The Impact of Fasting on the Interpretation of Triglyceride Levels for Predicting Myocardial Infarction Risk in HIV-Positive Individuals: The D:A:D Study

The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study[a]

We assessed whether fasting modifies the prognostic value of these measurements for the risk of myocardial infarction (MI). Analyses used mixed effect models and Poisson regression. After confounders were controlled for, fasting triglyceride levels were, on average, 0.122 mmol/L lower than nonfasting levels. Each 2-fold increase in the latest triglyceride level was associated with a 38% increase in MI risk (relative rate, 1.38; 95% confidence interval, 1.26–1.51); fasting status did not modify this association. Our results suggest that it may not be necessary to restrict analyses to fasting measurements when considering MI risk.

Although combination antiretroviral therapy is effective for the treatment of human immunodeficiency virus (HIV)-positive individuals, side effects, including elevated triglyceride levels, are common [1]. Although some studies have demonstrated that raised triglyceride levels may be an independent risk factor for cardiovascular disease (CVD) in the general population [2, 3], a recent large study found that triglyceride levels were not independently associated with the risk of CVD after controlling for other lipid measurements [4].

Although several studies have investigated whether the fasting status of the patient at the time of triglyceride levels are measured has an impact on their interpretation [5–10], this issue has not been adequately addressed in the HIV-positive population. The aims of this study were to describe the effect of fasting status on triglyceride levels in a large study of HIV-positive individuals, and to assess whether fasting status modifies the prognostic value of triglycerides for the risk of myocardial infarction (MI).

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[a] Members of the writing committee are listed in Appendix.
Correspondence: David A. Kamara, MSc Medical Statistics, Research Department of Infection and Population Health, Division of Population Health, UCL Medical School, Royal Free Campus, Rowland Hill St, London NW3 2PF, United Kingdom (d.kamara@ucl.ac.uk).

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METHODS

Study Population

The Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study is a prospective, observational study formed by the collaboration of 11 existing cohorts of HIV-positive persons. The data set used for the present analysis includes data on 33,308 individuals seen at 212 clinics in Europe, Australia, and the United States. Informed consents were obtained from all patients included in the study. The D:A:D study methodology has been described in detail elsewhere [11].

Data Collection

Patients are followed up prospectively during visits to outpatient clinics scheduled as part of regular medical care, with information collected on family history of coronary heart disease, prior history of CVD and diabetes mellitus, cigarette smoking, blood pressure, lipid-lowering and antihypertensive therapy, clinical signs of lipodystrophy, and serum lipid levels (total cholesterol, high-density lipoprotein cholesterol, triglyceride), as well as HIV-related information (antiretroviral therapy, CD4 cell counts, HIV viral loads, and dates of diagnoses of all AIDS-defining diseases). All study end points (including incident cases of MI) are reported to the coordinating office for validation and coding, as described in earlier reports [11]. The present analyses were performed on the data set merged in July 2008.

Statistical Methods

Impact of Fasting Status on Triglyceride Levels. Mixed effect models (SAS PROC MIXED), with an unstructured variance-covariance matrix, were used to describe the independent association of fasting status (fasting, nonfasting, or unknown) on triglyceride levels after controlling for potential confounders. Factors considered were sex, age, ethnicity, body mass index, smoking status, family history of CVD, previous CVD, diabetes mellitus, hypertension (systolic blood pressure, $\geq$150 mm Hg; diastolic blood pressure, $\geq$100 mm Hg; receipt of angiotensin-converting enzyme inhibitors or antihypertensive drugs), use of lipid-lowering drugs, hepatitis C virus serostatus, and calendar year. Of note, our aim was not to assess correlations with other lipid measurements, and thus these analyses did not incorporate the individual's total or high-density lipoprotein

cholesterol measurement. The analyses were repeated after log transformation of the triglyceride level; because the conclusions were unchanged, only the untransformed results are shown.

Triglyceride Levels and Fasting Status in Predicting MI. Using methods reported elsewhere [11], patients were followed up from the time of study entry until the first MI during prospective follow-up, date of death, 6 months after the last clinic visit, or 1 February 2008. Each patient's follow-up was split into a series of consecutive 1-month periods, and his or her covariate data were updated at the start of each month. Initial exploratory analyses stratified follow-up (and any events that occurred) into quintiles defined by both the latest triglyceride level and the fasting status of the measurement, and event rates were calculated for each stratum. Poisson regression models (SAS PROC GENMOD) were used to describe the association between the latest triglyceride level and risk of MI, with levels incorporated as a $\log_2$-transformed time-updated covariate; thus, relative hazards for triglyceride levels relate to a 2-fold increment (ie, a doubling) in the latest triglyceride level. We investigated whether the association between the triglyceride level and MI risk was modified by fasting status by incorporating interaction terms into the model. The analyses were then repeated after adjusting for the potential confounders listed previously. Of note, there was no evidence of overdispersion in the Poisson models.

