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1 **A Longitudinal Study of Systemic Inflammation and Recovery of Lean Body Mass among**  
2 **Malnourished HIV-infected Adults Starting Antiretroviral Therapy in Tanzania and**  
3 **Zambia**

4  
5 **Authors and Institutional affiliations**

6 George PrayGod<sup>1§</sup>, Meridith Blevins<sup>2,3</sup>, Susannah Woodd<sup>4</sup>, Andrea M Rehman<sup>4</sup>, Kidola  
7 Jeremiah<sup>1</sup>, Henrik Friis<sup>5</sup>, Paul Kelly<sup>6,7</sup>, John Changalucha<sup>1</sup>, Douglas C. Heimburger<sup>3,8</sup>, Suzanne  
8 Filteau<sup>4</sup>, John R. Koethe<sup>3,8</sup>

9  
10 <sup>1</sup>Mwanza Research Centre, National Institute for Medical Research, Mwanza, Tanzania

11 <sup>2</sup>Department of Biostatistics, Vanderbilt University School of Medicine, Nashville, TN, USA

12 <sup>3</sup>Vanderbilt Institute for Global Health, Nashville, TN, USA

13 <sup>4</sup>Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical  
14 Medicine, London, UK

15 <sup>5</sup>Department of Nutrition, Exercise, and Sports, University of Copenhagen, Copenhagen,  
16 Denmark

17 <sup>6</sup>University Teaching Hospital, Lusaka, Zambia

18 <sup>7</sup>Barts & the London School of Medicine, Queen Mary University of London, London, UK

19 <sup>8</sup>Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA

20

21 Running title: Inflammation and body composition during early ART

22

23 <sup>§</sup>Corresponding author: Dr George PrayGod, Mwanza Research Centre, National Institute for

24 Medical Research, Box 1462, Mwanza, Tanzania. Tel: +255 28 2503012. E-mail:

25 [gpraygod@yahoo.com](mailto:gpraygod@yahoo.com)

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35 **Abstract:**

36 **Background:** The effects of inflammation on nutritional rehabilitation after starting  
37 antiretroviral therapy (ART) are not well understood. We assessed the relationship between  
38 inflammation and body composition among patients enrolled in the Nutritional Support for  
39 African Adults Starting Antiretroviral therapy (NUSTART) trial in Tanzania and Zambia from  
40 2011-2013.

41

42 **Methods:** HIV-infected, ART-eligible adults with body mass index (BMI)  $< 18.5 \text{ kg/m}^2$  enrolled  
43 in the NUSTART trial were eligible for this study. Anthropometric and body composition data  
44 were collected at recruitment and 6 and 12 weeks post-ART and C-reactive protein (CRP) was  
45 measured at recruitment and 6 weeks. The relationships between CRP and body composition  
46 were assessed using multiple regression.

47

48 **Results:** Of 1815 trial participants, 838 (46.2%) had baseline and 6 week CRP measurements.  
49 Median age was 36 years, 55% were females, and median CD4 count was 135 cells/ $\mu\text{L}$ . A one-  
50 log reduction in CRP at 6 weeks was associated with increased mid-upper-arm circumference  
51 (0.45 cm; 0.30, 0.61), calf circumference (0.38 cm; 0.23, 0.54), waist circumference (0.98 cm;  
52 0.59, 1.37), BMI ( $0.37 \text{ kg/m}^2$ ; 0.24, 0.50), fat-free mass (0.58 kg; 0.26, 0.91), but not with fat  
53 mass (0.09 kg; -0.17, 0.34). Fat-free mass gains persisted at 12 weeks and were more closely  
54 associated with 6 week CRP values than with baseline values.

55

56 **Conclusions:** Reduction in CRP shortly after ART initiation was associated with higher fat-free  
57 mass gains. Further studies are warranted to determine whether interventions to reduce systemic  
58 inflammation will enhance the gains in fat-free mass.

