria. Receiver operating characteristics curves were constructed to assess cut-off values of WC.**

Results: **The crude prevalence of the metabolic syndrome was higher with the JIS definition (27.8%, age-standardised 14.1%) than with the ATP III definition (17.6%, age-standardised 8.7%). Optimal cut-off values of WC were 87.5 and 80.5 cm in men and women, respectively.**

Conclusions: **There was a high prevalence of the metabolic syndrome among our African subjects. Our data suggest different WC cut-off values for Africans in relation to other populations.**

Keywords: metabolic syndrome, waist circumference, Africans, Angola

The metabolic syndrome is characterised by the presence of multiple metabolic risk factors for cardiovascular (CV) disease[1] and type 2 diabetes mellitus.[2] In clinical practice, the metabolic syndrome is diagnosed by combinations of three or more of the following five risk factors: central obesity, elevated blood pressure, glucose intolerance, hypertriglyceridaemia and low high-density lipoprotein cholesterol (HDL-C).[3-6]

Worldwide the prevalence of the metabolic syndrome is increasing and becoming a pandemic, and this increase has been mainly attributed to sedentary lifestyle and obesity.[7] However, levels of prevalence may vary greatly according to cut-off points of diagnostic criteria and the ethnic group studied.[8]

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In sub-Saharan Africa, the majority of countries are experiencing a rapid demographic and epidemiological transition.[9,10] Available information from studies in African populations reported a prevalence of the metabolic syndrome ranging from 0 to as high as about 50% or more, depending on the population setting.[11] These data however, are limited to some countries,[12-21] since there are no available data for the majority of African countries.

Angola is a country in sub-Saharan Africa, which in last few years has undergone significant political changes, accompanied by a rapid economic growth and increased urbanisation. These changes may imply the increasing prevalence of factors contributing to the metabolic syndrome, such as obesity, insufficient physical activity, dyslipidaemia, high blood pressure and glucose intolerance. However, the prevalence of the metabolic syndrome and which factors contribution more to its occurrence in the Angolan population remain unknown.

Despite the efforts of several organisations to regulate the algorithm for a definition of the metabolic syndrome,[3-5] there is inconsistency on cut-off levels of waist circumference (WC) for defining the metabolic syndrome in several populations. The International Diabetes Federation (IDF)[5] recommended the use of ethnic or country-specific cut-off values of WC for the majority of populations, a recommendation reinforced in the Joint Interim Statement (JIS),[7] which tried to define different criteria for a definition of the metabolic syndrome.

These cut-off values were defined using different methods. For example, Western countries derived their cut-off values of WC from a correlation with body mass index (BMI),[4,22] whereas Asian groups tried to define WC cut-off values yielded by receiver operating characteristics (ROC) curve analyses.[23] Due to a lack of specific data from African populations, cut-off points of WC derived from the European population have been recommended,[5,7] although emerging data suggest that African-specific cut-off values would be different from the European cut-off points currently recommended by the IDF.[18,24,25] Therefore, definition of a more reliable cut-off point for WC is needed to build a consistent tool for diagnosis of the metabolic syndrome in sub-Saharan African populations.

The aim of this study was to determine the prevalence of the metabolic syndrome in a sample of Africans from Angola, using either the third report of the National Cholesterol Education Program Adult Treatment Panel (ATP III)[4] or the JIS[7] criteria. Additionally, this study tried to identify threshold WC levels that best predict other components of the metabolic syndrome.

Methods

This was a cross-sectional study on cardiovascular (CV) risk factors, conducted from 2009 to 2010 in employees of a public university in Luanda, Angola. Participants aged 20 years and older (n = 625) attended to the Department of Physiology, Faculty of Medicine of

Agostinho Neto University, Luanda, Angola to be submitted to clinical and laboratorial examinations to identify cardiovascular risk.

A total of 615 subjects with complete data were included in this study. Details of the study design are described elsewhere.[26,27] The study was conducted according to the tenets of the Declaration of Helsinki and participants signed an informed consent form approved by the Ethics Committee of the Faculty of Medicine, Agostinho Neto University.

Clinical examinations were performed between 08:00 and noon in temperature-controlled rooms (22–23°C) after a 12-hour fast. Participants were asked to refrain from smoking, physical exercise and caffeinated beverages for at least 12 hours before the visit. Venous blood samples were obtained from the forearm by standard techniques and processed immediately using commercially available kits (BioSystems SA, Costa Brava 30, Barcelona, Spain) for determination of levels of serum triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), glucose, creatinine and uric acid.

Biochemical parameters were analysed using enzymatic methods on a spectrophotometer (BioSystems BTS-310, Barcelona, Spain). In subjects with triglyceride levels < 400 mg/dl, low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula,[28] and very low-density lipoprotein cholesterol (VLDL-C) was calculated as previously described.[4]

Diabetes was defined as a fasting glucose level ≥ 126 mg/dl or the use of antidiabetic drugs.[29] Dyslipidaemia was defined as the presence of one or more of the following: total cholesterol ≥ 200 mg/dl, triglycerides ≥ 150 mg/dl, LDL-C ≥ 160 mg/dl, or HDL-C < 40 mg/dl (men), < 50 mg/dl (women).[4]

Demographics including socio-economic level, educational data and medical history were collected using a structured questionnaire. Participants were classified as non-smokers (never and ex-smokers) and current smokers (daily and occasional smokers).

Anthropometric measures included weight, height, WC and hip circumference (HC), obtained from individuals wearing underwear and no shoes. Weight was measured to the nearest 0.1 kg using a previously calibrated mechanical scale (SECA GmbH & Co, Germany) with a maximum capacity of 220 kg.

Height was measured with a precision of 0.5 cm using a stadiometer fixed to the SECA scale. WC and HC were each measured twice using an inextensible, 1-cm-wide tape measure. The WC was measured at the end of normal expiration, at the midpoint between the lower border of the rib cage and the top of the iliac crest,[30] and recorded nearest to the 0.1 cm. The waist:hip ratio (WHR) was calculated from the WC and HC.

