

2nd International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV



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MEETING REPORT: 2ND INTERNATIONAL WORKSHOP ON ADVERSE DRUG REACTIONS AND LIPODYSTROPHY IN HIV

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The 2nd International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV was held over 3 days from 13-15 September 2000 in Toronto, Canada. As the incidence of treatment-associated complications increases with the prolonged use of antiretroviral drugs, this annual Workshop has become a premier forum for the exchange of basic science and clinical data on the pathophysiology and management of this diverse and multi-factorial group of conditions.

The Workshop was opened with an introductory address by Dr Morris Schambelan (University of California San Francisco, USA) on the rapid identification of lipodystrophy and other adverse events related to antiretroviral therapy as important considerations in the management of HIV disease. In his speech, Dr Schambelan cited the recent emergence of lactic acidosis and osteopenia among HIV-infected individuals on therapy. He commented on the large increase in attendees and abstract submissions since the 1st International Workshop, held in San Diego last year. He also described how increasing cohort sizes have allowed the ongoing refinement of estimates for the prevalence of lipodystrophic changes since early reports of lipodystrophy on HAART in 1997.

Keynote address

Following Dr Schambelan's opening address, Dr Gerald Shulman (Howard Hughes Institute, Yale University, USA) gave an excellent keynote presentation, in which he

reviewed the clinical history of insulin resistance in type 2 diabetes and its association with elevated body weight. He presented data from an elegant series of studies involving NMR isotopic analysis of *in vivo* glucose metabolism in patients with type 2 diabetes, demonstrating that the rate-limiting step in this insulin resistance is an inhibition of intracellular glucose transport. This block is associated with elevated intracellular triglycerides and free fatty acids. Dr Shulman proposed, on the basis of recent investigations, that high intracellular lipid causes a serine kinase cascade that inhibits tyrosine phosphorylation of phosphoinositol 3 kinase — an important step in the activation of GLUT4 glucose transporters. Data were also reviewed, demonstrating that lack of adipose tissue in mice with lipodystrophy can also lead to insulin resistance and intracellular lipid accumulation in liver and muscle. This is caused by inappropriate storage outside of the adipocyte compartment, especially in the liver.

Animal models

Two transgenic mouse models of insulin resistance and lipodystrophy were described in invited lectures by Dr Marc Reitman (National Institutes of Health, USA) and Dr Iichiro Shimomura (University of Texas, USA), Dr Reitman described the A-ZIP/F-1 mouse, in which tissue-specific expression of a dominant negative inactivator of specific B-ZIP transcription factors results in adipose tissue ablation. These mice have virtually no white adipose tissue, reduced brown adipose tissue, a deficiency in the fat-derived, food intake and energy-regulating hormone leptin, severe insulin resistance and diabetes, and an accelerated adaptation to fasting. They also lack both adipose and non-adipose responses to the β_3 -adrenergic agonist, CL316243. Leptin treatment to physiological levels was shown to have little effect in these animals. However, transplantation of near-physiological amounts of wild-type adipose tissue into the A-ZIP/F-1 mice dramatically lowered insulin levels and improved muscle insulin sensitivity, as well as partially or completely reversing all other abnormal metabolic aspects of the A-ZIP/F-1 phenotype - including hyperphagia, hepatic steatosis and somatomegaly - in a dose-dependent manner. The results thereby demonstrate that the lack of adipose tissue is the cause of both the diabetes and other metabolic abnormalities of lipodystrophy in these mice. Dr Reitman indicated that the compound troglitazone improves levels of HbA_{1c}, triglycerides and free fatty acids in the treatment of both lipodystrophy and lipodystrophy. This compound may act through the adipose tissue itself, unlike leptin which targets the sympathetic nervous system, thus increasing metabolic rates. Clinical trials of troglitazone analogues may show an increase in body fat.

Dr Shimomura followed Dr Reitman's presentation with a discussion of a transgenic mouse line expressing a truncated version of the transcription factor nSREBP-1c (sterol-regulatory-element-binding protein-1c) under the control of the adipose-specific aP2 enhancer. This transcription factor is of major importance for control of adipocyte differentiation and cell response to insulin. Expression of the truncated form results in mice that mimic the symptoms of generalized lipodystrophy - reduced adipose tissue, insulin resistance, hyperinsulinaemia, hyperglycaemia and enlarged, fatty liver. Adipose tissue in this line is severely leptin-deficient, but unlike the A-ZIP/F-1 mice previously described, insulin resistance in these animals was found to respond to the continuous

systemic infusion of recombinant leptin, an effect that was not mimicked by chronic food restriction. Thus, it appears that leptin modulates insulin sensitivity and glucose disposal independently of its effect on food intake. Dr Shimomura also described the finding that despite the down-regulation of the important hepatic insulin signalling component, IRS-2, by the chronic hyperinsulinaemia in these mice — a situation resulting in insulin resistance — insulin continued to stimulate production of SREBP-1c. Since this transcription factor also activates fatty acid synthesis, the combination of inappropriate gluconeogenesis and elevated lipogenesis results in a vicious metabolic cycle that aggravates hyperinsulinaemia and insulin resistance. Dr Shimomura also mentioned that in their assay system primary hepatocytes had similar responses to the transgenic mice. He suggested that given the very low levels of plasma leptin found in lipodystrophic individuals, clinical trials of leptin treatment are warranted. In a question and answer session, Dr Reitman mentioned that a NIH study using leptin is underway, four patients have enrolled and one has been receiving the drug for a month.

