Proteomic Approaches to Predict Bioavailability of Fatty Acids and Their Influence on Cancer and Chronic Disease Prevention^{1–3}

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Abstract

A low intake of fish and PUFA and high dietary *trans*- and SFA are considered to be among the main preventable causes of death. Unfortunately, epidemiological and preclinical studies have yet to identify biomarkers that accurately predict the influence of fatty acid intake on risk of chronic diseases, including cancer. Changes in protein profile and post-translational modifications in tissue and biofluids may offer important clues about the impact of fatty acids on the etiology of chronic diseases. However, conventional protein methodologies are not adequate for assessing the impact of fatty acids on protein expression patterns and modifications and the discovery of protein biomarkers that predict changes in disease risk and progression in response to fatty acid intake. Although fluctuations in protein structure and abundance and inter-individual variability often mask subtle effects caused by dietary intervention, modern proteomic platforms offer tremendous opportunities to increase the sensitivity of protein analysis in tissues and biofluids (plasma, urine) and elucidate the effects of fatty acids on regulation of protein networks. Unfortunately, the number of studies that adopted proteomic tools to investigate the impact of fatty acids on disease risk and progression is quite small. The future success of proteomics in the discovery of biomarkers of fatty acid nutrition requires improved accessibility and standardization of proteomic methodologies, validation of quantitative and qualitative protein changes (e.g., expression levels, post-translational modifications) induced by fatty acids, and application of bioinformatic tools that can inform about the cause-effect relationships between fatty acid intake and health response. J. Nutr. 142: 1370S–1376S, 2012.

Introduction

Whereas some epidemiologic studies have suggested an inverse relationship between intake of fatty acids and cancer risk (1) and

mortality (2), other investigations have reported inconclusive results (3,4) or even suggested harmful associations (5). In general, animal and in vitro studies have been more conclusive about the protective and promoting effects of (n-3) and (n-6) fatty acids, respectively [for review, see (6–9)]. In addition, a reduction in cardiovascular diseases has been associated with dietary intake of (n-3) fatty acids (10). The beneficial and detrimental effects of fatty acids on cancer and other chronic diseases (e.g., inflammation) are influenced by the type of fatty acid (11), dose of intake (12), duration and timing of exposure (13), and target tissue (4). Therefore, there is a need to develop biomarkers that account for these sources of variation and predict how specific fatty acids influence disease risk.

Historically, tissue and blood fatty acids have been used as biomarkers of dietary fat intake (14). Because fatty acids influence protein networks that control biological processes (e.g., cancer and inflammation) (15), there is a growing interest in developing protein biomarkers related to the efficacy of fatty acids. However, fluctuations in protein concentrations in biofluids and tissues, inter-individual variability, and large differences in protein abundance and modifications are challenges to gathering protein data using traditional protein methodologies (16). Therefore, the objective of this article is to discuss observations from epidemiological and preclinical studies about

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the effects of dietary fatty acids on risk of cancer and other chronic diseases and highlight how proteomic technologies may be useful for the development of protein biomarkers linking fatty acid intake and disease risk.

Epidemiological Studies

A meta-analysis of cohort studies reported a significant protective association between the presence in biological samples of long-chain (LC)⁶ (n-3) PUFA and breast cancer risk (5). Conversely, an increased association with breast cancer risk was found for palmitic (16:0) and oleic [18:1(n-9)] acid and total MUFA. Total SFA were significantly associated with breast cancer risk in cohort studies only in postmenopausal women. A review and a meta-analysis of case-control and prospective cohort studies reported there was no evidence that a diet high in linoleic acid [18:2(n-6)] or PUFA increased the risk of breast, colorectal, or prostate cancer (17). The same study found positive associations between cancer rates and per capita intake of animal or SFA, whereas cancer risk was reduced in association with increased consumption of vegetable oil or PUFA. A meta-analysis of observational studies found an increased risk of prostate cancer in men with a high intake or blood levels of α -linolenic acid [18:3 (n-3)]. Conversely, a high intake of α -linoleic acid was associated with a reduced risk of fatal heart disease in prospective cohort studies (18). More recently, a meta-analysis indicated that a high intake and adipose tissue levels of α -linolenic acid were associated with an increased risk of prostate cancer (19).

