Stability of plasma indices of inflammation/coagulation and homeostasis after fatty and non-fatty meals in treated people with HIV

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Abstract

Objectives: The relationship between lipid levels in plasma and inflammatory indices is complex and fatty meals alter plasma inflammatory markers in people with diabetes. There is interest in monitoring the effects of interventions on plasma inflammatory and coagulation elements in people with HIV, as they have been linked to risk for morbid outcomes and HIV persistence. Understanding the effects of feeding and time of specimen acquisition is important for the correct scheduling of clinical sampling.

Methods: We examined the effects of feeding on plasma inflammatory, coagulation and homeostatic indices among 24 non-diabetic people with HIV, with controlled viraemia and on antiretroviral therapy after fasting and then 1, 3 and 6 hours after ingesting a fatty meal, and also approximately 1 week later after fasting and after an isocaloric non-fatty meal. Plasma levels of IL-6, IL-7, IP-10, sCD14, sCD163, sTNFrII and D-dimer were monitored by immunoassay.

Results: Fasting levels of all markers obtained approximately 1 week apart were significantly correlated ($P<0.001$). Mild alterations in plasma concentrations of inflammatory markers were observed after feeding but geometric means varied more than 10% from baseline for only IL-6 and IL-7. Meal type was differentially associated with changes in plasma levels for IL-7 only. Antiretroviral treatment regimen, body mass index and changes in plasma triglyceride levels were not linked to post-feeding changes in these biomarkers.

Conclusions: These plasma inflammatory, coagulation and homeostatic indices are relatively stable at fasting and are only minimally affected by feeding or time of day. These findings will aid in the monitoring of inflammatory and homeostatic indices that may contribute to control of HIV expression and its persistence.

Keywords: inflammation, coagulation, biomarkers, fasting, feeding

Introduction

Indices of immune activation and inflammation tend to persist in people living with HIV even when viraemia is suppressed with antiretroviral therapy (ART) (reviewed in [1]). Recent work from several groups has demonstrated that soluble markers of immune activation and inflammation predict the occurrence of serious morbidity and mortality in people living with HIV who are receiving suppressive ART [2,3] and may be linked to indices of HIV persistence [4]. While robust assays are available to measure these indices, the diurnal and day–to–day variabilities in levels of these markers are not well understood and may be affected by fatty meals as has been observed among persons with diabetes mellitus [5,6]. Perturbations in plasma lipid populations particularly among oxidised and saturated lipids have been linked to inflammatory indices in treated and untreated HIV infection [7]. A better understanding of the possible influence of feeding on inflammatory markers could help us determine optimal conditions for monitoring these indices in clinical trials. We therefore examined plasma levels of selected important inflammatory/coagulation and homeostatic markers in the fasting state, and at 1, 3 and 6 hours after ingestion of a fatty meal. Each subject returned, fasting, 1 week later and had blood drawn at baseline and then

at 1, 3 and 6 hours after an isocaloric non-fatty meal to examine the variability over time in these indices and to see if fat content drives changes in these indices. We found that fasting plasma levels of IL-6, IL-7, D-dimer, sCD14, sTNFrII, IP-10 and sCD163 are well correlated when drawn 1 week apart. After feeding fatty or non-fatty meals, plasma levels of these indices were also relatively stable over 6 hours and unaffected by different ART regimens or body mass index (BMI).

Methods

Population

This study was approved by the Institutional Review Board at University Hospitals Case Medical Center. After written informed consent was provided, blood samples were obtained via venepuncture from 24 non-diabetic participants living with HIV receiving care at the Special Immunology Unit at University Hospitals Case Medical Center. Each was receiving combination ART and had most recent plasma HIV RNA levels of <50 copies/mL. None had a diagnosis of diabetes; all had most recent random blood sugar levels of <120 mg/dL.