Adipocyte biology and protease inhibitors

In an interesting corollary to Dr Shimomura's description of the metabolic effects of expressing a truncated SREBP-1c gene, Dr Jacqueline Capeau (INSERM, Faculté de Médecine St Antoine, France) discussed altered ADD1/SREBP-1 maturation and adipocyte differentiation following treatment with indinavir. The clonal proliferation and insulin responsiveness of 3T3-F442 pre-adipocytes grown in the presence of indinavir were not affected. However, indinavir inhibited adipose conversion by 50-60%, as assessed by (i) a reduction in the number of newly formed adipocytes; (ii) a lower level of the adipogenic markers ADD1/SREBP-1, PPAR γ and insulin receptors by Western blotting; and (iii) the absence of immunoreactive transcription factors, ADD1/SREBP and PPAR γ , from most treated cell nuclei by confocal microscopy. This partial loss of adipose conversion was found to correlate with defective SREBP-1 maturation in the presence of indinavir, identified by elevated expression of a partially processed SREBP-1 fragment and SREBP-1 accumulation at the nuclear periphery. Indinavir treatment also rendered the adipocytes resistant to insulin for MAP kinase activation at a step distal to IRS-1 tyrosine phosphorylation. Thus, it appears that indinavir impairs adipocyte differentiation and may promote adipocyte insulin resistance by inhibiting proteolytic maturation of SREBP-1, a finding that may help explain the subcutaneous cellular fat loss in PI-associated lipodystrophy. This finding of altered maturation of at least one important transcription factor for adipocyte differentiation was of particular interest with respect to poster data by Dr Greg Stevens (Pfizer, USA). GeneChip array analysis of 11 000 mRNAs, from both murine 3T3-L1 and human pre-adipocytes converted adipocytes in the presence of nelfinavir or indinavir (5-20 μ M), demonstrated altered expression patterns affecting multiple metabolic pathways. Of particular relevance were observed changes in the expression of essential genes for adipocyte differentiation, including the induction of the C/EBP signalling inhibitors, calpain and GADD153, and a decrease in C/EBP delta — required for initial signalling and maintenance of the adipocyte phenotype. The data therefore suggest an alternative mechanism of inhibition of C/EBP activation that may contribute to impaired adipocyte differentiation by PIs.

Dr Rex Parker (Bristol-Myers Squibb, USA) discussed work that compared the effect of NRTIs and PIs on adipogenesis and metabolism in murine cell culture. Differentiating CB1 and 3T3-L1 adipocytes were incubated with a variety of NRTI and PI agents and assayed for mitochondrial function (ATP measurement) at day 5 and intracellular triglyceride accumulation at day 6. Fully differentiated cells were assayed for lipolysis (by glycerol release) after 4 days of drug exposure. In order of decreasing effect on triglyceride accumulation, the five available PIs ranked nelfinavir ($IC_{50}=7.0 \mu M$) > saquinavir ($24 \mu M$) > ritonavir ($67 \mu M$) > indinavir ($140 \mu M$) > amprenavir ($150 \mu M$). For the NRTIs tested, this ranking was fialuridine ($84 \mu M$) > abacavir ($100 \mu M$) > zidovudine ($110 \mu M$) > stavudine = lamivudine ($120 \mu M$ each). A similar pattern was observed for increases in lipolysis, but ritonavir/stavudine or ritonavir/zidovudine co-incubation augmented their effects on both triglyceride accumulation and lipolysis compared with either drug alone. IC_{50} values for ATP synthesis for the PIs ranged from $76 \mu M$ (saquinavir) to $>1000 \mu M$ (indinavir and amprenavir), while all those for the NRTIs tested were $>1000 \mu M$. Dr Parker concluded that, under the conditions of the experiment, PIs were more potent inhibitors of adipogenesis and promoters of lipolysis than NRTIs, and that NRTIs had a minimal effect on ATP production.

Finally, Dr Reitman returned to give a brief account of the Forum for Collaborative HIV Research, held in early August in Washington. This group is trying to assess the interplay of the following factors in lipodystrophy syndrome: the contribution of drugs, the effect of the virus, the immune and inflammatory responses, diet and genetic background. Further experimental work needs to be carried out on the most appropriate tissues and animal models. There is a pressing need to investigate insulin resistance and diabetes in a range of cohorts. These will include comparing the above parameters in infected and uninfected subjects, and in both treated and untreated HIV-infected individuals.

Mitochondrial toxicity, hyperlactatemia and lactic acidosis

An introductory address on the primary and secondary causes of mitochondrial dysfunction and their role in human disease was given by Dr Anthony Shapira (University College Medical School and Institute of Neurology, London, UK). Dr Shapira described the broad spectrum of mitochondrial and nuclear genomic abnormalities, and the range of mitochondrially targetted toxins, that may lead to alterations in function and the subsequent expression of a remarkable range of disease states. With particular reference to the loss of mitochondrial DNA (mtDNA) and experimental systems for its determination, Dr Schapiro described the resultant defects in respiration and oxidative phosphorylation believed to contribute to lactic acidosis and other pathologies clinically observed on combination HIV therapy.

Dr Schapira's introduction was followed by two presentations describing the loss of mtDNA from subcutaneous adipose tissue in lipodystrophic individuals on antiretroviral therapy. In a study of subcutaneous fat biopsies taken from the buttocks of 24 HIV-infected patients, five of whom were NRTI naïve (four on NNRTI/PI treatment and one untreated), Dr Ulrich Walker (Albert-Ludwig University, Germany) showed a significant loss of mtDNA in the 19 patients exposed to NRTIs, compared to the four treated patients

