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Serum E-Selectin, Antioxidant and Metabolic Biomarkers in Adults Consuming Sunflower Oil Versus Milk Fat

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Abstract. Dietary fat source may shift redox balance, lipid handling and endothelial activation, yet community data comparing a vegetable oil with milk-derived fat remain scarce. This study compared serum antioxidant, metabolic and endothelial markers between adults who reported sunflower oil and adults who reported free fat separated from cow milk as their principal household cooking fat. Sixty non-smoking adults aged 20 to 40 years from Samarra, Iraq, were classified into two groups of thirty in a comparative cross-sectional design. Fasting serum was analysed for C-reactive protein, glutathione, glutathione peroxidase activity, malondialdehyde, glucose, triglycerides, total cholesterol, high-density and low-density lipoprotein cholesterol, and soluble E-selectin by enzyme-linked immunosorbent assay. Groups were compared by Welch-corrected t-tests with false discovery rate control, and receiver operating characteristic analysis estimated how far soluble E-selectin separated the groups. Glutathione in the milk-fat group reached 10.4 ± 1.39 µmol/L against 4.4 ± 1.4 µmol/L in the sunflower oil group ($P < 0.001$). The sunflower oil group carried higher fasting glucose (8.69 ± 2.71 against 6.59 ± 1.76 mmol/L, $P = 0.001$), triglycerides (2.23 ± 1.09 against 1.68 ± 0.42 mmol/L, $P = 0.013$) and soluble E-selectin (980.83 ± 196.25 against 873.50 ± 32.27 pg/mL, $P = 0.006$), and lower high-density lipoprotein cholesterol (0.97 ± 0.44 against 1.23 ± 0.16 mmol/L, $P = 0.005$). C-reactive protein, glutathione peroxidase, malondialdehyde, total cholesterol and low-density lipoprotein cholesterol did not separate the groups. Soluble E-selectin discriminated the groups with an area under the curve of 0.731 (95% confidence interval 0.603 to 0.858), 66.7% sensitivity and 96.7% specificity. Reported household use of milk-derived fat coincided with a larger glutathione pool, a more favourable glucose, triglyceride and high-density lipoprotein pattern, and lower endothelial activation. The cross-sectional design fixes association only; controlled feeding studies with fatty acid profiling must decide whether fat composition itself drives these differences.

Keywords: Sunflower oil, Milk fat, E-selectin, Endothelial activation, Lipid peroxidation.

1. Introduction

Dietary fats supply the substrate for metabolic homeostasis, and different fat sources push oxidative stress and lipid metabolism in measurably different directions (Toncan et al., 2025). Lipids carry the fatty acids that cells use to assemble membranes and to generate energy (De Carvalho and Caramujo, 2018). Fatty acids serve not only as structural components of membranes but also as energy sources, where mitochondria degrade these molecules through beta-oxidation, converting their carbon chains into acetyl-CoA, which subsequently supports ATP synthesis (Dikalov et al., 2024).

Disorders of hepatic lipid handling drive the progression of chronic disease. The liver runs lipid synthesis, lipolysis, and the uptake and secretion of lipoproteins, which places it at the centre of whole-body lipid traffic (Zhang et al., 2022). Clinical and experimental work ties dysfunction in these hepatic routes to the build-up of atherosclerotic plaque and to cardiovascular disease (Yanai et al., 2023). In peroxidation, one of the byproducts of this damaging process is malondialdehyde (MDA), forming when double bonds of polyunsaturated acids are being attacked by reactive oxygen species like free radicals and the carbon chain is fragmented in some cases. Hence why it serves as the standard readout of lipid peroxidation. An increase of the levels of MDA correlates with an equivalent rise in oxidative stress and cell

damages. Cells would answer the attack with glutathione (GSH), a tripeptide which would donate an electron from its sulfhydryl group to neutralise peroxides (Cordiano et al., 2023), while the glutathione peroxidase family uses that donated reducing power to convert hydrogen peroxide and organic hydroperoxides into waters and alcohols (omova et al., 2024). C-reactive protein (CRP) sits alongside these as a readout of systemic inflammatory tone (Plebani, 2023).