BMI was calculated as the weight divided by the square of the height (kg/m^2). According to BMI values, individuals were classified as normal (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2) and obese (≥ 30.0 kg /m^2).[31]

Socio-economic status was classified into quartiles according to average monthly household income;[27] first quartile (low socio-economic class), second quartile (middle class), third quartile (upper middle class), and fourth quartile (upper class). Education was classified into three levels based on the number of years of education: low (≤ four years of education), middle (five to 12 years of education), and high (≥ 13 years of education).[27]

Blood pressure and heart rate were measured in triplicate in the non-dominant arm after five minutes of resting in a seated position with the arm at the level of the heart. These parameters were measured using a validated, automated digital oscillometric sphygmomanometer (Omron 705CP, Tokyo, Japan). The readings were repeated at three-minute intervals. The mean of the last two readings was recorded.

Table 1. Characteristics of the participants according to gender

Characteristics	All	Men	Women	*p*-value
Number (%)	615 (100)	294 (47.8)	321 (52.2)	0.392
Age (years)	44.5 ± 10.6	45.1 ± 11.1	44.0 ± 10.1	0.176
Weight (kg)	68.6 ± 15.3	68.0 ± 14.9	69.2 ± 15.7	0.349
Height (cm)	163.3 ± 7.9	167.4 ± 7.1	159.6 ± 6.6	< 0.001
WC (cm)	82.1 ± 13.3	80.1 ± 12.9	83.9 ± 13.5	< 0.001
HC (cm)	95.7 ± 11.3	91.5 ± 9.4	99.5 ± 11.4	< 0.001
WHR	0.86 ± 0.09	0.87 ± 0.08	0.84 ± 0.09	< 0.001
BMI (kg/m^2)	25.7 ± 5.4	24.1 ± 4.3	27.1 ± 5.8	< 0.001
SBP (mmHg)	134.7 ± 24.9	136.5 ± 22.7	133.0 ± 26.6	0.087
DBP (mmHg)	82.6 ± 14	82.7 ± 14.2	82.5 ± 13.8	0.862
PP (mmHg)	52.1 ± 14.9	53.8 ± 13.2	50.5 ± 16.2	0.007
MBP (mmHg)	100.0 ± 16.9	100.6 ± 16.4	99.4 ± 17.5	0.351
Heart rate (bpm)	68 ± 10	67 ± 10	69 ± 10	0.003
Glucose (mg/dl)	94.0 ± 21	94.9 ± 20	93.2 ± 21.8	0.313
Creatinine (mg/dl)	1.1 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	< 0.001
Uric acid (mg/dl)	5.4 ± 1.7	6.1 ± 1.7	4.8 ± 1.4	< 0.001
TC (mg/dl)	191.5 ± 38.9	189.5 ± 41.4	193. 2 ± 36.5	0.239
HDL-C (mg/dl)	46.0 ± 10.9	44.1 ± 10.3	47.6 ± 11.2	< 0.001
LDL-C (mg/dl)	125.5 ± 40.1	125.0 ± 41.8	125.9 ± 38.7	0.796
VLDL-C (mg/dl)	20.0 ± 8.0	20.4 ± 8.3	19.7 ± 7.7	0.339
TGL (mg/dl)	100.2 ± 40.0	101.8 ± 41.7	98.7 ± 38.4	0.339

Values are means ± standard deviation. WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MBP, mean blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TGL, triglycerides.

The pulse pressure (PP) was computed as the difference between basal systolic blood pressure (SBP) and diastolic blood pressure (DBP). Mean blood pressure (MBP) was computed as the DBP + (PP/3). Hypertension was defined as SBP ≥ 140 mmHg, and/or DBP ≥ 90 mmHg, and/or the use of antihypertensive drugs.

Both the ATP III[4] and JIS[7] criteria were used to define the metabolic syndrome. The ATP III definition was based on the presence of three or more of the following components: WC > 102 cm (men), 88 cm (women); SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg and/or BP-lowering treatment; fasting triglyceride levels ≥ 150 mg/dl or treatment for hypertriglyceridemia; HDL-C < 40 mg/dl (men), 50 mg/dl (women), or treatment for dyslipidaemia; fasting glucose level ≥ 110 mg/dl or on antidiabetic medication.

The JIS definition was based on the presence of three or more of the following components: WC ≥ 94 cm (men), 80 cm (women); SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg and/or BP-lowering treatment; fasting triglyceride levels ≥ 150 mg/dl or treatment for hypertriglyceridaemia; HDL-C < 40 mg/dl (men), 50 mg/dl (women) or treatment for dyslipidaemia; fasting glucose level ≥ 100 mg/dl or on antidiabetic medication.

Statistical analysis

Data were analysed using SPSS software, version 13.0 (SPSS Inc, Chicago, IL). Continuous variables are reported as mean ± standard deviation, and compared by gender using the independent-samples *t*-test. Categorical variables were expressed as proportions

and compared using the chi-square test or Fisher's exact test if appropriate. Prevalence of the metabolic syndrome was age-standardised by direct method using as reference the world population distribution as projected by the WHO for 2000 to 2025.[32] Age-specific prevalence of the metabolic syndrome was estimated per age decades (< 30, 30–39, 40–49, 50–59 and ≥ 60 years).

ROC curve analysis was performed to determine the appropriate cut-off points of WC for identifying subjects with two or more components of the metabolic syndrome (except for WC), as defined by the JIS criteria. For the purpose of this analysis, we considered the presence or absence of the metabolic syndrome as an outcome variable and WC as a testing variable.

Optimal values of WC were obtained from the Youden index [maximum (sensitivity + specificity – 1)].[33] Positive predictive values (PPV) and negative predictive values (NPV) were also presented. The kappa coefficient was used to assess the statistical agreement between the ATP III and JIS criteria for identify individuals with the metabolic syndrome. A p-value < 0.05 was considered statistically significant.

Results

A complete data set was collected for 615 subjects (52.2% women). Compared with women (Table 1), men had higher mean values for height, WHR, creatinine and uric acid levels (all $p < 0.001$), and PP ($p = 0.007$). Women had higher means values for HDL-C, WC, HC, BMI (all $p < 0.001$), and heart rate ($p = 0.003$). Age, weight, SBP, DBP, MBP, and glucose, total cholesterol, LDL-C, VLDL-C, and triglyceride levels were similar in both sexes.