After an overnight fast, morning blood samples were obtained between 0820 and 1000. Patients then broke their fast with a fatty meal containing 660 kcal (37 g fat, 67 g carbohydrates, and 15 g protein) and repeat blood samples were then obtained 1, 3, and 6 hours after the meal. Each patient returned, after another

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overnight fast, 6–11 days later and morning blood samples were obtained between 0810 and 1130. This time, the fast was broken with an isocaloric non-fatty meal (5.8 g fat, 137.5 g carbohydrates and 24 g protein), and blood was obtained 1, 3, and 6 hours after the meal.

Blood collection and analysis

Blood was collected in one serum separator tube (Beckton Dickinson, Franklin Lakes, NJ, USA), and in one EDTA-containing tube (Beckton Dickinson, Franklin Lakes, NJ, USA). Within 2 hours the tubes were centrifuged at 400xg for 10 minutes. Plasma was clarified with a second centrifugation at 400xg for 10 minutes. The serum separator tube was centrifuged at 800xg for 10 minutes and all samples were frozen at -80°C until cytokine levels and metabolic indices were analysed in batch. Serum glucose, insulin and triglyceride levels were measured in the University Hospitals of Cleveland clinical laboratories.

Levels of IL-6, sCD14, sTNFrII, sCD163, IP-10 and IL-7 were measured by enzyme linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) and D-dimer levels were also measured by immunoassay (Diagnostica Stago, Asnières-sur-Seine, France).

Statistical analysis

Correlations were assessed with the rank-based Spearman's rho and associated 95% confidence intervals (CI). Post-fasting changes in cytokine levels were analysed with repeated measures models. Because there is dependence between values after a meal and between the two meals an unstructured direct product structure across meals was used. Due to the skewed distribution, cytokine levels were log_{10} transformed prior to analysis. The comparison of baseline factors between participants with increases versus decreases in cytokine levels at 1 hour post-meal were made using Fisher's exact test and the Wilcoxon rank-sum test.

All statistical testing used a two-sided 5% alpha coefficient with no adjustment for multiple testing.

Results

A total of 24 patients were studied, 21 of whom were men and three women. The median age was 44 (range 31–60) years. Median CD4 T cell count was 711 (260–2151) cells/mm³. All had most recent plasma HIV RNA level <50 copies/mL. The duration of virological control ranged from 2 to 109 months (median 20.5 months). Twelve of the 23 participants were current smokers (information was not available for one subject). Twelve participants were African-American, 10 were white and two were Hispanic. All but one patient were receiving at least one nucleoside or nucleotide reverse transcriptase inhibitor (NRTI), 12 were receiving protease inhibitors, seven were receiving integrase strand transfer inhibitors (INSTI), 13 were receiving CYP3A4 inhibitors, eight were receiving non-nucleoside reverse transcriptase inhibitors (NNRTIs), one was receiving a CCR5 antagonist and one clinical trial participant was also receiving either a CCR5 antagonist or placebo. The range of BMI at the time of study was 20.3–35.8 kg/m² with a median of 23.6 kg/m².

Fasting inflammatory markers were correlated upon repeat testing

Correlations between the two fasting measurements of each inflammatory/coagulation marker for each of the 24 participants obtained approximately 1 week apart are shown in Figure 1. Fasting levels of all markers were significantly correlated (*P*<0.001) with rho values of 0.76 for D-dimer, 0.80 for IL-6, 0.71 for IL-7, 0.93 for IP-10, 0.80 for sCD14, 0.83 for sCD63 and 0.84 for sTNFrII.