The link between dietary fat and insulin resistance sits at the centre of metabolic research. Excess dietary fat stored as triglyceride can be released again as free fatty acids, and those fatty acids interfere with insulin signalling and trigger inflammatory responses (Elkanawati et al., 2024; Park and Seo, 2020). Milk-derived fat carries short- and medium-chain fatty acids that have been reported to improve insulin sensitivity (K et al., 2025). Vegetable oils loaded with omega-6 fatty acids can push the balance the other way when intake climbs (DiNicolantonio and O'Keefe, 2018).

Studies repeatedly find raised lipid peroxidation indicators such as MDA sitting next to depleted antioxidants (GSH, GPx) and raised inflammatory indicators such as CRP, and those shifts arrive alongside deteriorating glucose and lipid values in metabolic syndrome, obesity and diabetes (Picu et al., 2017). Other work reads this same clustering as one disease network rather than several, which makes the simultaneous measurement of MDA, GSH, GPx and CRP together with glucose and lipids a workable approach to estimating cardiovascular and metabolic risk tied to dietary fat (S et al., 2024; Vona et al., 2019).

Serum chemistry cannot tell whether the vessel wall has registered any of this. Endothelial activation precedes visible atherosclerotic change and is detectable in the circulation long before a structural lesion forms. E-selectin appears only on activated endothelium, where it tethers passing leukocytes and slows them into rolling contact with the vessel surface; cleavage then releases a soluble fragment (sE-selectin) into plasma, where it can be assayed (Roldán et al., 2003). Concentrations rise in hypertension, diabetes and hyperlipidaemia. Fatty acids act on this system directly. Linoleic and palmitic acids exert measurably different effects on cytokine-induced adhesion molecule expression in cultured human endothelial cells (Reissig et al., 2003; Sanadgol et al., 2012), and a controlled human dietary trial lowered circulating sE-selectin by replacing saturated with unsaturated fatty acids (Wong et al., 2021). We therefore added sE-selectin to the panel to ask whether the two dietary groups separate at the endothelium and not only in serum chemistry.

Sunflower oil and milk-derived free fat differ at the level of the fatty acid itself. Linoleic acid (C18:2 n-6) dominates sunflower oil and supplies the substrate from which peroxidation products arise (Lužaić et al., 2023; Machate et al., 2022). Milk-derived free fat delivers short- and medium-chain saturated fatty acids (Tadmor-Levi and Argov-Argaman, 2025). The present design reaches that contrast only indirectly. It compares two groups of people who report different household cooking fats; it neither administers a fat, nor quantifies intake, nor isolates fatty acid composition from the dietary and lifestyle pattern that travels with it.

The objective of this study was to compare serum redox (GSH, GPx, MDA), inflammatory (CRP), metabolic (glucose and lipid profile) and endothelial (sE-selectin) markers between adults reporting sunflower oil and adults reporting milk-derived fat as their predominant household cooking fat, and to estimate how well sE-selectin discriminates the two groups. We hypothesised that the two groups would show distinct profiles, and we tested this in a community sample from Samarra, Iraq, where household cooking-fat habits divide along these two lines.

2. Materials and Methods

2.1 Study Design and Participants

This comparative cross-sectional study ran from February to March 2025 in Samarra, Iraq. Sixty never-smoking adults of both sexes, aged 20 to 40 years, were recruited. Participants were neither randomised nor given any dietary intervention. They were classified into two groups of thirty according to the predominant household cooking fat reported at recruitment: G1 reported sunflower oil and G2 reported free fat separated from cow milk. The exposure variable throughout is this reported cooking-fat category, and the labels "sunflower oil group" and "milk-fat group" rest on that basis alone. Each group comprised 15 men and 15 women (Table 1). Eligibility required that the household had used the stated fat as its principal cooking medium for at least 12 months before sampling, established by direct questioning; no weighed intake record was collected and daily fat consumption was not quantified. Exclusion criteria removed anyone with a confirmed history of diabetes mellitus or cardiovascular disease, anyone taking lipid-lowering medication, and any current or former smoker.

The research ethics committee of the College of Applied Sciences, University of Samarra, reviewed and approved the study protocol. Written informed consent was obtained from every participant before sampling.

