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Adipokines and the insulin resistance syndrome in familial partial lipodystrophy caused by a mutation in lamin A/C

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Abstract *Aims/hypothesis:* Familial partial lipodystrophy (FPLD) and obesity are both associated with increased risks of type 2 diabetes and cardiovascular disease. Although adipokines have been implicated, few data exist in subjects with FPLD; therefore we investigated a family with FPLD due to a lamin A/C mutation in order to determine how abnormalities of the plasma adipokine profile relate to insulin resistance and the metabolic syndrome. *Methods:* Plasma levels of adiponectin, leptin, resistin, IL-1β, IL-6 and TNF-α in 30 subjects (ten patients, 20 controls) were correlated with indices of metabolic syndrome. *Results:* Compared with controls, FPLD patients had significantly lower plasma levels of adiponectin (3.7±1.0 in FDLP cases vs 7.1±0.72 μg/ml in controls, p=0.02), leptin (1.23±0.4 vs 9.0±1.3 ng/ml, p=0.002) and IL-6 (0.59±0.12 vs 1.04±0.17 pg/ml, p=0.047) and elevated TNF-α (34.8±8.1 vs 13.7±2.7 pg/ml, p=0.028), whereas IL-1β and resistin were unchanged. In both groups, adiponectin levels were inversely correlated with body fat mass (controls, r=−0.44, p=0.036; FDLP, r=−0.67, p=0.025), insulin resistance (controls, r=−0.62, p=0.003; FDLP, r=−0.70, p=0.025) and other features of the metabolic syndrome. TNF-α concentrations were positively related to fat mass (controls, r=0.68, p=0.001; FDLP, r=0.64, p=0.048) and insulin resistance (controls, r=0.86, p=0.001; FDLP, r=0.75, p=0.013). IL-6, IL-1β and resistin did not demonstrate any correlations with the metabolic syndrome in either group. *Conclusions/interpretation:* Low adiponectin and leptin and high TNF-α were identified as the major plasma adipokine abnormalities in FPLD, consistent with the hypothesis that low adiponectin and high TNF-α production may be mechanistically related, and perhaps responsible for the development of insulin resistance and cardiovascular disease in FPLD.

Keywords Adipokines · Adiponectin · Diabetes · Insulin resistance · Lipodystrophy · Obesity · TNF-α

Abbreviations FPLD: familial partial lipodystrophy · HIV: human immunodeficiency virus · HOMA-IR: homeostasis model assessment of insulin resistance · IQR: interquartile range

Introduction

Familial partial lipodystrophy (FPLD) of the Dunnigan variety is an autosomal dominant form of insulin resistance caused by a mutation of the lamin A/C (*LMNA*) gene, which encodes a component of the nuclear envelope [1]. Affected subjects have a normal phenotype at birth but develop gradual redistribution of adipose tissue after the onset of puberty, with loss of subcutaneous adipose tissue in the extremities and gluteal region and accumulation of increased cervicofacial and intra-abdominal visceral adipose tissue and intramyocellular lipid [1]. Despite an overall reduction in body fat, individuals with FPLD paradoxically demonstrate features of the metabolic syndrome typically seen in obesity, including insulin resistance, hyperinsulinaemia, type 2 diabetes, dyslipidaemia, and increased serum C-reactive protein and NEFA levels [2], leading to premature cardiovascular disease [1, 3]. The mechanisms underlying the pathogenesis of the metabolic phenotype in FPLD remain uncertain;

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however, it is possible either that the absolute loss and redistribution of adipose tissue, or intrinsic dysfunction of adipose tissue itself arising directly from the genetic defect, could lead to the development of the metabolic abnormalities.

