PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Lower Levels of High-density Lipoprotein Cholesterol in Urban Africans Presenting with Communicable Versus Non-communicable Forms of Heart Disease: The 'Heart of Soweto' hospital registry study
AUTHORS	Lyons, Jasmine; Sliwa, Karen; Carrington, Melinda; Raal, Frederick; Pretorius, Sandra; Thienemann, Freidrich; Stewart, Simon

VERSION 1 - REVIEW

REVIEWER	Anne E. Sumner National Institutes of Health
REVIEW RETURNED	08-Apr-2014

GENERAL COMMENTS	Lyons et al. in a cross-sectional study compare the prevalence of dyslipidemia, particularly low HDL-C in Africans from Soweto with non-communicable heart disease vs communicable heart disease. The authors conclude that low HDL-C is more common in Africans with communicable heart disease and this could potentially represent a long term cardiovascular. If their findings are confirmed, then pharmacological intervention should be considered. The leading reason for the low HDL-C in the women with communicable heart disease group is presumably inflammation as demonstrated by high hsCRP in the subset of Africans in whom hsCRP levels were measured. The challenges with this manuscript start with the abstract. Abstracts are critically important because that is how the scientific community is first exposed to the content of publication. The questions that arose in reading the abstracts as well as key omissions from the abstract include the following-some of which were answered in the actual text of the manuscript and some of which were not: 1) What were the levels of HDL-C that defined "low HDL" 2) What were the frequency of low HDL-C in the 2 groups after adjusting for sex, age and BMI? 3) What were the total CHOL levels? Are these low HDL-C levels in Africans a function of low total cholesterol and not pathological at all? 4) Were ratios evaluated including: chol/hdl, ldl/hdl and TG/HDL 5) The frequency of low HDL-C in the communicable disease group was 67% vs 58% in the noncommunicable disease group. This difference is statistically significant but is this <10% difference clinically significant when the overall percentage is greater than 50% in each group. 6) The abstract needs to provide in the Background/Objective section a sentence about biologically plausibility for why HDL-C might be lower in the communicable disease group-to justify the

	undertaking of the study. 7) The abstract makes no mention of the hsCRP analyses in the abstract-which is key strength of the manuscript and their argument. 8) Similarly the authors make no mention of the difference in frequency of HIV infection in the abstract in the 2 groups. Overall it is interesting that the authors report that African women had higher chol, higher LDL and no difference in TG than African men. This is opposite to findings in whites as well as in African Americans. In both whites and African-Americans, men have higher chol, TG and LDL levels than women. Why are there such major differences in the lipid profile by sex in South African blacks compared to whites or South Africans? The reviewer agrees with the authors' statement in the opening paragraph of the discussion (p.13) specifically: atherogenic LDL is low in this setting and that low HDL may not be indicative of increased risk at least in the short term. This is a cross-sectional comparison with all the weaknesses inherent in such a design. The authors do not fully acknowledge this as a weakness. But their argument that low HDL Is likely to be harmful would be much enhanced, novel and important if they looked at prospective data. Did the Africans in either the communicable or non-communicable disease with low HDL-C fare worse in 3 to 5 years than their counterparts with either communicable or communicable heart disease who did not have low HDL?

REVIEWER	Yvonne Commodore-Mensah Johns Hopkins University School of Nursing USA
REVIEW RETURNED	14-Apr-2014

GENERAL COMMENTS	Overall, this paper was very well written and has profound implications on atherosclerotic cardiovascular disease risk reduction in Sub-Saharan Africa. Title: I would suggest that the authors specify that lipid levels are compared for patients with communicable versus non-communicable disease. It appears that the whole paper is based on this comparison so the title should reflect this. Abstract: There was no mention of CRP in the abstract so it was confusing to read about it later on in the paper. Please introduce it in the abstract. Table 1: Please included percentage of communicable vs. non-communicable heart disease . Line 39: Under the heading of Lipid profiles, the authors stated that "There were significant reductions in TC, LDLC and HDLC in those with communicable forms of HD." This statement gives the false impression that those with communicable diseases were treated and the treatment led to significant reductions. I think what the authors meant to say was the those with communicable HD had significantly lower TC, LDLC, and HDLC. Figures and Legends: I would suggest that each legend is placed underneath the figure to avoid confusion. It was hard to figure out which legend corresponded with each figure because all the legends were provided separately from the figures.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Anne E. Sumner, National Institutes of Health

What were the levels of HDL-C that defined “low HDL”
Abstract amended to include:

‘Main outcome measures: … Low HDLC was defined as <1.0 mmol/L for males and <1.2mmol/L for females, according to applicable South African Clinical Guidelines.’