RESULTS

Characteristics of Patients and Distribution of Triglyceride Levels at Study Entry

At study entry, triglyceride measurements were available for 27,646 (83.0%) of the 33,308 patients (7072 fasting, 4403 nonfasting, 16,171 in an unknown fasting state). Most participants were of white ethnicity (56.1%), male (74.5%), did not have AIDS (74.0%), were antiretroviral-experienced (77.8%) and were not receiving lipid-lowering therapy (95.3%) at study entry. The median triglyceride level at study entry was 1.60 mmol/L (interquartile range [IQR], 1.04–2.62); levels were higher when triglycerides were measured in a nonfasting state (1.66 mmol/L; IQR, 1.07–2.70) than in a fasting state (1.47 mmol/L; IQR, 1.00–2.33) state, and levels measured in an unknown state were similar (1.66 mmol/L; IQR, 1.06–2.80) to those measured in a nonfasting state.

Associations Between Triglyceride Levels and Demographic, CVD, and HIV Infection Characteristics

Patients were followed up for a median of 7.1 years (IQR, 5.3–7.7 years; total follow-up, 180,176 person-years). Including the baseline measurement, a total of 405,756 triglyceride measurements were available from 30,669 individuals (median per patient, 12; IQR, 7–17). Differences between triglyceride measurements by fasting status were similar to those at study entry (Table 1).

In exploratory analyses, nonfasting levels were generally higher than fasting levels regardless of stratifying factor (Table 1). Median triglyceride levels were higher in men, increased with age, lower in those of black ethnicity, higher in those with higher body mass index, and higher in those with a previous CVD event, diabetes mellitus, hypertension, and in those receiving lipid-lowering drugs. In addition, median triglyceride levels were also higher in those who acquired HIV infection through sex between men, and in current and former smokers. Triglyceride levels, however, were lower in those coinfected with hepatitis C virus.

In mixed models adjusted for the factors listed above, fasting triglyceride levels were, on average, 0.122 mmol/L lower than nonfasting triglyceride levels (95% confidence interval [CI], .094–.149 mmol/L; $P < .001$), with triglyceride levels measured in an unknown fasting state being, on average, 0.041 mmol/L lower than those measured in a nonfasting state (95% CI, .008–.073 mmol/L; $P = .02$).

Impact of Fasting Status on Prognostic Value of Triglyceride Levels for MI Risk

Over a total of 178,836 person-years, 580 patients experienced an MI (rate, 3.2/1000 person-years; 95% CI 3.0–3.5). The risk of MI increased in those with higher triglyceride levels, regardless of fasting status. The risk of MI at a given triglyceride level was higher when measured in a nonfasting or unknown state than when measured after fasting (Figure 1).

In univariable analyses, each doubling in the latest triglyceride level was associated with a 67% increase in the risk of MI (relative rate [RR], 1.67; 95% CI, 1.54–1.80; $P < .001$). When fasting and nonfasting levels were considered separately, the increased risks were 74% and 70%, respectively (RR for fasting values, 1.74; 95% CI, 1.50–2.03 [$P < .001$]; RR for nonfasting values, 1.70; 95% CI, 1.40–2.06 [$P < .001$]). A formal test of interaction suggested that the difference between these values was nonsignificant ($P = .83$). After adjustment for potential confounders, the RR associated with triglyceride levels was reduced, both overall (1.38; 95% CI, 1.26–1.51; $P < .001$) and when fasting (1.37; 95% CI, 1.15–1.63; $P < .001$) and nonfasting (1.46; 95% CI, 1.17–1.82; $P < .001$) levels were considered separately, with a similar lack of evidence for an interaction between fasting status and triglyceride levels ($P = .88$).