2.2 Chemicals, Reagents and Equipment

Thiobarbituric acid and 5,5′-dithiobis-(2-nitrobenzoic acid) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Enzymatic kits for glucose, total cholesterol, triglycerides and high-density lipoprotein cholesterol were obtained from Biolabo (Maizy, France). Absorbance was read on an Azzota SM-1100 UV-VIS spectrophotometer (Azzota Corporation, USA). The CRP and sE-selectin ELISA kits were obtained from SunLong Biotech Co., Ltd. (Hangzhou, China).

2.3 Sample Collection and Serum Preparation

After an overnight fast of 8 to 12 h, 10 mL of venous blood was drawn with disposable syringes into plain gel tubes containing no anticoagulant. Tubes stood at room temperature for 30 min to clot and were then centrifuged at 4000 rpm for 10 min. Serum was divided into aliquots and held at −20 °C until analysis; each sample passed through a single freeze–thaw cycle. Samples from both groups were interleaved across assay plates so that no plate carried a single group, and all samples for a given analyte were run in one batch.

2.4 Determination of oxidative stress and inflammatory markers

MDA was measured as thiobarbituric acid reactive substances (TBARS): under heat and acid, MDA condenses with two molecules of thiobarbituric acid into a pink adduct read colorimetrically at 532 nm (Ohkawa et al., 1979). GSH was measured with Ellman's reagent, whose disulfide bond is reduced by free sulfhydryl groups, releasing the yellow thiolate anion (Luo et al., 2011; Riddles et al., 1983). GPx activity was estimated spectrophotometrically by a coupled assay in which the enzyme reduces hydrogen peroxide at the expense of glutathione, and the residual glutathione is quantified colorimetrically (Rotruck et al., 1973). Serum CRP was quantified by sandwich ELISA with a Human C-Reactive Protein ELISA kit (SunLong Biotech Co., Ltd., Hangzhou, China) according to the manufacturer's instructions, with absorbance read at 450 nm and concentrations interpolated from the kit standard curve.

2.5 Determination of Metabolic Parameters

Fasting glucose, total cholesterol and triglycerides were measured enzymatically by the GOD-PAP (Rascón-Careaga et al., 2021), CHOD-PAP (Allain et al., 1974) and GPO-PAP (McGowan et al., 1983) reactions, each terminating in a quinoneimine chromogen read at 500 nm. High-density lipoprotein cholesterol (HDL-C) was measured after phosphotungstic acid precipitated the apolipoprotein B-containing lipoproteins (Burstein et al., 1970). Very-low-density lipoprotein cholesterol (VLDL-C) was derived as triglycerides divided by five, and low-density lipoprotein cholesterol (LDL-C) as total cholesterol minus HDL-C minus VLDL-C, following the Friedewald equation (Friedewald et al., 1972). Both derivations were applied to each participant, in mg/dL, before group means were taken. All glucose and lipid values were then converted to SI units (glucose × 0.0555, cholesterol fractions × 0.0259 and triglycerides × 0.0113, giving mmol/L). VLDL-C and LDL-C are therefore calculated rather than measured quantities, and their comparisons carry no statistical information independent of the triglyceride, total cholesterol and HDL-C measurements from which they derive.

2.6 Determination of soluble E-selectin

Serum sE-selectin was quantified by sandwich enzyme-linked immunosorbent assay (ELISA) with a Human E-selectin (ES) ELISA kit (SunLong Biotech Co., Ltd., Hangzhou, China) whose stated working range is 8 to 500 pg/mL. Serum was diluted five-fold in the sample diluent supplied with the kit so that readings fell inside the calibrated portion of the curve. Standards and samples were run in duplicate, absorbance was read at 450 nm, and concentrations were interpolated from a four-parameter logistic curve fitted to the kit calibrators. Interpolated values were multiplied by the dilution factor and are reported in pg/mL.

