Published in final edited form as:
Am J Cardiol. 2014 January 15; 113(2): 279–284. doi:10.1016/j.amjcard.2013.08.057.

Usefulness of Serum Unbound Free Fatty Acid Levels to Predict Death Early in Patients with ST Segment Elevation Myocardial Infarction[From the TIMI II Trial]

Andrew H. Huber, PhD[a], **J. Patrick Kampf, PhD**[b], **Thomas Kwan, PhD**[b], **Baolong Zhu, PhD**[c], **Jesse Adams III, MD**[d], and **Alan M. Kleinfeld, PhD**[a,e]

[a]FFA Sciences LLC, San Diego, CA

[b]Astute Medical Inc. San Diego CA

[c]LS9 Inc. South San Francisco, CA

[d]University of Louisville and Jewish Hospital Heart and Lung Institute, Louisville, KY

[e]Torrey Pines Institute, San Diego, CA.

Abstract

Circulating total free fatty acids (FFA) are elevated early in myocardial infarction (MI) and are associated with an increase in mortality. We investigated the association of serum unbound free fatty acids (FFA_u) levels with mortality,in patients presenting with ST elevation myocardial infarction (STEMI) in the Thrombolysis in Myocardial Infarction (TIMI) II trial.TIMI II enrolled patients within 4 hours of chest pain. Patients were treated with recombinant tissue plasminogen activator within 1 hour of enrollment. The concentration of FFA_u was evaluated in serum samplesfrom 1834 patients obtained at baseline, before therapy.FFA_u was an independent risk factor for death as early as one day of hospitalization and continued to be an independent risk factor for the more than 3·8 years of follow up. When adjusted for other cardiovascular risk factors FFA_u levels in the fourth as compared to the first quartile remained an independent risk factor for death due to MI (hazard ratio, 5.0; 95 % confidence interval, 1.9-13.0), to all cardiac death (hazard ratio, 2.4; confidence interval, 1.3-4.4) and to all cause death (hazard ratio, 1.9, confidence interval, 1.2-3.1).Females were twice as likely to be in the upper two FFA_u quartiles and had approximately twice the rate of death as males. In conclusion, increased levels of FFA_u are one of the earliest molecular biomarkers of mortality in STEMI and are independent of other risk factors known to affect outcomes in STEMI.

Keywords

unbound free fatty acids; mortality; myocardial infarction; risk factors

Corresponding author: Alan M. Kleinfeld, PhD, Torrey Pines Institute for Molecular Studies 3550 General Atomics Court, San Diego, CA 92121, TEL: 858-455-3724, FAX: 858-455-3792, akleinfeld@tpims.org.

CONFLICTS OF INTEREST

AMK is the founder of and major stock holder in FFA Sciences LLC. AHH, JPK, TK and BZ either are (AHH) or were employees, are inventors on patents and applications assigned to FFA Sciences LLC and have profit interests in FFA Sciences LLC. JA is an advisor to FFA Sciences LLC.

Plasma free fatty acid (FFA) levels are elevated early after acute myocardial infarction (MI) and correlate with increased rates of arrhythmias and mortality, particularly within the first 12 hours.[1-4] The MI-associated FFA increase occurs primarily through catecholamine activation of adipose tissue lipolysis rather than FFA release from the ischemic cardiac tissue.[4] Although most plasma FFA is bound to albumin, a small fraction ($< 10^{-4}$) is unbound FFA (FFA_u). FFA_u levels increase exponentially with increasing ratio of total FFA to albumin and thus FFA_u are more sensitive to physiologic changes than total FFA.[5;6]FFA_ulevels increase rapidly, within 30 minutes of cardiac ischemia induced by balloon angioplasty.[7;8]We investigated whether FFA_ulevels from patients in the Thrombolysis in Myocardial Infarction (TIMI)Phase II trial of STEMI provide an independent assessment of risk for poor outcomes at timesas early as 24 hours after symptoms.

METHODS

The TIMI II trial treated 3262 patients,who presented within 4 hours of STEMIonset,with intravenous recombinant tissue plasminogen activator plus heparin.[9]After recombinant tissue plasminogen activatortherapy patients were randomly assigned to either a percutaneous coronary interventionor a conservative strategyin which only patients exhibiting ischemia (13%) received percutaneous coronary intervention. TIMI investigators recorded demographics, medical history and outcomes over a follow up period of 3.8 years. A Limited Access DataSetof patient parameters was available for this study.