It is now recognised that adipose tissue, besides its role as a depot for energy storage, is also an active endocrine organ, enhancing the effects of a large number of factors and hormones with important effects on fuel metabolism and inflammation [4]. Abnormalities in the production of these adipose tissue-derived products, collectively termed 'adipokines', have been postulated to play major roles in the pathogenesis of the metabolic syndrome related to obesity [4]. Among the adipokines secreted by adipose tissue are TNF-α, IL-6, IL-1β and the more recently described hormones adiponectin and resistin. In view of the paradoxical occurrence of the metabolic syndrome in individuals with depleted adipose tissue, FPLD is an interesting experiment of nature that may shed important light on how abnormalities of adipose tissue lead to metabolic disease. Recently, adiponectin levels were found to be reduced in patients with acquired and congenital lipodystrophies [2, 5] and there was a strong negative correlation between adiponectin and fasting insulin concentrations [5]. Moreover, the administration of adiponectin partially corrected the insulin resistance of lipoatrophic mice [6], suggesting that hypoadiponectinaemia may be an important mediator of insulin resistance in lipodystrophy. Thus, the low-adiponectin state may be the common defect in both obesity and lipodystrophy. However, increased plasma levels of various proinflammatory cytokines expressed by adipose tissue have also been observed in various groups of lipodystrophic subjects [7–11]. The aim of the present study was to investigate the interrelationships between the abnormalities of adiponectin and other adipokines, and to determine which defects are most closely predictive of the metabolic syndrome in a genetically and phenotypically well-characterised group of subjects with FPLD and unaffected controls.

Subjects and methods

Subjects

We studied ten patients from a single family with a history of FPLD. The ten patients were previously demonstrated to have a mutation (R482Q) in the *LMNA* gene [12, 13]. These subjects were matched for age, sex and BMI with four unaffected control subjects from the same family, but without the *LMNA* mutation. We also studied and included as additional controls 16 matched healthy volunteers with no family history of FPLD. Three of the FPLD group and four of the control group had type 2 diabetes, treated either with diet or with diet in combination with oral hypoglycaemic agents. The diagnosis of type 2 diabetes was made on the known clinical history and a fasting glucose of more than 7.0 mmol/l. The history of vascular disease was obtained from previous clinical accounts of cardiovascular or cerebrovascular ischaemic events. The use of antihypertensive and lipid-lowering drugs was also recorded. Subjects were asked to fast, drink only water and avoid smoking from 2200 hours on the night before the study, and refrain from strenuous physical exercise or alcohol in the 24 h prior to the study day. All subjects gave written informed consent. The study was approved by the local Research Ethics Committee and performed in accordance with the principles of the Declaration of Helsinki.

Measurements

BMI, waist and hip circumference were measured and the WHR was then calculated. Blood pressure was measured in the supine position from the right arm using an Omron HEM-705C (Omron International, Kyoto, Japan). The mean of three separate readings taken at 3-min intervals was used in the analysis. Body composition was assessed by whole-body electrical bioimpedance analysis (Bodystat 1500, Isle of Man, UK), allowing the measurements of total fat mass and percentage body fat. Blood samples were taken into plastic EDTA tubes and immediately separated by centrifugation at 4°C. Plasma samples were stored at −70°C until analysis.

Assay methods

Plasma glucose concentrations were measured in duplicate by the glucose hexokinase method using the Advia 1650 system (Bayer UK, Newbury, UK). Total cholesterol, HDL-cholesterol, directly obtained LDL-cholesterol and triglyceride levels were assayed by the routine biochemistry laboratory at Southmead Hospital, Bristol. Fasting plasma insulin was determined by an ELISA (BioSource Europe, Nivelles, Belgium), with a sensitivity of 0.15 IU/ml and intra-assay CV of 6.0%. Insulin resistance was estimated using the homeostasis model of insulin resistance (HOMA-IR) with the following formula: HOMA-IR=fasting insulin (IU/ml)×fasting glucose (mmol/l)/22.5 [14]. Plasma adiponectin was measured using an ELISA (B-Bridge International, supplied by Metachem Diagnostics, Northampton, UK) with a detection limit of 0.25 ng/ml and intra-assay CV of 4.1%. Plasma resistin levels were determined by an ELISA (BioVendor Medical Laboratory, Brno, Czech Republic) with a detection limit of 0.5 ng/ml and a mean intra-assay CV of 5.7%. Leptin was measured by ELISA (BioSource Europe), with a minimal detection limit of 1 ng/ml and an intra-assay CV of 4.9%. TNF-α and IL-1β were determined using ELISA (IBL, Hamburg, Germany) with detection limits of 0.6 pg/ml and intra-assay CV between 3.5 and 6.0%. IL-6 concentrations were measured using an ELISA (HS Quantikine; R&D Systems Europe, Abingdon, UK) with a minimal detection limit of 0.05 pg/ml and CV between 2.1 and 8.4%.

