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RESEARCH ARTICLE

Oat-enriched diet reduces inflammatory status assessed by circulating cell-derived microparticle concentrations in type 2 diabetes

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Scope: Inflammatory status can increase the risk of adverse cardiovascular events linked to platelet activity and involvement of microparticles (MP) released from platelets (PMP), leukocytes (LMP), and monocytes (MMP). These MP carry host cell-derived antigens that may act as markers of metabolic health. Subjects newly diagnosed with type 2 diabetes are offered appropriate standard dietary advice (SDA) but this may not be optimal as specific inclusion of other nutrients, such as oats, may add benefit. The effectiveness of such interventions can be tested by examination of MP activation markers.

Methods and results: Subjects ($n = 22$) with type 2 diabetes participated in a randomized cross-over trial involving 8 wk interventions with either an oat-enriched diet (OAT) or following reinforced SDA. Responses were also compared with preintervention habitual (HAB) intake. OAT reduced the concentrations and proportions of fibrinogen- and tissue factor-related PMP and MMP_11b. The main effect of SDA was to reduce fibrinogen-activated PMP. Regardless of chronic intake, a healthy test meal led to postprandial declines in total PMP as well as tissue factor-, fibrinogen-, and P-selectin-positive PMP.

Conclusion: OAT improved risk factors assessed by MP status, even in subjects with type 2 diabetes already well-controlled by diet and life-style alone.

Keywords:
Microparticles / Oats / Platelets / Standard dietary advice / Type 2 diabetes

1 Introduction

Although circulating microparticles (MP; small vesicles, ranging in size from 0.1 to 1.0 μm in diameter [1–3]) can be released from a range of cell types, including leukocytes, lymphocytes, erythrocytes, and endothelial cells, following activation or apoptosis, the majority (approximately 70–90%) are derived from platelets [4–6]. Following activation, through membrane fusion and internalization these MP transfer components, including proteins, lipids, and receptors, to various target cells with resultant contributions to inflammation, coagulation, and vascular dysfunction events [7]. Notably, MP create a surface environment favorable for thrombin formation and synthesis of cytokines, with impacts on platelet, endothelial, and monocyte activation that may contribute to the development of cardiovascular disease (CVD) [8]. For example, the increased plasma concentrations of cell-derived MP observed in patients with CVDs, or other ailments associated with vascular impairment [1,4,9–15] including diabetes mellitus [11,14,16], have been proposed as biomarkers of vascular health, or even prognostic markers for atherosclerotic disease [17–19]. Indeed, our previous study demonstrated that

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Abbreviations: CVD, cardiovascular disease; **GI**, glycemic index; **HAB**, habitual diet; **LMP**, leukocyte-derived microparticles; **MMP**, monocyte-derived microparticles; **MMP_11b**, CD11b-positive-monocyte-derived microparticles; **MP**, microparticles; **OAT**, oat-enriched diet; **PMP**, platelet-derived microparticles; **REML**, restricted maximum likelihood; **SDA**, standard dietary advice; **TF**, tissue factor

platelet-derived MP (PMP) markers, linked to pathways related to vascular inflammation, differed between healthy subjects and those with type 2 diabetes, independent of obesity status [20]. Such data suggest that both the concentrations and activation status of PMP may represent markers of subtle changes in subclinical inflammation and identify mechanisms that link inflammation to disease.

Dietary and life-style interventions are the first line management for a number of important chronic diseases, including newly diagnosed type 2 diabetes [21]. A large body of data suggest that low glycemic index (GI) diets can aid glycemic control [22–28]. Furthermore, postprandial hyperglycemia may be particularly harmful in terms of cardiovascular risk [29, 30], related to acute increases in plasma concentrations of markers of systemic inflammation and oxidative stress [31–34]. Platelet activation status can alter in response to consumption of single meals, although the responses have not been consistent [35–38]. These contradictory outcomes may depend on both subject status, e.g. with or without type 2 diabetes, and the type of meal offered. Furthermore, data on changes in MP from various origins in response to chronic dietary consumption are limited.

The current report examines the impact of chronic (8 wk) consumption of either an oat-enriched diet (OAT) or one based on the standard dietary advice (SDA) provided in the UK to subjects with type 2 diabetes, controlled by diet and lifestyle alone, on indices of MP. The OAT diet was investigated because, first, oats are considered a low GI food [39] and, as such, might be expected to modulate postprandial hyperglycemia [40]. Second, oats can exert direct anti-inflammatory and antioxidant properties [41, 42]. The hypothesis was that OATs would improve fasting inflammation status, as assessed by platelet markers, especially MP, compared with either the habitual (HAB) diet or SDA.