Measurements were performed on serum samples (baseline) drawn prior to recombinant tissue plasminogen activator and heparin therapy.[10]Serum wascollected in STEMI patients by the TIMI investigators [11] and maintained at −70°C by the National Heart Lung Blood Institute blood specimen repository. A subset of these specimens from 2500 patients was provided by the National Heart Lung Blood Institute. The linkage between patient information and blood specimen tube identification was maintained separately by the Maryland Medical Research Institute. After our results were deposited with the National Heart Lung Blood Institute, the linkage was un-blinded. Complete patient information from the Limited Access Data Setwas available for 1834 patients of the 2500 baseline blood specimens and results reported here are for the 1834 patients. This study complies with the Declaration of Helsinki and was approved by the Institutional Review Board committee of the Torrey Pines Institute for Molecular Studies.

Measurements of serum FFA_u concentrations were performed using the fluorescent probe ADIFAB2 (FFA Sciences) as described previously in cardiac ischemia and MI patients[8;12] but modified for 96-well plate fluorometry using a Flurolog 3 spectrofluorometer with a MicroMax plate reader (J.Y Horiba). Serumsamples were diluted to 1% (v/v) in 200 L of measuring buffer[13] in 96-well plates. Fluorescence was measured after addingADIFAB2 (1.5 mol/L) and the intensities were used to determine the ratio (R) of the fluorescence intensitiesat 550 to 457 nm,with background subtracted. Sample FFA_u concentrations (nmol/L) were calculated using $FFA_u=227(R-R_o)/(0.925 – R)$, where R_o is the ADIFAB2 fluorescence ratio in the absence of FFA and the numeric factors were determined as described previously [6]. All samples were measured in duplicate, yielding an average CV of 6.5 %.

FFA_u quartiles were determined using all baseline FFA_u values (Table 1). Correlations between FFA_u and patient baseline characteristics were carried out usingallcardiovascular confounders available in the limited data set. In TIMI II, patients with renal disorders were excluded, Killip class was not recorded and creatine kinase was the only cardiac biomarker

measured. Outcomes of death due to STEMI, to cardiac causes and to all causes were determined through committee adjudication by the TIMI investigators. Statistical analyses were performed using XLSTAT (Addinsoft, New York), p values ≤ 0.05 were considered significant.

RESULTS

Baseline FFA_u levels were measured in serum samples collected within 4 hours of initial symptoms. FFA_u concentrations ranged from < 1 nmol/L to > 500 nmol/L (Table 1). This range is larger than we reported previously for a cohort of nominally healthy subjects whose range was 0.6 to 4.5 nmol/L with a mean value of 1.5 nmol/L.[12]Correlations of FFA_u quartiles with all cardiovascular risk factors available from the limited access data set and for which patientswith the indicated risk factors comprised at least 10% of all patients are shown in Table 1.

Of the 1834 patients, 187 died from all causes, 125 from cardiac causes and 76 from MI (Table 2). A positive correlation of death from MI with FFA_u quartile was present as early as one day following enrollment and peaked at about 30 days post enrollment. Because most deaths within 30 days were due to MI, cardiac deaths and deaths from all causes also correlated strongly with baseline FFA_u. Peak (within 8 hours, Table 2), but not baseline (Table 1) creatine kinase levels,were correlated with FFA_u.

Relative to the total TIMI II population FFA_u levels correlated positively with female gender, age, and diabetes. Approximately twice as many females and diabetics were in the upper two FFA_u quartiles (Table 1). Deaths due to MI in non-diabetic females and males as well as in diabetics increased with increasing FFA_u quartile (Table 3). This correlation reached significance in non-diabetic females and males, but not obtained in diabetics. The lack of correlation in diabetics is likely due to small numbers and to two deaths within 5 hours in the first quartile, but no additional deaths in the first quartile over the following 3.8 years. The death rate for females and diabetics was almost 2-fold > for non-diabetic males,except for day 1, mostly due to larger death rates at 30 days in the 3rd and 4th FFA_u quartiles. Using subgroup interaction analysis death rates in Q3+Q4 were significantly (p=0.05) higher in non-diabetic females thanmales but not (p=0.07) inall diabetics versus non-diabetics[14].