Geometric mean levels (and 95% confidence intervals) of plasma markers at baseline and after both fatty and non-fatty meals are shown in Figure 2. Geometric mean (and 95% CI) levels of each marker tended to be stable over each period of study. On Figure 3 these data are reduced to show geometric mean fold changes (and 95%CI) of marker levels at each time point after feedings. In a repeated measures model, time after eating did affect levels of D-dimer (*P*=0.014), IL-6 (*P*=0.003), IL-7 (*P*=0.010), sCD14
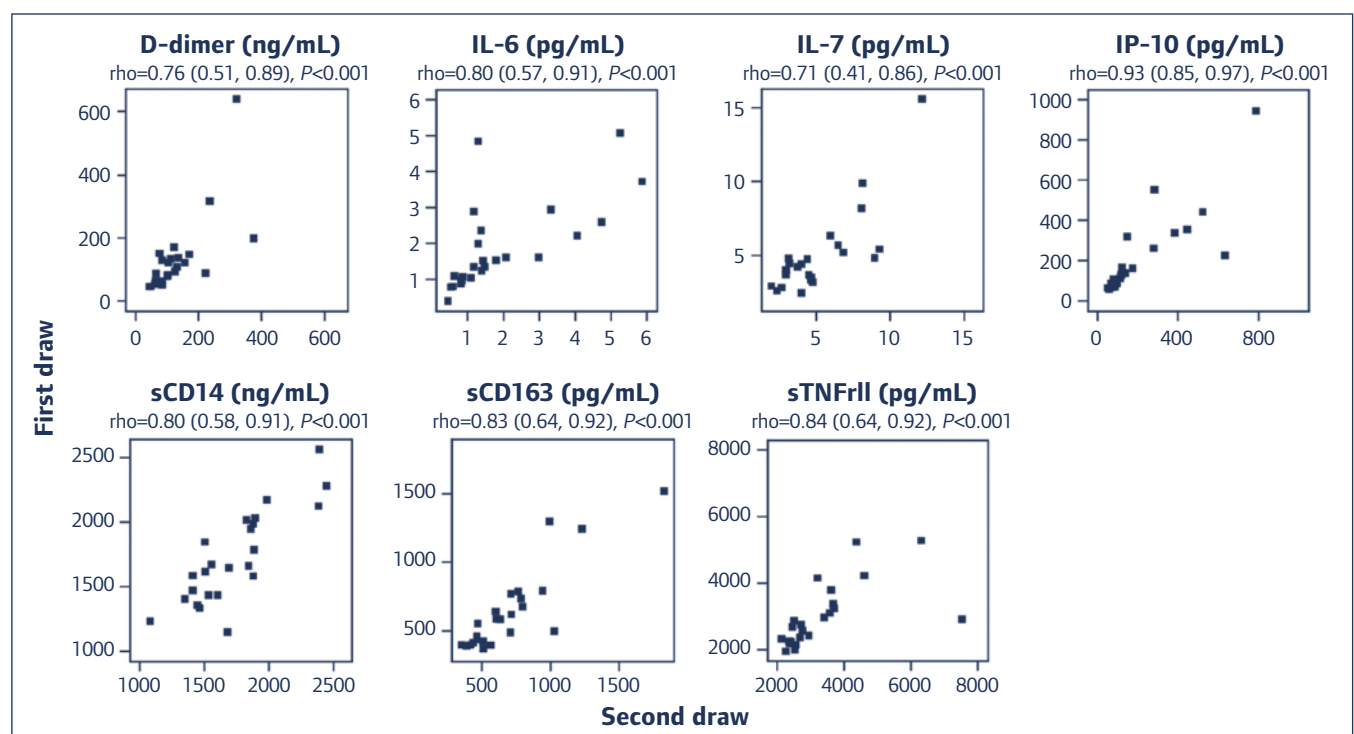