who were NRTI-naïve ($P=0.009$). Furthermore, treated patients with clinical evidence of lipoatrophy ($n=11$) showed a 38% reduction in mtDNA compared with treated patients without lipoatrophy ($n=12$; $P=0.04$). There was no difference between the mtDNA content in the four NNRTI/PI-treated patients and a group of eight HIV-negative controls. Dr Walker concluded that the evidence suggested a potential link between mitochondrial damage and lipoatrophy. Similar findings and conclusions were presented by Dr Cecilia Shikuma (University of Hawaii at Manoa, USA), who presented preliminary results from an ongoing study of subcutaneous fat biopsies from the necks, abdomens and thighs of four small groups of volunteers. Using a semi-quantitative PCR assay, a comparison of mtDNA levels between lipodystrophic ($n=8$) and non-lipodystrophic ($n=7$) subjects on HAART revealed reduced or absent mtDNA in 87 versus 45% of all samples combined ($P=0.018$). In comparison, HAART-naïve ($n=2$) and HIV-seronegative ($n=7$) study groups showed mtDNA loss in 0 and 30% of samples, respectively. There was no statistical significance for the incidence of mtDNA loss between the three non-lipodystrophic groups. Curiously, an analysis of the loss of mtDNA by adipose tissue site showed significant reductions for abdominal and neck samples from the lipodystrophic group but no significant loss from thigh tissue. There was also no difference between the four groups for mtDNA loss in peripheral blood mononuclear cells.

Also of relevance to this topic was a poster presentation by Dr Justin St John (University of Birmingham, UK), demonstrating significantly more mtDNA deletions in spermatozoal DNA obtained from the semen of a small sample of HIV-infected patients on NRTI-based HAART for more than 12 months, compared with those with less experience of treatment ($P<0.05$). No patients ($n=3$) with <12 months HAART presented with multiple deletions, whereas all four experienced patients showed a wide range of multiple deletions, with the deletion rate increasing in a time-dependent manner in one patient for whom multiple samples were available. Only one of five antiretroviral-naïve patients exhibited multiple deletions, with none at all observed in the remaining four. Dr St John suggested that spermatozoal mtDNA analysis may provide a non-invasive method of monitoring treatment-associated mitochondrial damage.

Two posters addressed the incidence of hyperlactatemia associated with NRTI-containing therapy. Dr F Blanco (Instituto de Salud Carlos III, Spain) reported a finding of elevated venous lactate (>16 mg/dl) in 60% of a cross-sectional study of 127 patients with >6 months of therapy. In univariate analysis the conditions associated with a statistically significant higher incidence of hyperlactatemia were: male gender (67% hyperlactatemic versus 33% normolactatemic); homosexual behaviour (54 versus 19%); time on HAART (25 ± 13 versus 17 ± 13 months); elevated triglycerides (286 ± 283 versus 161 ± 131 mg/dl) and cholesterol (236 ± 67 versus 205 ± 52 mg/dl); and morphological changes associated with lipodystrophy (75 versus 39%). Dr Tyler Lonergan (University of California San Diego, USA) presented an analysis of the incidence rate (IR) by NRTI components of reproducible hyperlactatemia with either or both abdominal symptoms, or elevated alanine aminotransferase nor explainable by other factors in 33 patients receiving NRTIs identified between 1998 and 2000. The relative rates (RR) of hyperlactatemia for each regimen were estimated against a zidovudine plus lamivudine-containing standard regimen. The incidence rate (per 1000 person-years) of any stavudine containing regimen

was found to be 25.6 versus 1.9 for NRTI-containing regimens without stavudine, with estimates of the relative rate compared to zidovudine/lamivudine of 11.6 ($P=0.018$) for stavudine plus lamivudine, 15.0 ($P=0.027$) for stavudine plus abacavir, 36.3 ($P=0.001$) for stavudine plus didanosine, and 147.9 ($P<0.0001$) for stavudine plus didanosine plus lamivudine. The relative rate for lamivudine as the only NRTI was 10.5, which failed to achieve statistical significance at the 5% level ($P=0.096$). Eleven patients were rechallenged with alternative NRTIs for >6 months and none have had a recurrence of the syndrome. A corollary to these findings was given in an oral presentation by Dr Matthew Law (NCHECR, Australia) in a set of patients taking zidovudine plus lamivudine ($n=21$), stavudine plus lamivudine ($n=35$), or stavudine plus didanosine ($n=28$) in addition to indinavir (Ozcombo I trial) or nevirapine (Ozcombo II). The incidence of peripheral lipodystrophy was significantly higher for stavudine plus didanosine across both studies ($P<0.05$). Central fat change differences were only apparent for the comparison of patients taking indinavir with those taking nevirapine ($P<0.001$).

Dr Mark Paulik (Glaxo Wellcome, USA) described a study of postprandial serum chemistries, liver weights and microarray analysis of liver mRNA for oxidative stress genes in AKR/J mice treated for 2 weeks with NRTIs or placebo, in the presence or absence of ascorbate and α -tocopherol. Mice treated with stavudine at 5 mg/kg twice daily displayed significantly elevated triglycerides and reduced non-esterified fatty acids ($P\leq 0.05$), three- to fourfold elevated triglyceride synthesis, a 50-60-fold increase in liver TNF- α and higher levels of serum lactate, compared with controls. Stavudine-treated mice also displayed increased liver weight and significant changes in 86/144 liver oxidative stress genes. Furthermore, stavudine reduced the expression of several genes involved in lipid metabolism in treated mice, and promoted mitochondrial β -oxidation in mouse primary hepatocyte culture. Treatment with ascorbate and α -tocopherol reduced or abolished the effects of stavudine on liver weight, lactate, lipid metabolism gene expression, TNF- α , AP and most (71%) of the affected oxidative stress genes. In contrast, Dr Paulik reported that mice treated with 5 mg/kg zidovudine twice daily showed no changes in triglyceride or NEFA, but added the caveat that lipid changes were observed at 50 mg/kg dosing. This led to criticism from the floor during the subsequent question period, concerning the comparative dosing of zidovudine and stavudine at 5 mg/kg with respect to their use in human HIV therapy and for the omission of data for stavudine at lower doses in this protocol.