Kaplan-Meier survival curves for deaths due to STEMI, to all cardiac causes and to all causes reveal striking dependencies on FFA_u quartiles (Figure 1). Log-rank tests indicate that the risk of death for all three categories increased significantly ($p < 0.001$) with increasing FFA_u quartile. For MI almost all deaths occurred within the first 30 days. For deaths from all causes the survival curve reveals a similar slope after 60 daysfor all 4 quartiles, consistent with a lack of correlation with FFA_u for non-cardiac mortality (Table 2). Cox proportional hazard modeling of the three sets of survival curves reveals hazard ratios that increase significantly with FFA_u quartile relative to the first quartile and are relatively unaffected by other risk factors (Table 4). The unadjusted hazard ratios for MI deaths increased from 2.3 for Q2 to 5.6 for Q4 and the corresponding adjusted hazard ratios were 2.9 and 5, respectively. Except for the Q1 to Q2 increase, the hazard ratios increases with FFA_u quartile were significant ($p \leq 0.03$) for all unadjusted and adjusted analyses.

DISCUSSION

Oliver and Opie recognized more than 40 years ago that serum levels of FFA increase rapidly following MI and that the elevated FFA levels may, by inducing arrhythmias, contribute to sudden cardiac death and death in MI.[1-4]Moreover, these studies found that

early death in MI was highly correlated with increasing total FFA levels [1]. Total FFA levels were also correlated with the long term (6.9 years) increased risk of sudden cardiac death inthe LURIC study.[15]Our results are consistent with and additive to LURIC.[15]Both the present and LURIC studies found strong baseline correlations between FFA_u or total FFA levels and female gender or a history of diabetes (Table 1).Hazard ratios for MI deaths in TIMI II were larger (≥3 fold) and less sensitive to other risk factors than in the LURIC study. Presumably, this increase in sensitivity is a reflection of the acute presentation of STEMI in TIMI II and the high degree of FFA_u sensitivity to cardiac ischemia [7;8].

The lower survival of females and diabetics as compared to non-diabetic men (Table 3) is consistent with other studies in STEMI patients.[16;17]In TIMI II females had higher FFA_u levels than men; median FFA_u for non-diabetic men and females were 3·7 nmol/L and 4.5 nmol/L, respectively.The higher FFA_u levels for femalesin TIMI II might reflect a gender specific difference in the response to STEMI or might be a related to the older (7 years) age of females in TIMI II, given that FFA_u increase with age (Table 1). However, the adjusted (see Table 4 legend) hazard ratio for death due to MI for females in Q4 versus Q1 is 9·7 (95% CI: 0.971 to 100) with p = 0.053. These results, and the higher death rates in Q3+Q4 for females, raise the possibility that FFA_u may be a more potent independent risk factor for females than for males.

Although these results demonstrate that FFA_u correlate with early death after STEMI, causation is unproven. The increase in circulating FFA_u may simply reflect the ischemia-induced activation of adipose lipolysis in proportion to the degree of ischemia and is probably largely generated from adipose tissue lipolysis stimulated by an ischemia-mediated increase in catecholamine levels.[3;4]In contrast, evidence for FFA having a causal role are studies in nonischemic animals in which increasing circulating FFA adversely affect myocardial metabolism, stimulate insulin resistance, induce arrhythmias and increase cardiac enzyme release.[18-21]Consistent with a causal role for FFA_uin TIMI II are the increase in peak (8 hours) but not baselinecreatine kinase levels with increasing baseline FFA_u(Table 3).

If FFA adversely affectmyocardial function, reducing FFA_u levels at times early after the ischemic event may reduce deaths and/or arrhythmias in MI.[3;4]Infusions of glucose-insulin-potassium have been, shown to be protective in the dog [22], were used to treat STEMI patients andreduced circulating total FFA acutely in STEMI patients.[23]Although the effect on outcome in STEMI patients has been mixed, it has been suggested that GIK treatment would be most effective if given at the earliest possible time.[24;25] This concept was implemented in the IMMEDIATE trial by treating STEMI patients with glucose-insulin-potassium in pre-hospital emergency medical settings.[25;26]The IMMEDIATE trial revealed that glucose-insulin-potassium significantly reduced FFA levels, reduced cardiac arrest plus in-hospital mortality and reduce infarct size as compared to patients not treated with glucose-insulin-potassium. Conceivably, therapeutic efficacy of such interventions, using glucose-insulin-potassium or inhibitors of lipolysis, would be most evident in those MI patients who present with the highest levels of FFA_u; for which further studies are necessary.