Figure 1. Fasting indices 1 week apart are correlated. rho=Spearman's correlation coefficient (95% CI)
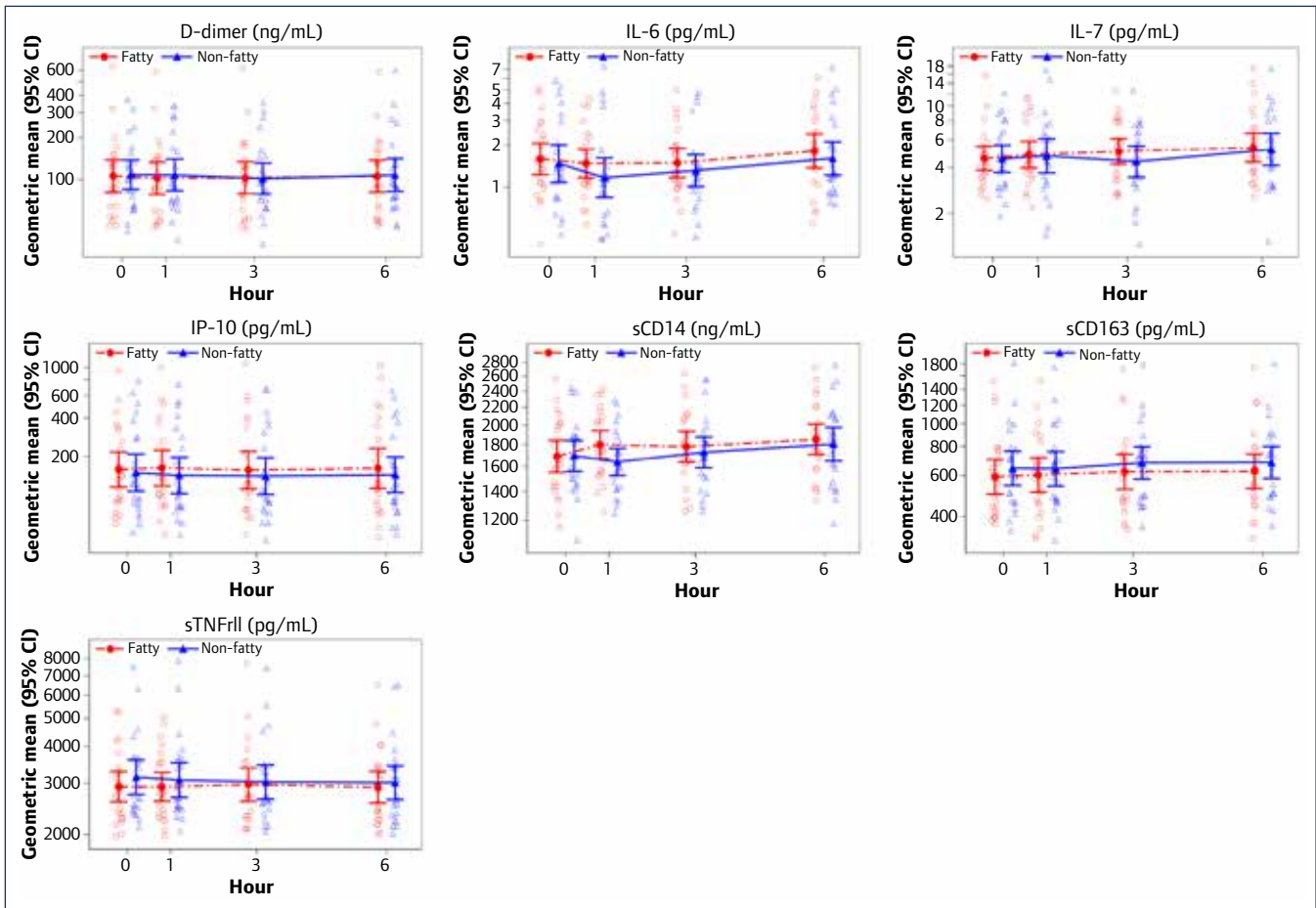