The cancer-modifying effects of fish and marine fatty acids are influenced by various factors, including fatty acid composition, gender, ethnicity, intake of dietary supplements, and type of cancer (20). For example, in the European Prospective Investigation into Cancer and Nutrition study (21), no associations were found among fish consumption, menopausal status, and breast cancer risk. On the other hand, fish intake was associated with a decreased risk of breast cancer in Asian populations (22,23). In the Physician's Health Study, an inverse association was observed between colorectal cancer risk and blood levels of LC (n-3) PUFA in men who were not taking aspirin (24). Moreover, the intake of LC (n-3) PUFA from fish was inversely associated with the risk of colorectal cancer (25). Unfortunately, the molecular mechanisms responsible for these differential responses to fish and LC (n-3) PUFA are not clearly understood.

Sources of variation in epidemiological studies

Wide differences among epidemiological studies in the cataloging of intake of total and specific fatty acids likely contribute to difficulties in drawing inferences about the health impact of dietary fatty acids (8). Sources of variation include the use of different FFQ and nutrient databases, recall bias, inconsistent case definitions, residual confounding, and other personal bias (26). For example, the estimation of fatty acid intake may not be accurate, because food composition and nutrient databases often lack information for specific fatty acids. Compared with estimates obtained with FFQ, direct measurements of fatty acid composition in biological samples (e.g., plasma, tissue) would offer better measurements of fatty acid intake and efficacy and reduce the bias associated with estimation of bioavailability (5).

In epidemiological studies, the strength of the observed protective or negative associations of fatty acid intake with

disease risk may be influenced by the type of fatty acid and biological pool targeted for analysis (e.g., plasma, adipose tissue) (Fig. 1). For example, the lipid fractions of adipose tissue and blood correlate well with dietary intake of exogenous fatty acids such as *trans*- or odd-chain fatty acids, linoleic acid, α -linolenic acid, and fish oil-derived LC (n-3) PUFA such as EPA [20:5(n-3)] and DHA [22:6(n-3)]. Analysis of adipose tissue would be ideal for long-term dietary interventions (i.e., longer than 6 mo) (26). Conversely, for short-term dietary intervention studies, analysis of erythrocytes would be preferable, because whole blood or plasma fatty acid composition are influenced by plasma FFA, which reflect adipose tissue composition (14). For example, increased levels of erythrocyte EPA plus DHA (i.e., the omega-3 index) have been related to reduced risk of coronary heart disease (27). Overall, more sensitive and systemic biomarkers are needed to predict bioavailability of fatty acids and their influence on cancer and other chronic diseases.

Preclinical Studies

In general, animal studies have not clearly elucidated the impact of fatty acid nutrition on the initiation phase of carcinogenesis and influence of timing of exposure (e.g., prepuberty or early adulthood) (9). A meta-analysis of data obtained from 97 rodent studies found that (n-6) PUFA had strong mammary tumor-enhancing effects compared with SFA, whereas no associations were found for MUFA (11). Most animal studies have documented that the administration of LC (n-3) PUFA during the promotion and progression phases of tumor development exerted a repressing effect on carcinogenesis. Unfortunately, the translational relevance of these findings to cancer prevention remains unclear, because the intake levels of LC (n-3) PUFA that exert protective effects in animal experiments would be difficult to attain in humans (28, 29).

The discrepancy between the results of preclinical and epidemiological studies of the cancer preventative properties of LC (n-3) PUFA (e.g., breast and colon) has been attributed in part to various confounding factors such as differences in the composition of the fat, the (n-3):(n-6) fatty acid ratio of the diet, bioavailability of fatty acids, and dose and timing of exposure (10). Better control of experimental conditions and improved analytical procedures are necessary to reduce inconsistencies between epidemiological and preclinical studies and to identify the mechanisms of action of specific fatty acids.

Mechanisms of action of fatty acids

Mechanisms through which fatty acids influence cellular response have been extensively reviewed (6–9). Fatty acids induce changes in membrane structure and function (6), influence gene expression (9), and regulate cell membrane Toll-like (TLR) and G-protein-coupled (GPR) receptors (30). Through these mechanisms, fatty acids influence several signaling pathways associated with inflammation. For example, SFA were reported to promote inflammation by activating the TLR4 on fat cells and macrophages (31). Conversely, LC (n-3) PUFA were shown to induce broad, antiinflammatory effects in monocytic RAW 264.7 cells and in primary i.p. macrophages through the activation of GPR120 (also known as O3FAR1). The antiinflammatory effects of LC (n-3) PUFA were antagonized by knockdown of GPR120/O3FAR1 (32). Therefore, proteomic investigations may be useful for discovering the effects of dietary fatty acids on networks downstream of GPR and TLR and the development of protein biomarkers of inflammation.