Table 1 Biochemical and anthropomorphic characteristics of FPLD and control subjects

	FPLD	Controls	*p* (difference)
Number	10	20	
Age (years)	38.9±6.28	44.5±3.08	NS
Sex (male/female)	3/10	6/20	
Diabetes (%)	30	20	NS
Vascular disease (%)	50	10	0.019
BMI (kg/m^2)	23.1±1.47	25.1±1.23	NS
WHR	0.854±0.01	0.85±0.024	NS
Fat mass (kg)	12.8±2.1	22.0±2.6	0.014
Body fat (%)	19.72±2.1	31.3±2.1	0.0024
Total cholesterol (nmol/l)	6.60±0.35	5.41±0.24	0.024
HDL-cholesterol (mmol/l)	1.12±0.11	1.63±0.08	0.004
LDL-cholesterol (mmol/l)	4±0.4	3.17±0.23	NS
Dyslipidaemia treatment	40%	0%	0.008
Fasting triglycerides (nmol/l)	4.8±1.9	1.16±0.13	0.001
Systolic BP (mmHg)	147.3±9.76	122.39±4.66	0.015
Diastolic BP (mmHg)	79.5±4.53	77.62±3.0	NS
Hypertension treatment (%)	30	10	NS
Fasting insulin (U/l)	10.93±2.89	5.86±0.78	0.030
HOMA-IR	3.1±0.6	1.6±0.3	0.016
Fasting glucose (mmol/l)	5.49±0.34	5.73±0.53	NS

NS not significant
Values are mean±SEM
Significance is defined as $p<0.05$

Statistical analysis

The Statistical Package for the Social Sciences version 11.5 (SPSS, Chicago, IL, USA) was used for data analysis. The distributions of the variables were tested for normality using the Shapiro–Wilk *W* test. Summary statistics are presented as mean±SEM. Because of small numbers in the group with a family history of FPLD (but without the *LMNA* mutation), these subjects were pooled with other unaffected controls for analysis. Comparisons between groups were made using two-tailed Student's *t* test or the Mann–Whitney *U* test as appropriate. Categorical variables were compared using Fisher's exact test. Plasma adipokine levels are illustrated as box plots showing the interquartile range (IQR) and range. The concentrations of the adipokines and HOMA-IR were positively skewed and were log-transformed to a normal distribution to calculate Pearson (*r*) correlation coefficients, in order to explore the relations between plasma adipokine concentrations and demographic and metabolic characteristics. Standardised regression coefficients (β) were calculated to determine associations between variables. Significance was defined at $p<0.05$.

Results

Demographic and clinical comparisons

Demographic and clinical characteristics for all subjects are shown in Table 1. The two groups were of similar age and sex. Despite similar BMI and WHR, FPLD patients had lower body fat content but higher prevalences of type 2 diabetes and cardiovascular disease. Although diastolic blood pressure and the use of antihypertensive therapy were not significantly different between the two groups, systolic blood pressure was higher in subjects with FPLD. Additionally, despite greater usage of lipid-lowering drugs in FPLD subjects, they had significantly higher serum total cholesterol and triglyceride levels and lower HDL-cholesterol levels than control subjects.

Adipokines

Mean leptin and adiponectin levels were significantly lower in FPLD than in control subjects (Table 2, Fig. 1a,b). Furthermore, diabetic subjects in both groups demonstrated

Table 2 Plasma concentrations of adipokines in FPLD and control subjects

	FPLD	Controls	*p* (difference)
Adiponectin (μg/ml)	3.7±1.0	7.1±0.72	0.02
Leptin (ng/ml)	3.0±0.4	9.0±1.3	0.002
Resistin (ng/ml)	27.6±2.4	30.7±4.1	NS
TNF-α (pg/ml)	34.8±8.1	13.7±2.7	0.028
IL-6 (pg/ml)	0.59±0.12	1.04±0.17	0.047
IL-1β (pg/ml)	1.45±0.26	1.77±0.23	NS

NS not significant
Values are mean±SEM
Significance is defined as $p<0.05$

lower levels of adiponectin compared with non-diabetic subjects for FPLD and controls, respectively. In contrast, resistin concentrations in the two groups did not differ (Table 2, Fig. 1c). Plasma TNF-α, but not IL-1β, was significantly elevated in FPLD compared with control subjects (Table 2, Fig. 1d,e), whereas levels of IL-6 were reduced in affected subjects (Table 2, Fig. 1f).