2.7 Statistical analysis

Data were analysed in IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA; released 2020) and are expressed as mean ± standard deviation (SD). Distributional shape was checked with the Shapiro–Wilk test and variance homogeneity with Levene's test before any comparison was run. Comparisons between the two groups were made with the independent samples t-test, applying the Welch correction wherever the assumption of equal variance did not hold, and confirmed with the Mann–Whitney U test where dispersion differed markedly between groups. Receiver operating characteristic (ROC) analysis was performed for sE-selectin, with area under the curve (AUC) reported alongside its 95 per cent confidence interval (CI) calculated by the Hanley–McNeil method (Hanley and McNeil, 1982); the operating point was selected by maximising the Youden index, and sensitivity and specificity are reported at that point. Because eleven comparisons were performed, the Benjamini–Hochberg procedure was applied across the nine directly measured markers to control the false discovery rate at 5 per cent; VLDL-C and LDL-C were excluded from that correction because both are deterministic functions of variables already tested. For every comparison the difference between group means is reported with its 95 per cent CI and with Hedges' g as a bias-corrected standardised effect size, so that the magnitude of each finding can be judged independently of its P value. The ROC operating point was derived from the same sample in which it was evaluated and was not validated in an independent dataset; the resulting sensitivity and specificity are therefore optimistic, and the analysis is presented as exploratory. Statistical significance was set at $P < 0.05$, and adjusted values are reported where the correction alters interpretation.

3. Results and Discussion

3.1 Oxidative stress and inflammatory markers

Regarding oxidative stress parameters, the results were as shown in Table 2. No statistically significant difference in CRP was detected between the two groups ($P = 0.286$), with a confidence interval for the difference that spans zero. CRP is a single acute-phase reactant, and a null result on it does not establish that systemic inflammatory tone is equivalent in the two groups; a panel including interleukin-6 and tumour necrosis factor alpha would be needed for that claim. Serum glutathione behaved very differently, climbing to more than double in the milk-fat consumers (10.4 ± 1.39 against 4.4 ± 1.4 μmol/L, $P < 0.001$), and the size of that gap points towards the biochemistry of the source rather than towards chance.

Synthesis of glutathione occurs through two ATP-dependent steps, and cysteine availability sets the ceiling on the whole process because glutamate–cysteine ligase cannot run faster than its scarcest substrate arrives (Gould and Pazdro, 2019). That places sulphur amino acid supply at the front of any explanation. Milk proteins, particularly the whey and casein fractions, are dense in cysteine and methionine (Haug et al., 2007), and methionine feeds the transsulfuration pathway, where it passes through homocysteine and cystathionine into cysteine and releases the sulfhydryl groups needed to build the gamma-glutamyl-cysteinyl-glycine tripeptide (McBean, 2012; Pajares, 2025). Plant proteins carry both amino acids at lower concentration and lower biological value, so a supply difference between the two diets is expected in principle.

The exposure studied here cannot carry that explanation. Clarified milk fat is not milk. Separation strips out the milk solids, and with them nearly all the casein and whey, leaving a product with under 0.3 g protein per 100 g. A residual amino acid contribution of that size cannot plausibly double a serum glutathione pool, which moves the explanation away from the protein fraction and towards the lipid fraction itself.

Butyric acid (C4:0) makes up roughly 3 to 4% of milk fat by weight (Haug et al., 2007) and activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Released from its cytoplasmic inhibitor Keap1, Nrf2 translocates to the nucleus and binds antioxidant response elements in the promoters of target genes, raising transcription of glutamate–cysteine ligase, glutathione reductase, the glutathione S-transferases and other phase II enzymes (Vomhof-DeKrey and Picklo, 2012). Butyrate-driven Nrf2 activation would expand glutathione synthetic capacity even at constant cysteine supply, with no need for a protein contribution.

The same literature holds a tension that we record rather than write around. Vomhof-DeKrey and Picklo (2012) report that lard-based high-fat diets, unlike soybean-oil-based diets, reduce mRNA expression of Nrf2 and its downstream targets, which places saturated fat on the opposite side of the Nrf2 argument. Chain length may reconcile the two

observations. Long-chain palmitic and stearic acids dominate lard, whereas the Nrf2-activating fraction of milk fat is short-chain butyrate, which lard lacks in meaningful quantity. Fatty acid profiling of the consumed fats, not performed here, offers the direct test and the clearest target for follow-up work.

Malondialdehyde carries the second half of the argument. GSH more than doubled while MDA did not separate the groups (2.84 ± 2.31 against 1.99 ± 2.22 μmol/L, P = 0.152), and that combination rules out one obvious alternative reading. Had the milk-fat group simply been carrying a heavier oxidative load, the expanded glutathione pool would read as a compensatory response to ongoing peroxidation, and MDA would have climbed alongside it (Zheng et al., 2021; Zheng et al., 2023). MDA did not climb. Numerically it sat lower in the milk-fat group, though dispersion in both groups is too wide to support a claim. The data fit greater glutathione availability better than a defensive reaction to damage already under way, but they do not establish it; separating the two requires the GSSG/GSH ratio, an estimate of synthesis rate, or expression data for the synthetic enzymes, none of which was obtained. GPx activity showed no separation either (P = 0.067), consistent with neither group clearing an unusual hydroperoxide load.

