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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
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20

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22

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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/ μL . 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 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/m²) and early mortality in this group is exceedingly high.^(1, 2) 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.^(3, 4) 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.⁽⁵⁾

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.⁽⁶⁻⁸⁾ 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.⁽⁹⁻¹¹⁾ 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.^(12, 13) 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.⁽¹⁴⁾ In prior studies,
83 aggressive parenteral nutrition in critically ill patients did not markedly improve lean body
84 mass,⁽¹⁵⁾ and weight gain during treatment for pulmonary tuberculosis was primarily due to gains
85 in adipose tissue rather than lean mass.⁽¹⁶⁾

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/m², 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 ⁽¹⁷⁾. 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).

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/ μ l (IQR 63, 225), and median BMI of 16.8 kg/m² (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/m² 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.⁽¹⁸⁾ 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.(4,
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.⁽⁶⁻⁸⁾ 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.⁽²²⁻²⁶⁾ 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.^(6-8, 27)

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 .^(15, 16)(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.^(9, 10) 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.⁽²¹⁾

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.⁽²⁹⁾ 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.⁽³⁰⁾ 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.⁽³¹⁾ 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/m² 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.⁽³²⁾ 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

REFERENCES

1. Liu E, Spiegelman D, Semu H, Hawkins C, Chalamilla G, Aveika A, et al. Nutritional Status and Mortality Among HIV-Infected Patients Receiving Antiretroviral Therapy in Tanzania. *J Infect Dis.* 2011 Jul;204(2):282-90.
2. Gupta A, Nadkarni G, Yang WT, Chandrasekhar A, Gupte N, Bisson GP, et al. Early Mortality in Adults Initiating Antiretroviral Therapy (ART) in Low- and Middle-Income Countries (LMIC): A Systematic Review and Meta-Analysis. *PLoS One.* 2011;6(12):e28691.
3. Grobler L, Siegfried N, Visser ME, Mahlungulu SS, Volmink J. Nutritional interventions for reducing morbidity and mortality in people with HIV. *The Cochrane database of systematic reviews.* 2013;2:CD004536.
4. Ndekha MJ, van Oosterhout JJ, Zijlstra EE, Manary M, Saloojee H, Manary MJ. Supplementary feeding with either ready-to-use fortified spread or corn-soy blend in wasted adults starting antiretroviral therapy in Malawi: randomised, investigator blinded, controlled trial. *BMJ (Clinical research ed.)* 2009;338:b1867.
5. Mulligan K, Harris DR, Monte D, Stoszek S, Emmanuel P, Hardin DS, et al. Obesity and dyslipidemia in behaviorally HIV-infected young women: Adolescent Trials Network study 021. *Clin Infect Dis.* 2010 Jan 1;50(1):106-14.
6. Marchetti G, Cozzi-Lepri A, Merlini E, Bellistri GM, Castagna A, Galli M, et al. Microbial translocation predicts disease progression of HIV-infected antiretroviral-naive patients with high CD4+ cell count. *AIDS.* 2011 Apr 18;25(11):1385-94.
7. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection. *J Infect Dis.* 2011 Jan 20;203(6):780-90.
8. Elia M, Goren A, Behrens R, Barber RW, Neale G. Effect of total starvation and very low calorie diets on intestinal permeability in man. *Clin Sci (Lond.)* 1987 Aug;73(2):205-10.
9. Yarasheski KE, Zachwieja JJ, Gischler J, Crowley J, Horgan MM, Powderly WG. Increased plasma gln and Leu Ra and inappropriately low muscle protein synthesis rate in AIDS wasting. *Am J Physiol.* 1998 Oct;275(4 Pt 1):E577-83.
10. Macallan DC, McNurlan MA, Milne E, Calder AG, Garlick PJ, Griffin GE. Whole-body protein turnover from leucine kinetics and the response to nutrition in human immunodeficiency virus infection. *Am J Clin Nutr.* 1995 Apr;61(4):818-26.