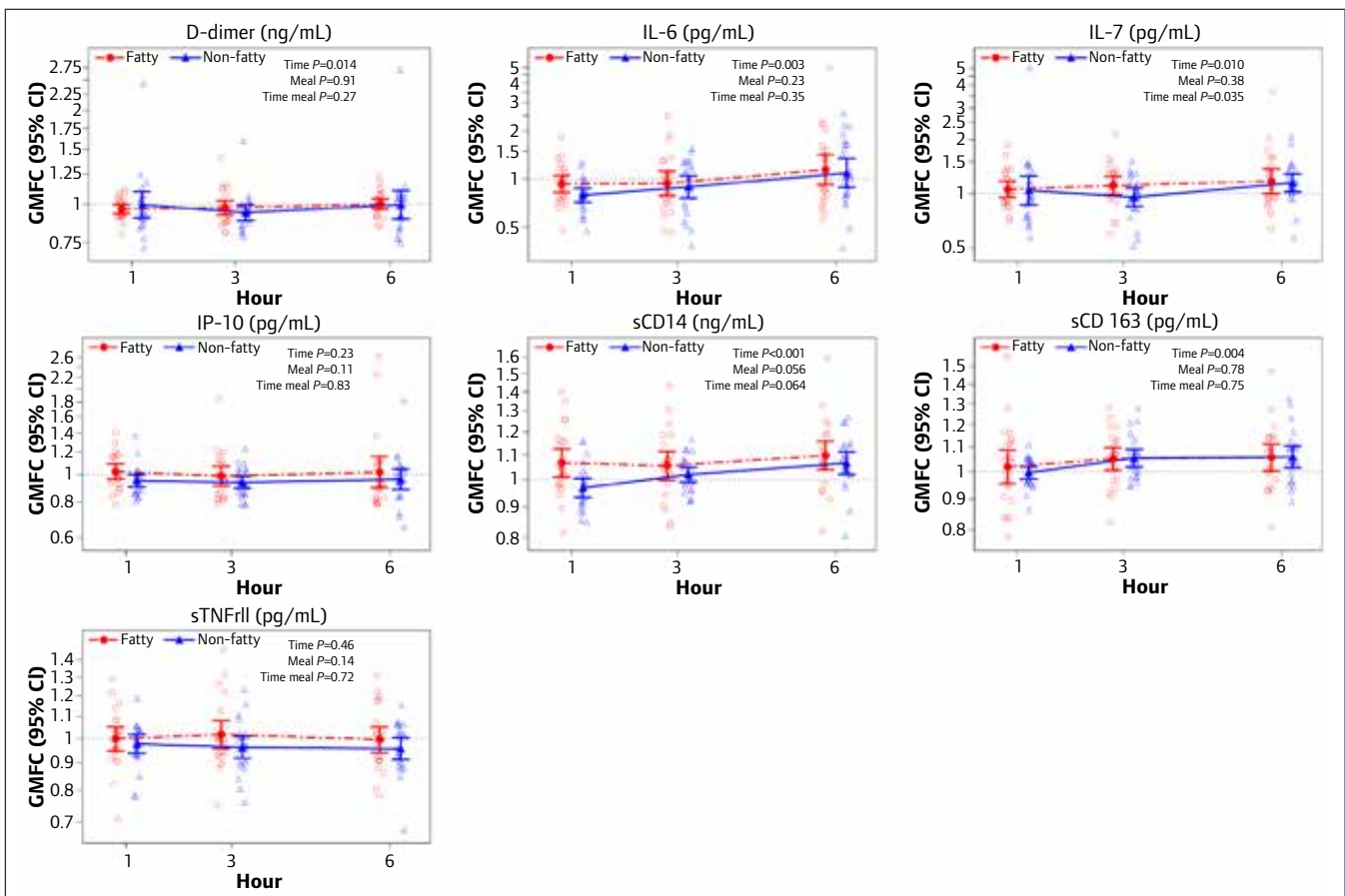**Figure 2.** Geometric mean (95% CI) plasma marker levels before and after feeding