Table 2 shows distribution of risk factors, socio-economic and educational characteristics of the study population. Current smoking was higher in men ($p = 0.035$), whereas prevalence of overweight, obesity and low HDL-C levels were higher in women (all $p < 0.001$). However, prevalence of hypertension, diabetes, hypercholesterolaemia, hypertriglyceridaemia and high LDL-C levels were similar in both sexes (Table 2).

Table 2. Risk factors, educational level and socioeconomic class of the study population

Characteristics	All	Men	Women	*p*-value
Hypertension, *n* (%)	278 (45.2)	136 (46.3)	142 (44.2)	0.615
Current smokers, *n* (%)	39 (6.3)	25 (8.5)	14 (4.4)	0.035
Diabetes, *n* (%)	35 (5.7)	16 (5.4)	19 (5.9)	0.799
Overweight, *n* (%)	180 (29.3)	80 (27.2)	100 (31.2)	< 0.001
Obesity, *n* (%)	120 (19.5)	27 (9.2)	93 (29.0)	< 0.001
High TC, *n* (%)	68 (11.1)	31 (10.5)	37 (11.5)	0.698
High TGL, *n* (%)	77 (12.5)	37 (12.6)	40 (12.5)	0.963
High LDL-C, *n* (%)	121 (19.7)	61 (20.7)	60 (18.7)	0.522
Low HDL-C, *n* (%)	308 (50.1)	108 (36.7)	200 (62.3)	< 0.001
Education level				0.926
Low, *n* (%)	213 (34.6)	110 (37.4)	103 (32.1)	
Medium, *n* (%)	150 (24.4)	69 (23.5)	81 (25.2)	
High, *n* (%)	252 (41.0)	115 (39.1)	137 (42.7)	
Socio-economic class				0.392
Low, *n* (%)	154 (25.0)	81 (27.6)	73 (22.7)	
Middle, *n* (%)	156 (25.4)	77 (26.2)	79 (24.6)	
Upper middle, *n* (%)	152 (24.7)	66 (22.4)	86 (26.8)	
Upper, *n* (%)	153 (24.9)	70 (23.8)	83 (25.9)	

Values are number of subjects (*n*) and percentages (%).

Table 3. Crude and age-standardised prevalence of the metabolic syndrome in men and women according to ATP III and JIS criteria

Age group (years)	*n*	ATP III	JIS
Men (*n* = 294)			
< 30	40	2 (5.0)	3 (7.5)
30–39	52	2 (3.8)	4 (7.7)
40–49	89	8 (9.0)	15 (16.9)
50–59	90	10 (11.1)	23 (25.6)
≥ 60	23	3 (13.0)	5 (21.7)
Total crude	294	25 (8.5)	50 (17.0)
Age-standardised	–	4.8	9.0
Women (*n* = 321)			
< 30	32	0 (0.0)	1 (3.1)
30–39	71	8 (11.3)	13 (18.3)
40–49	125	43 (34.4)	62 (49.6)
50–59	79	27 (34.2)	37 (46.8)
≥ 60	14	5 (35.7)	8 (57.1)
Total crude	321	83 (25.9)	121 (37.7)
Age-standardised (%)	–	12.6	19.2
Overall (*n* = 615)			
Crude	615	108 (17.6)	171 (27.8)
Age-standardised (%)	–	8.7	14.1

Values are *n* (%). ATP III, National Cholesterol Education Program Third Adult Treatment Panel; JIS, Joint Interim Statement.

The overall crude prevalence of the metabolic syndrome was 17.6% [age-standardised: 8.7%, 95% confidence interval (CI): 6.8–11.3] for the ATP III criteria and 27.8% (age-standardised: 14.1.0%, 95% CI: 11.6–17.1) for the JIS criteria. As expected, the crude prevalence was higher in women than in men, irrespective of the criteria used (Table 3). In both sexes, the prevalence of the metabolic syndrome increased with age, however, women showed a higher prevalence in all age groups from 30 years and older (Table 3). Regarding socio-economic class and educational level

Table 4. Prevalence of the metabolic syndrome from JIS criteria in men and women according to socio-economic class and educational level

	Number (%)	*p*-value
Men		
Socio-economic class		0.083
Low	8 (9.9)	
Middle	13 (16.9)	
Upper middle	11 (16.7)	
Upper	18 (25.7)	
Education level		0.444
Low	15 (13.6)	
Medium	12 (17.4)	
High	23 (20.0)	
Women		
Socio-economic class		0.199
Low	29 (39.7)	
Middle	28 (35.4)	
Upper middle	26 (30.2)	
Upper	38 (45.8)	
Education level		0.294
Low	45 (43.7)	
Medium	27 (33.3)	
High	49 (35.8)	

Values are number of subjects (*n*) and percentages (%).