Univariate correlations

The principal factors affecting adipokine concentrations were explored by linear regression analysis in controls and FPLD subjects separately (Table 3). In control subjects, plasma adiponectin levels correlated inversely with fat mass (Fig. 2a), BMI, WHR, triglycerides, fasting glucose and

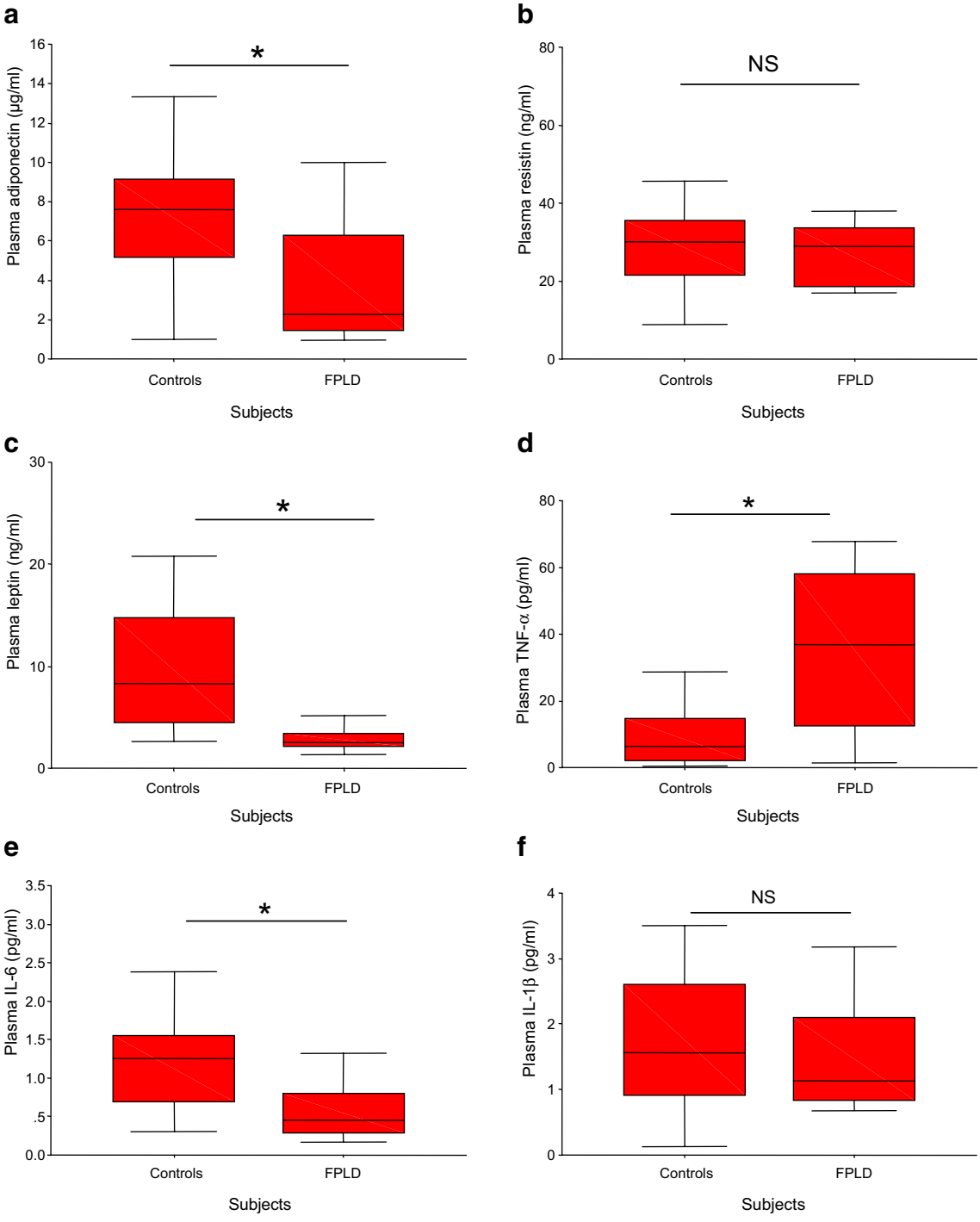

Fig. 1 Box plots illustrating plasma levels of (**a**) adiponectin, (**b**) resistin, (**c**) leptin, (**d**) TNF-α, (**e**) IL-6 and (**f**) IL-1β in FPLD and control subjects. *Boxes* represent median and interquartile ranges. *Error bars* represent 10th and 90th percentiles. Significance is defined as *$p<0.05$. *NS* not significant

insulin, HOMA-IR (Fig. 2b) and TNF-α concentrations (Fig. 2c), whereas there was a positive correlation with HDL-cholesterol concentrations. Furthermore, the levels of plasma TNF-α were strongly related to increasing fat mass, BMI, body fat percentage, HOMA-IR, plasma insulin and glucose, but not to WHR. In subjects with FPLD, plasma adiponectin also correlated inversely with fat mass (Fig. 2b), BMI, fasting insulin and glucose, HOMA-IR (Fig. 2a), triglycerides and plasma TNF-α (Fig. 2c), and HDL-cholesterol was also positively associated with adiponectin concentrations. TNF-α concentrations also positively correlated with fat mass and BMI, as well as HOMA-IR and fasting insulin levels.