Our own glutathione values need the same scrutiny. A serum concentration of 10.4 μmol/L exceeds the single-digit micromolar range usually quoted for plasma and serum in healthy adults (Detcheverry et al., 2023). As applied here, the Ellman procedure reports total acid-soluble thiol rather than reduced glutathione specifically (Riddles et al., 1979), so other low-molecular-weight thiols and protein-bound sulfhydryl groups incompletely removed at deproteinisation may contribute. Both groups were processed identically, which keeps the comparison valid, but the absolute concentrations depend on the method used.

Sunflower oil chosen for this comparison is based on its chemical substrate. substrate. It contains linoleic acid, which contains two double bonds separated by a bis-allylic methylene group separates them. Radicals primarily target the hydrogen at this specific position. Once this hydrogen is abstracted, a propagation chain begins and eventually forms MDA (Pratt et al., 2011; Sadoudi et al., 2014). A diet built on sunflower oil therefore loads the tissue lipid pool with the very molecules that peroxidation consumes (Ghendov-Moșanu et al., 2023). However, we must note a methodological limitation. The thiobarbituric acid assay is not entirely specific to malondialdehyde. It actually detects a broader group of reactive carbonyls (Ortega, 2025), so the values reported here are thiobarbituric acid reactive substances and are not a specific index of lipid peroxidation. That the sunflower oil group did not show significantly higher MDA, despite carrying more of the vulnerable substrate, deserves recording as a finding in its own right. The wide standard deviation in that group (2.31 on a mean of 2.84) suggests the group is not homogeneous, and a subset carrying high MDA may well be masked by the group average.

3.2 Glucose and Lipid Profile

Regarding metabolic parameters, the results were as shown in Table 3. The pattern in Table 3 is selective, and the selectivity is informative. Total cholesterol and LDL-C sat almost on top of each other in both groups, separated by roughly 0.05 mmol/L in each case, so no significant between-group difference was detected in total cholesterol or in calculated LDL-C. What did separate the groups were the three measures that track hepatic triglyceride handling and insulin action: fasting glucose (8.69 ± 2.71 against 6.59 ± 1.76 mmol/L, P = 0.001), triglycerides (2.23 ± 1.09 against 1.68 ± 0.42 mmol/L, P = 0.013), and VLDL-C, all higher in the sunflower oil consumers.

These three move together for a mechanistic reason. So, the liver delivers the triglycerides from it in a VLDL. So, the concentration of VLDL is simply a question about the amount of triglycerides the liver needs to dispatch. The higher blood fasting glucose as well higher triglyceride fit the image that the reduced control insulin has on production of VLDL from liver, insulin inhibits apolipoprotein B secretion by promoting its degradation (Van Zwol et al., 2024). This is an inference than the description. No insulin, C-peptide, or HOMA-IR measurement was made in this study, so the pathway proposed here rests on the pattern of the markers rather than on any direct index of insulin action.

HDL-C moved in the opposite direction and sat roughly 0.26 mmol/L higher in the milk-fat group (1.23 ± 0.16 against 0.97 ± 0.44 mmol/L, P = 0.005). In terms of cholesteryl ester transfer protein, triglyceride concentrations and HDL concentrations are linked. It catalyses the exchange of triglyceride from VLDL into HDL and cholesteryl ester from HDL into VLDL. This exchange gives HDL more triglyceride molecules which are then hydrolysed by hepatic lipase and cleared faster from the plasma (Chapman et al., 2010). Lower circulating triglyceride slows that exchange and preserves HDL particles. On that reading the HDL and triglyceride findings in Table 3 are not two independent results

but two faces of one process. Neither cholesteryl ester transfer protein activity nor hepatic lipase activity nor apolipoprotein B was measured in this study, so the pathway is invoked to explain the pattern, not demonstrated by it.