2 Materials and methods

2.1 Subjects

Measurements were made in a subset ($n = 22$) of patients with type 2 diabetes, controlled by diet and life-style alone, from a larger study [43]. Both male and postmenopausal females (age 40–75 years) with type 2 diabetes were recruited from two Scottish Health Boards, Grampian (centered at Aberdeen) and Highland and Islands (centered at Inverness). Inclusion criteria included either newly diagnosed type 2 diabetes or with the disease controlled by diet and life-style alone. Exclusion criteria included significant liver, renal, cardiovascular or psychiatric illness, plus medications that might invalidate the study results (including oral hypoglycemic drugs, corticosteroids, hormone replacement therapy anticoagulants, aspirin, and statins). Recruitment protocols included newspaper and poster advertisement plus approaches based on searches of patient databases followed by approval to contact from the appropriate General Practitioner. All volunteers provided signed consent. The study was approved by the North of Scotland Research Ethics Committee (07/S0802/163) and registered with the ISRCTN Register (ISRCTN12655129).

2.2 Experimental protocol

The full study details and subject characteristics have been provided elsewhere [43]. Briefly, the volunteers had all been diagnosed previously with type 2 diabetes by Aberdeen or Inverness Diabetes Clinics, part of the Scottish National Health Service, based on a combination of fasting (>7 mmol/L) or random glucose (>11 mmol/L) on at least two occasions or response to an oral glucose tolerance test (>11 mmol/L, 2 h after ingestion of 75 g glucose) but all had <64 mmol/mol glycosylated hemoglobin (HbA1c). All volunteers had been offered advice on diet and lifestyle at diagnosis and had used this to control their diabetes without need for specific medication. At recruitment, fasting glucose and insulin were 7.7 mmol/L and 11.0 mU/L, with HOMA-IR 3.9 [43]. At entry to the study, subjects were following their habitual diet (HAB) and were randomly allocated to a cross-over design with each arm of 8 wk that involved either their normal diet but with part of their carbohydrate intake substituted with a range of oat products (OAT) that were provided or by following a reinforcement of the SDA routinely offered at diagnosis and based predominantly on ''Eating well with diabetes'' provided by Diabetes UK. While on SDA, the subjects were asked to minimize their intake of oats (this decreased from 32 g/d HAB to 5 g/d SDA). For the OAT arm, subjects were provided with written guidelines on how to include a minimum of 70–100 g oat products into their daily diet (mean intake was 131 g/d). A range of suitable oat products available commercially were provided gratis to the subjects. Measurements were made on entry to the study (HAB) and at the end of the two intervention periods (OAT and SDA) and involved analysis of overnight fasted blood samples and the subsequent response to a standardized test meal [43]. This test meal followed the recommendation of Diabetes UK for healthy eating and allowed investigation of the postprandial effects on MP concentrations and activation in subjects with type 2 diabetes.

Overnight fasted blood samples were taken and then subjects were offered the healthy test meal, consumed within 15 min. The test meal comprised 3.3 MJ metabolizable energy as 28 g protein, 104.5 g carbohydrate (of which sugar 30.6 g), 29.1 g fat (of which saturates 8.6 g), and fiber 11.2 g [43]. The GI was estimated at 57.1, with available carbohydrate of 92.7 g and with a glycemic load of 52.9 g. Blood samples were withdrawn at 15 min intervals for 3 h following the meal [43], but only those taken at 0, 90, and 180 min were analyzed for platelet and MP characteristics. Other than dietary intervention, diabetes care and management of the subjects was not altered during the study.

2.3 Platelet activation status

Blood (10 mL) from the overnight fasted state was collected into sodium citrate tubes (S-Monovette, Sarstedt, Numbrecht, Germany) and the whole blood count for leukocyte, lymphocyte, and platelet quantities (counts/ μL) was analyzed with a Sysmex KX21 (Sysmex Ltd, UK). The proportions of platelets with activation markers for fibrinogen, tissue factor (TF), and CD62P were determined as described elsewhere [44].

2.4 Microparticle measurements

2.4.1 Isolation of MPs and flow cytometry detection

Blood (10 mL) was collected into sodium citrate S-monovette tubes and centrifuged at 2000 × *g* for 20 min to isolate platelet-poor plasma that was then stored at −80°C prior to isolation and measurement of MP as described previously [20]. To 20 μL of the MP suspension was added 80 μL HEPES buffer and the mixture was incubated in the dark for 20 min at room temperature together with appropriate monoclonal antibodies conjugated with separate fluorescent markers; fluorescein isothiocyanate (FITC), phycoerythrine (PE), peridinin chlorophyll protein (PerCP) or allophycocyanin (APC), and analyzed as described previously (see Table 1) [20].

2.5 Clinical and glycemic measurements

Total cholesterol, LDL, oxidized-LDL, high-density lipoprotein (HDL), triglycerides C-reactive protein (CRP), and interleukin-18 were measured as described for the larger study [43] as were oxygen radical antioxidant capacity (ORAC) and urinary isoprostanes.

2.6 Statistical methods

Unless otherwise stated, data were obtained from 22 volunteers, who received three chronic dietary interventions with a test meal measured at 3 time points at the end of each intervention. Data were analyzed by restricted maximum likelihood (REML) using Genstat 13th Edition Release 13.2 (VSN International, Hemel Hempstead, Herts, UK). Random effects consisted of volunteer, with week and time, plus their interaction, nested within volunteer. Fixed effects were site, gender, diet, time, and week with all interactions between diet, time, and week considered. Site and gender interactions were restricted to those with diet and week. Data are summarized as means ± SEM, based on the between-volunteer spread. To allow comparison of mean values the corresponding standard error of difference (SED) is also presented, obtained from the residual variance of the appropriate error stratum. Where significant responses ($p < 0.05$) were observed for main effects or interactions then posthoc *t*-tests were applied to determine which mean values differed.