⁶ Abbreviations used: 2D-GE, 2-dimensional gel electrophoresis; GPR, G protein-coupled receptor; LC, long chain; SEH, soluble epoxide hydrolase; TLR, Toll-like receptor.

FIGURE 1 Schematic overview indicating the role of blood/plasma, blood cells, and organ system samples for the measurement of intake proteomic biomarkers, or the development of systemic and cellular efficacy proteomic markers.

Pathways involved in the synthesis of eicosanoids are a major target for the prevention of inflammation and cancer. Upon release from the cell membrane phospholipid fraction by the action of various phospholipases, EPA, arachidonic acid [20:4 (n-6)], and dihomo- γ -linolenic acid [20:3(n-6)] are metabolized into various eicosanoids such as PG, thromboxanes, and leukotrienes (7,9). Eicosanoids produced from arachidonic acid are key stimulators of inflammatory and immune responses, promote tumor survival through inhibition of apoptosis (6), and stimulate cell proliferation, angiogenesis, and metastasis (9). Furthermore, some lipoxygenase products generated from arachidonic acid such as leukotriene-B₄ and 5-hydroxyeicosatetraenoic acid enhance the generation of reactive oxygen species, which damage DNA and contribute to cancer initiation (28). Conversely, EPA has been shown to compete with arachidonic acid, which is as a substrate for the cyclooxygenase-2, lipoxygenase, and P450 epoxygenase enzymes. Eicosanoid products of LC (n-3) PUFA are considered beneficial, because they do not induce the same level of inflammation as those synthesized from arachidonic acid (15). The combination of lipidomic platforms, which allows the identification and quantification of thousands of lipid species (33,34), with proteomic methodologies may be useful to dissect the role of food lipid-protein interactions in health and disease.

Proteomics in Fatty Acid Nutrition and Disease Prevention

Currently, we do not have reliable biomarkers to predict the health effects of dietary fatty acids. Proteomic platforms offer unparalleled opportunities to investigate complex food-protein interactions, discover novel molecular targets, and develop protein biomarkers of disease (Fig. 1) (16,35). Unfortunately, of the ~100 publications found in PubMed and Scopus related to the use of proteomics in fatty acid nutrition, only 14 studies with humans or animals have actually investigated the influence of dietary fatty acids on the cellular or biofluid proteome (36–49). Although the number of studies that have applied proteomics to the field of nutrition and disease prevention is small, their results clearly suggest proteomic-based approaches may be useful in the identification of protein targets of fatty acids that are related to disease development (Table 1).

Proteomics for discovery of biomarkers

The term biomarker has been defined as “a characteristic that is objectively measured and evaluated as an indicator of normal or

pathogenic processes or pharmacological responses to a therapeutic intervention” (50). This definition suggests at least 2 levels of investigation are needed for the development of protein biomarkers of fatty acid nutrition. First, proteomic research should focus on identifying changes in protein expression and post-translational modifications in response to dietary fatty acids. Second, candidate protein biomarkers should be validated in disease-specific models (e.g., inflammation, cancer) using other protein methodologies (e.g., Western blotting, ELISA) (51). For example, traditional protein methodologies have not effectively identified plasma proteins that can be used as biomarkers of disease risk, mainly because levels of proteins in plasma can vary by 10 orders of magnitude between individuals or between different states, i.e., healthy and diseased, control vs. experimental, etc. (52). Also, clinically relevant biomarkers may be present in blood at concentrations below detection levels (53). For example, in a controlled, single blind, randomized, multiple crossover trial, the 2-dimensional gel electrophoresis (2D-GE) technique was not effective in determining differences in plasma proteome following supplementation with 3 diets (3 wk each in random order) providing 7% of energy with *trans*-fatty acids, *cis*9-, *trans*11-CLA, or oleic acid (40). It is unclear whether differences in plasma proteome could not be detected because 2D-GE lacked sensitivity or because the plasma proteome was not affected by dietary fats. Conversely, in a double blind, randomized study in which healthy volunteers were assigned to a fish (3.5 g/d for 6 wk) or oleic sunflower (3.5 g/d for 6 wk) oil-based diet, 9 serum proteins were found to be differentially regulated by fish oil (39). Specifically, serum levels of APOA1, APOL1, zinc- α -2-glycoprotein, haptoglobin precursor, α -1-antitrypsin precursor, anti-thrombin III-like protein, serum amyloid P component, and hemopexin were down-regulated by fish oil compared with oleic acid-enriched or high-oleic acid sunflower oil supplementation. Results of the latter studies imply that fish oil may activate antiinflammatory and lipid-modulating mechanisms that alter early onset of cardiovascular disease. Similarly, proteomic studies with a rodent model reported a virgin olive oil-based diet lowered plasma concentrations of acute phase and antioxidant proteins during aging (44) (Table 1).