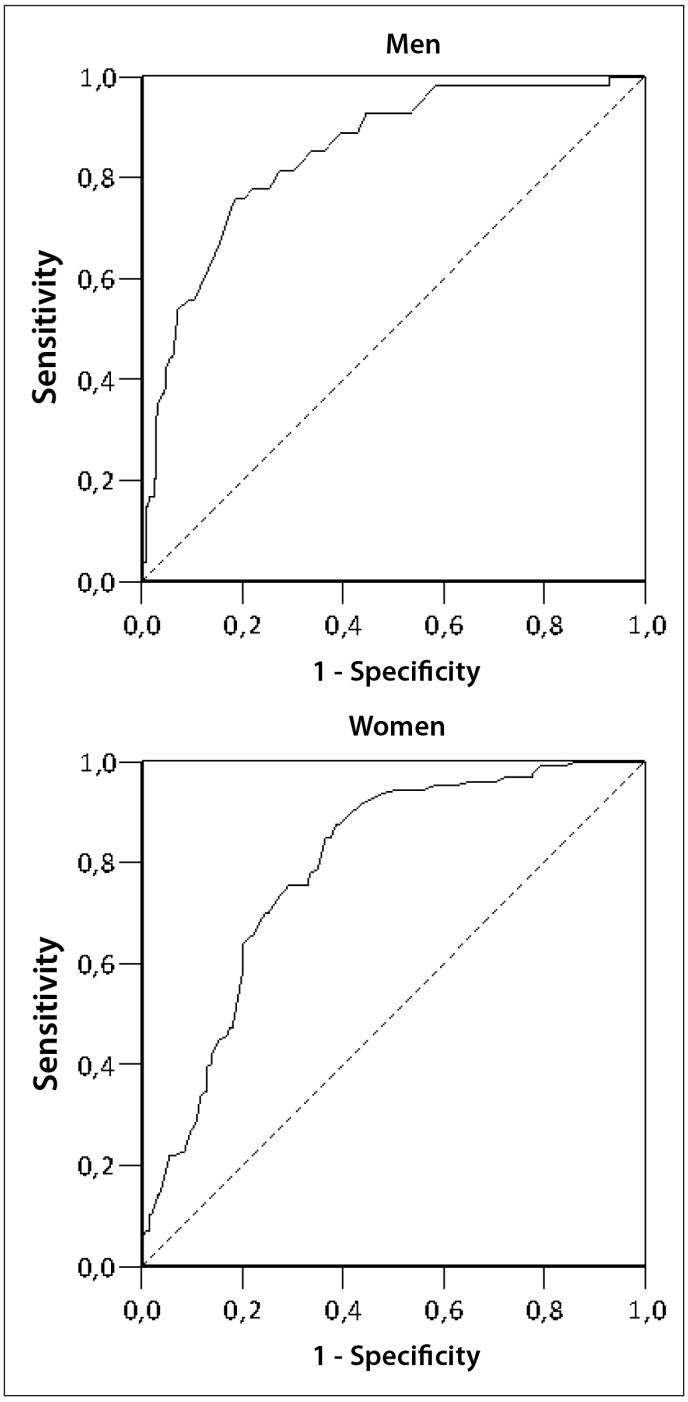

Fig. 1. Receiver operating characteristic (ROC) curves of waist circumference (WC) to detect the metabolic syndrome in men and women, according to the Joint Interim Statement definition. Area under the ROC curve: 0.85 in men and 0.79 in women. WC cut-off values in men: 87.5 cm (sensitivity 75.9%, specificity 81.2%) and 80.5 cm (sensitivity 88.4%, specificity 60.5%) in women.

(Table 4), there was no significant relationship of these factors with the metabolic syndrome in both sexes.

In individuals diagnosed with the metabolic syndrome from the JIS definition (n = 171), the most frequent components were elevated blood pressure: 52.5% (men 55.4% vs women 49.82%, p = 0.165), reduced HDL-C levels: 50.1% (men 36.7% vs women 62.3%, $p < 0.001$) and high WC: 39.8% (men 15.3% vs women 62.3%, $p < 0.001$). The less frequent components were elevated glucose levels: 23.4% (men 25.9% vs women 21.2%, p = 0.172) and raised triglyceride levels: 10.7% (men 12.6% vs women 9.0%, p = 0.155).

Although the prevalence of the metabolic syndrome diagnosed from the JIS criteria was higher than with the ATP III criteria, there was a good agreement between the two classifications in the overall sample [kappa = 0.712, ($p < 0.001$; 95% CI: 0.648–0.777)], as well as in men [kappa = 0.624 ($p < 0.001$; 95% CI: 0.493–0.755)] and in women [kappa = 0.731 ($p < 0.001$; 95% CI: 0.654–0.809)].

Fig. 1 shows results from the ROC curve analysis to identify subjects with two or more components of the metabolic syndrome using the JIS criteria. In men, the optimal cut-off value of WC to detect the metabolic syndrome with maximum sensitivity and specificity (Youden index = 0.563) was 87.5 cm (sensitivity 75.9%, 95% CI: 62.4–86.5; specificity 81.2%, 95% CI: 75.7–86; positive predictive value (PPV) 44.2%, 95% CI: 38.5–49.9 and negative predictive value (NPV) 94.2%, 95% CI: 91.5–96.9); whereas in women, the optimal cut-off value of WC (Youden index = 0.489) was 80.5 cm (sensitivity 88.4%, 95% CI: 81.3–93.5; specificity 60.5%, 95% CI: 53.4–67.3; PPV 57.5%, 95% CI: 52.1–62.9 and NPV 89.6%, 95% CI: 87.9–91.3).

There was good accuracy ($p < 0.001$) of the cut-off values of the WC to predict other components of the metabolic syndrome, as suggested by values of the area under the ROC curve [men: 0.85 (95% CI: 0.80–0.89) and women: 0.79 (95% CI: 0.74–0.84)].

Discussion

The main findings of this study were a high prevalence of the metabolic syndrome among our subjects and a different cut-off value for WC for the diagnosis of the metabolic syndrome from those recommended for Africans by other studies.[5,7] To our knowledge, this is the first study reporting the prevalence of the metabolic syndrome in Angolans.

Worldwide, the metabolic syndrome is increasingly becoming a pandemic,[7] the level of prevalence being estimated to be 17–25% in the general population. However, estimates in sub-Saharan African populations are scarce and inaccurate.[11] The crude prevalence in this study was in an intermediate point of the range (0–50%) reported for different African populations.[11]

The three most frequent components of the metabolic syndrome were elevated blood pressure, low HDL-C levels and elevated WC. A similar cluster of components was reported in an urban population in Kenya,[20] and in a study including West Africans (Nigeria and Ghana) and African-Americans.[34] Other studies reported a combination of high WC and low HDL-C levels as the most frequent components in Africans with high a prevalence of the metabolic syndrome.[14,18,25]

Although the underlying mechanisms are not fully understood, the increasing prevalence of the metabolic syndrome has been associated with a sedentary lifestyle and obesity.[7] Also, it has been reported that in contrast to developed nations, in some African nations, a higher socio-economic status has been associated positively with increased obesity.[35]

In our study, distribution of the metabolic syndrome according to socio-economic class, defined by average household monthly income, was not significant. However, this study also showed a high prevalence of both obesity and overweight (47.8%) and hypertension (45.2%). The three most common components

of the metabolic syndrome were elevated blood pressure, low HDL-C levels and high WC, suggesting a high risk for CV diseases in this occupational cohort. Therefore, considering the on-going socio-economic changes in Angola, the findings of this study may reflect the impact of the nutritional transition, behavioural and occupational changes, environmental risk factors and unhealthy lifestyle (mainly sedentary) with rapid weight gain, and the high consumption of salty and high caloric food.

Although this study showed a good concordance between the two criteria, the crude prevalence estimated with the JIS definition was 10.2% higher than that estimated with ATP III. This difference was mainly attributed to the different cut-off point for WC, which is lower for JIS than for ATP III criteria.