59

60 **Key Words:** HIV, inflammation, body composition, malnutrition, antiretroviral therapy

## 61 INTRODUCTION

62 Infection with Human Immunodeficiency Virus (HIV) continues to be a major public health  
63 problem in Sub-Saharan Africa. Despite efforts to promote early diagnosis and treatment  
64 initiation before the onset of advanced disease, over a third of HIV-infected patients initiate  
65 antiretroviral therapy (ART) after developing malnutrition (i.e., a body mass index [BMI] <18.5  
66 kg/m<sup>2</sup>) and early mortality in this group is exceedingly high.<sup>(1, 2)</sup> Prior studies in Africa  
67 investigating the effects of nutritional supplementation in the early ART period have not shown a  
68 mortality benefit, and some supplements may actually produce a disproportionate increase in fat  
69 mass.<sup>(3, 4)</sup> A greater recovery of lean mass, as opposed to fat mass, during the early HIV  
70 treatment period may improve survival and reduce the long-term risk of developing chronic  
71 diseases, but the factors influencing lean mass gains among malnourished adults starting ART  
72 are poorly understood.<sup>(5)</sup>

73

74 Malnutrition and HIV infection are accompanied by high levels of systemic inflammation, due in  
75 part to unchecked viremia, reduced mucosal defenses, and opportunistic infections.<sup>(6-8)</sup> In  
76 advanced HIV infection, an elevated rate of protein turnover and inappropriately low muscle  
77 protein synthesis prevent weight gain despite sufficient intake of calories and protein.<sup>(9-11)</sup> With  
78 the initiation of ART and suppression of viremia, systemic inflammation normalizes to varying  
79 degrees, with a concomitant reduction in resting metabolic expenditures and improved weight  
80 gain in most undernourished patients.<sup>(12, 13)</sup> However, abnormalities in factors related to body  
81 mass partitioning, such as an elevated rate of lipolysis, can persist in some patients despite viral  
82 suppression and may have effects on subsequent nutritional rehabilitation.<sup>(14)</sup> In prior studies,  
83 aggressive parenteral nutrition in critically ill patients did not markedly improve lean body  
84 mass,<sup>(15)</sup> and weight gain during treatment for pulmonary tuberculosis was primarily due to gains  
85 in adipose tissue rather than lean mass.<sup>(16)</sup>

86

87 We hypothesized that a failure to normalize systemic inflammation after starting ART impairs  
88 recovery of lean mass and biases weight gain towards adipose tissue deposition. Using data from  
89 malnourished HIV-infected patients enrolled in a nutritional supplementation trial in Tanzania  
90 and Zambia, we analyzed the relationships between C-reactive protein (CRP) and fat and fat-free  
91 mass immediately before and during first 12 weeks of ART.

92

### 93 **METHODS**

94 This study was conducted as part of the Nutritional Support for Africans Starting Antiretroviral  
95 Therapy (NUSTART) trial (registration # PACTR201106000300631), a randomized, double  
96 blind, controlled trial of a lipid-based nutritional supplement (LNS; prepared by Nutriset,  
97 Malauney, France) in 1815 malnourished HIV-infected patients starting ART in Mwanza,  
98 Tanzania and Lusaka, Zambia. The study was conducted between August 2011 and December  
99 2013. NUSTART participants were randomized to receive either the LNS alone (control arm) or  
100 fortified with additional vitamins and minerals (intervention arm; LNS-VM) in a two-stage  
101 nutritional intervention designed to mimic standard protocols for management of severe  
102 malnutrition in young children. From recruitment until 2 weeks after starting ART, participants  
103 received a low calorie (30 g) LNS, and during weeks 2-6 of ART participants received a high  
104 calorie (250 g or ~1400 kcal/d) LNS. Trial inclusion criteria were 18 years of age or older, ART-  
105 naive except for standard regimens to prevent maternal-to-child HIV transmission, BMI < 18.5  
106 kg/m<sup>2</sup>, and a CD4 count < 350 cells/μl or WHO stage 3 or 4 disease. Self-reported pregnancy  
107 was an exclusion criterion. In separate analysis LNS-VM compared to LNS did not increase fat  
108 mass or fat-free mass at 12 weeks of ART.