In the control group, as expected, plasma leptin levels correlated positively with BMI, WHR, fat mass, body fat percentage, fasting insulin and glucose, HOMA-IR, TNF-α and IL-6 (Table 3). In FPLD subjects, leptin concentrations positively correlated with BMI, fat mass and body fat percentage. However, plasma leptin and TNF-α concentrations were not correlated significantly (r=0.45, p=0.10), in contrast with control subjects (r=0.66, p=0.001).

In both groups, plasma IL-6 levels positively correlated with BMI, fat mass and body fat percentage as well as plasma leptin concentrations (Table 3). In control subjects, there were strong positive correlations between plasma IL-6 levels and HOMA-IR, fasting insulin and glucose. Moreover, plasma IL-6 was inversely related to adiponectin levels. In contrast, these relationships were not observed in the FPLD group. Similarly, although there was a significant positive correlation between IL-6 and TNF-α in the control group, this was not observed in FPLD. We were unable also to demonstrate any relationships between plasma IL-1β and any of the anthropometric or metabolic variables in either group of subjects. Plasma resistin levels also showed little or no relationship with these variables, with the exception that plasma resistin levels showed weak positive correlations with total cholesterol (r=0.64, p=0.047) and triglycerides (r=0.73, p=0.016) and a weak negative correlation with HDL-cholesterol (r=−0.61, p=0.049) in the FPLD group alone.

Multivariate analysis

Using multivariate regression analysis, HOMA-IR was inversely related with adiponectin levels in control subjects, after controlling for measures of obesity, including fat mass, BMI and WHR (β=−0.62, p=0.047). There was also a positive correlation between HDL-cholesterol and adiponectin concentrations, but this did not reach significance after controlling for the above measures of obesity (β=0.41, p=0.061). Subjects with FPLD demonstrated similar findings, whereby HOMA-IR was again related to adiponectin concentrations after correction for fat mass and BMI (β=−0.92, p=0.046). As with the control group, there was a trend towards positive correlation between HDL-cholesterol and adiponectin concentrations after adjusting for obesity-related variables (β=0.54, p=0.11).

Table 3 Correlation analyses of adipokine concentrations with metabolic and obesity-related indices

	Adiponectin				TNF-α				IL-6			
	FPLD		Controls		FPLD		Controls		FPLD		Controls	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
BMI	−0.72	0.019	−0.51	0.022	0.70	0.007	0.62	0.004	0.60	0.046	0.54	0.013
WHR	0.012	NS	−0.45	0.046	0.12	NS	0.22	NS	0.15	NS	0.41	0.019
Fat mass	−0.67	0.025	−0.44	0.036	0.64	0.048	0.68	0.001	0.74	0.014	0.58	0.007
Body fat (%)	−0.316	0.037	−0.26	0.028	0.37	NS	0.49	0.028	0.75	0.013	0.45	0.01
Total cholesterol	0.15	NS	0.03	NS	0.10	NS	0.05	NS	0.21	NS	0.09	NS
HDL-cholesterol	0.72	0.018	0.52	0.019	−0.54	NS	−0.4	NS	−0.475	NS	−0.38	NS
LDL-cholesterol	−0.09	NS	−0.09	NS	−0.05	NS	0.22	NS	0.02	NS	0.15	NS
Triglycerides	−0.61	0.016	−0.49	0.029	0.49	NS	0.37	NS	0.26	NS	0.3	NS
Systolic BP	−0.12	NS	−0.1	NS	0.50	NS	0.1	NS	0.39	NS	0.19	NS
Diastolic BP	−0.07	NS	−0.19	NS	0.39	NS	0.23	NS	−0.27	NS	0.28	NS
Fasting insulin	−0.78	0.009	−0.58	0.008	0.79	0.006	0.8	0.001	0.52	NS	0.57	0.009
Fasting glucose	−0.52	0.02	−0.53	0.015	0.56	NS	0.65	0.001	0.43	NS	0.62	0.003
HOMA-IR	−0.70	0.025	−0.62	0.003	0.75	0.013	0.86	0.001	0.59	NS	0.64	0.003
Adiponectin	–	–	–	–	−0.78	0.008	−0.53	0.017	−0.28	NS	−0.37	0.037
Leptin	−0.45	NS	−0.46	0.044	0.45	NS	0.66	0.001	0.71	0.001	0.49	0.025
TNF-α	−0.78	0.008	−0.53	0.017	–	–	–	–	0.37	NS	0.53	0.016
IL-6	−0.28	NS	−0.37	0.037	0.37	NS	0.53	0.016	–	–	–	–
IL-1β	−0.21	NS	−0.1	NS	0.06	NS	0.1	NS	−0.03	NS	−0.1	NS
Resistin	0.13	NS	−0.04	NS	0.08	NS	0.16	NS	0.36	NS	0.24	NS