It is known that WC reflects both visceral and subcutaneous fat depots, but it has been used as a crude but relevant index of visceral adiposity. The role of visceral adiposity in the development of each metabolic syndrome component has been shown in non-African populations.[36-39] In sub-Saharan African populations, a high WC was suggested as a key determinant for development of the metabolic syndrome.[14]

However, since country-specific cut-off values of WC still need to be defined for Africans, the cut-off values of WC derived from European population groups have been recommended for Africans.[5,7] Emerging data suggested that African-specific cut-off values would be different from European cut-off values currently recommended by the IDF.[18,24,25] In this study, the cut-off values for men were lower than that currently recommended for Africans (87.5 instead of 94 cm);[5,7] whereas for women, these cut-off values were similar to those recommended for European and African women (80.5 vs 80 cm).

A few studies have attempted to establish cut-off values of WC for African groups,[18,24,25] and they found different cut-off values from those currently recommended. In our study, the value of 87.5 cm for men is similar to that reported in South African studies of African men (86 cm),[18] but different for women.[18,25] However, our findings differed from those reported for men and women in another study of the same population (men: 90 cm, women: 98 cm).[24]

Discordant cut-off values of WC between different studies are to be expected since even in the same ethnic group, the WC may vary according to the country, as emphasised by the IDF[5] and the JIS.[7] Furthermore, it has been reported that variation in WC cut-off values obtained using the sensitivity and specificity approach were strongly correlated with mean levels of WC in the population.[40,41] The cut-off values increased linearly with increasing population means, independent of WC measurement techniques and regardless of whether the health outcome was hypertension, dyslipidaemia, hyperglycaemia or a cluster of multiple outcomes.[40] However, it remains to be clarified whether this variation was due to biological characteristics or the methodological approaches used to define the best cut-off point.[40]

In this study, women had higher mean values of WC than men (Table 1). It is known that the proportion of total fat in subcutaneous depots is higher in women than men.[42] Therefore there is a potential risk of misclassification of women as having excessive visceral adiposity by using values of WC to predict other components of the metabolic syndrome. To minimise this difficulty in this study and ensure a correct classification for only women with strong evidence of two or more components of the metabolic syndrome, we selected the best cut-off values of WC, as suggested by the higher values of the Youden index. Therefore, this study reinforces the opinion that definition of cut-off values of WC should be country- and gender-specific.

There was a potential limitation to this study. Because we studied a convenient sample consisting of staff of a public university, our findings may not apply to the Angolan population as a whole. As previously detailed,[27] however, participants were recruited from all higher education institutions, which represented university staff in the whole country. When this study was designed in 2009, all university staff were invited to take part. The study group included all occupational and socio-economic classes, including teachers and non-teaching workers.[26,27]

Conclusion

There was a high prevalence of the metabolic syndrome in this occupational cohort, with a higher prevalence among women. This study suggested that optimal cut-off values of WC of 87.5 cm and 80.5 cm would be appropriate for the diagnosis of the metabolic syndrome in men and women, respectively. This may imply that the prevalence would have been different from that reported in this study if these values had been used. Further investigation is therefore needed to confirm optimal cut-off values of WC in the general Angolan population, in order to consistently estimate the trends of cardiometabolic risk factors in African populations.

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Diabetes News

New culprit identified in metabolic syndrome

A new study suggests uric acid may play a role in causing the metabolic syndrome, a cluster of risk factors that increases the risk of heart disease and type 2 diabetes.

Uric acid is a normal waste product that is removed from the body by the kidneys and intestines and is released in the urine and stools. Elevated levels of uric acid are known to cause gout, an accumulation of the acid in the joints. High levels are also associated with markers of the metabolic syndrome, which is characterised by obesity, high blood pressure, and elevated blood sugar and cholesterol levels. But it has been unclear whether uric acid itself is causing the damage or it is simply a by-product of other processes that lead to the dysfunctional metabolism.

New research from the Washington University suggests that excess uric acid in the blood is no innocent bystander. Rather, it appears to be a culprit in disrupting normal metabolism. The research team states that uric acid may play a direct, causative role in the development of the metabolic syndrome. The work showed that the gut is an important clearance mechanism for uric acid, opening the door to new potential therapies for preventing or treating type 2 diabetes and the metabolic syndrome.

Recent research by the senior author, Kelle H Moley, the James P Crane professor of obstetrics and gynecology, and her collaborators has shown that a protein called GLUT9 is an important transporter of uric acid. The team studied mice to learn what happens when GLUT9 stops working in the gut, essentially blocking the body's ability to remove uric acid from the intestine. In this study, the kidney's ability to remove uric acid remained normal.

Eating regularly, mice missing GLUT9 only in the gut quickly developed elevated uric acid in the blood and urine compared with control mice. And at only six to eight weeks of age, they developed the hallmarks of the metabolic syndrome: high blood pressure, elevated cholesterol and blood insulin levels, and fatty liver deposits, among other symptoms.

The researchers also found that the drug allopurinol, which reduces uric acid production in the body and has long been used to treat gout, improved some but not all of the measures of metabolic health. Treatment with the drug lowered blood pressure and total cholesterol levels.

Exposure to uric acid is impossible to avoid because it is a normal byproduct of cell turnover in the body. But there is evidence that diet may contribute to uric acid levels. Many foods contain compounds called purines that break down into uric acid. Adding to growing concerns about fructose in the diet, evidence suggests that fructose metabolism in the liver also drives uric acid production.

Switching to foods heavy-laden with fructose over the past 30 years has been devastating, according to Moley. 'There's a growing feeling that uric acid is a cause, not a consequence, of the metabolic syndrome. The medical community now knows that fructose directly makes uric acid in the liver. With that in mind, the laboratory is doing further research to study what happens to these mice on a high-fructose diet.'

Source

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Low-density lipoprotein cholesterol does not predict cardiac risk in diabetes

Low-density lipoprotein cholesterol (LDL-C) level wasn't a good predictor of cardiovascular disease in type 1 diabetes, but the total cholesterol-to-high-density lipoprotein cholesterol (HDL-C) ratio appeared more reliable, an observational study has shown. Dr Christel Hero, of Sahlgrenska University Hospital in Gothenburg, Sweden, and colleagues reported that LDL-C had modest associations with the development of cardiovascular disease but no consistent dose response above the 100-mg/dl threshold for statin treatment in this population (American Diabetes Association 2014; Abstract 301-OR).