Based on visual inspection of the residual plots, data for MP numbers and activation status were log-transformed in order to stabilize the variance. For these data the means are presented after back-transformation based on first order Taylor approximation. The corresponding SEM and SED were obtained from back transformation of the residual variance from the appropriate error stratum of the REML output. For outcomes related to PMP no significant diet × time interactions were observed. Therefore, for these outcomes only the main effects of either diet or time are presented from the full analysis (but which still included diet × time interactions). In contrast, there were significant diet × time interactions for certain leukocyte-derived microparticles (LMPs) and MMP outcomes. These interactions are detailed in the text, while the main effects for either diet or time are included in the various tables.

For overnight fasted data, relationships between MP markers (log transformed) and other inflammatory markers (untransformed) were investigated with random effects regression using REML with subject as a random effect. Biological meaning was only ascribed to regression relationships at $p < 0.01$.

3 Results

3.1 Concentrations of MP

The concentrations of the various MP varied considerably between volunteers, as shown in Figs. 1A and 1B which are based on the means of raw data for the nine observations from each subject, i.e. the three dietary interventions (HAB, OAT, and SDA) and the three sample times accompanying the test meal (0, 90, 180 min) are combined. The between-volunteer variability necessitated log transformation of the data prior to statistical analysis.

3.2 Effects of chronic dietary intervention

3.2.1 Platelet count and platelet activation status

Only at the Aberdeen site was it possible to measure platelet count, volume, and activation status ($n = 14$). Neither baseline platelet counts nor platelet volume were impacted by chronic dietary status (Table 2). In terms of platelet activation status, the proportion of baseline samples that contained tissue factor-activated platelets was lower (−23%, $p = 0.004$) after intervention with OAT than for either HAB or SDA.

3.2.2 MP count and MP activation status

Both absolute counts (gate counts/μL) and proportion of MP were decreased ($p < 0.05$) for CD11b-positive MMP,

Table 1. Antibodies used and the microparticles targeted

Antibody	Targeted microparticle	Name of antigen
CD61 + [a)]	PMP	Integrin beta-3
CD61+/fibrinogen+ [a),c)]	Fibrinogen-positive PMP	Integrin beta-3/Fibrinogen
CD61+/CD62P+ [a),b)]	P-selectin-positive PMP	Integrin beta-3/P-selectin
CD61+/CD142+ [c),d)]	TF-positive PMP	Integrin beta-3/Tissue Factor
CD45+ [e)]	LMP	Leukocyte common antigen
CD45+/CD14+ [e),f)]	MMP	Leukocyte common antigen/Monocyte differentiation antigen CD14
CD45+/CD11b+ [e),g)]	CD11b-positive-LMP	Leukocyte common antigen/Integrin alpha M
CD45+/CD14+/CD11b+ [e),f),g)]	CD11b-positive-MMP	Leukocyte common antigen/Monocyte differentiation antigen CD14/ Integrin alpha M

a) anti-CD61-PerCP (BD Bioscience, San Jose, CA, USA); b) anti-CD62P-APC (BD Pharmingen, BD Bioscience, San Diego, CA, USA); c) anti-fibrinogen-FITC (DakoCytomation, Glostrup, Denmark); d) anti-tissue factor-FITC (American Diagnostica, Stamford, CT, USA); e) anti-CD45-PE (BD Pharmingen, BD Bioscience); f) anti-CD14-APC (BD Bioscience); g) anti-CD11b-FITC (Beckman Coulter, Paris, France). FITC-, PE-, PerCP- and APC-conjugated isotype controls (IgG1, BD Bioscience) were used to define the background noise of the labeling.

fibrinogen-positive PMP, and tissue factor-positive PMP after OAT intervention compared with HAB (Table 3). The concentrations and proportion of fibrinogen-positive PMP also decreased ($p < 0.01$) after the SDA intervention compared with HAB. There were no differences, however, for MP concentrations and activation status between the OAT and SDA interventions. The chronic diet intervention had no effect on LMP counts (data not shown).

3.3 Postprandial effects

Counts of platelets, leukocytes, and lymphocytes within blood all decreased ($p < 0.01$; Table 4) 90 min after meal ingestion but either returned to premeal values by 180 min (platelets and lymphocytes) or increased to above baseline (leukocytes; $p < 0.01$), In all cases the postprandial decreases at 90 min exceeded any response due to hemodilution (based on −1.8% changes in red blood cell and hemoglobin concentrations, data not shown). The mean platelet volume was increased slightly at 90 min (+2%, $p = 0.003$) and remained elevated at 180 min ($p = 0.001$; Table 4).