Evidence-based guidelines currently recommend the rapid application of re-perfusion therapy with primacy given to percutaneous coronary artery intervention, with limited use of tissue plasminogen activator,the therapy in TIMI II. Nevertheless,the30 day death rate in TIMI II (4.2%) was not significantly differentthan the 5.4% in contemporary STEMI patients.[16]Adjustments for confounders that might affect hazard ratios for FFA_uwere limited because important prognostic factors[27],includingKillip class, baseline heart rate, number of diseased vessels, ejection fraction,smokingand troponin, were not available or not recorded

in the limited access data set. ACE inhibitors and statins were first approved for use in 1981 and 1987, respectively. TIMI II enrollment wasbetween 1986 and 1988 and therefore most patients were unlikely to have been treated with these medications. Catecholamines are associated, weakly, with mortality in STEMI [28] but were not measured. The beneficial effects of glucose-insulin-potassium suggest that FFA not catecholamines areimportant contributors to mortality in STEMI.TIMI II protocols did not anticipate determination of FFA levels and therefore blood samples may have had higher *ex vivo* than *in vivo* FFA as a consequence of lipoprotein lipolysis.[29]However, this *ex vivo* effect would have obscured rather than enhanced the observed FFA_u correlations with outcome. The determination of FFA_u concentrations requires knowledge of the relative distribution of the different FFA_u present in serum and was estimated from the distribution of total FFA as described in references.[6;13]Differences in the FFA_u distributions such as those that may occur in acute coronary syndromes[30] are not expected to alter the levels of total FFA_u significantly.[6]The study was borderline under powered for female patients, suggesting the need for further studies.

Acknowledgments

We thank the TIMI investigators, the NHLBI and the Maryland Medical Research Institute.

FUNDING

This work was supported by in part by the National Institutes of Healthgrants DK070314, DK058762 andin part by FFA Sciences LLC.

References

1. Oliver MF, Kurien VA, Greenwood TW. Relation between serum-free-fatty acids and arrhythmias and death after acute myocardial infarction. Lancet. 1968; 291:710–714. [PubMed: 4170959]
2. Gupta DK, Jewitt DE, Young R, Hartog M, Opie LH. Increased plasma-free-fatty-acid concentrations and their significance in patients with acute myocardial infarction. Lancet. 1969; 294:1209–1213. [PubMed: 4187809]
3. Oliver MF, Opie LH. Effects of glucose and fatty acids on myocardial ischaemia and arrhythmias. Lancet. 1994; 343:155–158. [PubMed: 7904009]
4. Oliver MF. Sudden cardiac death: the lost fatty acid hypothesis. QJM. 2006; 99:701–709. [PubMed: 16935924]
5. Richieri GV, Anel A, Kleinfeld AM. Interactions of long chain fatty acids and albumin: Determination of free fatty acid levels using the fluorescent probe ADIFAB. Biochemistry. 1993; 32:7574–7580. [PubMed: 8338853]
6. Richieri GV, Kleinfeld AM. Unbound free fatty acid levels in human serum. J Lipid Res. 1995; 36:229–240. [PubMed: 7751810]
7. Kleinfeld AM, Prothro D, Brown DL, Davis RC, Richieri GV, DeMaria A. Increases in serum unbound free fatty acid levels following coronary angioplasty. Am J Cardiol. 1996; 78:1350–1354. [PubMed: 8970405]
8. Cantor WJ, Hoe Kim H, Jolly S, Moe G, Burstein JM, Mendelsohn A, Kleinfeld AM, Fitchett D. B-Type Natriuretic Peptide and Serum Unbound Free Fatty Acid Levels after Contemporary Percutaneous Coronary Intervention. J Invasive Cardiol. 2008; 20:186–188. [PubMed: 18398236]
9. The TIMI Study Group. Comparison of invasive and conservative strategies after treatment with intravenous tissue plasminogen activator in acute myocardial infarction. Results of the thrombolysis in myocardial infarction (TIMI) phase II trial. New Engl J Med. 1989; 320:618–625. [PubMed: 2563896]
10. Passamani E, Hodges M, Herman M, Grose R, Chaitman B, Rogers W, Forman S, Terrin M, Knatterud G, Robertson T. The Thrombolysis in Myocardial Infarction (TIMI) phase II pilot study: tissue plasminogen activator followed by percutaneous transluminal coronary angioplasty. J Am Coll Cardiol. 1987; 10:51B–64B.

