pute the possibility that CD38-deficient neutrophils could be defective in other functional assays. Thus, if β_2 integrin–induced Ca^{++} release is shown to be cADPR dependent, it would represent a good candidate for another CD38-dependent signaling process in neutrophils.

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Frances E. Lund, Troy D. Randall & Santiago Partida-Sánchez
Trudeau Institute
Saranac Lake, New York, USA
Email: flund@trudeauinstitute.org

Dyslipidemia due to retroviral protease inhibitors

To the editor—The biochemical basis for the development of lipodystrophy in HIV-1-infected patients treated with retroviral protease inhibitors (PIs) is unclear. Liang et al.[1] demonstrate that the PIs ritonavir and saquinavir affect fat metabolism through alterations in neutral lipid synthesis and the secretion of apolipoprotein B (ApoB). The authors also propose that inhibition of proteasomal chymotryptic activity by ritonavir and saquinavir contributes to the observed intracellular accumulation of ApoB *in vitro*. Whereas the latter effect requires drug concentrations in the region of 50–100 μM, the former effects occur at 5–15 μM. For several reasons, we question the relevance of effects on proteasome function to the development of metabolic abnormalities associated with PI-induced lipodystrophy *in vivo*.

First, although we agree that ritonavir and saquinavir inhibit the chymotryptic activity of isolated 20S proteasomes *in vitro*, two other HIV-1 PIs, indinavir and nelfinavir, do not[2,3]. The use of either indinavir or nelfinavir would therefore have provided relevant controls for the experiments attributing intracellular accumulation of ApoB to inhibition of proteasomal activity. Further, although ritonavir does seem particularly likely to induce severe dyslipidemia, this metabolic complication of therapy is a feature of all retroviral PIs. Thus, it is difficult to attribute PI-associated lipodystrophy primarily to proteasome inhibition.

Second, the authors claim that the effects on ApoB metabolism attributed to inhibition of the proteasome were seen at therapeutically relevant concentrations of ritonavir and saquinavir. The *in vitro* IC$_{90}$ (90% inhibitory concentration) of ritonavir is approximately 100 nM and that of saquinavir is less than 50 nM (ref. 4). Maximum plasma concentrations (C$_{max}$) of these drugs *in vivo* are also well below the levels used by Liang et al. The C$_{max}$ of ritonavir is approximately 15 μM (ref. 4). The C$_{max}$ for saquinavir, even in ritonavir-boosted regimens, is less than 10 μM, and in non-boosted regimens is 10-fold lower[5]. Although hepatocytes might be exposed to higher drug concentrations before systemic redistribution, such exposure is likely to be transient. Moreover, as both drugs are highly protein bound, any extrapolation of drug effects from *in vitro* to *in vivo* must take into account differences in protein concentrations. For any given total drug concentration, the pharmacologically active free drug levels are likely to be significantly higher *in vitro*, where serum concentrations are typically around 10%.

Third, we have found that concentrations greater than 10 μM of ritonavir and saquinavir compromise the viability of several B- and T-cell lines *in vitro*[3]. The validity of drug-mediated cellular effects detected under such conditions is questionable.

The inhibition of both neutral lipid synthesis and ApoB secretion reported by Liang et al. occurs at concentrations of ritonavir and saquinavir that are feasible *in vivo*. Interestingly, the authors state that indinavir also causes similar alterations to neutral lipid synthesis. It may be that these *in vitro* drug effects provide insight into the generation of the lipid abnormalities that complicate the treatment of HIV-1 infected individuals with retroviral PIs. We would, however, caution against overemphasizing the role of proteasome inhibition in the development of lipodystrophy for the reasons outlined above.

Anthony D. Kelleher[1], Andrew K. Sewell[2] & David A. Price[3]
[1]*National Centre in HIV Epidemiology and Clinical Research, Darlinghurst, NSW, Australia*
[2]*The Peter Medawar Building for Pathogen Research, South Parks Road, Oxford, UK*
[3]*Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford, UK*

Sturley et al. reply—The major issue raised by Kelleher et al. regards the pharmokinetics of HIV protease inhibitors such as