In type 1 diabetes patients already on statins, LDL-C levels didn't have any significant link to subsequent cardiovascular disease in the Swedish National Diabetes Registry data. The total cholesterol-to-HDL-C ratio had similarly modest links to cardiovascular disease in patients on or off lipid medications, but with a consistent rise in risk across categories. Hero added, 'The ratio of total cholesterol to HDL-C is a more reliable marker for risk when considering primary prevention.'

Dr Fernando Ovalle, director of the Comprehensive Diabetes Centre of the University of Alabama in Birmingham, commented, 'The findings emphasised how much remains unknown about cardiovascular disease in type 1 diabetes. We made a lot of assumptions and jumped to a lot of conclusions that the markers of cardiovascular disease and treatments for prevention of cardiovascular diseases will be the same in type 1 diabetes as in type 2, and that just may not be the case. This could potentially change how we see the use of statins and the assessment of cardiovascular risk in general.'

Dr Elizabeth Seaquist, president for medicine and science and a moderator at the session, cautioned, 'Don't toss out LDL-C in clinical practice just yet.' Dr Seaquist continued by saying that LDL-C may not be as strong a predictor for cardiovascular disease as in type 2 diabetes, as has been suspected from prior studies, but further research is needed to determine what to use in the clinic. 'These patients are still at great risk for cardiovascular events, and we need to make certain that we're doing the right things to prevent that', she said. 'It will help us if we were to do a trial to determine the benefits of lipid-lowering in type 1 patients.'

This study was published as an abstract and presented at a conference. These data and conclusions should be considered preliminary until published in a peer-reviewed journal.

Source: *http://www.diabetesincontrol.com/articles/53-/16480-ada-ldl-doesnt-predict-heart-risk-in-diabetes*

Cardiac death risk in diabetic haemodialysis patients increased due to thyroid problems

A prospective study found that diabetic haemodialysis patients' subclinical hyperthyroidism and euthyroid sick syndrome might increase the risk of sudden cardiac-related deaths. Dr Christiane Drechsler, of University Hospital Würzburg in Würzburg, Germany, and colleagues conducted a study that included 1 000 patients undergoing haemodialysis for diabetes. Of those patients, 78.1% had euthyroidism, 13.7% had subclinical hyperthyroidism, 1.6% had subclinical hypothyroidism and 5.4% had euthyroid sick syndrome.

Patients with euthyroidism were compared with those who had subclinical hyperthyroidism and euthyroid sick syndrome, with regard to which group showed an increased short-term (within a 12-month period) risk of sudden cardiac death. It showed that patients who had euthyroidism had a 2.0-times increased short-term risk of sudden cardiac death, and those who had subclinical hyperthyroidism and euthyroid sick syndrome had a 2.7-fold increase.

The results showed that euthyroid sick syndrome was associated with a three-fold increased risk of short-term mortality, but in the long term (two to four years) it showed no increased risk. The study revealed that subclinical hypothyroidism was not associated with cardiovascular events or all-cause mortality, which revealed thyroid disorders had no influence on the risks of myocardial infarction and stroke.

This study led researchers to conclude, 'Regularly assessing a patient's thyroid status may help estimate the cardiac risk of dialysis patients.'

Source: *http://www.renalandurologynews.com/thyroid-problems-up-cardiac-death-risk-in-diabetic-hd-patients/article/348571/*

Painful neuropathy and increasing blood pressure

Painful diabetic neuropathy (PDPN) has been associated with higher nocturnal blood pressure in patients, according to a study by D'Amato *et al*. published in the 10 July 2014 issue of *Diabetes Care*. PDPN can cause obstructive sleep apnoea (OSA) and affect quality of life. As this condition is often underdiagnosed, researchers conducted a study focusing on the increasing cardiovascular risk associated with neuropathic pain.

The study included a total of 113 diabetes patients with PDPN (n = 34), patients with painless diabetic polyneuropathy (n = 33), and those without diabetic polyneuropathy (n = 46). Neuropathic pain, risk of obstructive sleep apnoea, autonomic function and blood pressure were all assessed using the Douleru Neuropathique en 4 Questions (DN4).

Nocturnal systolic blood pressure was significantly higher in patients with PDPN (130.4 ± 15.6 mmHg) than in those without diabetic polyneuropathy (119.9 ± 10.6 mmHg; $p <$ 0.0001) and in those with painless polyneuropathy (124.2 ± 12.3 mmHg; $p < 0.05$). The PDPN group also experienced less change in systolic and diastolic blood pressure overnight when compared to those without diabetic polyneuropathy ($p < 0.05$). The 'non-dipping' decrease in blood pressure overnight was seen in eight patients, which was highly correlated to PDPN status ($p = 0.007$).

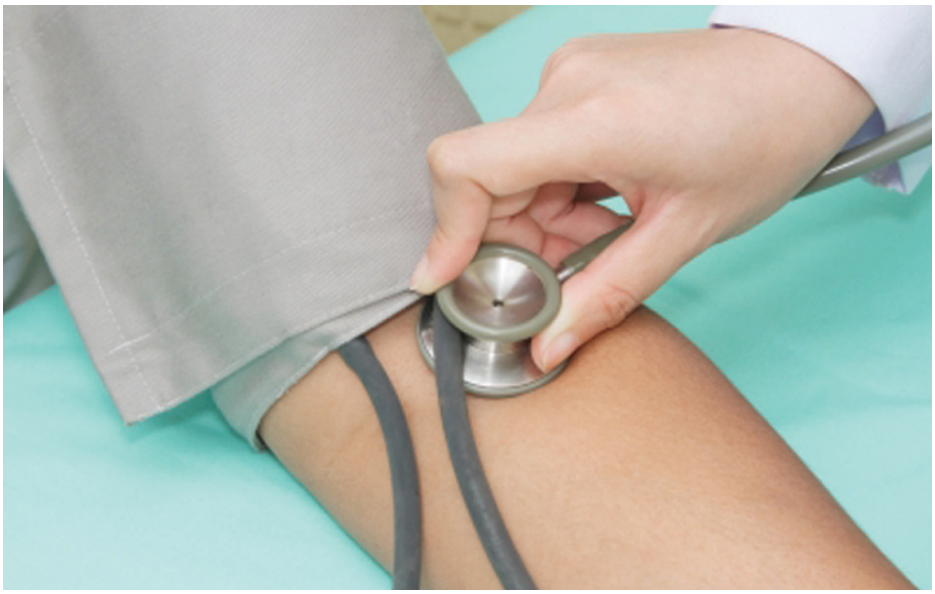

Image courtesy of Praisaeng at FreeDigitalPhotos.net

The researchers concluded that PDPN is associated with higher nocturnal blood pressure that is independent of pain-related sleep problems and other diabetes-related co-morbidities. The theory is that neuropathic pain acts as a stressor, which induces sympathetic response during the night and inhibits the blood pressure from falling. This highlights the importance of managing the patient's cardiovascular risk more closely while attempting to treat the neuropathic pain at the same time.