Compared with premeal values, postprandial declines ($p < 0.01$) over 3 h were observed for concentrations of total PMP and LMP, tissue factor-positive PMP and P-selectin-positive PMP (Table 5). For the latter three, the decline was fully established by 90 min. There was also a decrease ($p < 0.05$) in fibrinogen-positive PMP between 90 and 180 min. In contrast, the proportions of both CD11b-positive LMP and fibrinogen-positive PMP were increased ($p < 0.01$) at both

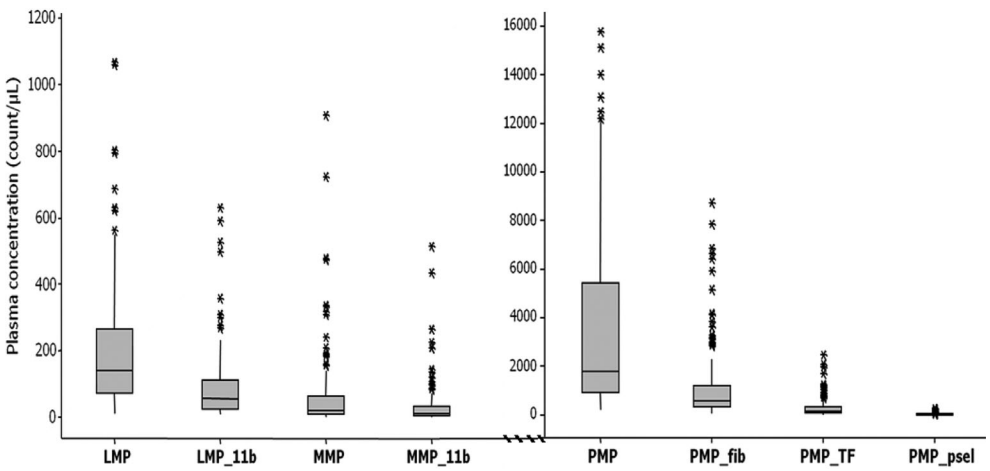

Figure 1. Plasma quantities (counts/μL) of microparticle (MP) for individual subjects. Leukocyte-derived microparticles (LMP), monocyte-derived microparticles (MMP), CD11b-positive leukocyte-derived microparticles (LMP_11b), CD11b-positive monocyte-derived microparticles (MMP_11b), platelet-derived microparticles (PMP), fibrinogen-positive platelet-derived microparticles (PMP_fib), tissue factor-positive platelet-derived microparticles (PMP_TF) and P-selectin-positive platelet-derived microparticles (PMP_psel). Data are based on raw observations, combined for the 9 samples taken for each subject (3 diets each with 3 time points). In boxplot format the shaded box encloses the 25–75 percentile of the data, with the median indicated by a bar. Whiskers extend to 1.5 times of the box height or the most extreme data point, whichever is smaller. The asterisks indicate those volunteers who fall outside these limits.

Table 2. Overnight fasted plasma platelet count (counts/μL), mean platelet volume (MPV; fL) and the percentage of platelets that express P-selectin (%CD62P), fibrinogen (%fib), and tissue factor (%TF) following 8 wk of each dietary intervention ($n = 14$)

Diet	Platelet 10^3/μL	MPVfL	%CD62P	%fib	%TF
HAB	203 ± 13	8.5 ± 0.15	12.5 ± 2.6	18.1 ± 5.9	54 ± 6[a]
OAT	196 ± 10	8.6 ± 0.14	9.3 ± 1.6	17.6 ± 4.8	41 ± 5[b]
SDA	211 ± 10	8.4 ± 0.13	13.6 ± 3.1	14.4 ± 6.5	52 ± 5[a]
SED	13.7	0.23	2.7	7.5	4.3
p	NS	NS	NS	NS	0.004

Data are shown as means ± SEM (based on between-volunteer spread). SED, standard error of difference within volunteer, values within a column with unlike superscripts differ by $p < 0.05$, NS not significant ($p > 0.05$). HAB, preintervention habitual diet; OAT, oat intervention; SDA, standard dietary advice.

Table 3. Plasma activated microparticle amounts (gate counts/μL) or as percentage of appropriate MP population following each dietary intervention ($n = 22$)

Diet	MMP_11b counts/μL	PMP_fib counts/μL	PMP_TF counts/μL	MMP_11b%	PMP_fib%	PMP_TF%
HAB	17.7 ± 4.1	1095.8 ± 150.3	176.5 ± 37.2	58.0 ± 2.4	58.9 ± 4.4	17.1 ± 3.3
OAT	6.4 ± 1.4	480.9 ± 54.8	101.9 ± 16.8	43.0 ± 2.2	33.1 ± 3.1	7.5 ± 0.9
SDA	8.8 ± 1.8	585.4 ± 60.8	126.2 ± 21.6	44.8 ± 2.4	39.4 ± 3.6	8.5 ± 0.9
SED	5.14	185.01	18.95	4.21	5.31	4.45
p	0.043	<0.001	0.007	0.001	<0.001	0.036
HAB versus OAT	0.015	0.002	0.013	< 0.001	<0.001	0.040
HAB versus SDA	NS	0.013	NS	0.004	<0.001	NS
OAT versus SDA	NS	NS	NS	NS	NS	NS

Mean ± SEM (based on between-volunteer spread for the mean value of 3 time points on each test meal day). For absolute concentrations the calculations (mean, SEM) and statistical analyses (SED, *p*-values) are based on the log-transformed data for monocyte (MMP) and platelet (PMP) microparticles activated for fibrinogen (fib) or tissue factor (TF). The results presented are obtained from back transformation of the means and appropriate residual variances. The percentage values and corresponding analyses are based on untransformed data. *p* values within columns compared for overall diet effects and between each pair of chronic diets, NS not significant ($p > 0.05$). HAB, pre-intervention habitual diet; OAT: oat intervention; SDA: standard dietary advice.