Candidate biomarkers of fatty acid efficacy discovered through proteomics should be further characterized using different protein technologies and then validated in small human populations and larger groups before they can be used as a surrogate for a specific clinical outcome (e.g., increased or reduced

TABLE 1 Proteomic studies on tissues and biofluids to identify molecular targets of dietary fatty acids in vivo in animal models or humans¹

Reference	Intervention	Technology	Number of regulated proteins	Regulated pathways	New biomarkers
42	Effects of CLA-enriched beef or beef supplemented with <i>cis9-</i> , <i>trans11-</i> CLA compared with linoleic acid, for 4 wk, on the adipose, hepatic, and skeletal muscle proteome in <i>M ob/ob</i> mice (8 mice/group)	2D-GE and MS	43 proteins in epididymal adipose tissue, 79 proteins in hepatic tissue, and 79 proteins in skeletal muscle	Cellular/oxidative stress, cytoskeletal integrity, glucose metabolism, fatty acid metabolism, energy metabolism	EHD2, CDC42, serine/threonine-protein phosphatase 2A, HSP
40	Effects of complete diets providing 7% of energy as industrial <i>trans</i> -fat or <i>cis9-</i> , <i>trans11</i> -CLA compared with oleic acid, for 2 wk, on the plasma proteome in healthy M (<i>n</i> = 12)	2D-GE and MS	Of the 818 resolved plasma proteins, 21 were significantly affected by the nature of the dietary intervention, 275 by period, and 177 by subject effects		
43	Effects of a diet rich or low in (n-3) fatty acid status, for 6 mo, on brain DHA status and the synaptic plasma membrane proteome (3 mice/group)	2D-GE and (16)O/ (18)O labeling	Of the 384 proteins from the SPM fraction (196 plasma membrane, 21 mitochondrial membrane, and 11 membrane-associated proteins), 18 synaptic proteins showed differential expression	Synaptic integrity, synaptic neurotransmission, caspase-3 pathways	CREB
44	Effects of dietary virgin olive oil, compared with sunflower oil, for 6 mo (young animals) or 24 mo (old animals), on the plasma proteome in Wistar rats (10 rats/group)	2D-GE and MS	54 of 144 plasma proteins	Acute phase proteins, antioxidant proteins, coagulation, lipid metabolism	
45	Effects of EPA and arachidonic acid, compared with oleic acid, for 6 wk on the colon proteome of <i>IL-10</i> gene-deficient mice (6 mice/ group)	2D-GE and MS	41 proteins from colon	Energy metabolism, signaling proteins, inflammatory/immune response proteins, cytoskeletal proteins, stress proteins	HSP-90AB1
47	Effects of dietary CLA, compared with no CLA, on the longissimus muscle proteome of finishing pigs (6 animals/group)	2D-GE and MS	26 of 598 proteins from muscle tissue	Energy metabolism, fatty acid oxidation, amino acid metabolism, defense, transport	Carbonic anhydrase-3 aspartate aminotransferase
41	Effects of DHA or fish oil, compared with high oleic acid sunflower oil, for 2 d and 1, 2, 4, and 10 wk on the hepatic proteome in apoE knockout mice (25 mice/group, 5 mice per time point)	2D-GE and MS	35 proteins from liver	Lipid metabolism, oxidative stress	SEH, APOA1, peroxiredoxin 3
46	Effects of oleic acid, compared with linoleic acid for 6 wk on the colon proteome of IL-10 gene-deficient and C57Bl/6 mice (6 mice/group)	2D-GE and MS	22 proteins from colon (16 in IL10 ^{-/-} mice and 7 in C57Bl/6 mice)	Metabolism, cytoskeletal, processes, immune system, apoptosis	FABP4 and FABP6, Calmodulin 1, TPM3
48	Effects of a diet rich or low in unsaturated fatty acids in F rats during gestation and lactation on the hepatic proteome of 3-4-old M pups (6 pups/diet)	2D-GE and MS	11 of >800 resolved hepatic proteins	Gluconeogenesis, redox balance, NO signaling	Argininosuccinate synthase
39	Effects of fish oil, compared with high oleic acid sunflower oil for 6 wk on the serum proteome of 81 healthy subjects	2D-GE and MS	9 of 350 resolved serum proteins	Lipid metabolism, inflammation	APOA1, APO11, zinc- α -2-glycoprotein, α -1-antitrypsin
36	Effects of a diet containing Pical or Arbequina olive oil, compared with palm oil, for 10 wk on the hepatic proteome in F <i>APOE</i> ^{-/-} mice (8 mice/ group)	2D-GE and MS	80 cytosolic proteins from liver	Lipid metabolism, glucose metabolism, redox balance	Adipophilin, betaine homocysteine methyl transferase