Source: *http://www.diabetesincontrol.com/articles/diabetes-news/16624-painful-neuropathy-and-increasing-blood-pressure*

Diabetic neuropathy improved with vegan diet

A randomised, controlled trial has indicated that a vegan diet may be beneficial in relieving diabetic nerve pain.

Diabetic peripheral neuropathy, which occurs in about half of all patients with type 2 diabetes, is underdiagnosed, partly because physicians aren't able to offer anything to treat the underlying cause of this condition. Current therapies treat only the pain.

The vegan diet is a plant-based diet, and studies show that it can help ease the pain caused by diabetic neuropathy. In an earlier observational study conducted by Crane and Sample, 21 type 2 diabetes patients with nerve pain were put on a low-fat, high-fibre vegan diet for one month, and 81% of the participants achieved complete pain relief and lost around 5 kg on average. Additionally, the diet enabled most of these patients to reduce their diabetes and blood pressure medications.

Anne Bunner, PhD, and Caroline Trapp, MSN, of the Physicians Committee for Responsible Medicine, sought to see whether these same benefits could be seen in a randomised, controlled trial. They conducted the Dietary Intervention for chronic diabetic Neuropathy pain (DINE) study, in which 15 patients with type 2 diabetes and diabetic neuropathy were randomised to either a low-fat, high-fibre vegan diet and vitamin B_{12} supplementation or vitamin B_{12} supplementation alone.

The patients had a mean age of 57 years, half of them were female, and half had a college education or higher. Bunner noted that there tended to be a deficiency in vitamin B_{12} in diabetic patients, especially those taking metformin. The participants who were put on the diet had to attend 20 weekly nutrition classes involving nutrition education, social support, cooking demonstrations and food product sampling, eat plant-based foods that had a low glycaemic index, get at least 40 g of fibre per day, and limit their consumption of fatty foods, such as oils and nuts, to 20–30 g per day. Since high-fibre foods are low in calories, there were no portion limits.

Five out of the seven patients who were put on the vegan diet were fully adherent. According to Bunner and Trapp, with good adherence, the participants who were put on the diet along with vitamin B_{12} reported greater improvements in McGill Pain Questionnaire pain scores ($p = 0.04$) and significantly greater reductions in body mass index ($p = 0.01$) when compared with the control group.

The results of the study indicated that there were also improvements in cholesterol and HbA_{1c} levels, neuropathy symptom scores (NTSS-6), and quality-of-life scores in which the changes differed significantly from the baseline. These improvements were not significantly greater in the diet group when compared to the control group.

There was a greater decrease in cholesterol and HbA_{1c} levels in the diet group, but many of the patients in the diet group discontinued their lipid and diabetes medications, while those in the control group were put on more lipid and diabetes medications, so the graphs were artificially lowered. Participants on the vegan diet had significant improvement in NTSS-6 and similar changes in quality-of-life scores not matched by the control group, but at the end of the trial, the differences among both groups were not significant, which Bunner believes may possibly have been due to the small number of patients or maybe even the effect of participating in the study in the control group.

The researchers plan to follow their study participants for one year to examine the long-term effects observed in these patients. They believe the study has shown that this particular dietary intervention can provide promising potential for treating diabetic nerve pain.

Source: *http://www.diabetesincontrol.com/index.php?option=com_content&view=article&id=16788&catid=1&Itemid=17*

Type 2 diabetes management more difficult with early onset

Younger age groups may be at higher risk of developing complications of type 2 diabetes, possibly due to statin medications only being added to their treatment regimen much later on, a study from Asia has found.

Type 2 diabetes can occur at a wide range of ages. Recent data have shown that the success rate of managing the disease may vary depending on the age of diagnosis.

According to an ongoing prospective study from the Joint Asia Diabetes Evaluation (JADE), patients diagnosed with type 2 diabetes at a younger age had poorer management of their disease than patients who were diagnosed at a later age. (People who developed diabetes before the age of 40 years are considered 'young onset' while those who developed diabetes aged 41 years and older are defined as 'late onset' in the study.)

The study also found that people diagnosed with diabetes before age 40 were more likely to develop complications. Compared to patients developing diabetes after the age of 40, these patients had a higher HbA_{1c} level and the chances of their achieving HbA_{1c} levels less than 7% were very unlikely. They also had high LDL cholesterol levels and were at higher risk of developing diabetic retinopathy.

Researchers believe the reason behind those with younger-onset diabetes developing more complications is associated with statin therapy. Younger-onset patients were less likely to receive statins or renin angiotensin system inhibitors for organ protection.

A more aggressive approach is suggested for patients who develop diabetes before the age of 40 years. Younger patients have to live with the disease for longer. Statin therapy should therefore be initiated in all diabetic patients, regardless of the age of diabetes onset, to prevent associated long-term complications. Findings from the JADE study suggested the results were consistent across all the countries participating in the study.

Source: *Yeung RO, Zhang Y, Luk A, et al. Metabolic profiles and treatment gaps in young onset type 2 diabetes in Asia: a cross sectional study of a prospective cohort. Lancet Diabetes Endocrinol 2014; July 28. http://www.diabetesincontrol.com/index.php?option=com_content&view=article&id=16786&catid=1&Itemid=17*

The potential of low-carbohydrate diets to reduce cardiovascular risk

Consuming low-carbohydrate diets can reduce inflammation in patients with type 2 diabetes, which may decrease the risk of patients developing cardiovascular disease (CVD). According to a Swedish study [*Diabetologia* 2012; **55**(8): 2118–2127], eating a low-carbohydrate diet can reduce inflammation in patients with type 2 diabetes.