Table 4. Mean platelet volume (MPV; fL) and blood numbers (gate counts/μL) of platelets, leukocytes, and lymphocytes during the post-prandial period after the test meal ($n = 14$)

Time (min)	Platelet 10^3/μL	MPVfL	Leukocytes 10^3/μL	Lymphocyte 10^3/μL
0	206.9 ± 7.2	8.4 ± 0.1	5.8 ± 0.2	1.9 ± 0.1
90	197.0 ± 7.8	8.6 ± 0.1	5.5 ± 0.2	1.7 ± 0.1
180	203.0 ± 7.7	8.6 ± 0.1	6.2 ± 0.2	1.9 ± 0.1
SED	2.09	0.058	0.108	0.063
p	<0.001	<0.001	<0.001	<0.001
0 versus 90	<0.001	0.003	0.012	<0.001
0 versus 180	NS	0.001	0.005	NS
90 versus 180	0.008	NS	<0.001	<0.001

Data are shown as means ± SEM (based on observed between-volunteer spread for each of the three sampling times (0, 90, 180 min) following the test meal combined across all 3 dietary interventions (HAB, OAT and STD). *p* values within columns compared for overall effect of time and between each time pair, NS not significant ($p > 0.05$).

90 and 180 min after the test meal. Diet × time interactions were observed for concentrations of LMP ($p < 0.001$), CD11b-positive-leukocyte-derived microparticles (LMP_11b) ($p = 0.009$) and MMP ($p = 0.040$). These responses were driven mainly by a continued decline in concentration over the 180 min of the test meal for OAT whereas for the HAB diet an initial decline at 90 min was then followed by an increase at 180 min that exceeded the initial (time 0) value (Fig. 2).

3.4 MP regressed against other markers

In agreement with data from the main study [43], there were no diet-related effects on glycemic control nor the glycemic or insulinemic responses to the test-meal for the current subpopulation (data not shown). In addition, neither oxidative stress or inflammation were altered between OAT and SDA (data not shown), again in agreement with the larger

Table 5. Plasma microparticle concentrations (gate counts/μL) or as percentage of appropriate MP population during the postprandial period after the test meal ($n = 22$)

Time (min)	LMP counts/μL	PMP counts/μL	PMP_fib counts/μL	PMP_TF counts/μL	PMP_psel counts/μL	LMP_11b%	PMP_fib%
0	157.6 ± 15.3	3145 ± 414	686.1 ± 87.4	161.2 ± 33.2	17.9 ± 3.5	41.6 ± 2.5	32.3 ± 3.5
90	122.2 ± 15.0	2160 ± 282	731.8 ± 95.2	122.8 ± 21.4	6.4 ± 1.4	47.7 ± 2.5	48.1 ± 4.2
180	124.1 ± 15.6	1515 ± 157	588.7 ± 72.4	112.5 ± 19.3	4.7 ± 0.8	46.7 ± 2.7	49.2 ± 3.7
SED	12.96	250.77	191.75	7.70	1.85	1.58	2.32
p	0.005	<0.001	0.032	<0.001	<0.001	0.005	<0.001
0 versus 90	0.003	0.007	NS	0.003	<0.001	<0.001	<0.001
0 versus 180	0.005	<0.001	NS	<0.001	<0.001	0.003	<0.001
90 versus 180	NS	0.011	0.037	NS	NS	NS	NS

Mean ± SEM (based on between-volunteer spread observed in each of the three sampling times (0, 90, 180 min) following the test meal and combined across all diets). For absolute concentrations the calculations (mean, SEM) and statistical analyses (SED, *p*-values) are based on the log-transformed data. The results presented are obtained from back transformation of the means and appropriate residual variances. Data for the percentages of LMP and PMP are based on untransformed values. NS, not significant ($p > 0.05$).

population [43]. Regression analysis (Table 6) showed that concentrations of total PMP were negatively associated with oxidized LDL ($p = 0.041$). Concentrations of P-selectin-positive PMP were negatively associated with oxygen radical antioxidant capacity ($p = 0.004$). LMP and CD11b-positive LMP concentrations were positively associated with amounts of oxidized LDL ($p < 0.001$) and total cholesterol ($p = 0.024$), but negatively associated with urinary F2-isoprostanes ($p = 0.039$). MMP and CD11b-positive MMP concentrations were positively associated with amounts of oxidized LDL ($p < 0.001$). Those relationships with $p < 0.01$ for activated MP are also presented in graphical form (Fig. 3). No significant relationships were observed between the various MP and either C-reactive protein or interleukin-18.