What were the frequencies of low HDL-C in the 2 groups after adjusting for sex, age and BMI?
We have provided fully adjusted analysis (age, sex, BMI) for each dyslipidaemia class (High TC, high LDLC and low HDLC) in communicable compared to non-communicable HD in Table 2.

What were the total CHOL levels?
Mean values of total cholesterol are detailed in Table 1.

Are these low HDL-C levels in Africans a function of low total cholesterol and not pathological at all?
This is a critical argument in the paper. Certainly low HDLC can merely reflect low TC. However we’ve focused on low HDLC specifically as it is often overlooked in sub-Saharan African literature, presumably because it has not been a traditionally prevalent CVD risk factor. We do not know that isolated low HDLC is pathological in African populations without appropriate prospective evidence.

We have added this to the introduction:

‘… However, it remains uncertain whether low HDLC is causal or just a cardiovascular risk marker[1]. If we are to extrapolate from studies in Western and Asian populations[2, 3, 4], isolated low HDLC is associated with increased risk for CVD in the long-term… Although it has been shown that those of African descent largely show a favourable lipid profile characterised by high HDLC levels [5], it is unlikely that they can remain athero-protective during an infected state[6].’

From the discussion:

‘… While traditionally uncommon[7], dyslipidaemia, in particular low HDLC, is becoming more prevalent in sub-Saharan Africa[8]. The low lipid levels present in the majority of cases with communicable HD reflects the dramatic changes to lipid metabolism observed in infection and is therefore, anticipated. We acknowledge that atherogenic LDLC is also low in this setting and that low HDLC may not be indicative of particularly increased disease risk, at least in the short-term. However we still deem this as highly clinically relevant given that low HDLC is associated with a higher risk of atherosclerotic forms of HD, even at very low LDLC levels[4], a finding that has been replicated in diverse populations[2].’

Were ratios evaluated including: chol/hdl, ldl/hdl and TG/HDL
Thank you for this important point, and we agree that lipid ratios can be very good discriminators of CVD risk in many populations. We had reported TC:HDL values in Table 1 and now have added TG:HDL and LDL:HDL ratios to Results.

The frequency of low HDL-C in the communicable disease group was 67% vs 58% in the noncommunicable disease group. This difference is statistically significant but is this <10% difference clinically significant when the overall percentage is greater than 50% in each group.

Thank you for this comment. We have added to the discussion:

'… We have shown that despite largely favourable lipid profiles, there are clear differences according to underlying aetiology of HD in urban Africans however, overall low HDLC was the most prevalent metabolic abnormality observed in this cohort. Younger Africans with communicable HD have particularly low levels of HDLC that, if maintained in the longer term, may leave them at increased risk of atherosclerotic disease. This is physiologically plausible in chronic infection; however low HDL at hospital admission could also simply reflect similarly low levels of TC/LDL and may not be indicative of increased long-term CVD risk. That uncertainty can only be resolved by well-powered studies with adequate follow-up, to provide sufficient evidence to address current gaps in evidence and, ultimately, guide clinical practice. Nevertheless if proven, targeted prevention programs that identify and actively manage individuals with a history of communicable HD (particularly an active case) and with low levels of HDLC may be indicated.'

The abstract needs to provide in the Background/Objective section a sentence about biologically plausibility for why HDL-C might be lower in the communicable disease group-to justify the undertaking of the study.
Abstract amended to:

Objectives: To investigate if urban Africans displayed lower levels of atheroprotective high-density lipoprotein cholesterol (HDLC) when presenting with communicable versus non-communicable forms of heart disease (HD) as both acute infection and chronic inflammation can reduce HDL-C levels.

The abstract makes no mention of the hsCRP analyses in the abstract-which is key strength of the manuscript and their argument.
Thank you for this suggestion. We had amended to include:

Participants: … Serum inflammatory marker C-reactive protein (CRP) was measured in a sub-set of 367 patients (31% of all cases).