109

110 NUSTART participants underwent detailed body composition and laboratory studies as part of  
111 an intensive visit schedule. After recruitment, patients came to the clinic weekly until the start of  
112 ART, and again at weeks 1, 2, 4, 6, 8, and 12 after starting ART. Height was measured at  
113 recruitment using a stadiometer fixed to the wall and weight at each visit using a digital balance.  
114 At recruitment, 2, 6, and 12 weeks after starting ART patients underwent additional  
115 anthropometric evaluation. Waist circumference, mid-upper arm circumference (MUAC), hip  
116 and calf circumferences were measured using a flexible tape, and triceps and sub-scapular  
117 skinfold thickness using a caliper in Lusaka only. All measurements were done in triplicate and  
118 the median value was recorded for analyses. Participants also underwent bioelectrical impedance  
119 analysis (BIA) to estimate fat mass and fat-free mass (Tanita, Tokyo, Japan). Venous blood  
120 samples were taken at all scheduled visits for laboratory analyses. Serum CRP was measured at  
121 recruitment and week 6 by ELISA (AssayPro, St. Charles, MO, USA), and hemoglobin was  
122 measured by Hemocue and CD4 count by local central clinical services at recruitment. We did  
123 not determine viral loads due to the limited availability of testing at our sites, the high cost, and  
124 because testing is not routinely available for clinical care in these settings. Furthermore, while  
125 providers at clinical sites recorded their diagnoses of suspected opportunistic infections, the  
126 diagnostic capacity was very limited and confirmatory testing was often not available, and  
127 therefore these data were not included in this analysis.

## 128 Sample size

129 As part of the main study, we recruited 1876 patients <sup>(17)</sup>. This number was sufficient to detect, at  
130 5% significance, 90% power and 25% attrition by 12 weeks due to death or loss to follow-up,  
131 differences of 0.18 of a standard deviation in secondary continuous outcomes measured at 12  
132 weeks. Since this was part of the secondary analyses we did calculate sample size a priori  
133

134 Analyses were conducted using Stata 12.1 and R-software 3.0.2 ([www.r-project.org](http://www.r-project.org)).

135 Demographics and clinical characteristics of the cohort were presented as percentages or



136 medians with interquartile ranges (IQR). Participants included in the analysis cohort versus those  
137 deceased/lost prior to 6 weeks after starting ART or without complete laboratory values were  
138 compared using the Kruskal-Wallis and Chi-square tests. CRP and body composition  
139 measurements were compared pairwise across baseline and 6 week, and baseline and 12 week,  
140 time points using the Wilcoxon signed-rank test.

141

142 The primary analysis for this paper assessed the relationship between the change in CRP from  
143 baseline (pre-ART) to 6 weeks post-ART and the change in anthropometric and bioelectric  
144 impedance measurements over the same period using linear regression. CRP was log-  
145 transformed while the anthropometric and BIA outcome measurements remained on a linear  
146 scale. Models were adjusted for age, sex, CD4+ count, hemoglobin, treatment arm, country, and  
147 whether the subject was receiving treatment for tuberculosis before starting ART. Hemoglobin  
148 was missing for 8% of cases and was multiply imputed. To account for possible non-linear  
149 associations, continuous variables were modeled using restricted cubic splines with 4 knots. We  
150 also adjusted for the number of days between enrollment and ART initiation to reduce bias  
151 associated with longer pre-ART periods on supplement.

152

153 A second analysis assessed the effect of changes in CRP at 6 weeks with body composition at 12  
154 weeks to determine whether CRP measurements during the LNS intervention predicted longer  
155 term nutritional status after the intervention ended. Using linear regression models, we first  
156 tested for a three-way interaction effect between CRP at enrollment, 6 weeks, and the  
157 intervention arm, but the interaction term was not statistically significant in any of the models  
158 ( $p > 0.10$  for all except calf circumference [ $p = 0.08$ ]). We then modeled a two-way interaction  
159 between CRP values and included the intervention arm as an additive effect. The regression  
160 coefficients for baseline and 6 week columns represent the average difference in 12 week body  
161 composition for a one-log difference around the median baseline and 6 week log-CRP values,

162 respectively. Models were adjusted for age, sex, CD4+ count, hemoglobin, treatment for  
163 tuberculosis, site, and the number of days between enrollment and ART initiation, and  
164 continuous variables were modeled using restricted cubic splines with 4 knots.

165

166 The NUSTART trial was conducted according to guidelines laid down in the Declaration of  
167 Helsinki and all procedures were approved by the ethics committees of the London School of  
168 Hygiene and Tropical Medicine, the University of Zambia, and the National Institute for Medical  
169 Research, Tanzania. All patients provided written or thumbprint informed consent.