11. Bovill EG, Terrin ML, Stump DC, Berke AD, Frederick M, Collen D, Feit F, Gore JM, Hillis LD, Lambrew CT. Hemorrhagic events during therapy with recombinant tissue-type plasminogen activator, heparin, and aspirin for acute myocardial infarction. Results of the Thrombolysis in Myocardial Infarction (TIMI), Phase II Trial. Ann Intern Med. 1991; 115:256–265. [PubMed: 1906692]
12. Apple FS, Kleinfeld AM, Adams JE. Unbound Free Fatty Acid Concentrations Are Increased in Cardiac Ischemia. Clinical Proteomics. 2004; 1:41–44.
13. Huber AH, Kampf JP, Kwan T, Zhu B, Kleinfeld AM. Fatty acid-specific fluorescent probes and their use in resolving mixtures of different unbound free fatty acids in equilibrium with albumin. Biochemistry. 2006; 45:14263–14274. [PubMed: 17128966]
14. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ. 2003; 326:219. [PubMed: 12543843]
15. Pilz S, Scharnagl H, Tiran B, Wellnitz B, Seelhorst U, Boehm BO, Marz W. Elevated plasma free fatty acids predict sudden cardiac death: a 6.85-year follow-up of 3315 patients after coronary angiography. Eur Heart J. 2007; 28:2763–2769. [PubMed: 17766282]
16. Donahoe SM, Stewart GC, McCabe CH, Mohanavelu S, Murphy SA, Cannon CP, Antman EM. Diabetes and mortality following acute coronary syndromes. JAMA. 2007; 298:765–775. [PubMed: 17699010]
17. Jneid H, Fonarow GC, Cannon CP, Hernandez AF, Palacios IF, Maree AO, Wells Q, Bozkurt B, Labresh KA, Liang L, Hong Y, Newby LK, Fletcher G, Peterson E, Wexler L. Sex differences in medical care and early death after acute myocardial infarction. Circulation. 2008; 118:2803–2810. [PubMed: 19064680]
18. Kurien VA, Yates PA, Oliver MF. Free fatty acids, heparin, and arrhythmias during experimental myocardial infarction. Lancet. 1969; 294:185–187. [PubMed: 4183137]
19. de Leiris J, Opie LH, Lubbe WF. Effects of free fatty acid and glucose on enzyme release in experimental myocardial infarction. Nature. 1975; 253:746–747. [PubMed: 1113869]
20. Vik-Mo H, Mjos OD. Influence of free fatty acids on myocardial oxygen consumption and ischemic injury. Am J Cardiol. 1981; 48:361–365. [PubMed: 6115579]
21. Gruzdeva O, Uchasova E, Dyleva Y, Belik E, Kashtalap V, Barbarash O. Relationship between free fatty acids, insulin resistance markers, and oxidized lipoproteins in myocardial infarction and acute left ventricular failure. Diabetes Metab Syndr Obes. 2013; 6:103–111. [PubMed: 23550027]
22. Maroko PR, Libby P, Sobel BE, Bloor CM, Sybers HD, Shell WE, Covell JW, Braunwald E. Effect of glucose-insulin-potassium infusion on myocardial infarction following experimental coronary artery occlusion. Circulation. 1972; 45:1160–1175. [PubMed: 5032816]
23. Chaudhuri A, Janicke D, Wilson M, Ghanim H, Wilding GE, Aljada A, Dandona P. Effect of modified glucose-insulin-potassium on free fatty acids, matrix metalloproteinase, and myoglobin in ST-elevation myocardial infarction. Am J Cardiol. 2007; 100:1614–1618. [PubMed: 18036357]
24. Kloner RA, Nesto RW. Glucose-insulin-potassium for acute myocardial infarction: continuing controversy over cardioprotection. Circulation. 2008; 117:2523–2533. [PubMed: 18474824]
25. Selker HP, Beshansky JR, Sheehan PR, Massaro JM, Griffith JL, D'Agostino RB, Ruthazer R, Atkins JM, Sayah AJ, Levy MK, Richards ME, Aufderheide TP, Braude DA, Pirrallo RG, Doyle DD, Frascone RJ, Kosiak DJ, Leaming JM, Van Gelder CM, Walter GP, Wayne MA, Woolard RH, Opie LH, Rackley CE, Apstein CS, Udelson JE. Out-of-hospital administration of intravenous glucose-insulin-potassium in patients with suspected acute coronary syndromes: the IMMEDIATE randomized controlled trial. JAMA. 2012; 307:1925–1933. [PubMed: 22452807]
26. Grossman AN, Opie LH, Beshansky JR, Ingwall JS, Rackley CE, Selker HP. Glucose-insulin-potassium revived: current status in acute coronary syndromes and the energy-depleted heart. Circulation. 2013; 127:1040–1048. [PubMed: 23459576]
27. Morrow DA, Antman EM, Charlesworth A, Cairns R, Murphy SA, de Lemos JA, Giugliano RP, McCabe CH, Braunwald E. TIMI risk score for ST-elevation myocardial infarction: A convenient, bedside, clinical score for risk assessment at presentation: An intravenous nPA for treatment of infarcting myocardium early II trial substudy. Circulation. 2000; 102:2031–2037. [PubMed: 11044416]