4 Discussion

Associated with type 2 diabetes are a number of comorbidities, including several that impact on cardiovascular events. For some patients changes in diet and lifestyle can aid control of glycemia without the need for pharmaceutical medications. In terms of diet, recommendations include reduced intake of fat and simple sugars and increased intake of complex carbohydrates. Certain of the recommended foods may have additional benefits as they contain compounds that may mitigate some of the comorbidities. As oats are reported to exert anti-inflammatory and antioxidant properties [41, 42] the impact of high intakes has been examined in the current study based on changes in specific MP populations that have been associated with atherosclerotic and cardiovascular risk. There is no standard method for the MP detection and many techniques have been used, including flow cytometry (FCM), nanosight (nanoparticle tracking analysis), ELISA, and electron microscopy [45–49]. These analytical methods give different values for MP concentrations, a problem exacerbated by between-laboratory differences in procedures such as MP storage and isolation, plus antibody specificity. The current study used carefully standardized procedures [20] for plasma collection, MP storage, and isolation plus use of

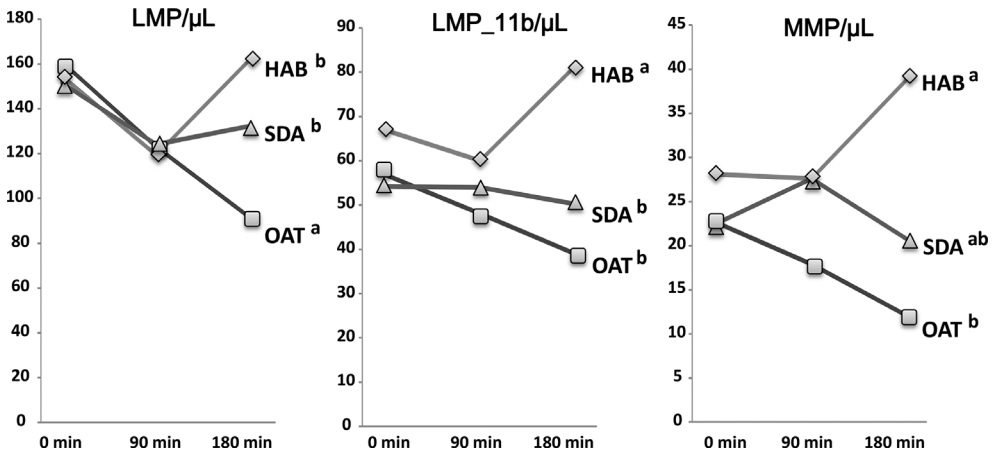

Figure 2. Plasma microparticle concentrations during the postprandial period after the test meal ($n = 22$). Data based on log-transformed data with mean values obtained by back transformation for each of the three sampling times (0, 90, 180 min) following the test meal and combined across all diets. Between diet comparisons at the same time point that are significantly different ($p < 0.05$) are shown by unlike superscripts.

Table 6. Linear regression outcomes between MP (log transformed) and other inflammatory markers (untransformed)

		Cholesterol	OxLDL	ORAC	Isoprostanes
LMP	*p*	0.024	<0.001	NS	0.039
	slope	0.139	0.016		−0.0003
LMP_11b	*p*	0.049	<0.001	NS	NS
	slope	0.131	0.016		
MMP	*p*	NS	<0.001	NS	NS
	slope		0.021		
MMP_11b	*p*	NS	<0.001	NS	NS
	slope		0.026		
PMP	*p*	NS	0.041	NS	NS
	slope		−0.008		
PMP_fib	*p*	NS	NS	NS	NS
	slope				
PMP_TF	*p*	NS	NS	NS	NS
	slope				
PMP_pesl	*p*	NS	NS	0.004	NS
	slope			−0.031	

Random effects linear regression with MP markers (counts/μL, values log-transformed) as dependent variables against various independent variables (untransformed), cholesterol (mmol/L), oxygen radical antioxidant capacity (ORAC; μM Trolox equivalent), oxidized LDL (OxLDL; U/L) and urinary isoprostanes (pg/mL). These were analyzed with Restricted Maximum Likelihood (REML) with subject set as a random factor. There were no significant relationships observed for interleukin-18.

specific antibodies coupled with flow cytometry detection. This and the cross-over within-subject design meant that treatment effects could be detected with confidence.