The absolute glucose concentrations require a frank comment, because they place a constraint on how this cohort may be described. A group mean of 8.69 mmol/L in G1 exceeds the diagnostic threshold for diabetes mellitus (7.0 mmol/L), and the G2 mean of 6.59 mmol/L falls inside the range for impaired fasting glucose. Our exclusion criterion removed participants with a confirmed history of diabetes, which by construction leaves undiagnosed dysglycaemia in the sample. This cohort is therefore not a healthy population, and we do not describe it as one. A substantial fraction of participants in both groups carry unrecognised hyperglycaemia, which is itself a finding worth reporting in a community sample of adults aged 20 to 40. It also complicates interpretation, since hyperglycaemia independently depletes glutathione and independently induces endothelial adhesion molecule expression (Kanikarla-Marie et al., 2019), and we return to that point below.

One further caution belongs with these numbers. The standard deviations differ substantially between groups for HDL-C and for triglycerides, and this heterogeneity of variance is why the Welch correction was applied. Wide dispersion in the sunflower oil group indicates that dietary fat source is not the only variable operating within it, and unmeasured contributors, total energy intake, physical activity and body mass index among them, remain candidates.

3.3 Endothelial activation and discriminatory performance of sE-selectin

Regarding endothelial activation and its discriminatory performance, serum sE-selectin in the two groups is shown in Table 4. Endothelial activation ran higher in the sunflower oil consumers by roughly 107 pg/mL, a separation of about 12 per cent above the milk-fat group mean (980.83 ± 196.25 against 873.50 ± 32.27 pg/mL, $P = 0.006$; adjusted $P = 0.013$). Shapiro–Wilk raised no objection to normality in either group ($P = 0.98$ and $P = 0.94$), so the parametric estimate can be reported as the primary result.

Levene's test rejected variance homogeneity outright ($F = 30.85$, $P < 0.001$), which is why the Welch correction was applied and why the non-parametric test was run alongside it ($U = 657.5$, $P = 0.002$). Both tests agree, so the conclusion does not rest on a distributional assumption. The actual direction of this follows the chemistry of the fatty acids vs. saturated, Linoleic acid which sunflower's oil has. Linoleic acid and its hydroperoxide derivatives act on the machinery that regulates adhesion molecule expression, with NF-κB being the meeting point to control this gene and for cells not to attach or for cells to grow. Peroxidation products generated from n-6 substrate function as the oxidised signal that drives this induction, which places the sunflower oil group at the receiving end of a pathway its own diet supplies the substrate for. Milk-derived fat, dominated by short- and medium-chain saturates, contributes far less of that substrate (Ayala et al., 2014; Panda et al., 2017; Zhang et al., 2025).

ROC analysis measured how far sE-selectin separates the two dietary groups (Figure 1). The AUC was 0.731 (95% CI 0.603 to 0.858). The Youden-optimal cut-off of 940 pg/mL gave 66.7% sensitivity and 96.7% specificity for identifying sunflower oil consumers.

The curve in question merits some comment, since a specificity of 96.7 per cent in conjunction with a sensitivity of 66.7 per cent is not characteristic of a well-balanced test. One individual from the milk-fat group reached the threshold, whereas one-third of those in the sunflower oil group fell below it. A value above 940 pg/mL thus identifies a sunflower oil consumer with few false positives; below this level, however, no separation is achieved because the lower half of the sunflower oil distribution lies within the range occupied by the milk-fat group. This asymmetry is a direct result of the variance structure shown in Table 4: the milk-fat group is confined to a band of 125 pg/mL whereas the sunflower oil group extends over 810 pg/mL, so the two distributions overlap throughout the lower range and only diverge in the upper tail. Since the lower confidence limit of 0.603 is close to 0.5, the discriminating ability of this marker at this sample size has to be described as modest.

3.4 Limitations

The cross-sectional design fixes an association and cannot establish that dietary fat caused the differences observed. The direction of any causal arrow is equally open: people with differing metabolic or socioeconomic characteristics may select different cooking fats. Convenience recruitment from one city admits selection bias and limits generalisation. The exposure itself carries the heaviest qualification. What separates the groups is a reported household

cooking-fat category, not a measured fatty acid intake. No gas chromatographic analysis of the consumed fats and no serum phospholipid fatty acid profiling were performed, so the compositional assumptions behind the mechanistic discussion were assumed rather than verified. Consumption outside the household was not captured, and two groups differing in cooking fat may also differ in whole dietary pattern. Body mass index, waist circumference, total energy intake and physical activity were not recorded, although each independently influences glutathione status and the glucose–triglyceride axis; residual confounding should therefore be assumed present. Thirty participants per group was a convenience sample rather than the product of an a priori power calculation, so the non-significant results for CRP, GPx, MDA, total cholesterol and LDL-C represent absence of evidence and not evidence of absence.