28. Ostrowski SR, Pedersen SH, Jensen JS, Mogelvang R, Johansson PI. Acute myocardial infarction is associated with endothelial glycocalyx and cell damage and a parallel increase in circulating catecholamines. Crit Care. 2013; 17:R32. [PubMed: 23433357]

29. Zambon A, Hashimoto SI, Brunzell JD. Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. J Lipid Res. 1993; 34:1021–1028. [PubMed: 8354949]

30. Bhardwaj A, Truong QA, Peacock WF, Yeo KT, Storrow A, Thomas S, Curtis KM, Foote RS, Lee HK, Miller KF, Januzzi JL Jr. A multicenter comparison of established and emerging cardiac biomarkers for the diagnostic evaluation of chest pain in the emergency department. Am Heart J. 2011; 162:276–282. [PubMed: 21835288]

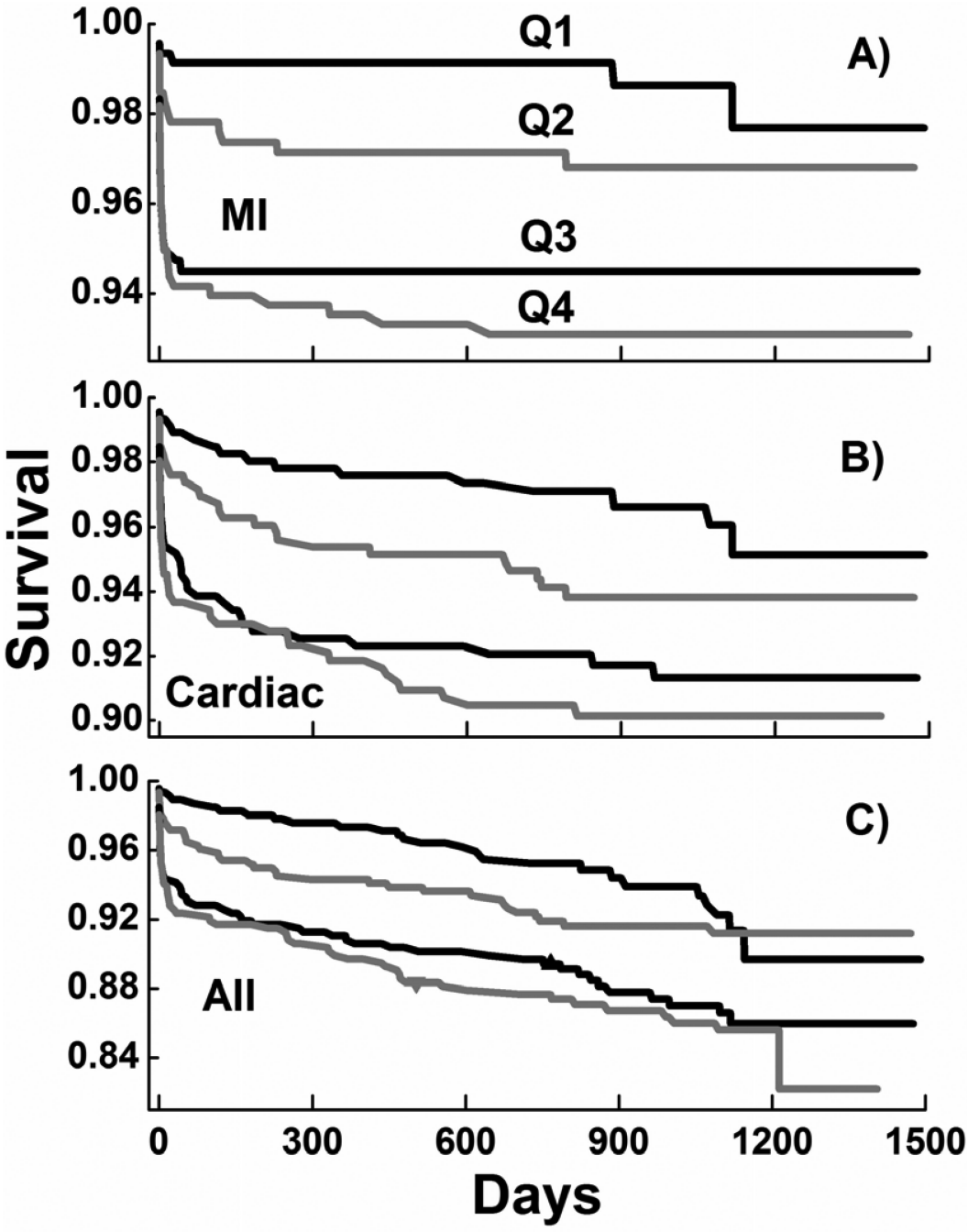

Figure 1. Kaplan-Meier survival curves for death by quartiles of baseline FFA_u

Survival curves for Q1 to Q4 for death due to A) MI, B) all cardiac causes and C) all causes. Log-rank probabilities were < 0·001.

Table 1

Baseline parameters for TIMI II patients as a function of unbound free fatty acid quartiles.

Number in quartiles	Quartile1 458	Quartile2 458	Quartile3 459	Quartile4 459	p Value
Unbound free fatty acids (nmol/L)	1.9 (0.09 – 2.6)	3.2 (2.6 – 3.9)	4.9 (3.9 – 6.4)	10.2 (6.46 – 523)	
Age (years)	53.9	53.8	55.3	55.4	0.008
Men	398(87%)	389(85%)	344(75%)	372(81%)	<0.0001
White	398(87%)	398(87%)	409(89%)	398(87%)	0.62
Body Mass Index (kg/m^2)	27 (15-40)	27 (18-46)	27 (16-54)	27 (17-54)	0.55
Diastolic blood pressure (mm Hg)	81 (42 -130)	81 (50 -110)	80.2 (40-120)	78.2 (40-118)	0.053
Systolic blood pressure (mm Hg)	130 (56 -180)	130 (60-183)	129 (80-210)	126 (50 -190)	0.044
Creatine Kinase (IU/L)	105 (5 -1760)	104 (12 -3590)	94 (11 -4422)	99 (11-3654)	0.080
Diabetes mellitus	41(9%)	37(8%)	73(16%)	78(17%)	<0.0001
Prior MI	60(13%)	64(14%)	69(15%)	73(16%)	0.50
Prior hypertension	174(38%)	156(34%)	188(41%)	179(39%)	0.20