NS not significant
Relationships between various adipokines were also explored and are shown at the bottom of the table
Significance is defined as $p<0.05$

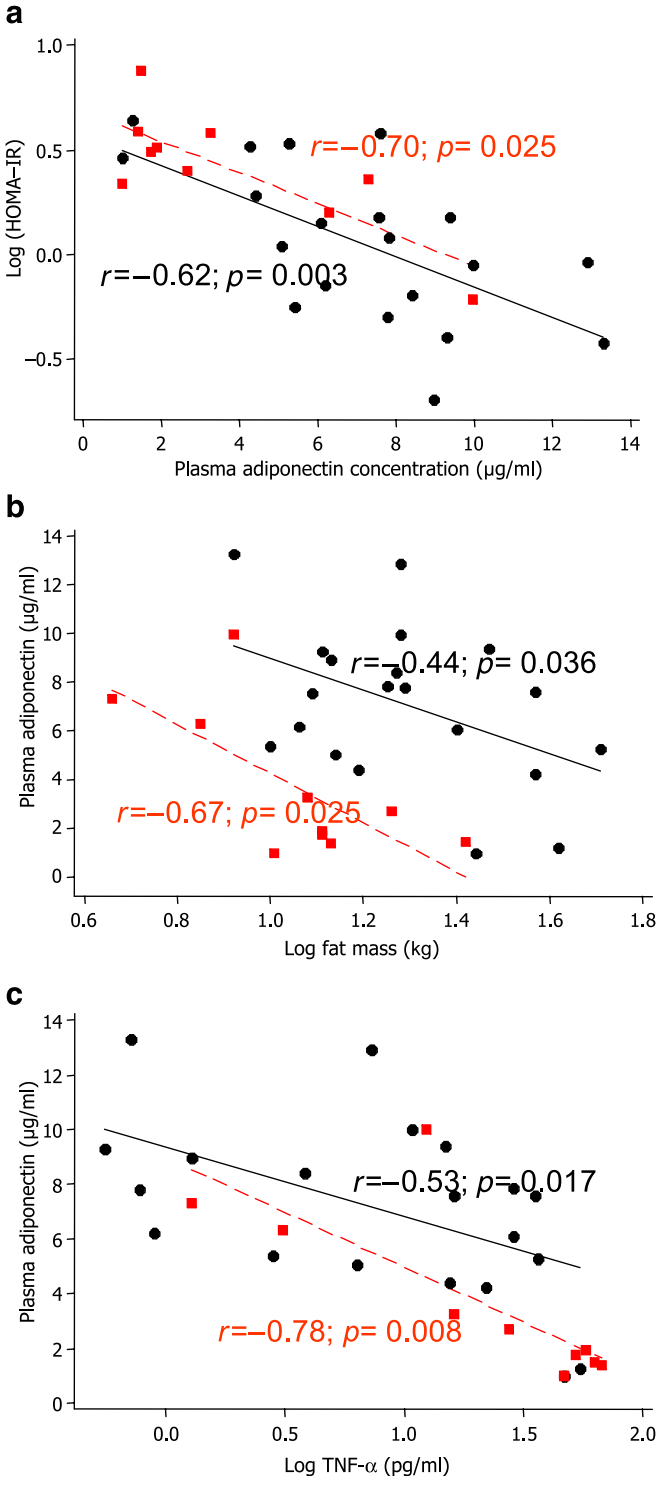

Fig. 2 Relationships between plasma adiponectin and (**a**) HOMA-IR, (**b**) fat mass and (**c**) plasma TNF-α levels in FPLD and unaffected subjects. *Circles*, controls; *squares*, FPLD. HOMA-IR, fat mass and plasma TNF-α were log-transformed to normalise distribution. Significance is defined as $p<0.05$