DISCUSSION

On average, triglyceride levels are lower when measured in a fasting rather than a nonfasting state, even after controlling for factors thought to have an impact on triglyceride levels. Although the association between triglyceride levels and MI risk was slightly lower when triglyceride levels were measured in a fasting state (37% increased risk of MI per doubling) than in a nonfasting state (46% increased risk), there was no evidence of

Table 1. Fasting and Nonfasting Triglyceride Levels, Stratified by Demographic, Cardiovascular, and Human Immunodeficiency Virus (HIV) Infection Characteristics

Characteristic	Fasting status		Nonfasting status		Unknown status	
	Patients, no.	Triglyceride level, median (IQR), mmol/L	Patients, no.	Triglyceride level, median (IQR), mmol/L	Patients, no.	Triglyceride level, median (IQR), mmol/L
All patients	108,785	1.61 (1.08–2.57)	52,683	1.76 (1.13–2.83)	244,288	1.74 (1.10–2.80)
Sex						
Male	80,060	1.78 (1.17–2.82)	37,291	1.94 (1.25–3.13)	186,576	1.90 (1.20–3.07)
Female	28,725	1.28 (0.89–1.91)	15,392	1.40 (0.94–2.12)	57,712	1.26 (0.85–2.00)
Age, years						
<30	6325	1.19 (0.82–1.80)	3447	1.24 (0.84–1.90)	16,775	1.20 (0.80–1.84)
30–40	41,671	1.50 (1.00–2.36)	17,222	1.56 (1.02–2.50)	81,571	1.60 (1.00–2.60)
40–50	39,781	1.69 (1.12–2.68)	20,162	1.85 (1.20–3.00)	89,291	1.80 (1.20–3.00)
>50	21,008	1.92 (1.29–3.00)	11,852	2.11 (1.40–3.30)	56,651	2.00 (1.32–3.23)
Ethnicity						
White	97,554	1.63 (1.09–2.58)	43,416	1.85 (1.20–2.97)	50,844	1.83 (1.20–2.94)
Black	3855	1.20 (0.80–1.85)	4774	1.12 (0.80–1.76)	25,629	1.19 (0.81–1.82)
Other	2849	1.68 (1.11–2.60)	2488	1.60 (1.07–2.45)	4064	1.89 (1.23–2.83)
Not known	4527	1.73 (1.10–2.84)	2005	1.69 (1.10–2.80)	163,751	1.80 (1.18–3.00)
BMI, kg/m^2						
<18	3042	1.35 (0.91–2.03)	2130	1.56 (1.09–2.30)	7648	1.45 (1.00–2.35)
18–26	76,386	1.57 (1.04–2.48)	38,120	1.70 (1.10–2.73)	151,449	1.73 (1.09–2.80)
26–30	14,997	1.89 (1.23–2.94)	7529	2.09 (1.30–3.38)	30,435	2.00 (1.20–3.20)
>30	4461	1.92 (1.24–3.02)	2530	2.23 (1.36–3.58)	11,032	1.80 (1.10–2.97)
Not known	10,899	1.64 (1.10–2.60)	2374	1.52 (1.00–2.42)	43,724	1.60 (1.05–2.60)
Previous CVD						
No	106,374	1.60 (1.07–2.55)	51,151	1.74 (1.12–2.80)	237,057	1.71 (1.08–2.80)
Yes	2411	2.27 (1.50–3.69)	1532	2.33 (1.50–3.70)	7231	2.20 (1.45–3.49)
Diabetes						
No	102,959	1.60 (1.06–2.50)	50,645	1.73 (1.12–2.80)	231,084	1.68 (1.07–2.79)
Yes	5826	2.29 (1.48–3.73)	2038	2.47 (1.40–4.20)	13,204	2.54 (1.57–4.25)
Hypertension						
No	90,567	1.55 (1.04–2.44)	40,118	1.67 (1.10–2.64)	199,049	1.61 (1.04–2.65)
Yes	18,218	2.02 (1.34–3.50)	12,565	2.13 (1.34–3.50)	45,239	2.11 (1.31–3.50)
Use of LLDs						
No	52,987	1.45 (0.99–2.20)	2097	1.67 (1.10–2.60)	80,393	1.37 (0.93–2.11)
Yes	20,466	2.70 (1.74–4.24)	10,437	2.80 (1.77–4.43)	55,934	2.68 (1.70–4.40)
Not known	35,332	1.50 (1.02–2.25)	40,149	1.60 (1.06–2.47)	107,961	1.60 (1.05–2.60)
Mode of HIV infection						
Sex between men and men	39,693	1.90 (1.22–3.00)	20,910	1.99 (1.28–3.21)	124,497	2.00 (1.20–3.20)
Sex between men and women	28,040	1.46 (1.00–2.19)	10,459	1.61 (1.10–2.50)	27,127	1.57 (1.05–2.40)
IDU	34,738	1.50 (0.99–2.37)	19,170	1.60 (1.01–2.60)	71,106	1.40 (0.93–2.34)
Not known	6314	1.71 (1.13–2.71)	2144	1.89 (1.20–3.10)	21,558	1.65 (1.09–2.60)
Smoking status						
Current	48,686	1.60 (1.08–2.50)	25,020	1.74 (1.16–2.74)	51,346	1.75 (1.12–2.81)
Ex-smoker	24,981	1.65 (1.08–2.64)	10,704	1.90 (1.20–3.10)	70,297	1.90 (1.20–3.10)
Never	26,253	1.60 (1.05–2.60)	15,047	1.71 (1.07–2.90)	65,903	1.60 (1.00–2.62)
Not known	8865	1.70 (1.12–2.74)	1912	1.50 (1.00–2.34)	56,742	1.65 (1.07–2.62)
Presence of hepatitis C virus						
No	60,477	1.70 (1.10–2.73)	38,022	1.80 (1.14–2.99)	166,878	1.76 (1.09–2.81)
Yes	29,785	1.47 (1.01–2.20)	13,233	1.63 (1.11–2.49)	35,347	1.56 (1.03–2.40)
Not known	18,523	1.70 (1.10–2.70)	1428	1.66 (1.10–2.79)	42,063	1.84 (1.20–3.10)