170

## 171 **RESULTS**

172 838 NUSTART participants survived beyond 6 weeks of ART and had serum CRP  
173 measurements performed at baseline and 6 weeks post-ART. The analysis cohort was 55%  
174 female with a median age of 36 years (IQR 30, 42), median pre-ART CD4+ T-cell count of 135  
175 cells/ $\mu$ l (IQR 63, 225), and median BMI of 16.8 kg/m<sup>2</sup> (IQR 15.9, 17.6) (**Table 1**). Participants  
176 were equally distributed between the intervention and control arms, with a higher percentage  
177 (59%) enrolled in Lusaka (similar to the full cohort). Among those not included in the analysis  
178 cohort, 340 had died at 12 weeks, 156 had withdrawn or were lost to follow-up, and the  
179 remaining 481 alive at 12 weeks either did not have a baseline or 6 week CRP measurement  
180 (**Table 2**). In comparison to the analysis cohort, the excluded participants were more likely to be  
181 male, younger, and had a lower median CD4+ T cell count and lower median BMI ( $p < 0.01$  for  
182 all).

183

184 **Table 3** shows median serum CRP at baseline and 6 weeks of ART, and anthropometric and BIA  
185 measurements at baseline and 6 and 12 weeks. Median CRP only decreased from 38.2 mg/l (IQR

186 8.9, 124) to 34.8 mg/l (IQR 12.2, 94.5) from baseline to six weeks, which was not statistically  
187 significant ( $p=0.91$ ). The paired change from baseline to 6 weeks of ART for all of the body  
188 composition measurements was statistically significant ( $p<0.001$  for all), and the paired change  
189 from 6 to 12 weeks was also significant ( $p<0.01$  for all).

190

191 The intra-individual changes in serum CRP from baseline to 6 weeks were inversely associated  
192 with changes in several of the body composition measurements over the same period (**Table 4**).  
193 A one-log reduction in CRP was associated with a 0.37 kg/m<sup>2</sup> increase in BMI, a 0.45 cm  
194 increase in mid-upper arm circumference, a 0.98 cm increase in waist circumference, and 0.58 kg  
195 increase in fat-free mass at 6 weeks of ART ( $p<0.001$  for all). Other anthropometric  
196 measurements were also inversely related to the change in CRP with the exception of BIA fat  
197 mass. These relationships appeared non-linear. While greater reductions in log-CRP were  
198 generally associated with greater body composition changes, a failure to reduce log-CRP or a  
199 rise in log-CRP on ART was generally associated with little change (**Figure**). The relationships  
200 of CRP at baseline and 6 weeks with body composition at 12 weeks were assessed using linear  
201 regression models incorporating baseline and 6 week values, in addition to a two-way interaction  
202 term between CRP values (**Table 5**). For the purpose of calculating the effects on body  
203 composition, the model for baseline CRP was adjusted to a median log-CRP value of 3.5, and the  
204 model for 6-week CRP was adjusted to a median log-CRP of 3.6. A one-log higher CRP at  
205 baseline was significantly associated with lower mid-upper arm and waist circumference at 12  
206 weeks. However, a one-log higher 6- week CRP was significantly associated with lower BMI,  
207 mid-upper arm, waist, hip and calf circumference, and triceps skinfold thickness at 12 weeks.  
208 The relationship between 6 week CRP and 12 week BIA fat-free mass approached significance  
209 ( $p=0.06$ ), while there was little evidence of an association of CRP with 12 week scapular  
210 skinfold thickness and BIA fat mass.

211

212 Due to the complicated nature of interaction effects, we summarized the statistical models of the  
213 combined effect of enrollment and week 6 log-CRP values on the change in 12 week body  
214 composition measurements using heat maps (**Supplementary Figure**). In these figures, deeper  
215 shades of blue represent larger increases in body composition measurements at 12 weeks  
216 corresponding to a pair of enrollment (x-axis) and week 6 (y-axis) log-CRP values, while deeper  
217 shades of violet represent smaller increases (or negative changes in some variables). The change  
218 in CRP was associated only with mid-upper arm, waist and hip circumference and triceps  
219 skinfold thickness ( $p < 0.05$ ), and approached significance for BMI ( $p = 0.05$ ; p-values refer to the  
220 effect of the two-way interaction term [log-CRP at enrollment and week 6] on outcome  
221 measurements). In general, larger increases in lean body mass metrics were seen in patients with  
222 moderate-to-high baseline CRP and lower 6 week CRP.