People with type 2 diabetes have a higher level of inflammation than those without diabetes, and this may play a role in the increased risk of CVD associated with diabetes. The Linkoping University study included 61 participants with type 2 diabetes. The participants were randomly divided up and given either a low-carbohydrate or low-fat diet. The study method was a retrospective follow-up study.

The low-carbohydrate and the low-fat diet participants were compared over the course of two years. Additionally, the researchers studied how the diets impacted on inflammation by checking the inflammation levels in the blood of each patient.

The results showed that both the low-carbohydrate and low-fat diets helped participants lose weight, roughly around nine pounds (4 kg), but when it came to which diet produced reduced inflammatory markers in the blood, the low-carbohydrate diet succeeded. Additionally, glucose levels dropped more in the low-carbohydrate diet groups.

In respect of cardiovascular risk, the researchers recommended aiming for a carbohydrate energy intake of 20% as a treatment alternative for at-risk patients.

Source: http://www.diabetesincontrol.com/articles/diabetes-news/16329-the-potential-of-low-carbohydrate-diets-to-reduce-cvd-risk.

Diabetes researchers track cells' ability to regenerate

Vanderbilt University scientists have found evidence that the insulin-secreting beta-cells of the pancreas, which are either killed or become dysfunctional in the two main forms of diabetes, have the capacity to regenerate. The surprising finding, posted online by *Cell Metabolism* earlier this year, suggests that by understanding how regeneration occurs, scientists may one day be able to stop or reverse the rising tide of diabetes. 'The study provides clues to how we might learn what signals promote beta-cell regeneration in type 1 and type 2 diabetes', said Dr Alvin Powers, the senior author and director of the Vanderbilt Diabetes Center.

In the past three months, the Powers group at Vanderbilt, in four separate articles, has reported important findings about the 'microenvironment' of the insulin-secreting beta-cells and glucagon-secreting alpha-cells, which are among four types of cells clustered in islets in the pancreas. Both hormones are important in regulating blood glucose levels and ensuring that glucose is delivered to the muscles and brain to be used as fuel, and stored in the liver. Powers called the islets a 'mini-organ' because they are highly vascularised and innervated, and exist within a specialised environment.

In type 1 diabetes, the beta-cells are destroyed and glucose levels rise in the blood because not enough insulin is being produced. In type 2 diabetes, a frequent consequence of obesity, tissues become resistant to insulin, again causing blood glucose to rise. Beta-cell function also becomes abnormal.

In two articles in the journal *Diabetes* and one each in *Development and Cell Metabolism*, the researchers described four main findings about islet vascularisation and innervation. First, vascular endothelial growth factor A (VEGF-A) is important for development of the islets' blood supply and for beta-cell proliferation. Blocking the growth factor early in development in a mouse model ultimately reduced beta-cell mass and insulin release and impaired glucose clearance from the bloodstream.

Second, VEGF and other 'signals' released by the endothelial cells lining the islet blood vessels consequently stimulated growth of islet nerves in mice that connected to the brain. 'If the islets don't become vascularised properly, they don't become innervated properly', Dr Marcela Brissova, who was co-author on three of the four articles, said. 'These signals also promote beta-cell growth.'

Third, VEGF-A was not involved when the beta-cell mass increased in an obese mouse model of type 2 diabetes in response to rising glucose levels. Unlike tumours, which sprout new blood vessels as they grow, the beta-cell tissue increased its blood supply by dilating existing vessels.

Finally, too much VEGF-A can lead to beta-cell death. But that sets up a regenerative micro-environment involving an interaction of vascular endothelial cells and macrophages, which, in turn, leads to beta-cell proliferation both in mice and human islets. 'That's very unusual because islet cells are like neurons; once they're dead, they don't usually regrow', Brissova said. 'We think that the endothelial cells and macrophages that are recruited from bone marrow create an environment that promotes the proliferation and regeneration of those beta-cells.'

Source: *http://medicalxpress.com/news/2014-03-diabetes-track-cells-ability-regenerate.html*

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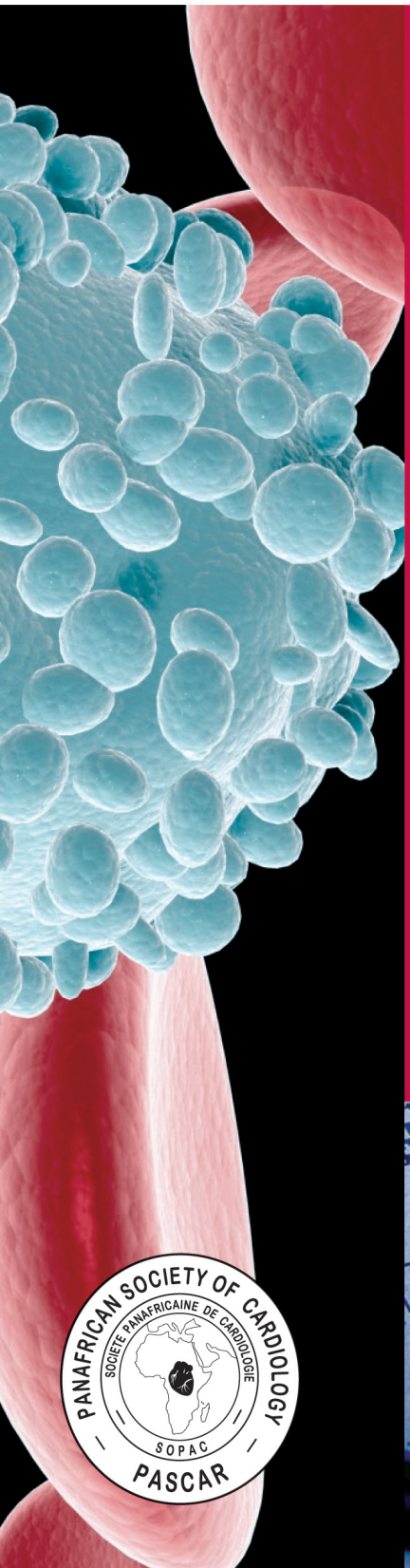

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