Results: … We also observed a strong relationship between low HDLC and higher risk levels of CRP, but only in females.

Conclusions: … The female-only inverse association between HDL-C and CRP warrants further investigation.

Similarly the authors make no mention of the difference in frequency of HIV infection in the abstract in the 2 groups.
We have not included the data on patients with confirmed HIV in the abstract as the prevalence was low- even in those with communicable HD. However we have made this comparison more distinct in the Results text by adding to the 'Clinical Profile' section:

'… Overall, 76 (6%) were confirmed HIV-positive:s 15 (2%) and 61 (12%) patients were confirmed HIV-positive in non-communicable and communicable HD groups respectively ($P<0.001$).

Overall it is interesting that the authors report that African women had higher chol, higher LDL and no difference in TG than African men. This is opposite to findings in whites as well as in African Americans. In both whites and African-Americans, men have higher chol, TG and LDL levels than women. Why are there such major differences in the lipid profile by sex in South African blacks compared to whites or South Africans?
This is a very interesting comment. Endogenous oestrogens have a favorable effect on lipid metabolism, which usually corresponds to higher levels of HDLC, but its impact on total cholesterol is

not clear. However one very large survey reported higher TC in women both globally and in sub-Saharan Africa. We quote from Farzadfar et al.

'… In 2008, age-standardised mean total cholesterol worldwide was 4·64 mmol/L (95% uncertainty interval 4·51—4·76) for men and 4·76 mmol/L (4·62—4·91) for women. …. It was lowest in sub-Saharan Africa at 4·08 mmol/L (3·82—4·34) for men and 4·27 mmol/L (3·99—4·56) for women.'

The authors suggest that:
'… trends in dietary fats, adiposity, and, in high-income countries, statin use are the likely drivers of the polarised worldwide trends.'

http://www.thelancet.com/journals/lancet/article/PIIS0140-6736%2810%2962038-7/fulltext

We have added to the discussion:
'… Additionally, we speculate that the higher lipid levels in women may be the result of much higher rates of obesity (50% compared with 26% in men) as the driver of elevated total cholesterol, which has been suggested by authors of a worldwide systematic analysis on high TC [9].'

We do not have data on dietary saturated fats, but can confirm that none of the patients were on lipid-lowering medication at the time of the survey.

The reviewer agrees with the authors' statement in the opening paragraph of the discussion (p.13) specifically: atherogenic LDL is low in this setting and that low HDL may not be indicative of increased risk at least in the short term.
Thank you for this comment. We have added to the discussion:

'… We have shown that despite largely favourable lipid profiles, there are clear differences according to underlying aetiology of HD in urban Africans however, overall low HDLC was the most prevalent metabolic abnormality observed in this cohort. … This is physiologically plausible in chronic infection; however low HDL at hospital admission could also simply reflect similarly low levels of TC/LDL and may not be indicative of increased long-term CVD risk. That uncertainty can only be resolved by well-powered studies with adequate follow-up, to provide sufficient evidence to address current gaps in evidence and, ultimately, guide clinical practice. Nevertheless if proven, targeted prevention programs that identify and actively manage individuals with a history of communicable HD (particularly an active case) and with low levels of HDLC may be indicated. …'

This is a cross-sectional comparison with all the weaknesses inherent in such a design. The authors do not fully acknowledge this as a weakness.
We have included some terms to 'Study limitations' in the Discussion to state:

'… Finally, owing to the cross-sectional design of this study we were not able to investigate the possible effect of the magnitude and timing of the contributing infection on lipid levels, beyond the data collected at admission. Given the transient, dynamic processes of lipid metabolism over the course of acute and chronic diseases, only longitudinal studies of lipid levels and subsequent outcomes can fully elucidate the clinical importance of our findings.'

But their argument that low HDL Is likely to be harmful would be much enhanced, novel and important if they looked at prospective data. Did the Africans in either the communicable or non-communicable disease with low HDL-C fare worse in 3 to 5 years than their counterparts with either communicable or communicable heart disease who did not have low HDL?

Regrettably we are not conducting any prospective follow-up on this cohort but await results from

further longitudinal studies in Africa. Please also refer to the two responses above.