NOTE. BMI, body mass index; CVD, cardiovascular disease; IDU, injection drug user; IQR, interquartile range; LLDs, lipid-lowering drugs.

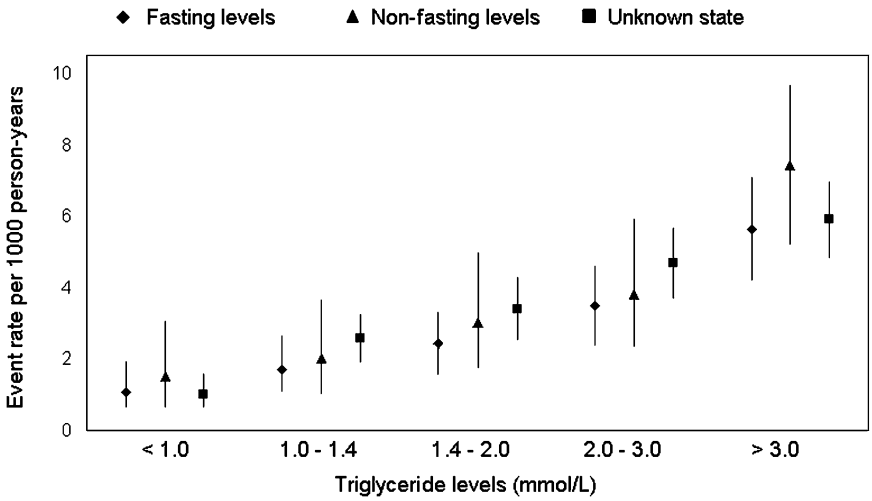

Figure 1. Incidence of myocardial infarction per 1000 person-years (with 95% confidence intervals) in individuals with different triglyceride levels, stratified according to whether triglyceride levels were measured in a fasting, nonfasting, or unknown state.

a significant interaction between fasting status and triglyceride levels. Thus, it is possible that this difference is simply a chance finding. Our results suggest that, at least within a cohort setting such as this, in which the timing of triglyceride measurement may vary from individual to individual, it may not be necessary to restrict analyses to triglyceride measurements that are only taken in a fasting (or nonfasting) state when considering the risk of MI. However, in absolute terms the triglyceride levels were low regardless of fasting status. Furthermore, our findings may not be generalizable to settings where triglyceride measurements are taken at more regular times, such as just after a meal, where fasting status may have a greater impact on overall results.

Elevated triglyceride levels have a substantial effect on lipoprotein metabolism, which explains much of the controversy about the role of serum triglyceride as a risk factor for CHD [12]. Postprandial lipids may play a more important role in the pathogenesis of CVD—in particular early atherosclerosis—than fasting lipids, because postprandial lipoproteins, including the triglyceride-rich ones, can penetrate the endothelial cell layer, where they can contribute to the formation of foam cells [13]. It has been suggested that HIV-positive patients have delayed triglyceride clearance [14], which may prolong the postprandial phase, leading to an accumulation of atherogenic particles [15].

Several studies have considered whether the fasting status of the patient when triglyceride levels are measured has an impact on the interpretation of the measurements. Some literature reviews of triglyceride as a vascular risk factor concluded that the evidence supports a potential role for both fasting and nonfasting triglycerides [5], although others [6–8] have concluded that nonfasting triglyceride may replace fasting triglyceride in assessing CVD risk factors.