Figure 3. Geometric mean fold change (95% CI) in plasma marker after feeding. GMFC: geometric mean fold change
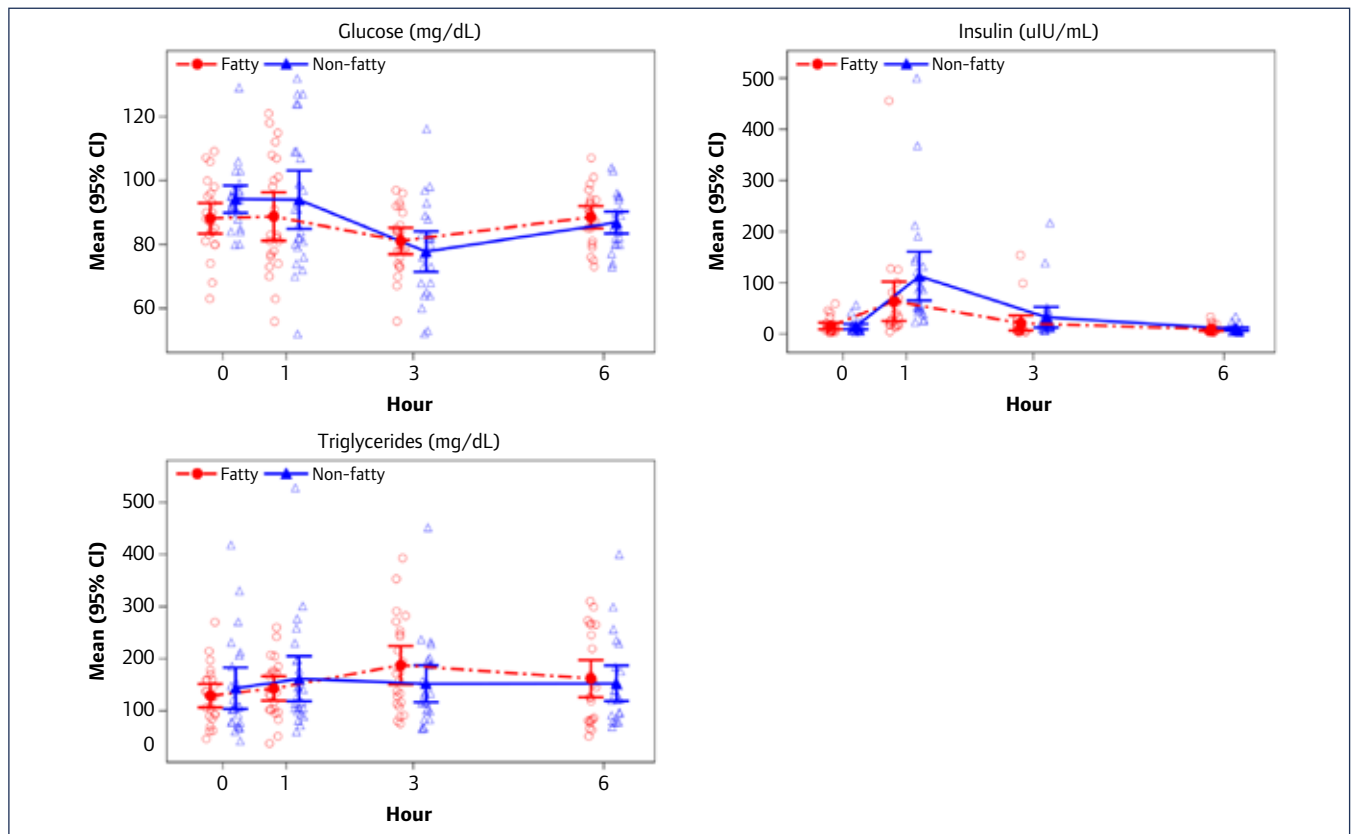
Figure 4. Serum glucose, insulin and triglycerides following fatty and non-fatty meals