223

## 224 **DISCUSSION**

225 In this study of undernourished HIV-infected patients starting ART, we found that a failure to  
226 reduce excessively high levels of CRP in the early weeks following treatment initiation is  
227 associated with failure to accrue lean mass as measured by both anthropometry and BIA.  
228 Furthermore, the accumulation of adipose tissue did not appear dependent on CRP reduction,  
229 suggesting that weight gain in the setting of uncontrolled inflammation may actually represent an  
230 unhealthy shift towards adiposity, with potential consequences for metabolic disease in the  
231 future. These findings suggest the monitoring of inflammatory biomarkers in undernourished  
232 ART patients during the early treatment period, and additional interventions to identify and treat  
233 sources of inflammation, could improve the nutritional and other health outcomes of this  
234 population.

235

236 Restoring individuals with advanced HIV disease and malnutrition to health requires both the  
237 recovery of effective immune protection and the rebuilding of adequate stores of metabolically  
238 active muscle and other lean tissues.<sup>(18)</sup> While the initiation of ART by undernourished HIV-  
239 infected adults is usually accompanied by weight gain to varying degrees, the composition of the  
240 newly deposited tissue is also an important factor in nutritional rehabilitation and the  
241 normalization of metabolic processes. Prior studies in diverse HIV-infected populations have  
242 found mixed effects of ART on body composition, with some showing no effect on fat and lean  
243 mass, and others suggesting that ART may lead to preferential increases in lean or fat mass.<sup>(4,</sup>  
244 19-21) However, the factors responsible for this heterogeneous response have not been  
245 previously explored in detail.

246

247 Our baseline and follow-up levels of CRP were higher than those reported from studies in  
248 resource-rich settings and may reflect to the combination of untreated viremia, secondary  
249 infections related to immunosuppression or local factors (e.g., parasites), and enteropathy related  
250 to both HIV infection and malnutrition.<sup>(6-8)</sup> HIV infection depletes lymphoid cells in the  
251 gastrointestinal mucosa integral to defense against bacterial, fungal, and parasitic pathogens, and  
252 impairs tight junctions between epithelial cells, resulting in altered intestinal integrity and  
253 increased translocation of microbes from the intestinal lumen to the circulation.<sup>(22-26)</sup> Increased  
254 microbial translocation is posited as a major contributor to elevated, chronic inflammation in  
255 HIV-infected individuals, which is likely compounded in the setting of chronic malnutrition due  
256 to similar impairments in intestinal mucosal integrity and the adaptive immune response in the  
257 gut.<sup>(6-8, 27)</sup>

258

259 Our observation that a higher CRP level is associated with lower lean mass gains may explain  
260 the finding in prior nutritional supplementation trials that some patients gained no weight or

261 mainly fat mass during early ART, which has also been reported in patients with similar  
262 proinflammatory states such as tuberculosis, severe trauma and cancer .<sup>(15, 16)</sup>(28) and indicates  
263 that across the spectrum of infectious and non-infectious diseases, inflammation may be a key  
264 determinant of nutritional depletion and recovery.

265 Elevated circulating inflammatory cytokines such as TNF-alpha and interleukin-6 are associated  
266 with reduced muscle protein synthesis and deposition, and may stimulate apoptosis in muscle  
267 cells precursors, suggesting that lack of lean mass gain associated with high inflammation may  
268 actually be due to failure of protein synthesis rather than excessive protein breakdown.<sup>(9, 10)</sup> The  
269 finding that inflammation and lean mass recover are closely linked will be important for  
270 interpreting findings of future nutritional intervention trials in low-income settings, and it may be  
271 the case that any meaningful effects of nutritional interventions on lean mass will depend on first  
272 reducing inflammation. Of note, a recent trial in Ethiopia found that that presence of persistent  
273 HIV-1 viremia at 3 months was associated with preferential fat mass gain, while viral  
274 suppression was associated with lean mass gain.<sup>(21)</sup>

275

276 In the present study, we noted that CRP levels were not closely associated with 6 or 12 weeks  
277 post-ART measurements of fat mass, which may indicate that the accrual of fat mass is driven by  
278 other factors independent of inflammation. In higher BMI populations, CRP is positively  
279 associated with fat mass.<sup>(29)</sup> A similar relationship between fat mass and CRP was not observed  
280 in our patients; and we hypothesize that any contribution of adipose tissue to circulating  
281 inflammatory mediators may have been obscured by the more pronounced effect of advanced  
282 malnutrition and HIV infection on systemic inflammation. However, further work is needed to  
283 understand the directionality of the relationships between inflammation and body fat mass in low  
284 versus normal and high BMI individuals.