4.1 Chronic diet intervention

Although many studies document increased concentrations of circulating MP in various autoimmune, inflammatory and CVD states, few have attempted to directly manipulate these, or the proportions of associated functional surface molecules, and certainly not by use of dietary interventions. For the current study the MP markers selected included those recently reported to differ between subjects with or without type 2 diabetes [20] and that may reflect inflammatory status and level of vascular dysfunction. Compared with HAB, the OAT diet lowered both the plasma concentration and proportion of CD11b-positive-monocyte-derived microparticles (MMP_11b) (Table 3). The antibody used targets integrin alpha M (Mac-1), a protein subunit within the integrin alpha-M beta-2 molecule expressed on the surface of many leukocytes. This mediates inflammation by regulating both leukocyte migration and adhesion to the vascular wall [50]. LMP and other MMP are involved in the preclinical stage of atherosclerotic disease, with a link between LMP and the burden of subclinical atherosclerosis within the arterial tree [51,52]. The current data indicate that chronic ingestion of an OAT diet may improve the status of early stage vascular inflammation,

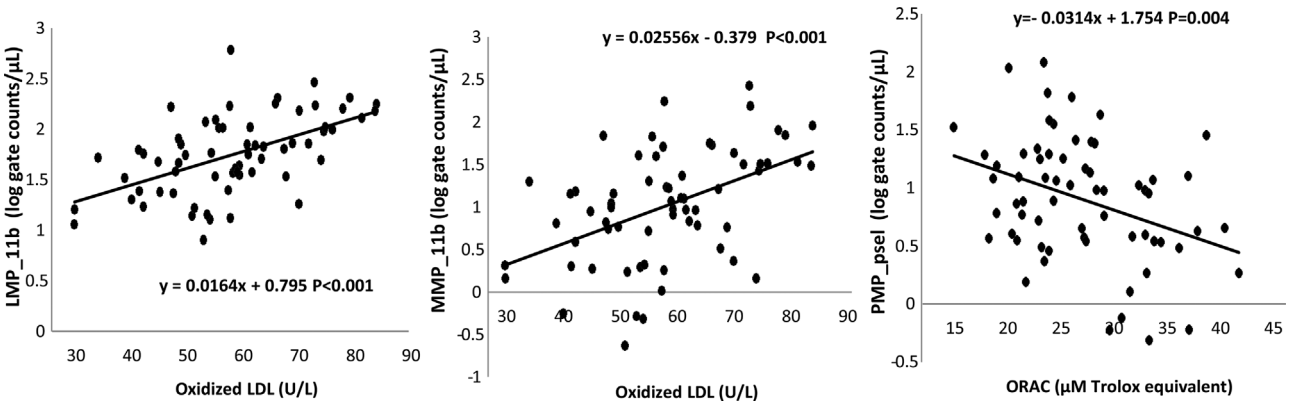

Figure 3. Relationships between clinical parameters in subjects with type 2 diabetes and log-transformed concentrations of activated microparticles: CD11b-positive leukocyte-derived microparticles (LMP_11b), CD11b-positive monocyte-derived microparticles (MMP_11b) and p-selectin positive platelet-derived microparticles (PMP_psel). Points shown are the overnight fasted values for 22 volunteers for each of the 3 diets (HAB, OAT, SDA). Data were analyzed by random effects regression using REML and with subject set as random factor to allow for within-volunteer effects to be examined.

possibly by downregulation of the recruitment of leukocytes and LMP for endothelial cells, although this needs to be tested. On their HAB diet the subjects consumed, on average, 30 g oats daily but did gain an improvement, based on MP parameters, when this was increased by 100 g/d. Importantly, SDA conferred similar advantages to OAT intervention. At diagnosis of type 2 diabetes, the subjects had been provided with information on the diet inclusions and exclusions recommended by Diabetes UK and they achieved reasonable control of their condition by diet and life-style alone. Nonetheless, reinforcement of the SDA as part of inclusion within the current study did reduce the activated MMP status (MMP_11b; Table 3) and suggests that the subjects may have ''drifted'' from following the ideal advice within their habitual intake. Therefore, although these subjects were able to exert adequate glycemic control without the need for medication there was still scope for further improvement. This emphasizes the need for regular reeducation in terms of diet (and other aspects of lifestyle) as part of the monitoring of the disease. Emphasis on higher inclusion of oats in the diet may also provide a simple and readily-identifiable means for patients who have been diagnosed with type 2 diabetes, or are at risk of such a diagnosis, to improve some of the associated comorbidities.

Positive effects on health also may accrue from the lower plasma concentrations and proportions of fibrinogen-positive PMP (PMP_fib) after OAT and SDA intervention compared with HAB. Fibrinogen is a soluble plasma glycoprotein, capable of binding to the receptor integrin αIIbβ3 on the surface of activated platelets and PMP. This firms the adhesion between platelets and the endothelium and provides the initial base for leukocyte recruitment and subsequent inflammation [53]. The reductions observed in response to OAT and SDA, therefore, may modulate the platelet adhesion to the endothelium that occurs during the early stages of inflammation and development of atherosclerosis. In future studies, this hypothesis should be tested by measurement of endothelial activation status.

In the current study, both the concentration and proportion of TF-positive PMP were also reduced after OAT intervention compared with HAB (Table 3). Tissue factor is a main activator of blood coagulation in the extrinsic pathway and plays an important role in the formation of thrombin [54–56]. Furthermore, TF may be involved in processes other than coagulation, including transcellular signaling or angiogenesis [57]. The MP can transport TF via the negatively charged phospholipids on the surface, with tissue factor-positive-platelet-derived microparticles (PMP_TF) strongly associated with hypercoagulation in the presence of vascular problems or inflammation, such as occurs in diabetes or cancer [50, 57, 58]. Therefore, reductions in the concentration of PMP_TF and the proportion of platelets with TF-activation may indicate that chronic high oat consumption provides an anti-inflammatory function.