Insulin resistance, carbohydrate metabolism and protease inhibitors

Dr Ralph Germinario (McGill University AIDS Centre and Lady Davis Institute for Medical Research, Canada) presented data on the effects of protease inhibitors on glucose transport and insulin binding in a variety of cell lines. Saquinavir (1 μ M) was found to increase basal transport of tritiated 2-deoxyglucose (2DG) in Jurkat lymphocytes, human fibroblasts and L6 myotubes, resulting in a reduced insulin-mediated:control transport ratio in both fibroblasts and myocytes, but had no effect on 2DG transport in 3T3 L1 adipocytes. This elevation was observed in Jurkat cells for up to 49 days in the presence of saquinavir. Saquinavir, ritonavir and indinavir all reduced specific binding of 125 I-

insulin in adipocytes over the period of adipocyte induction, with treated cells showing 40-50% of control cell binding at full induction (15 days drug exposure).

Dr Mustafa Noor (Veterans Affairs Medical Center and University of California San Francisco, USA) reported the results of a study of the metabolic effects of indinavir (800 mg three times daily) in the absence of HIV infection in a group of 10 healthy, HIV-negative male volunteers over a period of 4 weeks. Carbohydrate metabolism, fasting lipid profile and body composition were measured at baseline and at the end of the 4-week study period. No changes in plasma lipids and lipoproteins were observed over this period, but significant insulin resistance was observed. Fasting plasma glucose and insulin were both increased, as were the insulin/glucose ratio and HOMA insulin resistance index. In an oral glucose tolerance test, both glucose and insulin levels were increased at 2 h, and a significant decrease was observed in insulin-mediated glucose disposal rate during euglycaemic hyperinsulinaemic clamp. A small decrease in total body fat (DEXA) was noted, but visceral abdominal fat (CT) was unchanged. In contrast to these findings, and further underscoring the probably differing pathogenic mechanisms of glucose and lipid dysregulation, a prospective study of the effects of amprenavir-based therapy was presented as a poster by Dr Michael Dubé (Indiana University, USA). Here it was found that a significant increase in fasting cholesterol was observed after 8 weeks but no detectable effect on glucose metabolism was observed in a group of 10 HIV-infected, previously PI-naïve individuals on amprenavir plus lamivudine and abacavir.

Dr Kees Brinkman (OVLG-Amsterdam) rounded off the session with a brief summary of the major recommendations from the recent Forum on Mitochondrial Toxicity held in Washington in June of this year. The importance of evaluating simple biochemical tests, developing a case definition for further studies and the establishment of a registry for mitochondrially-linked diseases was discussed. The Forum participants also called for a number of other introductions to the field; including the standardization of assays and animal models, more research into dysfunction not linked to DNA polymerase γ and other contributing factors, and the development of methods to assess clinical interventions.

Body composition

Dr Ronnen Roubenoff gave his plenary lecture on the methods currently available to measure body composition. He reminded the audience that the conditions of wasting, cachexia and sarcopenia may need consideration in the context of body mass changes observed in patients with HIV. The first involves loss of mass from all body compartments, while cachexia may involve loss of cell mass and is associated with an alteration in metabolites and cytokine concentration and sarcopenia is the normal process of muscle loss with increasing age. He indicated that the methods of measuring body mass have improved, but all rely on assumptions that bias the results. Anthropometry is still a preferred method for measuring regional fat when carried out at a single site by the same investigator. The use of newer scanning techniques (DEXA, CT and MRI) make fewer assumptions and reduce bias. Although these techniques are expensive, they allow the study of large cohorts and can identify small but significant differences within the patient population. Dr Roubenoff stressed that all these techniques have to be used in

sequential longitudinal studies over a long period of time for any improvements or changes in condition to be meaningful.

Dr Steven Grinspoon (Massachusetts General Hospital, USA) described studies of growth hormone (GH) in patients with HIV lipodystrophy syndrome. His data indicated that increased visceral abdominal fat is linked to a reduction in GH levels. Patients with the HIV lipodystrophy syndrome ($n=21$) were compared with HIV-infected, non-lipodystrophic individuals ($n=20$) and uninfected controls ($n=20$) matched for age and BMI. Groups were compared for visceral abdominal fat levels (VAF), ratio of VAF to subcutaneous abdominal fat (SAF), mean overnight GH levels (from 20 min sampling at 20.00 h to 08.00), baseline GH and peak GH levels. In all cases there was a significant difference ($P<0.05$) between patients with lipodystrophy and those without and controls. However, in a multivariate regression model, controlling for age, BMI, body fat and visceral fat, only visceral fat was a significant predictor of reduced mean GH concentration ($P=0.0036$, $r^2=0.40$). On a related note, the value of low-dose GH treatment for lipodystrophy was discussed by Dr Joan Lo (University of California San Francisco, USA) in a study of seven HIV-infected individuals with fat accumulation (four buffalo hump and three abdominal obesity). Subjects were given GH (3 mg/day) for 6 months, with glucose metabolism, substrate utilization and body composition assessed at baseline and months 1 and 6. GH was discontinued in one subject at week 3 for hyperglycaemia, baseline oral glucose tolerance results indicated pre-existing diabetes despite normal fasting glucose, demonstrating glucose intolerance as a risk factor for GH-induced hyperglycaemia, and resulted in the exclusion of subsequent subjects with overt glucose intolerance. Of the five subjects who completed the trial, all showed clinical improvement in body composition, with a mean fat mass decrease of 4.4 kg (3.7 kg trunk fat) and a lean body mass increase of 5.4 kg. Over the treatment period, insulin sensitivity and glucose tolerance worsened initially, but improved towards baseline by the end of the trial. Dr Kenneth Lichtenstein discussed the changes that occur in HIV-associated fat maldistribution over time. A total of 1077 patients in the Hospital Outpatient Study were surveyed for the presence of fat maldistribution in the last quarter of 1998. Signs of atrophy included sunken cheeks, and fat loss on extremities, hips and buttocks and signs of fat accumulation via facial fat over parotid glands, dorsal cervical fat pad and abdominal visceral fat accumulation. Of these patients, 307 (28.5%) returned to their clinics in April–May 2000 and were once again evaluated. The increases in fat maldistribution for these 307 patients over the 20 months were generally modest, but a comparison of atrophy versus accumulation revealed markedly greater incidence of new lipodystrophy at follow-up (24.8%) versus accumulation (7.9%). Improvement rates for those patients with maldistribution at the initial survey time point were also poorer for atrophy (17%) versus accumulation (40%). However, it should be noted in this study that less than half the cohort had returned for reassessment by the time of the presentation, so changes observed are difficult to interpret.