MDA showed coefficients of variation of 81% in G1 and 112% in G2; a coefficient above 100% in a variable bounded at zero signals pronounced right skew, and median with interquartile range would describe this marker better than mean ± SD. Fasting glucose was measured once, without HbA1c, so the dysglycaemia noted above could not be confirmed on a second occasion as diagnostic criteria require.

Three points specific to the sE-selectin measurement need recording. The absolute concentrations obtained here, roughly 0.87 to 0.98 ng/mL, sit well below the range reported for healthy adults in the published literature, where values around 50 ng/mL are typical. Differences in kit calibrator material and antibody epitope between manufacturers account for part of this, and sample handling accounts for another part: serum was held at −20 °C, whereas studies of soluble adhesion molecules conventionally store at −80 °C, and prolonged storage at the warmer temperature can degrade the epitope and depress recovery. Both groups were collected, stored and assayed under identical conditions, so the between-group comparison remains internally valid, but the absolute values should not be compared against external reference ranges and no clinical cut-off should be derived from them. The dispersion of the milk-fat group is also unusually narrow, with a coefficient of variation of 3.7 per cent across thirty individuals, which is tighter than the intra-assay variability typically quoted for kits of this class.

4. Conclusion

In this cross-sectional community sample, adults reporting milk-derived fat as their predominant household cooking fat had higher serum glutathione and HDL-C, and lower fasting glucose, triglycerides, VLDL-C and sE-selectin, than adults reporting sunflower oil. The glutathione difference was the largest observed, more than twofold, with an effect size exceeding four standard deviations. MDA, GPx, CRP, total cholesterol and calculated LDL-C did not differ significantly, and the confidence intervals for those comparisons are wide enough that meaningful differences cannot be ruled out at this sample size.

These findings establish association, not causation, and do not identify which component of the two fats, or of the diets that accompany them, is responsible. The mechanisms discussed, butyrate-driven Nrf2 signalling and linoleic acid-derived endothelial activation, remain hypotheses: neither pathway was measured, the fatty acid composition of the consumed fats was not verified analytically, and the sE-selectin difference cannot be separated from the concurrent difference in fasting glucose. Controlled feeding studies with quantitative dietary assessment and fatty acid profiling are needed to determine whether these associations arise from the composition of the dietary fats themselves.

5. Declaration of Interest, Funding and Acknowledgements

Declaration of interest: The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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Table 1.0. Characteristics of the two study groups

Variable	G1 sunflower oil (n = 30)	G2 milk-derived fat (n = 30)
Age (years)	20–40	20–40
Sex (male/female)	15/15	15/15
Smoking status	Never-smokers	Never-smokers
Duration of reported fat use	≥ 12 months	≥ 12 months

G1 = sunflower oil group; G2 = milk-derived fat group. Group membership reflects the predominant household cooking fat reported at recruitment.

Table 2.0. Serum oxidative stress and inflammatory markers in the sunflower oil group (G1) compared with the milk-derived fat group (G2)

Parameter	G1 sunflower oil	G2 milk fat	Δ (95% CI)	g	P (Padj)
CRP (mg/L)	1.35 ± 0.49	1.22 ± 0.44	0.13 (−0.11 to 0.37)	0.27	0.286 (0.322)
GSH (µmol/L)	4.4 ± 1.4	10.4 ± 1.39	−6.00 (−6.72 to −5.28)	−4.25	< 0.001* (< 0.001*)
GPx (U/L)	528.17 ± 90.9	488.0 ± 74.7	40.17 (−2.86 to 83.20)	0.48	0.067 (0.100)
MDA (µmol/L)	2.84 ± 2.31	1.99 ± 2.22	0.85 (−0.32 to 2.02)	0.37	0.152 (0.195)

Values are mean ± SD; n = 30 per group. CRP = C-reactive protein; GSH = glutathione; GPx = glutathione peroxidase; MDA = malondialdehyde measured as thiobarbituric acid reactive substances. Δ = difference between group means (G1 − G2); CI = confidence interval; g = Hedges' g. P from the Welch-corrected independent-samples t-test. Padj = Benjamini–Hochberg adjusted P across the nine measured markers. * Significant at $P < 0.05$.