(P<0.001) and sCD163 (P=0.004) but not levels of IP-10 or sTNFrII. For sCD14 there was an apparent effect of meal type that approached significance (P=0.056) and a suggestion that the time effect varied by meal type (interaction P=0.064). For IL-7 the effect of time varied by meal type (P=0.035). As shown on the figures, these effects, while statistically significant, were modest as the geometric means change by more than 10% only for IL-6 and for IL-7 (down 21% at 1 hour after a non-fatty meal and up 14% 6 hours after a fatty meal for IL-6; and up 11% and 18% at 3 and 6 hours after a fatty meal and up 15%, 6 hours after a non-fatty meal for IL-7).

Serum glucose, insulin and triglyceride levels at baseline and following feeding with fatty and non-fatty meals are shown in Figure 4. As expected, insulin levels rose with feeding, peaking at 1 hour after feeding and more so after the non-fatty meal that included a higher quantity of carbohydrates. Glucose levels fell at 1 and 3 hours after feeding (metabolic inhibitors were not included in the serum separator tubes) and triglyceride levels increased at 1 hour after feeding, peaking at 3 hours and more after the fatty meal than after the non-fatty meal.

We next considered if any patient characteristics (age, CD4 T cell count, smoking status [current, past or never] BMI or baseline measures of glucose, insulin, triglycerides) could be linked to the baseline inflammatory/coagulation markers or whether any of these characteristics could be linked to increases in these markers 1 hour after feeding. As there were only three women in the study, analysis of sex effect was not conducted. In order to be considered significant, baseline correlations were required to meet nominal levels of significance (P<0.05) for both blood draws. Homeostatic model assessment of insulin resistance (HOMA-IR) was correlated with baseline plasma levels of sCD163 (r=0.45 and P=0.03 and r=0.44, P=0.04 for the two blood draws). Patients who had increased D-dimer levels 1 hour after fatty meals had

higher baseline insulin and HOMA-IR levels than those who did not (12.6 *vs* 2.4 ng/mL, P<0.002 and 2.5 *vs* 0.4 ng/ml, P=0.005 respectively); those with increased IL-6 levels 1 hour after fatty meals had lower baseline glucose levels than those who did not (64 *vs* 68 mg/dL, P=0.042). Participants who had increased IP-10 levels 1 hour after a fatty meal were older than those who did not (38 *vs* 32 years, P=0.010), while patients with increased IP-10 levels 1 hour after a non-fatty meal tended to be younger than those who did not (37 *vs* 46 years, P=0.021). Patients with increased sTNFrII levels after non-fatty meals had higher serum glucose levels at baseline than those who did not (96 *vs* 91 g/dL, P=0.024).

BMI was not related to fasting levels or 1 hour post-feeding plasma levels of any of these markers except fasting plasma levels of sCD163 that were nominally correlated with BMI before non-fatty meals (r=0.42, P=0.039) and before fatty meals (r=0.35, P=0.10). Fasting, plasma markers and their 1-hour post-feeding changes did not differ among participants receiving protease inhibitor (PI), NNRTI, or INSTI-containing ART regimens. We considered whether the 1-hour changes in plasma levels of triglycerides would predict the 6-hour changes in plasma marker levels anticipating a delay between cellular exposure to these lipids and the induction of cytokine expression. Only sCD163 levels demonstrated such a relationship for triglyceride levels 1 hour after a non-fatty meal (r=0.39, P=0.05) but not after a fatty meal (r=-0.17, P=0.43). Triglyceride changes 1 hour after a fatty meal or 3 hours after either meal did not predict any marker changes at 6 hours.

Discussion

Recent work from several groups including our own has demonstrated that soluble markers of immune activation and inflammation predict the occurrence of serious morbidity and mortality

in people living with HIV who are receiving suppressive ART [2,3,8,9]. While robust assays are available to measure these indices, the week-to-week variability in levels of these markers is not well known and might be affected by recent meals especially among persons with diabetes mellitus [10,11]. We find here that among non-diabetic people with HIV with controlled viraemia and on ART, there is a strong correlation between fasting levels of inflammatory/homeostatic/coagulation indices IL-6, IL-7, IP-10, D-dimer, sTNFrII, sCD14 and sCD163 in two samples obtained approximately 1 week apart.