Risk and prevalence of lipodystrophy

Dr. Esteban Martinez (Hospital Clinic Universitari, Spain) discussed the risk factors for lipodystrophy (LD) in 494 previously treatment-naïve patients who initiated HAART

with two NRTIs plus at least one PI between October 1996 and September 1999. Fat changes were categorized as subcutaneous lipoatrophy (SL), central obesity (CO) or both. Both increasing age (relative hazard 1.5 per 10 years older; 95% CI 1.2-1.8) and duration of therapy (RH 1.5 per 6 months extra; 95% CI 1.2-1.7) were associated with increased risk of any LD, but not the use of any individual drug. Risk factors for LD with SL or LD with CO were similar to those associated with any LD. However, in the case of LD with CO, a trend was observed towards duration of indinavir use but not duration of HAART as an additional risk factor (RH 1.3 per 6 months extra; 95% CI 1.0-1.6, $P=0.07$). It was also found that long-term use of stavudine increased the risk for LD with SL (RH 1.2 per 6 months extra; 95% CI: 1.0-2.0).

Three brief oral presentations described the results of surveys for lipodystrophy in patients from Asia and Africa. Dr Nicholas Paton (Communicable Disease Centre, Singapore) discussed the prevalence of lipodystrophic changes in a study of Asian outpatients with HIV infection. In the 319 evaluable individuals from the predominantly Chinese (82%) male (85%) cohort studied, 30% were treatment-naïve, 34% were on NRTIs plus NNRTI regimens without PIs, and 36% were on a PI-based regimens. Self-assessed fat loss between the three groups was similar (39, 39 and 43%, respectively; $P=0.79$). However, fat accumulation (25, 34 and 53%; $P<0.01$.) and mixed accumulation and atrophy (6, 7 and 18%; $P=0.0006$) were significantly more common in PI-treated patients. Dr Paton pointed out that the relatively high incidence of body changes in the treatment-naïve group underscores the difficulty of separating drug-induced lipodystrophy from natural variations due to the disease course, even after excluding cases of wasting and opportunistic infection. Dr Ernest Ekong (Military Hospital, Lagos, Nigeria) also concluded that drug-induced lipodystrophy, particularly truncal obesity and wasting of buttocks, was most common in PI-containing regimens. The data were obtained from a cohort of 84 individuals, of whom 75% were male. Fifty-nine patients received PIs and 30% of these were also receiving NRTIs. The average exposure time for PIs and NRTIs was 4 and 7 months, respectively. Fifteen percent of those patients who were receiving NRTIs experienced changes in body shape, compared with 35% of those receiving PIs. Interestingly, patients' assessment of changes were lower (48%) than that of the physicians monitoring the study (61%). A similar study was reported by Helen Fraser (AIDS Clinical Centre, International Medical Centre of Japan, Toyko). She pointed out that the lower serum lipids and BMI observed in Japanese people might be expected to influence the prevalence of HIV-associated lipodystrophy in this group, when compared with the Western cohorts in which most research has been done. However, a cross-sectional study of 192 patients (95% male) presenting at the clinic between January and May 2000 revealed a similar prevalence of lipodystrophic changes to those observed in Western cohorts. The overall incidence of central fat accumulation was 46%, of which 81, 12 and 7% of the total was for individuals taking PIs, those taking ARV without PIs and those ARV-naïve, respectively. Peripheral lipoatrophy was found at a prevalence of 51% (75, 15 and 10%, respectively) and mixed atrophy/accumulation at 64% (75, 14 and 11%, respectively). In contrast with the findings in the Nigerian cohort, the prevalence of physician-assessed changes in body habitus for this Japanese group (62%) was similar to the patient-assessed figure (59%).

Dyslipidaemia

Dr Ronald Krauss gave an introductory lecture on the complex interplay between factors for coronary heart disease, beginning with the historical association of raised triglycerides and lowered HDL with an increased incidence of coronary heart disease (CHD). He went on to describe how elevated triglycerides lead to a change in the nature of LDL particles, making them smaller and reducing their clearance rates, and described the mechanisms by which hypertriglyceridaemia (HTG) generates these smaller particles and the metabolic factors involved in their production and potential atherogenicity. Pointing out that patients on PI drugs show the same reduction in LDL particle size as those members of the normal population historically at risk of CHD, he described how genetic and dietary factors may combine to affect the clinical outcome in these individuals.