Overall, the current data suggest that the potential health benefits with the OAT diet might involve reduced release of MP with inflammatory properties. Oats contain specific polyphenols, the avenanthramides, known to possess positive health benefits [59] through antioxidant, antigenotoxic, antiatherogenic, and anti-inflammatory properties [42, 60–63]. The presence of avenanthramides may explain why the OAT diet was superior to SDA in terms of beneficial effects on numbers and activation status of MP. Nonetheless, both OAT and SDA caused improvement compared with the habitual intake of these subjects with type 2 diabetes controlled by diet and life style alone. Indeed, the reductions observed for both MMP_11b, and PMP_fib returned subjects to values reported for those without type 2 diabetes [20]. Therefore, either OAT or a reinforced SDA strategy should be encouraged because these may help control progression of the disease and reduce the risk of adverse comorbidities.

4.2 Postprandial responses

Previous studies have reported postprandial effects on platelet activation. For example, in healthy subjects, either high carbohydrate plus low fat intake or insulin infusion decreased both platelet activation and aggregation [35, 36]. In contrast, others have reported that meal ingestion or postprandial hyperglycemia is associated with enhanced platelet activation in patients with type 2 diabetes mellitus [37, 38]. In response to the ''healthy'' test meal provided in the current study, the decline in platelet numbers at 90 min was associated with preferential loss of the smaller platelets, based on the observed changes in mean volume (Table 4). Interestingly, the recovery in platelet numbers 3 h after the meal appeared to involve release of larger platelets into the blood, based on the maintained mean size. Although this initial decrease was followed by an increase in platelet numbers, there was a continued reduction in both total and activated forms of PMP, including those with response elements to TF, p-selectin, and fibrinogen (Table 5). The lower concentrations of the latter, alone or in combination with the other activated forms, might be beneficial for reducing platelet coagulation, platelet adhesion to the endothelium [53], and the early risk of plaque formation and thus emphasize the benefits of a ''healthy'' meal.

Leukocytes responded to the healthy test meal with a decline after 90 min, similar to platelets, but then recovered to values greater than preingestion by 3 h. The LMP response showed similarity with PMP in that total numbers declined in the postprandial period but the concentrations in activated form were unchanged and so increased as proportion of total LMP (Table 5). From other studies, fat loading is known to increase leukocyte number (including monocytes, neutrophils, and lymphocytes) and activation status (based on CD62L and CD66B) for up to 8 h [64–67]. This was considered a reflection of acute postprandial activation of inflammation but with the mechanisms involved unclear [68]. In contrast, the meal offered to the current volunteers was designed deliberately to meet Diabetes UK guidelines and, as such, contained low

amounts of fat. Whether the fat contents of the test meals ingested, or other factors, account for the difference in response between the current study and the earlier reports [64–67] is unknown, although not only the amount but also the form of dietary fat can have an impact on postprandial endothelial cell function and decrease the concentrations of vascular adhesion molecules [69].Whatever the mechanism, although the current data suggest that a healthy (low fat) meal reduces LMP numbers this does not extend to the activated forms and so it is unclear whether this will confer a direct health benefit. Nonetheless, as high fat meals produce harmful responses [64–67] the lack of response to the healthier type of meal is obviously to be preferred, especially for subjects who might be at risk of cardiovascular events.

4.3 Relationships between MP and biomarkers of disease

Although both OAT and SDA interventions failed to alter glycemia, inflammation, antioxidant capacity or oxidative stress [43], there were significant associations between certain of these indices and levels of MP, suggesting that the latter may provide sensitive markers to reflect subtle changes in additional health risks during the early stages of type 2 diabetes.

In this study, LMP markers and MMP markers were positively associated with oxidized LDL while PMP markers showed a negative association (Table 6 and Fig. 3). Oxidized LDL can affect monocytes, platelets, and endothelial cells, and those interactions are important in the development of vascular complications in thrombotic diseases. Increased concentrations of MP, especially LMP, have been associated with greater oxidized LDL in various patient groups [70, 71], possibly due to the capacity for carriage of oxidized LDL by MP. The current results may indicate that LMP, but not PMP, can act as a carrier of oxidized LDL.

This study confirms and extends other observations that demonstrate the sensitivity of platelet number, activity, and associated circulating MP activation to various dietary interventions. A healthy chronic diet, but particularly one rich in oats, appears to have positive effects in terms of lesser numbers of circulating MP that contain features linked to inflammatory or antioxidant processes. Even the ingestion of a single healthy meal can have positive effects, in contrast to the negative responses observed by other studies where high fat meals were provided. The long-term impact of such healthy eating on other metabolic parameters, especially those linked to markers of inflammation and CVD risk, needs to be evaluated and, coupled with information obtained from platelet and MP analysis, used to help advise patients with type 2 diabetes of the best dietary means to help control their condition.

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