Among diabetic patients studied elsewhere, administration of fatty meals was linked to increases in selected inflammatory indices (hsCRP, TNF-α, and IL-6; [12]), (TNF-α, IL-6, VCAM-1, and ICAM-1 [6]) in some studies, but not in all studies. In one study conducted among type II diabetics, plasma levels of TNF-α and IL-6 fell after meals of potato starch plus eggs irrespective of addition of saturated or monounsaturated fatty acids [11]. As metabolic perturbations have been linked to inflammation in both diabetes and in HIV infection, we asked whether fatty meals might also increase levels of inflammatory/homeostatic and coagulation indices in non-diabetic persons with HIV and controlled viraemia on ART, and whether these changes could be attenuated by isocaloric feeding of a low-fat meal.

BMI was related to fasting plasma levels of sCD163 but did not predict 1-hour post-feeding changes in plasma levels of any marker studied. The inclusion of PIs, NNRTIs or INSTIs in an ART regimen did not seem to affect either fasting levels of these markers or their post-feeding levels; however, the numbers of subjects in each of these groups was limited. Although changes in plasma triglycerides 1 hour post feeding were linked to 6-hour changes in sCD163 levels after non-fatty meals, we suspect this finding is spurious as no relationship was found for any markers and triglyceride levels that were induced 1 or 3 hours after fatty meals.

We did find that over 6 hours following feeding, levels of D-dimer, IP-10, sTNFrII sCD14 and sCD163 varied but only modestly, with geometric mean indices varying less than 10% from baseline. More post-meal variation was seen in plasma levels of IL-6 and IL-7. With the information available here, we cannot determine whether these small differences are related to diurnal variations in levels of these markers or are related to feeding itself, but in any event, feeding of a fatty meal compared to an isocaloric non-fatty meal was related to modest differences in levels of IL-7 only and non-significant differences in levels of sCD14. Franchimont *et al.* found that corticosteroids increased expression of the IL-7 receptor [13] and Shimba *et al.* showed that, in mice, glucocorticoid receptor signalling drove T cell expression of IL-7R and CXCR4 that supported diurnal variation in receptor density and T cell distribution in blood and tissues [14]. Alternatively, serum levels of IL-7 manifest a circadian rhythm in humans during wakefulness but this is less clear over a regular sleep–wake cycle where significant variation was not seen during daylight hours. [15]

Conceivably, feeding and the systemic translocation of microbial products that is induced by feeding accounts for small increases in monocyte/macrophage-related inflammatory indices (sCD14, SCD163) found after 6 hours. Consistent timing of blood draws in people with HIV (morning or afternoon) could further minimise these slight variabilities in monocyte/macrophage inflammatory indices.

As inflammatory and homeostatic indices contribute to the expression and persistence of HIV in people undergoing treatment for HIV [16], these findings will aid in the monitoring of studies to understand or alter the determinants of HIV persistence.

There are limitations to this work. This study was performed among non-diabetic individuals with HIV in order to directly examine the effects of feeding in in people with HIV without the potential confounding of diabetes and its metabolic and inflammatory effects. Thus, these findings cannot be extended to the common setting of HIV infection complicated by diabetes mellitus. Also, we cannot be confident that the few modest changes observed after feeding reflect an effect of feeding or diurnal variation in levels of these inflammatory indices.

In summary, fasting plasma levels of IL-6, IL-7, sTNFrII, D-dimer, sCD14, IP-10 and sCD163 are significantly correlated when drawn 1 week apart. After feeding fatty or non-fatty meals, levels of most of these indices were reasonably stable over 6 hours with only modest, but nominally significant, effects of time or feeding on levels of D-dimer, IL-6, IL-7, sCD14 and sCD163. It is unclear whether these increases are related to feeding or reflect diurnal variation in regulation.

Acknowledgements

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