Several investigators presented differing or complementary mechanisms that may contribute to the pathogenesis of PI-associated HTG. Dr Henry Ginsberg (Columbia University, USA) reported the results of an *in vitro* study of the effects of protease inhibitors on the secretion of apolipoprotein B (apoB), the major component of very low-density lipoprotein, from HepG2 and McArdle RH7777 hepatoma cells. Treatment of either line for 2 h with 5-100 μ M saquinavir or ritonavir significantly reduced proteosomal degradation of apoB and was associated with a block in apoB secretion in the absence of elevated lipid synthesis promoted by co-incubation with oleic acid (OA). Co-incubation with PI and 0.4 mM OA elevated HepG2 apoB secretion by 65% compared to cells treated with OA alone and with the same density distribution, while a 45% induction over 2 h of OA treatment was also observed with sequential exposure of cells for 90 min with ritonavir, followed by removal of PI and addition of OA. Dr Ginsberg concluded that elevated secretion of apoB may provide a molecular basis for PI-associated HTG.

The following oral presentation by Oliver Distler (University of New South Wales, Australia) continued this story by demonstrating the inhibition of sterol esterification by ritonavir and, to a lesser extent, saquinavir. Reduced esterification was observed both in HepG2 cells and in a cell-free microsomal assay via the acyl-coenzymeA-cholesterol O-acyltransferase (ACAT) reaction. This effect in cells was transient, as determined by the rapid restoration of baseline esterification on removal of PI, and was significantly reduced by co-incubation with 0.4 mM OA. Microsomal triglyceride transfer protein activity was also found to be partially blocked by these PIs, as was SREBP activity, with a corresponding drop in SRE-mediated transcription. Dr Distler hypothesized that these findings of intracellular apoB accumulation and reduced sterol esterification, both reversible by fatty acid exposure, may result in post-prandial bursts of LDL secretion. He also suggested that inhibition of MTP, the ACAT reaction and SREBP activity may affect multiple aspects of lipoprotein formation and lipid metabolism, further contributing to PI-associated dyslipidaemia.

While dual-NRTI therapy is known to be associated with body composition changes that are clinically indistinguishable from PI-associated lipodystrophy, some interesting differences in the effects on lipid metabolism of NRTIs versus PIs in lipodystrophic

patients were presented by Dr Lisa Ware (University of Southampton, UK). Using ^{13}C -labelled palmitic acid as a metabolic tracer, Dr Ware and her colleagues measured total triacylglycerol (TAG) and non-esterified fatty acids (NEFA) before, and for 7 h after a meal, in a group of seven PI-naïve lipodystrophic patients on dual-NRTIs, and compared results to those observed for six patients with PI-associated lipodystrophy. While the dual-NRTI group had a threefold smaller area under the post-prandial concentration–time curve (AUC) for TAG than PI-treated subjects ($P<0.05$), they also had a 1.5-fold increase in the AUC for NEFA ($P<0.05$). The difference in the form in which dietary lipid is retained in the circulation for individuals taking NRTIs compared with those on PIs, led Dr Ware to conclude that body composition changes may be influenced by different metabolic effects for the two groups.

Peripheral lipoatrophy with the accumulation of trunk fat was found to be associated with mild elevations of triglycerides, but not cholesterol, in a study of prepubertal HIV-infected children presented by Dr Stephen Arpadi (Columbia University, USA). Eight out of 28 (29%) children exhibited lipoatrophy of arms and legs along with trunk fat accumulation (ARF+) in a longitudinal, observational study between 1994-1999, associated with the use of PIs (6/8 ARF+ versus 6/20 ARF-; Odds Ratio 7.0; 95%CI 1.1-45.2; $P=0.044$) and current stavudine use (6/8 versus 5/20; OR 9.0; 95%CI 1.4-59.8; $P=0.03$). There were no statistically significant differences in mean triglyceride or cholesterol between the ARF+ and ARF- groups at baseline or follow-up at a mean interval of 1.2 years. However, there was a trend towards changes in triglyceride within the ARF+ group, with 38% of ARF+ children experiencing an elevation from <130 to >130 mg/dl during the study compared with 0.05% of ARF- children (Fisher exact=0.058). This elevation was associated with PI treatment (Fisher exact=0.024) but not with other ARV agents or immunological parameters.

Complementary to the risk of dyslipidaemic factors for heart disease, Dr Colleen Hadigan (Harvard Medical School, USA) reported significantly elevated levels of fibrinolytic markers that might also contribute to increased cardiovascular disease risk in HIV-infected patients with lipodystrophy. In a case-controlled study (three controls per case), 86 HIV-positive individuals with fat redistribution on stable ART were compared to 258 gender-, age- and BMI-mapped controls from the Framingham Offspring Study. Both tPA and PAI-1 antigen levels (both indicative of impaired fibrinolysis) were higher in the lipodystrophic cohort ($P<0.0001$). A 12-week, double-blind controlled trial of metformin (500 mg orally, twice daily; $n=11$) versus placebo ($n=14$) was undertaken in two groups of lipodystrophic subjects. PAI-1 and tPA levels were significantly reduced in the metformin group at 12 weeks ($P\leq 0.03$) and the tPA level change was highly correlated with the change in the insulin AUC ($r=0.43$; $P=0.03$). Dr Hadigan concluded that, while patients with fat redistribution display markers consistent with increased CVD risk, the risk may be improved by treatment with metformin.

Atherosclerosis and cardiovascular disease

Two plenary talks introduced this session on the incidence of cardiovascular disease, which is being recognized more frequently in the treatment of HIV with antiretroviral

drugs. Dr Frank Ruschitzka gave a detailed account of the role of nitric oxide (NO) in maintaining the normal responses of endothelial tissue and its role in inhibiting aggregation and adhesion in arteries. He then concentrated on the role of endothelin-1 in heart disease and how oxidized-LDL blocks NO activity. This stimulates endothelin-1 release, causing a large range of conditions including hypertension, heart failure and atherosclerosis. If antiretroviral drugs produce lipid abnormalities, then the use of endothelin antagonists may help to resolve atherosclerosis. Dr Mathias Egger (University of Bristol, UK) discussed the concept of risk benefit of cardiovascular complications in treating patients with HAART. He argued that it is essential to contrast the adverse effects of cardiovascular risk with the benefits of HAART. Furthermore, although lipodystrophy is associated with an increase in the risk for chronic heart disease, it is essential to develop a paradigm that, as well as including the drug regimen, also includes the patients age, their sex, lipid profile, insulin resistance, smoking habits and family history.