β blocker within 24 hours	87(19%)	87(19%)	78(17%)	73(16%)	0.68

Values for ordinal parameters are percent and p values were calculated by χ^2. Continuous parameters are median values and intra-quartile ranges. p values were determined by the Kruskal-Wallis test. Ages were grouped to protect confidentiality and are proportional to the grouped mean for each quartile. Baseline creatine kinase levels were elevated above the upper limit of normal in fewer than 20 % of patients. Mean time from chest pain symptom to treatment initiation was virtually identical (2.6 h) for each FFA_u quartile (data not shown). Demographic and clinical parameters in this table are representative of the entire TIMI II population.[10]

Table 2

Death and outcomes according to baseline unbound free fatty acid quartiles

Median unbound free fatty acid (nmol/L)	Quartile1 1.9	Quartile2 3.2	Quartile3 4.9	Quartile4 10.2	Total	p Value	p Trend
Death due to:							
Myocardial Infarction at 1 day	3	7	11	12	33 (1.8%)	0.1	0.015
Myocardial Infarction at 7 days	3	7	19	21	50 (2.7%)	0.0002	<0.0001
Myocardial Infarction at 30 days	4	10	21	29	64 (3.5%)	<0.0001	<0.0001
Myocardial Infarction at 3.8 years	6	14	24	32	76 (4.1%)	<0.0001	<0.0001
All cardiac causes at 1 day	3	7	11	12	33 (1.8%)	0.1	0.015
All cardiac causes at 7 days	3	7	19	21	50 (2.7%)	0.0002	<0.0001
All cardiac causes at 30 days	5	11	21)	29	66 (3.6%)	<0.0001	<0.0001
All cardiac causes at 3.8 years	16	27	38	44	125 (6.9%)	0.0006	<0.0001
All causes at 1 day	3	8	13	13	37 (2.0%)	0.056	0.0095
All causes at 7 days	3	9	24	23	59 (3.2%)	<0.0001	<0.0001
Al causes at 30 days	5	13	26	34	78 (4.3%)	<0.0001	<0.0001
All causes at 3.8 years	29	38	57	63	187 (10%)	0.0004	<0.0001
Non cardiovascular causes at 3.8 years*	13	9	13	15	50 (2.7%)	0.67	0.530
Other outcomes							
Peak Creatine Kinase (8 hours) (IU/L)	1628	1957	2039	2156		<0.0001	