11. Powanda MC, Beisel WR. Metabolic effects of infection on protein and energy status. *J Nutr*. 2003 Jan;133(1):322S-7S.
12. Grunfeld C, Pang M, Shimizu L, Shigenaga JK, Jensen P, Feingold KR. Resting energy expenditure, caloric intake, and short-term weight change in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *Am J Clin Nutr*. 1992 Feb;55(2):455-60.
13. Shevitz AH, Knox TA, Spiegelman D, Roubenoff R, Gorbach SL, Skolnik PR. Elevated resting energy expenditure among HIV-seropositive persons receiving highly active antiretroviral therapy. *AIDS*. 1999 Jul 30;13(11):1351-7.
14. van der Valk M, Reiss P, van Leth FC, Ackermans MT, Endert E, Romijn JA, et al. Highly active antiretroviral therapy-induced lipodystrophy has minor effects on human immunodeficiency virus-induced changes in lipolysis, but normalizes resting energy expenditure. *J Clin Endocrinol Metab*. 2002 Nov;87(11):5066-71.
15. Streat SJ, Beddoe AH, Hill GL. Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients. *The Journal of trauma*. 1987 Mar;27(3):262-6.
16. Schwenk A, Hodgson L, Wright A, Ward LC, Rayner CF, Grubnic S, et al. Nutrient partitioning during treatment of tuberculosis: gain in body fat mass but not in protein mass. *The American journal of clinical nutrition*. 2004 Jun;79(6):1006-12.
17. Filteau S, PrayGod G, Kasonka L, Woodd S, Rehman AM, Chisenga M, et al. Effects on mortality of a nutritional intervention for malnourished HIV-infected adults referred for antiretroviral therapy: a randomised controlled trial. *BMC medicine*. 2015;13:17.
18. Mupere E, Malone L, Zalwango S, Chiunda A, Okwera A, Parraga I, et al. Lean tissue mass wasting is associated with increased risk of mortality among women with pulmonary tuberculosis in urban Uganda. *Annals of epidemiology*. 2012 Jul;22(7):466-73.
19. McDermott AY, Shevitz A, Knox T, Roubenoff R, Kehayias J, Gorbach S. Effect of highly active antiretroviral therapy on fat, lean, and bone mass in HIV-seropositive men and women. *The American journal of clinical nutrition*. 2001 Nov;74(5):679-86.
20. Silva M, Skolnik PR, Gorbach SL, Spiegelman D, Wilson IB, Fernandez-DiFranco MG, et al. The effect of protease inhibitors on weight and body composition in HIV-infected patients. *AIDS (London, England)*. 1998 Sep 10;12(13):1645-51.
21. Olsen MF, Abdissa A, Kaestel P, Tesfaye M, Yilma D, Girma T, et al. Effects of nutritional supplementation for HIV patients starting antiretroviral treatment: randomised controlled trial in Ethiopia. *Bmj*. 2014;348:g3187.

22. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med*. 2004 Sep 20;200(6):749-59.
23. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med*. 2004 Sep 20;200(6):761-70.
24. Sankaran S, George MD, Reay E, Guadalupe M, Flamm J, Prindiville T, et al. Rapid onset of intestinal epithelial barrier dysfunction in primary human immunodeficiency virus infection is driven by an imbalance between immune response and mucosal repair and regeneration. *J Virol*. 2008 Jan;82(1):538-45.
25. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. *Blood*. 2008 Oct 1;112(7):2826-35.
26. Epple HJ, Schneider T, Troeger H, Kunkel D, Allers K, Moos V, et al. Impairment of the intestinal barrier is evident in untreated but absent in suppressively treated HIV-infected patients. *Gut*. 2009 Feb;58(2):220-7.
27. Welsh FK, Farmery SM, MacLennan K, Sheridan MB, Barclay GR, Guillou PJ, et al. Gut barrier function in malnourished patients. *Gut*. 1998 Mar;42(3):396-401.
28. McMillan DC. Systemic inflammation, nutritional status and survival in patients with cancer. *Curr Opin Clin Nutr Metab Care*. 2009 May;12(3):223-6.
29. Reingold J, Wanke C, Kotler D, Lewis C, Tracy R, Heymsfield S, et al. Association of HIV infection and HIV/HCV coinfection with C-reactive protein levels: the fat redistribution and metabolic change in HIV infection (FRAM) study. *J Acquir Immune Defic Syndr*. 2008 Jun 1;48(2):142-8.
30. Koethe JR, Blevins M, Nyirenda C, Kabagambe EK, Shepherd BE, Wester CW, et al. Nutrition and inflammation serum biomarkers are associated with 12-week mortality among malnourished adults initiating antiretroviral therapy in Zambia. *Journal of the International AIDS Society*. 2011;14:19.
31. Furstenberg A, Davenport A. Comparison of multifrequency bioelectrical impedance analysis and dual-energy X-ray absorptiometry assessments in outpatient hemodialysis patients. *Am J Kidney Dis*. 2011 Jan;57(1):123-9.

32. Madec Y, Szumilin E, Geneviev C, Ferradini L, Balkan S, Pujades M, et al. Weight gain at 3 months of antiretroviral therapy is strongly associated with survival: evidence from two developing countries. *AIDS (London, England)*. 2009 Apr 27;23(7):853-61.