Dr James Stein (University of Wisconsin, USA) described a study of the effect of PI therapy on flow-mediated vasodilation (FMD) of the brachial artery using high-resolution ultrasound. HIV-infected individuals on stable PI (PI+; $n=22$) and non-PI (PI-; $n=15$) regimens for ≥ 6 months were compared. The two groups were well matched for age, gender, time since HIV diagnosis and CD4 cell count. Impaired FMD — indicating significant endothelial dysfunction, and thereby possible risk of atherosclerosis and cardiovascular disease (CVD) — was observed in the PI+ group ($2.6 \pm 4.6\%$), whereas FMD was normal in PI- subjects ($8.1 \pm 6.7\%$, $P=0.005$). Cholesterol (total, HDL- and LDL-associated), fasting glucose and systolic blood pressure were similar in both groups, but weight and triglycerides were higher in PI+ individuals ($P=0.069$ and 0.023 , respectively). In a separate study of CVD risk in PI-treated individuals, Dr Renato Maserati (Infectious Disease Department, Cremona, Italy) presented the results of ultrasound assessment of the carotid intima media thickness (IMT) in PI-treated ($n=28$; ≥ 18 months treatment) and treatment-naïve ($n=15$) HIV-infected individuals and in uninfected controls ($n=16$). All subjects were matched for age, sex, risk factors for HIV infection and cigarette use. The mean carotid IMT was significantly greater in the PI-treated group compared with the naïve and uninfected groups (0.63, 0.45 and 0.5 mm, respectively; $P=0.00033$), suggesting an increased risk of coronary artery disease in PI-based therapy. In addition, a correlation between triglycerides, apoB, HDL cholesterol and IMT was observed across the whole cohort, indicating that the measurement of blood lipid profiles may be a safe and efficient means of identifying those at risk from atherosclerosis.

Dr Frank Goebel (Ludwig-Maximilians-Universität, Germany) presented an assessment of the risk factors for CVD and atherosclerosis associated with PI-induced hypercholesterolaemia. Measurements of cardiovascular function, atherosclerosis and plaque formation were made in a group of HAART-treated individuals ($n=11$; ≥ 24 months treatment) with serum cholesterol >300 mg/dl, and compared with HIV-positive, treatment-naïve subjects ($n=4$) and uninfected controls ($n=10$; both latter groups with cholesterol <200 mg/dl). Positron emission tomography was used to determine heart rate (HR), rate pressure product (RPP), myocardial blood flow (MBF) and coronary vascular

resistance (CVR), both before and after adenosine-induced stress. Electron beam tomography was used to assess calcium density in the coronary arteries, from which an age-adjusted calcium score was determined. The structural pathology of the coronary arteries, as assessed by calcium score, was not affected in the HAART group, with only three out of eight subjects studied showing mild elevations compared with normal age-adjusted values. However, this group showed a significantly blunted response to adenosine-induced stress for HR, RPP and MBF, as well as a lesser decrease in CVR on administration of adenosine. Interestingly, the treatment-naïve group showed a comparable reduction in HR and RPP response to adenosine stress to that observed in the HAART group, when compared against the uninfected controls. Dr Goebel concluded that while 2 years of HAART-associated hypercholesterolaemia does not significantly affect the structural pathology of the coronary arteries, both HAART and untreated HIV infection may promote cardiovascular dysfunction.

Switching antiretrovirals

In an invited lecture, Dr William Powderly (Washington University School of Medicine, USA) highlighted the fact that many of the studies reported concerning the improvement of metabolic toxicity on substituting antiretroviral drugs are uncontrolled or of short duration. It appears that some metabolic changes are reversible with time. For example, switching from a PI to a NNRTI or a third NRTI generally lowers triglyceride levels and insulin resistance, although it is not usually associated with reversals or improvement in fat redistribution or bone disease.

A number of poster presentations addressed alterations in metabolic parameters and body composition after changes in antiretroviral therapy. Among them, two posters by Dr Kevin Yarasheski (Washington University Medical School, St Louis, USA) discussed the improvements in lean body mass and insulin tolerance observed when patients were switched to nevirapine from PI treatment. At 6 months from baseline, insulin tolerance improved in 14 study participants ($P=0.03$), although the statistical significance fell to 0.10 at month 9 after therapeutic switch. Furthermore, HDL-cholesterol increased and triglycerides declined ($P<0.05$) at both timepoints and fasting glucoregulatory hormones were normalized. Despite an increase in whole body and thigh lean mass at 9 months ($P<0.01$), trunkal lean mass and abdominal lean tissue area did not increase, and the observed improvements did not appear to be due to muscle tissue. It was speculated that removal of PIs from regimens augment non-contractile protein mass (connective tissue, collagen and skin) and fluid accumulation. Thus, PIs may block the normal development of non-contractile proteins. Switching to nevirapine may augment macronutrient absorption or utilization. Dr F Blanco (Institute de Salud Carlos III, Spain) presented a retrospective analysis of 165 outpatients switched from stable PI-based therapy to an NNRTI triple combination in which 45% of the 65% of those presenting with body-shape changes at time of switch reported some improvement after 12 months on the new regimen. However, there were no significant differences observed in mean lipid values or the percentage of those with hypercholesterolaemia or hypertriglyceridaemia at 12 months. Dr Nick Paton (Tan Tock Seng Hospital, Singapore) discussed the changes observed between previously naïve 2-NRTI-treated (group A; $n=10$) and 2-NRTI plus PI