Reviewer 2: Yvonne Commodore-Mensah, Johns Hopkins University School of Nursing

Title I would suggest that the authors specify that lipid levels are compared for patients with communicable versus non-communicable disease. It appears that the whole paper is based on this comparison so the title should reflect this.
Thank you for this suggestion. We have changed the title to:

'Lower Levels of High-density Lipoprotein Cholesterol in Urban Africans Presenting with Communicable Versus Non-communicable Forms of Heart Disease: The 'Heart of Soweto' hospital registry study'

Abstract There was no mention of CRP in the abstract so it was confusing to read about it later on in the paper. Please introduce it in the abstract. Thank you for this suggestion. We had amended to include:

Participants: … Serum inflammatory marker C-reactive protein (CRP) was measured in a sub-set of 367 patients (31% of all cases).

Results: … We also observed a strong relationship between low HDLC and higher risk levels of CRP, but only in females.

Conclusions: … The female-only inverse association between HDL-C and CRP warrants further investigation.

Table 1 Please included percentage of communicable vs. non-communicable heart disease
Amended as per suggestion

Line 39 Under the heading of Lipid profiles, the authors stated that "There were significant reductions in TC, LDLC and HDLC in those with communicable forms of HD." This statement gives the false impression that those with communicable diseases were treated and the treatment led to significant reductions. I think what the authors meant to say was that those with communicable HD had significantly lower TC, LDLC, and HDLC. Thank you for this comment. Amended as per suggestion

Figures and Legends
I would suggest that each legend is placed underneath the figure to avoid confusion. It was hard to figure out which legend corresponded with each figure because all the legends were provided separately from the figures.
We assume this is a result of the Scholar One manuscript formatting for reviewers as we adhered to all BMJ article requirements upon submission.

VERSION 2 – REVIEW

REVIEWER	Anne E Sumner National Institutes of Health
REVIEW RETURNED	05-Jun-2014

GENERAL COMMENTS	The manuscript is much improved. I have 2 recommendations. 1) it is my preference that all references to "males" and "females" be changed to "men" and "women". 2) I suggest 2 references be added, both are by our group as they will demonstrate that beyond South Africa isolated low HDL-cholesterol has also been seen in African-Americans and Africans from Ghana and Nigeria. The references are: 1) Sumner, AE, COMMENTARY: “Half the Dyslipidemia of Insulin Resistance is the Dyslipidemia of Insulin-Resistant Blacks, Ethn Dis 2009;19:462-465 2) Sumner, AE, Zhou, J, Doumatey A, Imoisili, OE, Amoah A, Acheampong J, Oli J, Johnson T, Adebamowo C, Rotimi CN, Elevated triglyceride concentration is uncommon in West Africans and African Americans with the Metabolic Syndrome, CVD Prevention 2010;5:75-80

REVIEWER	Yvonne Commodore-Mensah Johns Hopkins University USA
REVIEW RETURNED	16-Jun-2014

GENERAL COMMENTS	The authors have addressed the reviewers' comments appropriately.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1: Anne E. Sumner, National Institutes of Health
Reviewer comment: Author response

The manuscript is much improved.
Thank you for your positive feedback.

It is my preference that all references to "males" and "females" be changed to "men" and "women".
Changed as per suggestion throughout the manuscript.

2) I suggest 2 references be added, both are by our group as they will demonstrate that beyond South Africa isolated low HDL-cholesterol has also been seen in African-Americans and Africans from Ghana and Nigeria.
The references are:
1) Sumner, AE, COMMENTARY: “Half the Dyslipidemia of Insulin Resistance is the Dyslipidemia of Insulin-Resistant Blacks, Ethn Dis 2009;19:462-465
2) Sumner, AE, Zhou, J, Doumatey A, Imoisili, OE, Amoah A, Acheampong J, Oli J, Johnson T, Adebamowo C, Rotimi CN, Elevated triglyceride concentration is uncommon in West Africans and African Americans with the Metabolic Syndrome, CVD Prevention 2010;5:75-80
Thank you for suggesting these important publications. We have added the second citation by

Sumner, Zhou et al. as it specifically focuses on low HDL-C as a common lipid pattern in West African populations.

Reviewer 2: Yvonne Commodore-Mensah, Johns Hopkins University School of Nursing

Reviewer comment Author response
The authors have addressed the reviewers' comments appropriately. Thank you for your positive feedback.