All other outcomes are numbers of patients. The number of deaths from all causes is equal to all cause cardiac plus non-cardiac plus hemorrhage. Values in parenthesis are % of total number of patients (1834). p values were determined by the χ^2 test for all but peak creatine kinase for which the Kruskal-Wallis test was used. p trend values were calculated using the Cochran-Armitage trend test.

*An additional 12 patients died due to hemorrhage over the 3·8 year follow up period (4 at day 1). Adding all cardiac plus non-cardiovascular deaths, plus one death without cause sums to 187.

Table 3

Myocardial infarction deaths in females, males and diabetics relative to baseline unbound free fatty acids.

Unbound free fatty acid Quartiles	Quartile1	Quartile2	Quartile3	Quartile4	Total	p Value	p Trend
Non Diabetic Females (n=283):							
By day 1	0	1	1	3	5(1.8%)	0.02	0.10
By 7 days	0	1	5	6	12(4.2%)	0.08	0.009
By 30 days	0	2	5	8	15(5.3%)	0.04	0.004
By 3.8 years	1	2	6	8	17(6.0%)	0.1	0.06
Non Diabetic Males (n=1322):							
By day 1	1	6	7	6	20(1.5%)	0.9	<0.022
By 7 days	1	6	9	10	26(2.0%)	<0.014	<0.0018
By 30 days	2	7	11	14	34(2.6%)	<0.003	<0.0003
By 3.8 years	3	10	12	17	42(3.2%)	<0.002	<0.0002
Diabetics (n=229):							
By day 1	2	0	3	3	8(3.5%)	0.11	0.86
By 7 days	2	0	5	5	12(5.2%)	0.44	0.37
By 30 days	2	1	5	7	15(6.6%)	0.57	0.23
By 3.8 years	2	2	6	7	17(7.4%)	0.79	0.32

Data are numbers of deaths and percent of each subgroup (n). p values were determined by χ^2 and p trend by the Cochran-Armitage trend test.

Table 4

Cox proportional hazard ratios for deaths relative to the first unbound free fatty acid quartile

	Quartile[a]	Hazard Ratio	CI (95%)	p Value
Myocardial Infarction Deaths				
Unadjusted	Q2	2.3	(0.9 – 5.9)	0.094
	Q3	4	(1.6 – 9.8)	0.002
	Q4	5.6	(2.3 – 13)	< 0.001
Adjusted	Q2	2. 9	(1.0 – 8.2)	0.043
	Q3	4.2	(1.6 – 11)	0.002
	Q4	5	(1.9 – 13)	0.001
All Cause Cardiac Deaths				
Unadjusted	Q2	1.7	(0.9 – 3.1)	0.103
	Q3	2.4	(1.3 – 4.3)	0.003
	Q4	2.8	(1.6 – 5.0)	0.000
Adjusted	Q2	1.7	(0·9 – 3.2)	0.108
	Q3	2.1	(1.2 – 4.0)	0.015
	Q4	2.4	(1.3 – 4.4)	0.004
All Cause Deaths				
Unadjusted	Q2	1.3	(0.8 – 2.1)	0.324
	Q3	1.9	(1.2 – 3.0)	0.004
	Q4	2.2	(1.4 – 3.4)	0.001
Adjusted	Q2	1.3	(0.8 – 2.1)	0.374
	Q3	1.7	(1.0 – 2.7)	0.032
	Q4	1.9	(1.2 – 3.1)	0.004

[a]Cox proportional hazard model generated hazard ratios and corresponding 95 % confidence interval (CI) and Wald's χ^2 probabilities. The model was adjusted for age, gender, race, body mass index, diastolic and systolic blood pressure at baseline, history of diabetes, MI and hypertension and use of β blockers within 24 hours of enrollment. This choice of factors was dictated by the limited access data set, known cardiac risk factors associated with more than 10 % of the 1834 patients and previous observed associations with free fatty acids.