(group B; $n=7$) treated groups, and a 2-NRTI plus PI group switched from previous treatment with NRTIs only (group C; $n=5$). Regional body composition was measured by DEXA and skin fold measurements at baseline, 5 and 15 months. At 5 months trunk fat levels rose in groups A and B (1.43 ± 1.25 and 1.37 ± 1.39 kg, respectively), but dropped in group C (-0.57 ± 1.12 kg; $P=0.024$ between groups). A similar pattern was also observed for appendicular fat distribution. All three groups showed similar increases in both appendicular and trunk lean tissue at 5 months. At 15 months, however, there was no significant difference between them for any parameter. Dr Paton suggested that underlying fat metabolism may be disturbed by the switch of an NRTI, introduction of a PI, or an improvement in virological control associated with the transfer from dual-nucleoside therapy to HAART, leading to a distinct early pattern of increased lean and decreased fat.

Switching from PI to abacavir was discussed in a poster by Dr Frank Goebel (Ludwig-Maximilians-Universität, Germany). This study was a randomized open pilot study with patients who were on their first PI-containing HAART regimen (two NRTIs + one PI). The follow-up period was 12 months. Thirty-one patients with PI-associated metabolic disturbance were enrolled and 16 were switched from PI to abacavir. Insulin and fasting plasma lipids were measured at baseline and at months 3, 6, 9 and 12. Improvement in median insulin sensitivity occurred in the abacavir group (+25, +59, +63 and +58 mmol/l/min, respectively), whereas it decreased in the PI group (-11, -31, -7 and -6 mmol/l/min). Both triglyceride and total cholesterol levels dropped significantly in the abacavir group, which was not apparent in PI-treated patients.

Bone demineralization and osteopenia

Two oral presentations from the Washington University Medical School identified evidence for elevated bone turnover in patients on PI-based therapy and provided a potential mechanism by which it may occur. Dr Pablo Tebas reported DEXA evidence of bone demineralization in 73 patients receiving PI-based treatment, with evidence for an increased rate of bone turnover as assessed by elevated urinary calcium, pyridinoline and deoxypyridinoline excretion and elevated serum bone alkaline phosphatase (SBAP) and osteocalcin. Both SBAP and urine n-telopeptides were inversely correlated with DEXA t - and Z -scores for the lumbar spine. Dr Tebas then presented *in vitro* data demonstrating that high physiological concentrations of ritonavir (11 $\mu\text{g/ml}$), indinavir (10 μM) and nelfinavir (6 $\mu\text{g/ml}$) impair bioactivation of vitamin D in the THP-1 monocyte-macrophage cell line. Enzymatic conversion of 25(OH)-vitamin D₃ to the more bioactive 1,25(OH)₂-vitamin D₃ by the cytochrome P450 mixed function oxidase 1 α -hydroxylase, was inhibited by 80% in the presence of ritonavir, 66% by indinavir and 31% by nelfinavir. However, plasma levels of 25-OH D₃ and 1,25-(OH)₂ D₃ were not reduced in the 73 patients previously described, posing a question as to the significance of these *in vitro* findings.

Dr David Nolan (Royal Perth Hospital and Murdoch University, Australia) reported a positive correlation between changes in bone mineral density (BMD; Δ lumbar spine Z -score/year by DEXA) and in percentage subcutaneous fat (mean Δ leg fat/year; $P=0.008$)

in a longitudinal study of 64 men on stable regimens from the Western Australia HIV cohort. The use of indinavir was independently associated with an elevated gain in BMD compared to nelfinavir-based regimens, irrespective of subcutaneous fat changes ($P=0.0369$). Cross-sectional analysis of 171 male individuals from the same cohort identified relatively higher rates of osteopenia (49%) and osteoporosis (17%) and lower BMD in those on PIs, findings similar to those of previous studies. On a similar theme, Dr Andrew Carr (St Vincent's Hospital, Australia) presented evidence of an association between osteopenia and elevated serum lactate (odds ratio 2.39 per 1 mmol/l increase; 95%CI 1.39-4.11; $P=0.002$) and lower body weight prior to therapy (OR 0.94 per 1 kg increase; 95%CI 0.90-0.98; $P=0.006$) in a cohort of 221 men recruited to a lipodystrophy prevalence survey between 1998 and 1999. No independent association was found with other parameters assessed, such as lipodystrophy or type/duration of antiretrovirals.

In the final oral presentation of the Workshop, Dr Marshall Glesby (Cornell University Medical College, USA) discussed the findings of a small-scale case-control study (two matched controls/case) of avascular necrosis (AVN) of the femoral head in 14 HIV-infected individuals diagnosed between 1992 and 2000. In matched univariate analyses, only prior *Pneumocystis carinii* pneumonia was significant at the 5% level for association with AVN (OR 6.3; 95%CI 1.3-31; $P=0.02$), with CD4 increase >50 cells/mm³ (OR 4.3), known prior corticosteroid use (OR 6.6) and PI use (OR 4.0) or stavudine use (OR 2.5) at time of AVN diagnosis all carrying P values of 0.08-0.11.

The Workshop concluded with a brief summary by Dr Jane Aubin of the recent *Forum for Collaborative HIV Research* meeting on bone biology. The conclusions of the Forum included the need for standard measurements to be developed and incorporated into new and ongoing studies as well as a determination of the prevalence of bone disorders in HIV disease. More clinical research was called for, particularly long-term prospective studies and studies in healthy volunteers taking antiretroviral drugs. The need for a better understanding of the phases of T cell function and how these affect bone resorption was also stressed as a priority for further research.

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