Table 3.0. Fasting serum glucose and lipid profile in the sunflower oil group (G1) compared with the milk-derived fat group (G2)

Parameter (mmol/L)	G1 sunflower oil	G2 milk fat	Δ (95% CI)	g	P (Padj)
Glucose	8.69 ± 2.71	6.59 ± 1.76	2.10 (0.92 to 3.29)	0.91	0.001* (0.004*)
Triglycerides	2.23 ± 1.09	1.68 ± 0.42	0.55 (0.12 to 0.99)	0.66	0.013* (0.023*)
Total cholesterol	4.19 ± 0.97	4.25 ± 0.83	−0.05 (−0.52 to 0.41)	−0.06	0.819 (0.819)
HDL-C	0.97 ± 0.44	1.23 ± 0.16	−0.26 (−0.43 to −0.08)	−0.77	0.005* (0.013*)
VLDL-C†	1.02 ± 0.50	0.77 ± 0.19	0.25 (0.06 to 0.45)	0.66	0.013
LDL-C†	2.20 ± 0.64	2.25 ± 0.52	−0.05 (−0.35 to 0.25)	−0.09	0.736

Values are mean ± SD; n = 30 per group. Values were calculated in mg/dL and converted to mmol/L (glucose × 0.0555; cholesterol fractions × 0.0259; triglycerides × 0.0113). HDL-C, VLDL-C and LDL-C = high-, very-low- and low-density lipoprotein cholesterol. Δ = difference between group means (G1 − G2); CI = confidence interval; g = Hedges' g. P from the Welch-corrected independent-samples t-test. Padj = Benjamini–Hochberg adjusted P across the nine measured markers. † Calculated by the Friedewald equation and excluded from the false discovery rate correction. * Significant at $P < 0.05$.

Table 4.0. Serum soluble E-selectin in the sunflower oil group (G1) compared with the milk-derived fat group (G2)

Parameter	G1 sunflower oil	G2 milk fat	Δ (95% CI)	g	P (Padj)
sE-selectin (pg/mL)	980.83 ± 196.25	873.50 ± 32.27	107.33 (33.23 to 181.43)	0.75	0.006* (0.013*)

Values are mean ± SD; n = 30 per group. sE-selectin = soluble E-selectin. Δ = difference between group means (G1 − G2); CI = confidence interval; g = Hedges' g. Welch-corrected t-test (t = 2.96, df = 30.6); the Mann–Whitney U test returned the same conclusion (U = 657.5, P = 0.002). Padj = Benjamini–Hochberg adjusted P across the nine measured markers. * Significant at $P < 0.05$.

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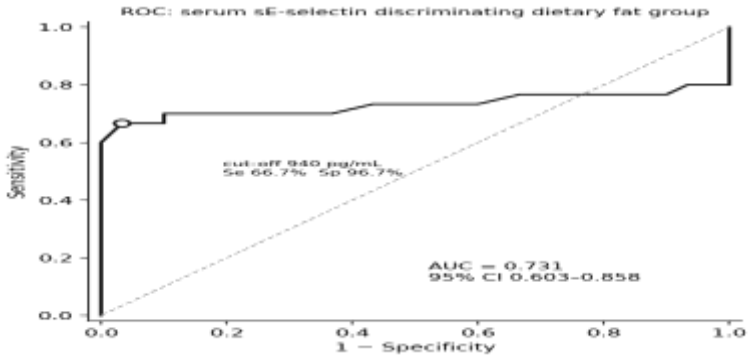


Figure 1: *Receiver operating characteristic curve for serum soluble E-selectin*

Curve discriminating the sunflower oil group from the milk-derived fat group (n = 30 per group). The solid line traces the empirical curve; the dashed diagonal marks chance discrimination. The open circle marks the Youden-optimal cut-off of 940 pg/mL (sensitivity 66.7%, specificity 96.7%). AUC = 0.731 (95% CI 0.603 to 0.858, Hanley–McNeil method).