(Continued)

TABLE 1 Continued

Reference	Intervention	Technology	Number of regulated proteins	Regulated pathways	New biomarkers
49	Effects of fish oil, compared with corn oil, for 3 mo on the lung proteome in F344 rats treated with or without nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (6 rats/group)	2D-GE and MS	38 of 500 protein peaks		APOA1, Clara cell 17 kDa
37	Effect of <i>cis9</i> , <i>trans11</i> -CLA and <i>trans10</i> , <i>cis12</i> -CLA, compared with linoleic acid, for 12 wk on the hepatic proteome of <i>APOE</i> ^{-/-} mice (9 or 10 mice/group)	2D-GE and MS	113 cytosolic proteins from liver	Lipid metabolism, glucose metabolism, redox balance	HSP-70, MIF
38	Effects of fish oil, <i>trans10</i> , <i>cis12</i> -CLA or <i>trans9</i> -elaidic acid (18:1) on the hepatic proteome of <i>APOE</i> ^{*3} Leiden transgenic mice (8 mice/group)	2D-GE and MS	74 cytosolic and 14 membrane proteins from liver	Lipid metabolism, glucose metabolism, redox balance	LC acyl-CoA thioester hydrolase protein, adipophilin, sepiapterin reductase, cysteine sulfonic acid decarboxylase

¹ 2D-GE, 2-dimensional gel electrophoresis; LC, long chain; MIF, macrophage migration inhibitory factor; SEH, soluble epoxide hydrolase.

risk of cancer, inflammation). Although concerns persist about the complexity of developing biomarkers for specific diseases (53), proteomic technologies have the potential to clarify how fatty acids influence disease states.

Discovering new mechanisms of action of fatty acids

Several studies have successfully used proteomic technologies to study how fatty acids influence pathways involved in glucose and fatty acid metabolism, antioxidant defense and redox states, and inflammation (Table 1) (36–49). In a proteomic study that used a rodent model, the dietary supplementation (20% wt:wt) with extra virgin olive oil for 10 wk induced (1.5- to 4.0-fold) hepatic protein levels of thioredoxin peroxidase-2, peroxiredoxin-3, superoxide dismutase, and glutathione-S-transferase compared with control oil (palm oil) (36). These findings suggested that components in olive oil diminished oxidative stress elicited by hepatic steatosis while reducing the risk of atherosclerosis. In this case, the use of proteomics allowed the detection of changes in the expression of proteins specific to pathways involved in inflammation. It is likely these changes would not have been observed using traditional protein methodologies.

Proteomic tools have also been successfully used to characterize the mechanisms through which LC (n-3) PUFA modulate eicosanoid metabolism and inflammation (41). An intervention with fish oil (2% wt : wt), but not DHA, was found to lower the protein levels of the hepatic soluble epoxide hydrolase (SEH) in male *APOE*^{-/-} mice. The differential effects of DHA and fish oil suggested that EPA may be responsible for lowering the expression of hepatic SEH, which converts epoxides to their corresponding inactive diols (15). Therefore, by reducing hepatic SEH protein, EPA may increase the levels of cardioprotective and antiinflammatory epoxyeicosatrienoic acids.