Recent publications recommend nonfasting triglyceride measurements over fasting triglyceride measurements for predicting CVD risk [9, 10]. These studies may suffer from bias, however, if fasting samples are selectively collected in specific patient groups whose CVD risk differs from that of patients whose triglyceride measurements are taken in a nonfasting state. In exploratory analyses of cohorts in the D:A:D study, we found that the tendency to measure triglyceride levels in a fasting state varied from cohort to cohort, as did the factors that were associated with measurement of triglyceride in a fasting status.

Our data set has some additional limitations, notably the fact that the study does not specify a particular method or assay for measurement of triglyceride, and no standardized meal requirements are specified. Some of the triglyceride levels reported as fasting may have been misclassified; interestingly, triglyceride levels measured in an unknown fasting state were slightly lower than those measured in a nonfasting state, suggesting that some of these measurements may actually have been taken after fasting, possibly explaining why levels did not differ greatly from those known to be measured in a nonfasting state. Despite having an impact on the absolute triglyceride level, however, the clinical impact of fasting status on predictions of the risk of MI was minimal.

In conclusion, although fasting triglyceride levels were lower than nonfasting levels, nonfasting triglyceride levels gave a similar indication of the risk of MI as fasting levels among HIV-positive individuals in the D:A:D study. Our results suggest that it is not necessary to take account of fasting status when considering the risk of MI in an individual, emphasizing the importance of considering all triglyceride measurements (whether fasting or nonfasting) in analyses of this risk.

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References

1. Morse CG, Kovacs JA. Metabolic and skeletal complications of HIV infection: the price of success. JAMA **2006**; 296:844–54.
2. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA **2007**; 298:299–308.
3. Sarwar N, Danesh J, Eiriksdottir G, et al. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 western prospective studies. Circulation **2007**; 115:450–8.
4. Di AE, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA **2009**; 302:1993–2000.
5. Ridker PM. Fasting versus nonfasting triglycerides and the prediction of cardiovascular risk: do we need to revisit the oral triglyceride tolerance test? Clin Chem **2008**; 54:11–3.
6. Stalenhoef AF, de GJ. Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. Curr Opin Lipidol **2008**; 19:355–61.
7. Warnick GR, Nakajima K. Fasting versus nonfasting triglycerides: implications for laboratory measurements. Clin Chem **2008**; 54:14–6.
8. Eberly LE, Stamler J, Neaton JD. Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. Arch Intern Med **2003**; 163:1077–83.
9. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation **2008**; 118:993–1001.
10. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. JAMA **2007**; 298:309–16.
11. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med **2003**; 349:1993–2003.
12. Toth PP, Maki KC. 2008 Practical lipid management: concepts and controversies. Oxford: Wiley-Blackwell.
13. Wilhelm MG, Cooper AD. Induction of atherosclerosis by human chylomicron remnants: a hypothesis. J Atheroscler Thromb **2003**; 10:132–9.
14. Stein JH, Merwood MA, Bellehumeur JB, McBride PE, Wiebe DA, Sosman JM. Postprandial lipoprotein changes in patients taking antiretroviral therapy for HIV infection. Arterioscler Thromb Vasc Biol **2005**; 25:399–405.
15. Ginsberg HN. New perspectives on atherogenesis: role of abnormal triglyceride-rich lipoprotein metabolism. Circulation **2002**; 106:2137–42.

Appendix

Writing Committee Members

David A. Kamara, MSc, Research Department of Infection and Population Health, UCL, London, United Kingdom;
Signe W. Worm, PhD, Copenhagen HIV Programme, University of Copenhagen, Denmark;
Peter Reiss, PhD, HIV Monitoring Foundation, Academic Medical Center, Amsterdam, the Netherlands;
Martin Rickenbach, MD, II University Hospital, Lausanne, Switzerland;
Andrew Phillips, PhD, Research Department of Infection and Population Health, UCL, London, United Kingdom;
Ole Kirk, DMSc, Copenhagen HIV Programme, University of Copenhagen, Denmark;
Antonella D'Arminio Monforte, DMSc, Hospital San Paolo, University of Milan, Italy;
Mathias Bruyand, PhD, ISPED, Université Victor Segalen, Bordeaux, France;
Matthew Law, PhD, National Centre in HIV Epidemiology and Clinical Research, Sydney, Australia;
Stephane De Wit, PhD, CHU Saint-Pierre Hospital, Department of Infectious Diseases, Bruxelles, Belgium;
Colette J. Smith, PhD, Research Department of Infection and Population Health, UCL, London, United Kingdom;
Christian Pradier, PhD, CHU Nice Hopital de l'Archet, France;
Jens Lundgren, DMSc, Copenhagen HIV Programme, University of Copenhagen, Denmark;
Caroline Sabin, PhD, Research Department of Infection and Population Health, UCL, London, United Kingdom