In control subjects, using similar multiple regression models with adjustment for fat mass, BMI and HOMA-IR, neither plasma TNF-α nor IL-6 concentration correlated with adiponectin levels. However, in FPLD subjects TNF-α was independently related to plasma adiponectin concentrations after adjusting for BMI and fat mass and HOMA-IR ($\beta=-0.91$, $p=0.02$).

Discussion

This study shows that FPLD is characterised by marked abnormalities in the adipokine profile, principally reduced circulating levels of adiponectin and leptin and increased levels of TNF-α, but with little change in concentrations of several other adipokines, including IL-6, resistin and IL-1β. These abnormalities were closely related to insulin resistance and the metabolic syndrome. We also show that plasma adiponectin levels were inversely related to indices of insulin resistance and obesity, in agreement with previous work [5, 15, 16]. Both increased TNF-α and reduced adiponectin levels have been implicated in the pathogenesis of the metabolic syndrome [17–20] and may therefore contribute to the development of this syndrome in FPLD. In this respect, subjects with FPLD behave as if they were obese, even though they are deficient in adipose tissue.

Adiponectin is expressed exclusively by adipocytes and, in contrast to other adipokines, plasma levels decline with increasing adiposity [16, 21, 22] and increase with weight loss [21, 23]. Recent work suggests that adiponectin levels are influenced more by intra-abdominal (visceral) than by subcutaneous adipose tissue [22, 24], and that plasma levels correlate with insulin sensitivity [16, 25, 26]. Low adiponectin levels are also associated with type 2 diabetes [15, 27], dyslipidaemia [28] and cardiovascular disease [29]. The best evidence that adiponectin deficiency plays a causal role in the pathogenesis of insulin resistance is from lipoatrophic mice with low levels of adiponectin. In this model, insulin resistance was partially ameliorated by administration of adiponectin [6]. Thus, the present data support the proposition that adiponectin deficiency contributes to the pathogenesis of insulin resistance in human FPLD.

There have been relatively few studies examining other proinflammatory cytokines in human lipodystrophies. TNF-α is of particular interest, having been closely linked to obesity and the metabolic syndrome [20]. In contrast to our data, Hegele et al. [2] found no change in plasma TNF-α in FPLD, although a recent case report of an individual with acquired idiopathic generalised lipodystrophy reported elevated plasma TNF-α in the presence of reduced adiponectin and leptin levels [10]. The relationship between adiponectin and the TNF-α system is of particular interest. TNF-α suppresses adiponectin gene expression in cultured human and rodent adipocytes [19, 30–32] and plasma levels of adiponectin are inversely related to plasma TNF-α and adipocyte TNF-α gene expression [7, 17, 33]. Furthermore, adiponectin knockout mice exhibit increased levels of TNF-α in adipose tissue and plasma [34]. Conversely, adiponectin inhibits TNF-α production in cultured macrophages [35] and myotubes [34]. Animal models of lipodystrophy also exhibit upregulation of TNF-α. Thus, mice transgenic for aP2-SREPB (adipocyte

specific enhancer/promoter-sterol regulatory element-binding protein 1c) demonstrated elevated expression of TNF-α in adipose tissue [36, 37], and conjugated linoleic acid supplementation elicits a lipodystrophic phenotype with enhanced TNF-α expression in adipocytes [38]. Furthermore, a recent study demonstrated increased subcutaneous adipose tissue TNF-α expression in HIV-related lipodystrophy concomitantly with reduced adiponectin levels [11]. Thus, the present observation of a strong inverse relationship between adiponectin and TNF-α is consistent with the idea that TNF-α and adiponectin are reciprocally related, and that this relationship may result directly from reciprocal regulatory effects of TNF-α and adiponectin on adipocytes, and of adiponectin on macrophage TNF-α expression. It is interesting to speculate that FPLD somehow leads to amplification of this reciprocal relationship, with harmful consequences for insulin resistance. It is currently unclear whether the increased TNF-α production in FPLD comes from visceral adipose tissue, macrophages or other tissues. It is possible that abnormal redistribution of fat in FPLD, rather than the absolute degree of adiposity, may account for the elevated levels of TNF-α. Circulating levels of adiponectin also appear to be more closely related to visceral adiposity [22, 39], although large lipid-rich visceral adipocytes also appear to produce less adiponectin [39]. Thus, although FPLD leads to expanded visceral adipose tissue depots [40], it is possible to speculate that this does not compensate for the loss of peripheral subcutaneous adipose tissue, and that overall systemic adiponectin production falls.