Proteomic studies revealed that heat shock proteins (HSP) are molecular targets for CLA and LC (n-3) PUFA (37,42,45). Specifically, *cis9*- and *trans11*-CLA were reported to increase hepatic levels of several post-translationally modified HSP-70, whereas the protein levels of HSP-70 were not altered by *trans10*- and *cis12*-CLA (37). These proteomic studies highlighted the differential effects of CLA isomers on HSP-70 expression. The induction of HSP-70 expression by *cis9*- and *trans11*- CLA may protect against subsequent exposure to severe stresses (54), possibly through attenuation of proinflammatory NFκB- (55) or cyclooxygenase-2- (56) regulated pathways. Other proteomic experiments revealed dietary EPA reduced the expression of HSP-90AB1 in colonic mucosa of IL10^{-/-} mice, a model of inflammatory bowel disease (45). Decreased levels of HSP-90B1, HSP-A5, and HSP-E1 were linked to lower endoplasmic reticulum stress (45), which is involved in the activation of various proinflammatory responses (57) and suppression of T cell tolerance (58). Because endoplasmic reticulum, oxidative, and inflammatory responses are regulated by multiple, likely overlapping protein networks, these examples clearly indicate proteomic tools are ideal to unravel how fatty acids regulate complex biological processes.

Future of Proteomics and Dietary Fatty Acid Intervention Studies

Proteomics platforms provide unprecedented protein separation and mass spectrometric detection methods to identify protein targets for dietary fatty acids. However, the future success of proteomics research applied to fatty acid nutrition and disease therapy requires progress in several areas. First, there is a need to

standardize procedures for the acquisition and preparation of samples from tissue and biofluids. The need for standardized methodologies has been emphasized by results of a proof-of-principle initiative funded by the European Nutrigenomics Organization, a Network of Excellence funded by the European Commission (59,60). The objective of this initiative was to compare differences in plasma proteomes of participants who underwent prolonged fasting using a 2D-GE technique or an assay of 87 plasma antigens (Fluorokine Multianalyte Profiling Assay). Although the 2 methods had comparable average CV, the number of differentially expressed plasma proteins detected with the Fluorokine Multianalyte Profiling Assay was significantly higher than that observed using the 2D-GE approach (B. de Roos, unpublished observations). This example clearly highlights the need for standardization of methodologies in nutritional proteomics. Second, because proteomic studies identify novel molecular targets for dietary fatty acids in tissues (e.g., the liver, adipose, colon) and biofluids (e.g., plasma, urine), validation is necessary to confirm their usefulness to predict disease risk and discriminate between responders and nonresponders. Third, proteomic approaches applied to fatty acid research should adopt “multiplexed” approaches, which allow the simultaneous measurement of multiple proteins through the use of triple quadrupole-derived MS technologies and ion traps (60). Unquestionably, the adoption of multiplex protein assays would accelerate the quantitative and qualitative analysis of peptides in complex biological mixtures such as tissues and biofluids. The ability to detect changes in low-abundance proteins may also be improved through the adoption of multiple reaction monitoring approaches. By adding calibrated, isotopically labeled reference peptides, precise quantitative information can be obtained about the effects of fatty acids on protein networks (16,61). Indeed, the combination of MS and multiple reaction monitoring platforms should enhance monitoring of subtle changes in low-abundance proteins in response to fatty acid intervention. Finally, chemical proteomics represents yet another MS-based affinity chromatography approach that may prove very valuable in fatty acid nutrition. In chemical proteomics, protein lysates from plasma, blood cells, or tissues are incubated with an affinity matrix containing an immobilized bioactive compound (e.g., fatty acid). The bound and subsequently eluted proteins can be separated and identified using SDS-PAGE or shotgun proteomics and nano-electrospray ionization-tandem MS (62). The latter approach is very useful for the development of fatty acid-protein interaction profiles. In summary, multiplex methods should be used in high-throughput assays to identify and validate newly discovered protein biomarkers in human plasma, blood cells, and tissues. The combination of proteomic tools with bioinformatics holds great potential to assess how dietary fatty acids influence the risk of cancer and other chronic diseases.

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