285

286 Prior studies in Africa have shown elevated CRP, interleukin-6, and other markers of systemic  
287 inflammation are associated with increased mortality on ART, but there are fewer data on the  
288 link between inflammation and long-term outcomes.<sup>(30)</sup> As the capacity of health systems to  
289 identify and treat cardiovascular, metabolic and other non-communicable diseases in HIV  
290 patients improves in sub-Saharan Africa, epidemiologic studies are needed to determine how  
291 very high levels of inflammation affect long-term health outcomes.

292

293 In this study we included in the analysis about half of the patients recruited for the trial. Patients  
294 not included in the analysis because of loss to follow-up, or death tended to be those who were  
295 severely malnourished, immunocompromised judged by CD4 count and had higher median CRP.  
296 Although a higher baseline CRP, in the excluded survivors would have potentially resulted in a  
297 large reduction in CRP from zero to 6 weeks, assuming that the patterns of correlation remained  
298 the same, this would probably not have significantly changed the associations given that we  
299 modelled the relation between CRP and body composition parameters on log rather than normal  
300 scale.

301

302 The strength of our study was the prospective design and large sample size, which permitted the  
303 assessment of longitudinal relationships between CRP and body composition between referral  
304 for ART and 6 and 12 weeks post-ART. The BIA method we used was well suited to clinical  
305 care in Africa and has been shown to correlate well with more complicated radiographic  
306 assessments in healthy patients, but there are fewer data comparing BIA versus DEXA and other  
307 radiographic methods in malnourished, HIV patients.<sup>(31)</sup> However, a longer follow-up period  
308 may have also provided additional insights on trends of body composition changes during ART  
309 among undernourished patients on ART. Furthermore, our study could not assess the long-term  
310 implications of early changes in body composition on ART. We observed a 0.37 kg/m<sup>2</sup> rise in

311 BMI at 6 weeks in patients with a one-log CRP reduction over the same period, which represents  
312 an approximately 2%-2.5% BMI increase (depending on the baseline BMI value). Prior studies  
313 have shown modest early increases in BMI are clinically important for long-term survival, but  
314 additional studies are warranted to understand how body composition, inflammation, and other  
315 nutritional factors interact to influence health outcomes.<sup>(32)</sup> Although additional LNS received  
316 by patients in the trial may have made them different from the rest of HIV population on ART,  
317 this difference would have disappeared a few weeks after starting ART as patients on treatment  
318 regained appetite and started consuming nutritionally diverse food. Thus, these findings can be  
319 generalized to all malnourished HIV-infected patients starting ART.

320

## 321 **CONCLUSIONS**

322 In conclusion, among HIV-infected adult patients, reductions in CRP over the first six weeks of  
323 ART were associated with higher lean body mass gains; and patients with lower CRP at 6 weeks  
324 continued to have greater lean mass up to 12 weeks. Promoting lean body mass gains in  
325 malnourished HIV patients starting ART is important for nutritional rehabilitation and may  
326 impact long-term survival and chronic disease risk. Future trials should consider interventions  
327 addressing both nutritional recovery and inflammation, to elucidate mechanisms and optimize  
328 outcomes in malnourished patients. Lastly, further studies on the effect of persistent high level of  
329 inflammation as well as fat mass gains on long-term chronic disease risk, including diabetes  
330 mellitus and cardiovascular conditions, are needed in sub-Saharan Africa.

331

## 332 **Figure legends**

333 **Figure.** Relationship of the change in C-reactive protein and body composition measurements  
334 between baseline and 6 weeks of antiretroviral therapy.



335 Models adjusted for sex, treatment arm, country, and age, CD4+ count, hemoglobin, and receipt  
336 of anti-tuberculosis therapy at treatment initiation. CRP is log-transformed. Abbreviations: BMI,  
337 body mass index.

338

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355

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357

358 Supplementary information is available at the European Journal of Clinical Nutrition website

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