In contrast to TNF-α, levels of IL-6 were lower in FPLD subjects than in control subjects (0.59 vs 1.04 pg/ml, p=0.047). A possible explanation is that adipose tissue is a major site of IL-6 production, accounting for up to 35% of circulating levels [41]; the reduced IL-6 concentrations in FPLD may reflect reduced total fat mass. Consistent with previous work, however, IL-6 levels were positively related to indices of obesity and insulin resistance [42] and inversely with adiponectin levels in the control group [19, 42, 43]. By contrast, in FPLD, although there was a correlation with obesity-related indices, we failed to find strong relationships of IL-6 with metabolic indices, although this was probably influenced by the small sample size. However, although IL-6 and TNF-α are both proinflammatory cytokines, plasma levels of IL-6 in this situation may be a marker of fat mass rather than being directly related to the metabolic syndrome in a mechanistic fashion [44]. In support of this, the study by Vozarova [45] demonstrated that although IL-6 was positively related to insulin resistance and fat mass, there was no correlation between IL-6 and insulin action, after adjustment for adiposity. A recent study also found that although there was no relationship between insulin sensitivity, as measured by hyperinsulinaemic–euglycaemic clamp and plasma IL-6 levels, there was a very strong correlation between IL-6 and BMI [46]. While our findings are in accordance with previous studies [19, 42, 43], it is possible that plasma IL-6 levels may be a reflection of fat mass rather than a mediator of insulin resistance.

We also failed to observe any change in plasma IL-1β in FPLD, or relationships with other metabolic variables. IL-1β is an important immune cytokine released by a large variety of cells, including adipocytes [47], in which it may have a paracrine effect. IL-1β has been shown to inhibit adipocyte maturation and lipid accumulation [48] and to suppress expression of adiponectin [49] and leptin [50]. Thus, although the present data do not exclude significant autocrine/paracrine interactions of IL-6 and IL-1β in the regulation of adiponectin and TNF-α production within adipose tissue, from these data at least the former of the two adipokines appears less likely to be important circulating mediators of the metabolic syndrome. Likewise, plasma resistin levels were similar in both FPLD and control subjects and there were no demonstrable relationships with any of the anthropometric variables or indices of the metabolic syndrome. However, resistin levels were found to be positively related to total cholesterol and triglyceride levels and negatively to HDL-cholesterol in the FPLD group only. Resistin is an adipokine that has been proposed to be an important link between insulin-resistant states and obesity. Circulating levels of resistin are elevated in diet-induced and genetic forms of obesity in mice (*ob*/*ob* and *db*/*db*) and are reduced in response to insulin-sensitising thiazolidinediones [51]. A recent study demonstrated that resistin knockout mice exhibit lowered hepatic glucose output and improved fasting glycaemia [52]. However, reports of resistin in humans remain more controversial [53]. Thus, although some studies demonstrate that circulating resistin [54] or resistin gene polymorphism [55] have significant positive relationships with obesity and insulin resistance, in others findings have been discordant [56]. Despite the lack of correlation of resistin with most of the anthropometric and metabolic variables, in the FPLD group, those with total and HDL-cholesterol as well as triglycerides were significant. However, the majority of the present findings suggest that resistin plays a less important role than adiponectin and TNF-α in the metabolic syndrome, which may be related to species differences in the influence of resistin on metabolic processes [53].

Finally, it is important to note that this was a cross-sectional study which was quite clearly limited by the small numbers of subjects studied. Additional studies with a more substantial number of subjects and, if possible, interventional experiments would be required to confirm the relationships proposed here and to determine cause-and-effect relationships.

In conclusion, this study has shown that FPLD is a state of low adiponectin and raised TNF-α production, and that these abnormalities are closely and inversely related to each other and to the metabolic syndrome. In contrast, little or no relationship was observed between metabolic parameters and circulating levels of IL-6, IL-1β or resistin. Adiponectin and TNF-α may play direct roles in the pathogenesis of cardiovascular disease and diabetes in FPLD.

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